Proteomic Body Fluid Assay Validation

Validation of Molecular Serology's proteomic mass spectrometry assay for the identification of blood, saliva, and semen follows SWGDAM DNA validation guidelines and was performed on a SCIEX Eksigent high performance liquid chromatography (HPLC) instrument and QTRAP 6500 mass spectrometer.

A brief overview of the validation and definitions of the statistical methods precedes a description of the work conducted. Additional validation documents include:

1) Supplemental Table 1 - Summary of Experiments & Samples Numbers
2) Supplemental Table 2 - Volunteer Samples (anonymized but including demographic data)
3) Supplemental Table 3 - Standards & Body Fluid Marker Peptides and Transition Ions & Species Summary
4) Supplemental Document 1 - Description of Classification Assay Metrics
5) Body Fluid Proteomics SOP
6) LCMS MRM Interpretation SOP
7) Body Fluid Proteomics System Suitability

NB: This validation addresses all applicable SWGDAM Validation Guidelines. Descriptions of all experimental designs are given below. Benchwork details for all experiments – including the numbers of samples run, sample concentrations, sample dilutions, etc. are given in detail in Supplemental Table 1 Summary of Experiments & Samples Numbers.

ABBREVIATIONS

ANOVA - analysis of variance
BCA - bicinchoninic acid
BSA - bovine serum albumin
CI – confidence interval
CTS – Collaborative Testing Services
CV – coefficient of variation
HPLC - high performance liquid chromatography
LOD - limit of detection of the assay
LOQ - limit of quantitation
LOB – limit of blank
MRM – multiple reaction monitoring
MS - mass spectrometry
PT –Proficiency Test
QA – Quality Assurance
QC – Quality Control
SDC – sodium deoxycholate
SOP – Standard Operating Procedure
SWGDAM - Scientific Working Group on DNA

Analysis Methods

Overview: This validation encompasses the entire Proteomic Body Fluid Assay for blood, saliva, and semen from sample extraction through MS and body fluid identification. Due to the nature of protein assays - protein expression varies between individuals - it was important to validate the assay with large numbers of different individuals in order to accurately evaluate results. Consequently, this validation tests samples from 20 individuals for blood, 25 for saliva, and 27 for semen for a total of 72 individual samples. Two analysts performed the validation. The numbers of samples tested and the numbers of times they were processed may be found in Supplemental Table 1 and in the Summary of Samples Tested section below. Both neat and mock samples were evaluated. Neat samples were collected under NYC's Department of Health

and Mental Hygiene IRB No. 18-018. Mock samples were obtained from the Department of Forensic Biology's QA/QC group and consisted of DNA proficiency tests from Bode Technologies (Lorton, VA, USA), and Collaborative Testing Services, Inc. (Sterling, VA, USA) received by FBio between the years 2009 to 2020.

The assay has two parts. The first is a quantitative determination of the amount of protein in a sample. The second is a classification system that determines what body fluid is present in a sample. Data analysis was performed for each assay to evaluate sensitivity, accuracy, and precision. It is important to note that the metrics for accuracy and precision for quantitative and classification assays differ, and the quantitative and classification assays will be evaluated by their respective metrics (see "Quantitation Assay - Definition of Statistical Terms" page 2, "Classification Assay Performance Metrics" page 4, as well as Supplemental Document 1 "Description of Classification Assay Metrics").

Validation Criteria - This validation was based on the Scientific Working Group on DNA Analysis Methods (**SWGDAM**) Validation Guidelines approved in December 2012 (most recent) and evaluated six criteria:

1. Sensitivity
2. Accuracy
3. Precision and Repeatability
4. Mixed Samples
5. Non-Targeted Samples (vaginal fluid & non-human blood, saliva and semen) *
6. Sample Degradation

*****SWGDAM validation criterion No. 5 addresses sample contaminants that can diminish DNA detection – e.g., inhibitors of DNA polymerase. As there are currently no known contaminants that inhibit protein detection by mass spectrometry, criterion No. 5 addresses non-targeted body fluids (e.g., vaginal fluid and non-human blood, saliva and semen).

Assay Workflow – The molecular proteomic serology assay has three main steps:

1. Sample protein extraction and quantitation
2. Protein processing and digestion
3. Peptide separation by HPLC and identification by MS

Assay Validation – Two analysts performed the validation. Methods followed the Proteomic Body Fluid SOPs. As discussed, the validation was performed in two parts: **i)** protein quantitation, and **ii)** marker peptide identification by mass spectrometry. Supplemental Table 1 (Excel) summarizes experiments performed and gives a complete list of the number of samples extracted, number of replicates, and number of runs.

PART 1 – Protein Quantitation Validation

QUANTITATION ASSAY - DEFINITION OF STATISTICAL TERMS – There is some ambiguity in the literature regarding what exactly is being measured by precision, repeatability, and reproducibility. For the purpose of this validation these terms are defined below.

- **PRECISION** - Precision for a quantitative assay is defined as the measurement of the extent of variation in repeat measurements of the same sample by the same method.
- **REPEATABILITY** - Repeatability is a subset of precision, measuring variation in measurements obtained by the same method in a short time frame.
- **INTERMEDIATE PRECISION** (frequently confused with reproducibility) - Intermediate precision, evaluates variation in measurements that occur when specific changes are made to a method one at a time. Here we assess effects on assay results obtained on different days by the same analyst as well as by different analysts.
- **REPRODUCIBILITY** - Precision and accuracy of results (quantitative and/or qualitative) among different operators and/or instruments are evaluated.

Materials –

- 20 samples of blood, 24 of saliva and 27 of semen from anonymized females and males of different ages and ethnicities (Table 2).
- Pierce BCA Protein Assay Kit (C/N 23225/23227; Thermo Fisher Scientific)
- BSA Pierce (C/N 23225; Thermo Fisher Scientific, Waltham, MA)
- 500 mM tris 2-carboxyethyl phosphine (TCEP), pH 7.0 (Sigma, 646547-10X1ML)
- Promega, Trypsin Gold MS Grade, V5280
- MS Quantitation Standard Cytochrome C Digest (Fisher Scientific, 161089)
- 10% Sodium deoxycholate (SDC, Fisher Scientific 50-255-884)
- QA PT Test Kits from Bode Technologies and Collaborative Testing Services, 2009-2020

Validation of Protein Quantitation: Bicinchoninic acid (**BCA**) assay results are measured spectrophotometrically at a wavelength of 562 nm. Validation of the BCA assay was done in two parts. Part A used the BSA standard to determine the sensitivity, precision, and repeatability of the quantitation assay. Part B used a subset of blood, saliva, and semen samples to evaluate precision and repeatability of measuring sample proteins. Body fluid samples were extracted following the Proteomic Body Fluid SOPs. See Supplemental Table 1 for details.

1A – Protein Quantitation Assay using Standard Protein BSA

1A.1. SENSITIVITY – The BCA assay has a linear working range from 20 to 2,000 ng protein/µL[1]. To validate sensitivity, BSA standard was serially diluted from 2,000 ng/µL to 10 ng/µL (i.e., below the manufacturer's stated limit of detection) as described below. Samples were run in triplicate on 6 different days by two analysts (468 total samples; Supplemental Table 1). These data were used to establish the linear range, limit of quantitation (**LOQ**), and limit of detection of the assay (**LOD**). See Supplemental Table 1 for details.

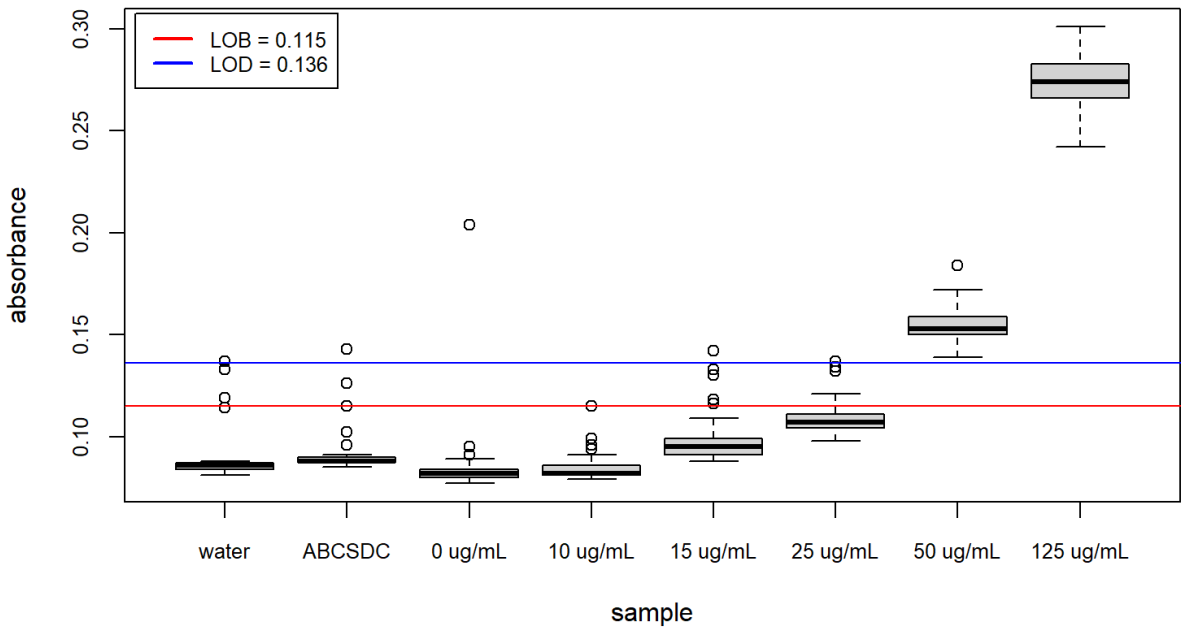
Protein Standard Serial Dilution ng protein/µL

2,000

1,500	
1,000	
750	
500	
250	
125	
50	
25	
15	
10	
Negative Control (Buffer Blank)	
Water Blank	

Measurements of 0 µg/mL of BSA (water and buffer (ABC/SDC only) were used to establish the highest expected absorbance in the absence of protein in a sample (limit of blank, **LOB**). Variation at low concentrations of BSA standards was used to establish limit of detection (LOD) above LOB.

The LOD for BSA was determined to be ≥ an absorbance of 0.136, which is equivalent to ~50 µg/mL of BSA.



Based on the low CV for 50 µg/mL BSA preparations, the LOQ is functionally the same as LOD at 50 µg/mL of BSA.

1A.2. REPEATABILITY & PRECISION – Repeatability (a subset of precision, measuring variation in measurements obtained by the same method in a short time frame) was evaluated by analysis of variance (**ANOVA**) using the BSA sensitivity validation data generated above. To evaluate the repeatability of BSA standard and BCA plate preparation, only measurements of BSA batches that were run on BCA plates on three days were used.

The CV between batch variation and between plate variation are low, indicating that these procedures do not introduce unacceptable variation to the measurement. Total variation is within tolerance.

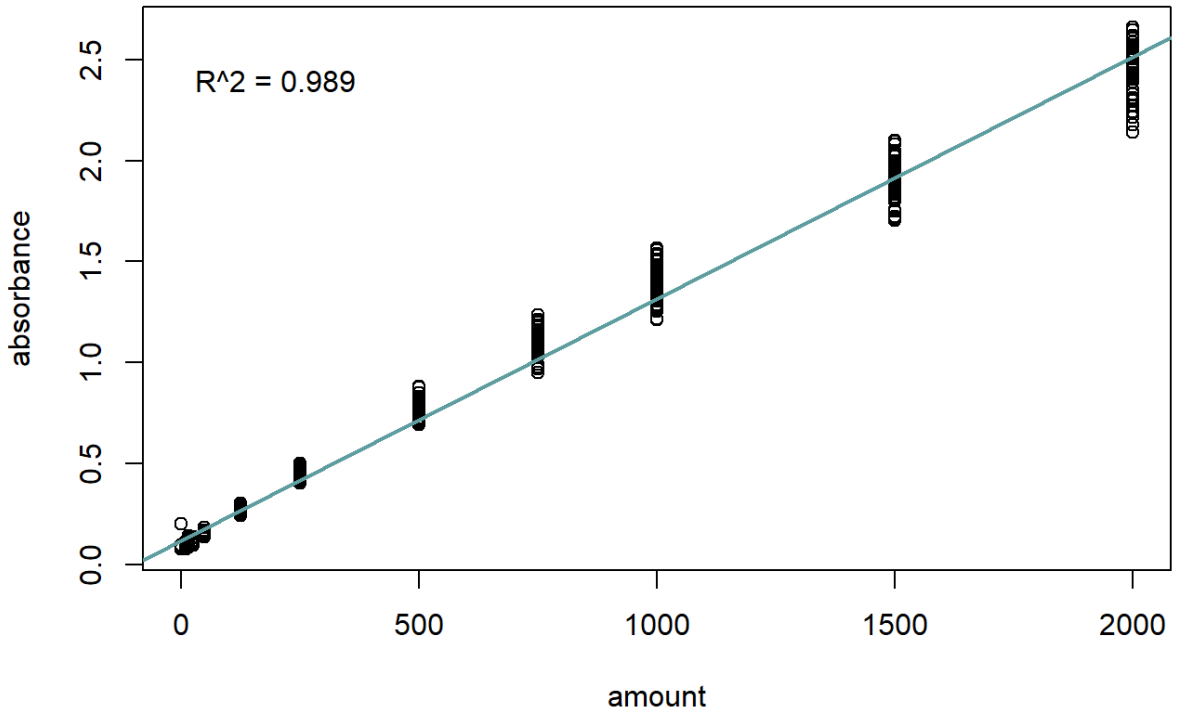
	CV (%)	95% CI Lower Limit	95% CI Upper Limit
Within Group	10.304	6.960	19.741
Between BCA plates	2.004	1.339	14.266
Between BSA batch preparations	2.361	1.046	9.449
Total	10.497	10.096	17.617

Considering repeat measurements of the same preparation of BSA on the sample plate, variation is higher at the lowest BSA concentrations. However, only 0 µg/mL had a CV above 15%.

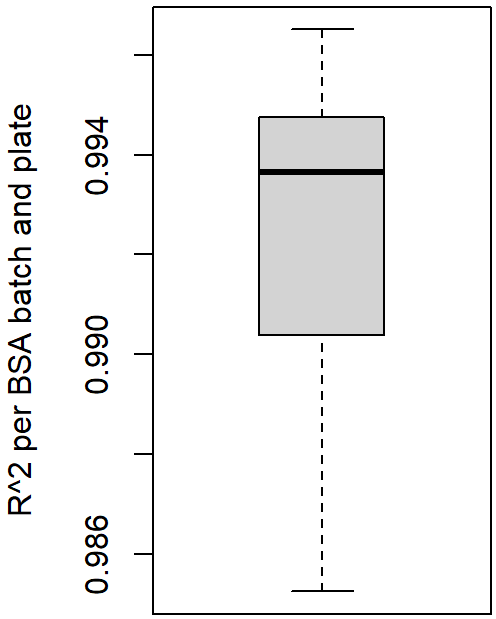
CV (%)	95% CI Lower Limit	95% CI Upper Limit	Mean Absorbance	BSA concentration(µg/mL)
5.443	4.633	7.111	2.431	2000
5.680	4.614	7.945	1.908	1500
6.221	5.138	8.535	1.395	1000
5.931	4.892	8.152	1.096	750
5.515	4.561	7.555	0.791	500
5.025	4.146	6.905	0.455	250
4.810	3.932	6.682	0.274	125
5.132	4.318	6.858	0.155	50
7.194	6.069	9.567	0.109	25
11.360	9.636	14.957	0.098	15
7.405	6.232	9.889	0.084	10
20.022	17.097	25.930	0.085	0

Linearity

The R^2 value for all standard measurements across multiple BSA preparations and plate runs is 0.989, as shown in the figure below.



The mean R^2 value per BSA batch and plate, which are the conditions in which the standards curve for sample evaluation is established, was 0.993.



1B – **Protein Quantitation Assay using Samples of Blood, Saliva, and Semen –** Sensitivity, precision, and repeatability of the BCA assay were measured using neat samples of blood, saliva, and semen (see Supplemental Table 1 for details). Samples were extracted following Proteomic Body Fluid SOPs. All analyses were performed on supernatants of extracted samples.

1B.1. SENSITIVITY – The amount of protein in blood, semen, and saliva varies on a volume basis with neat blood having ~250 ng protein/µl, semen ~25 ng protein/µl, and saliva ~1.5 ng protein/µl. The protein concentration of saliva is significantly more variable than semen or blood. Consequently, two donor samples were used to assay protein content in blood and semen while three donor samples were used for measurements in saliva. It is important to note that the focus of protein quantitation is to measure the amount of protein prior to performing HPLC in order not to overload HPLC columns with an excessive amount of protein which would result in poor peptide separation and high column carryover leading to increased background. In this assay, four dilutions of each body fluid were prepared: 3 dilutions within the BCA assay's working range and one near the LOQ established using BSA in Section 1A.1. Each dilution of each sample was performed in triplicate on 6 different days by two analysts (see Supplemental Table 1). Samples were analyzed and used to establish linearity and approximate (since exact body fluid protein content is unknown) LOQ and LOD for all body fluids.

Based on the LOD established in section 1A.1, measurements of semen, saliva, and blood were assigned as detectable as defined by an absorbance greater than 0.136 and predicted concentration above 50 µg/mL. The tables below show the percentage of semen, saliva, and blood measurements that were detectable for different dilutions of extracts. Again, it should be noted that all samples, regardless of quant, are processed through LC-MS. Quantitation is performed to ensure LC columns are not overloaded.

Semen

Dilution Factor	Percent Detectable (%)
1	100
2	100
4	89.8
8	55.9
10	89.8
20	61.9
32	0
64	0

Semen samples diluted at 32x and 64x are too dilute to read within the limits of the assay.

Saliva

Dilution Factor	Percent Detectable (%)
1	89.70%
2	38.70%
3	16%
4	0.94%
5	0%
10	0%
20	0.78%

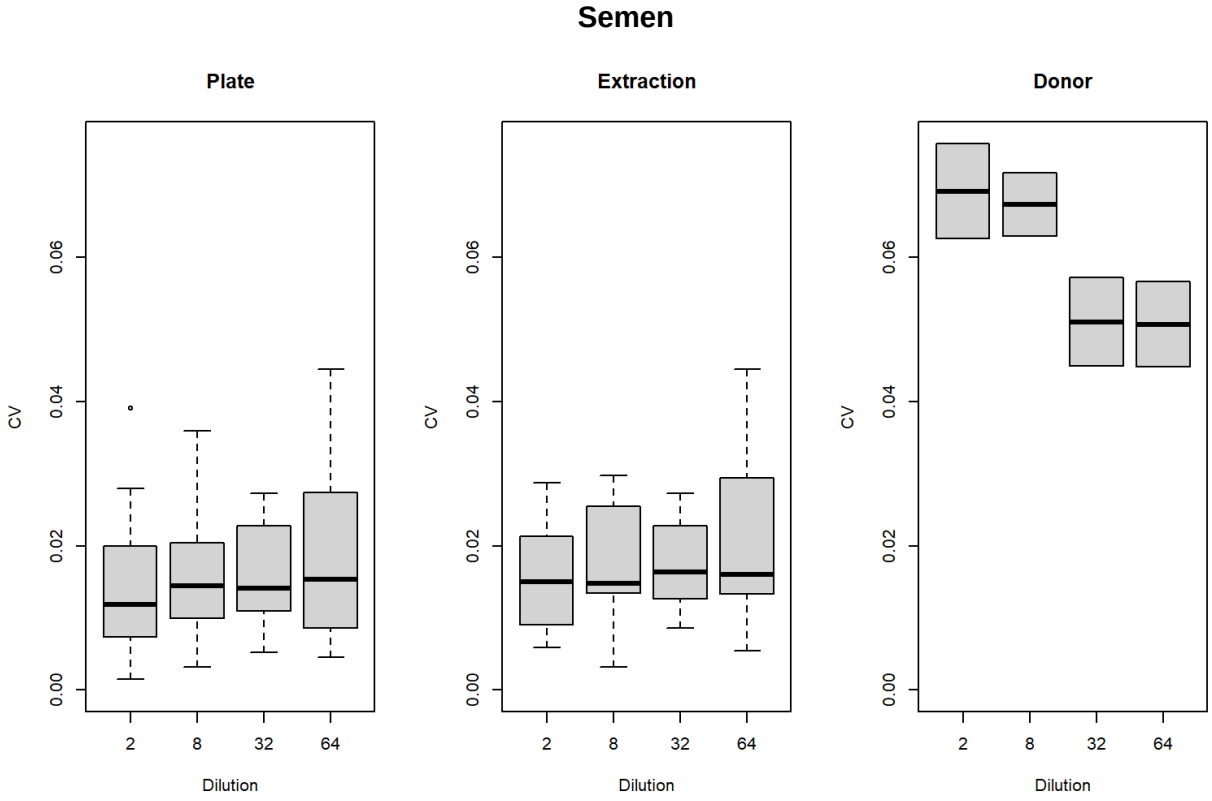
Protein concentration in saliva is more variable than semen and blood, however undiluted saliva extraction shows the best sensitivity for the assay.

Blood

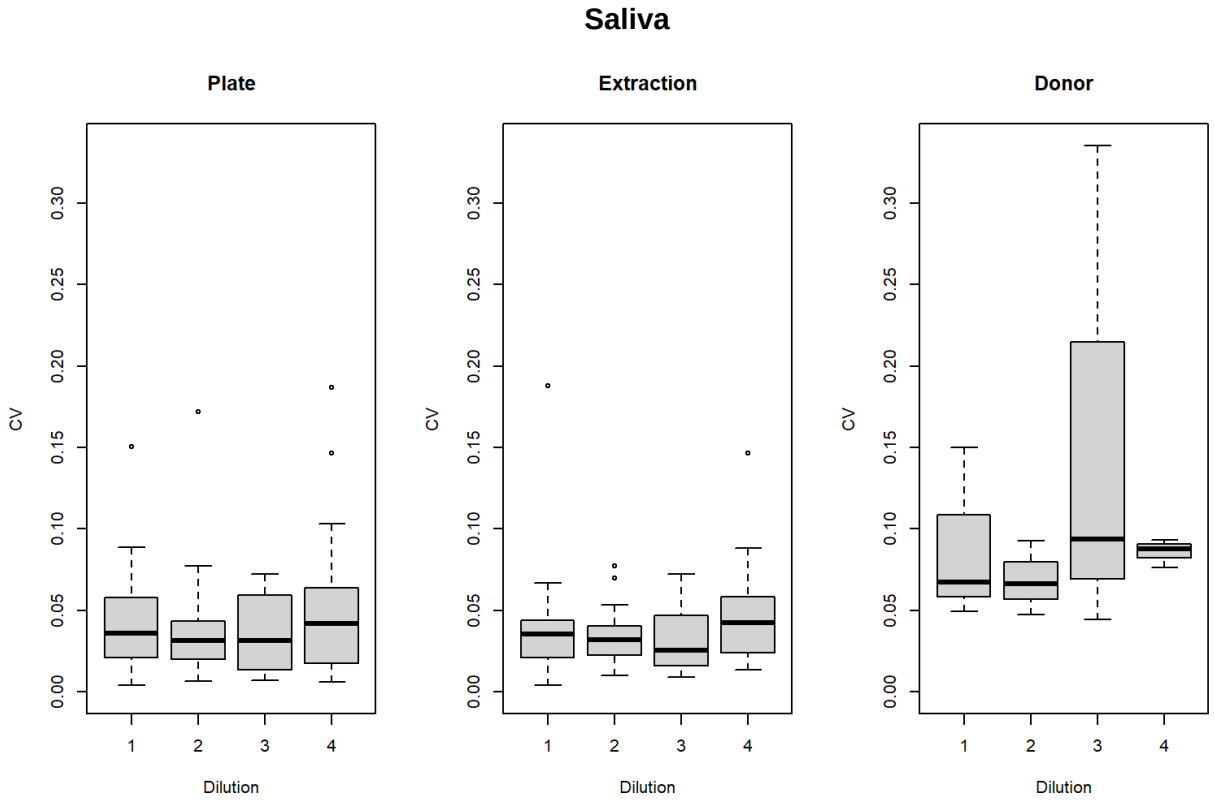
Dilution Factor	Percent Detectable (%)
5	100
10	100
20	100
40	100
200	8.33

1B.2. PRECISION AND REPEATABILITY – Precision and repeatability analyses were done on all samples from Section 1B.1 above in the manner described in Section 1A.2 (see Supplemental Table 1).

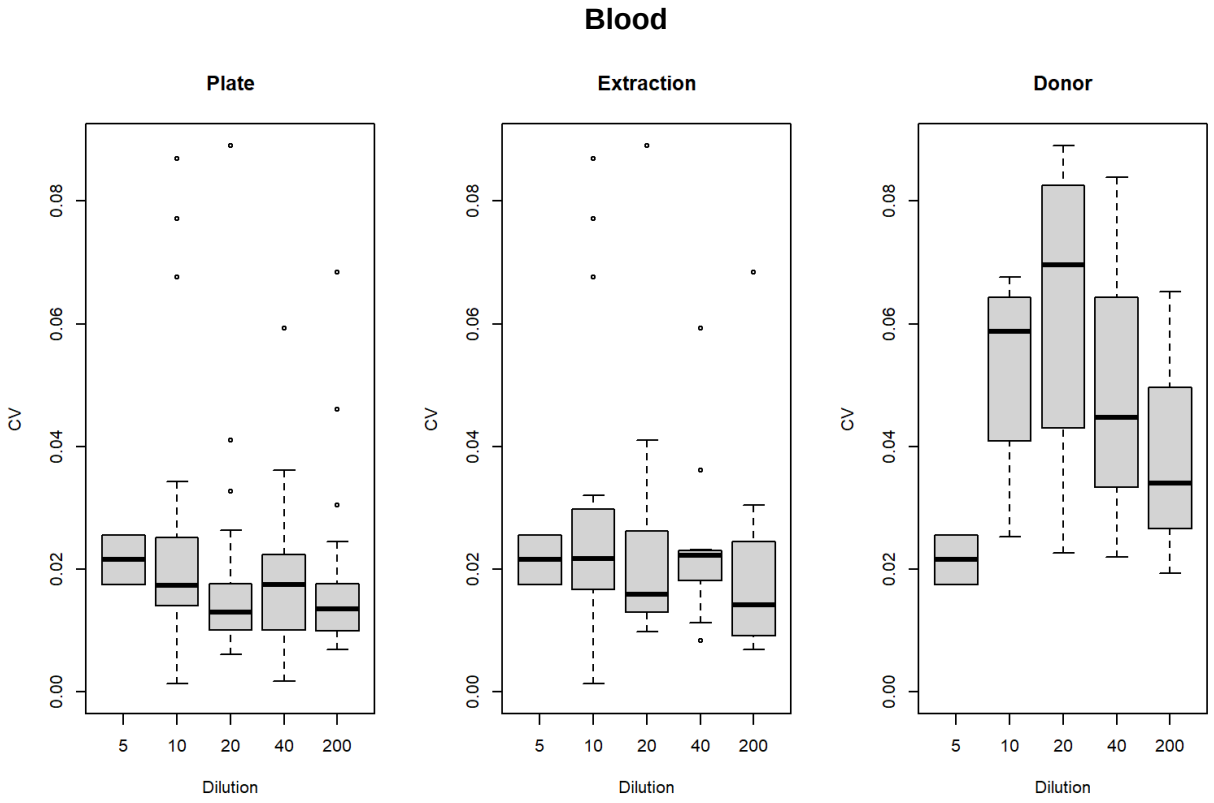
For samples where the same extraction and dilution were measured on the same plate at least 3 times, CVs were calculated for plate, extraction, and donor.



While absorbance CV increases for different extractions from the same semen donor, all are below 10%.



Absorbance CV for saliva samples is low for repeat measurements of the same extraction, but variation increases more dramatically for different extractions from the same donor.



Similar to semen, blood absorbance CV, while higher for different extractions, is consistently under 10%.

PART 2 – Body Fluid Identification / Classification Assay

Section 2 – Body Fluid Marker Identification - Following protein extraction and quantitation, samples were processed (reduced and alkylated) and then digested with trypsin (Proteomic Body Fluid SOPs). Tryptic peptides were then separated by HPLC and analyzed by mass spectrometry. Body fluid identification is based on detection of body fluid specific peptide markers and their fragment ions (see Table 3). A positive body fluid identification is made when all marker peptides and all peptide transition ions are detected. Validation of body fluid marker identification was performed on 72 body fluid samples, 20 of blood, 24 of saliva, and 27 of semen. Accuracy, sensitivity, specificity, and repeatability were assessed.

CLASSIFICATION ASSAY PERFORMANCE METRICS & DEFINITION OF STATISTICAL TERMS – Classification assays are not quantitative. They are based on identifying a sample result as a

true positive (positive sample with positive test result), a false positive (negative sample with positive result), a true negative (negative sample with negative result), or a false negative (positive sample with negative result). For each body fluid in this validation, positive samples are all known samples of that body fluid. Negative samples are the two other body fluids plus negative controls and non-target samples (e.g., vaginal fluid and non-human blood, saliva, and semen). True positives and true negatives are correct results, false positives and false negatives are errors. Classification metrics based on these results are ratios (often reported as percentages), and defined as follows:

- **ACCURACY:** The total correct classifications / total samples tested
- **SENSITIVITY:** Sensitivity is total true positives / total positive samples tested (true positives and false negatives), and is the inverse of the false negative rate.
- **FALSE NEGATIVE RATE:** The false negative rate is total false negatives / total positive samples tested and is the inverse of sensitivity.
- **SPECIFICITY:** Specificity is total true negative / all negative samples tested (true negatives and false positives) and is the inverse of false positive rate.
- **FALSE POSITIVE RATE:** The false positive rate is the is the total false positives / all negative samples tested and is the inverse of specificity.
- **FALSE DISCOVERY RATE:** The false discovery rate is the total false positives / all positive results obtained, and the primary metric used in this validation to understand false positives (or in this case lack thereof) and the confidence that can be applied to that result.
- **REPEATABILITY:** Repeatability in classification assays is the ability of the assay to obtain consistent classification results for replicate measurements of the same sample. Repeatability of the assay will be assessed at the level of analyst, extraction batch, and HPLC column injection using the samples described above.

SAMPLE PREPARATION & HPLC/MS RUNS: Neat samples from five individuals for each body fluid (blood, saliva, and semen) were extracted three separate times by two analysts (see Supplemental Table 1) following Proteomic Body Fluid SOPs. The remaining 15 samples of each blood, saliva, and semen were extracted once each by two analysts. An extraction negative control was included in each extraction batch. Sample supernatants from each extraction were analyzed from protein quantitation through HPLC-MS (Supplemental Table 1). Each sample and negative control were spiked with a cytochrome C peptide (to measure HPLC sample loading). Each sample and negative control were run twice by HPLC-MS, once through each of the two HPLC's columns. Total numbers of samples and HPLC/MS runs are outlined in Supplemental Table 1.

Mock body fluid samples consisted of DNA proficiency tests samples received by the Department of Forensic Biology between the years 2009 to 2020. It is important to note that while the type of body fluid used in preparing these samples was known, the amount of protein they contained was not. Additionally, these kits only provided samples for semen and blood. Saliva mock samples were created by collecting buccal swabs from volunteers. Known and non-probative/mock evidence samples of blood, saliva, and semen were also prepared by pipetting 20 µg, 10 µg, and 2 µg of protein (measured from neat body fluid samples) onto cotton swabs. Mock samples from 5 donors for each body fluid were extracted 3 times each according

to Proteomic Body Fluid SOPs and run by two analysts. Each sample was run twice through HPLC-MS (once through each HPLC column, see Supplemental Table 1).

MIXED SAMPLES - In order to assess the classification assay's performance in the case of a sample containing a mixture of body fluids, mixtures of blood/semen, blood/saliva, and semen/saliva were prepared. Three samples from three different individuals of each blood, semen, and saliva were mixed in a 1:1 protein ratio, extracted, processed, digested, and prepared for HPLC-MS according to Proteomic Body Fluid SOPs. In addition, one sample from one individual was used to make mixtures of blood/semen, blood/saliva, and semen/saliva in 1:4, 1:8, 1:12, 12:1, 8:1, and 4:1 protein ratios, and blood/saliva/semen mixtures in 1:1:1, 1:1:10, 1:10:1, and 10:1:1 protein ratios. Each mixture was run twice through HLPC-MS, once through each column (see Supplemental Table 1 for details). Classification results for blood, semen, and saliva were analyzed as described above for neat samples.

NON-TARGETED SAMPLES - Non-targeted samples were human body fluids other than blood, semen, and saliva, as well as non-human body fluids. Human samples tested were vaginal fluid (expected negative for blood, saliva, and semen), and menstrual blood (expected positive for blood). Non-human samples were blood from Canis lupus (dog), Felis catus (cat), Bos taurus (cow), Sus scrofa (pig), and Gallus gallus (chicken), saliva from Canis lupus (dog) and Felis catus (cat), and semen from Sus scrofa (boar) and Bos taurus (oxen). All samples were extracted and analyzed by HPLC-MS according to Proteomic Body Fluid SOPs (see Supplemental Table 1 for details).

AGED SAMPLES - Aged samples were OCME DNA proficiency test kits from Bode Technologies and Collaborative Testing Services, Inc. prepared from whole body fluids on cotton swabs, cards and fabric dating back to 2009. Aged mock samples were extracted according to Proteomic Body Fluid SOPs and run by two analysts. Each sample was run twice through HPLC-MS, once through each column. See Supplemental Table 1 for details.

DATA ANALYSIS: This assay functions as three binary classification tests, one each for blood, semen, and saliva (it is possible to have positive results for more than one classification in the case of a mixed sample). The performance metrics reported below are from results for neat, known, and non-probative/mock evidence samples (aged and non-aged), non-target samples, and mixed samples as described above.

The specific cutpoints for all peptide metrics (i.e. the threshold that must be met for each metric to determine that the peptide is present in the sample) were determined by values that optimized precision and sensitivity on multiple subsets of metric data for each peptide and averaging the results. The resampling and averaging process prevents overfitting. Expected ion ratios for each target body fluid peptide were generated from 396 HPLC-MS runs of labeled synthesized peptides. The ion ratios were largely consistent over all runs, with narrow distributions. The median over all runs was selected as the expected ratio for each fragment ion which minimizes the effects of outliers. Decision criteria for identification of a peptide using the cutpoints, and cutpoints themselves, are found in the LCMS MRM Interpretation SOP.

Summary of Samples Tested

The validation includes a total of 1496 LCMS runs, each with associated preceding cleaning solution run. Of these, 3 runs were negative with no detection of Cytochrome C control, indicating failed or incomplete LCMS injection, and were removed from the data set. This results in a total of 1493 LCMS runs of 691 sample extractions.

Number of sample extractions and LCMS injections by sample type

Sample Type	Number of Extractions	Number of Injections
Blood	175	358
Blood Saliva	11	22
Blood Saliva Semen	4	12
Blood Semen	18	36
Menstrual Blood	4	8
Negative Control	130	337
Non-Human Blood	11	22
Non-Human Saliva	6	12
Non-Human Semen	10	20
Saliva	144	293
Saliva Semen	11	24
Semen	163	341
Vaginal Fluid	4	8

Neat Body Fluid Samples

Neat Single Source Samples

A total of 244 neat single-source body fluid samples were tested.

Number of individual donors and sample extractions of neat single source body fluid samples

Sample Type	Number of Individuals	Number of Extractions
Blood	20	76
Menstrual Blood	2	4
Saliva	25	74
Semen	27	90

Neat Mixture Samples

A total of 37 neat mixture body fluid samples were tested, shown in the table below by sample type. For each 2-component body fluid mixture, five 1:1 mixtures of different individual donors were tested, as well as 1 mixture at each of the following mixture ratios: 1:4,1:8,1:12,4:1,8:1, and 12:1. Ratios are by total protein amount. In addition, 3-component Blood Saliva Semen mixtures were tested at 1:1:1, 1:1:10, 1:10:1, and 10:1:1 protein ratios.

Number of individual donors and sample extractions of neat body fluid mixture samples

Sample Type	Number of Individuals per BF	Number of Extractions
Blood Saliva	3	11
Blood Saliva Semen	1	4
Blood Semen	3	11
Saliva Semen	3	11

Mock-Casework Type Body Fluid Samples

A total of 249 mock casework type samples of human body fluid were tested.

Swabs with known amounts of body fluid protein

A total of 180 mock samples with known amounts of protein were tested. Number of swabs extracted for each body fluid, per total amount are shown below.

Extractions of dried stain swabs with known amounts of body fluid deposited

	Blood	Saliva	Semen
0.05ug	6	6	6
0.1ug	6	6	6
0.25ug	6	6	6
0.5ug	6	6	6
1ug	6	6	6
2ug	10	10	10
10ug	10	10	10
20ug	10	10	10

Number of individual donors used to prepare swabs

Body Fluid	Number of Individuals
Blood	5
Saliva	6
Semen	6

Mock Samples with Unknown Amount of Protein

A total of 69 mock samples with unknown/uncontrolled amount of protein deposited were tested, including blood cards, blood and semen stains on fabric and swabs, and buccal swabs.

Extractions of mock-type samples of known body fluids with unknown/uncontrolled amounts of protein

Sample	Card	Fabric	Swab
Blood	31	8	0
Blood Semen	0	7	0
Saliva	0	0	10
Semen	0	0	13

Non-Target Samples

Non-target samples do not contain any of the body fluids targeted by this assay and are tested to confirm lack of false positive results. A total of 161 non-target samples were tested, including vaginal fluid, non-human body fluids, and extraction negative controls.

Sample Type	Number of Extractions
Negative Control	130
Non-Human Blood	11
Non-Human Saliva	6
Non-Human Semen	10
Vaginal Fluid	4

Body Fluid Identification Performance Summary

Limits of Detection from Dried Stains

Reliable detection of most body fluid marker peptides can be achieved with approximately 20 ng of digested body fluid protein injected into the LCMS. When a sample contains enough protein for a reliable protein quant determination by BCA, 200 ng of digested total protein is injected into the LCMS for reliable, robust detection. However, extraction from dried stains frequently do not yield a high enough concentration of protein for detection with BCA, but a body fluid marker profile can still be obtained by LCMS, which is more sensitive. To determine the minimum amount of dried body fluid material needed to obtain a profile from LCMS after extraction, quantitation, and digestion, known amounts of protein were deposited on cotton swabs and allowed to dry overnight before extraction.

The table below shows the sensitivity of detection at each protein amount of blood, semen, and saliva.

Known amount of semen and saliva dried overnight on swabs

Protein Amount (µg)	Blood	Saliva	Semen
0.05	0%	0%	0%
0.10	0%	0%	83.3%
0.25	0%	33.3%	100%
0.50	16.7%	66.7%	100%
1.00	83.3%	83.3%	100%
2.00	100%	100%	100%
10.00	100%	90%	100%
20.00	100%	100%	100%

Different body fluids have different amounts of protein per same unit volume – e.g. blood has approximately 10 times more protein per µl then semen, and about 167 times more protein than an equivalent volume of saliva. The table below shows average protein concentrations of undiluted neat body fluids. It should be noted however, that the natural variation in protein concentration of saliva and semen is considerably greater than that of blood.

Protein Concentrations of Body Fluid

Body Fluid	Protein Concentration
Blood	250 µg/µL
Semen	25 µg/µL
Saliva	1.5 µg/µL

The lowest amount of deposited protein for which a full peptide profile was obtained in 100% of samples was 2 µg for blood and saliva, and 0.25 µg for semen. This corresponds to ~8 nL of blood, ~1.3 µL of saliva, and ~10 nL of semen.

Blood

The table below shows the performance of blood identification in 212 extractions of known human blood samples and 479 extractions of known non-blood or non-human blood samples. Non-blood samples include human saliva, semen, vaginal fluid, and negative controls. Non-human blood samples include those from livestock and domestic pets and one gorilla.

For a sample to be identified as positive for blood, that sample must have all nine blood peptide markers present, 3 from each blood marker protein.

Sample		95% CI Lower	95% CI Upper	N
Sensitivity	87.7%	82.4%	91.69%	212
False Discovery Rate	0.0%	0.0%	2.52%	186
Accuracy	96.2%	94.5%	97.48%	691
Injection Repeatability	100.0%	99.3%	100.00%	691

The sensitivity reported above includes all known blood samples, even those with very small known amounts of protein (to determine limits of detection, described above). Sensitivity is therefore also reported below for different types of known blood samples.

Sensitivity of Blood Detection in Different Types of Known Blood Samples

Sample		95% CI Lower	95% CI Upper	N
Neat Single	100.0%	94.3%	100.0%	80
Neat Mixture	96.2%	78.4%	99.8%	26
Mock - Known Amount 1µg and less	20.0%	8.4%	39.1%	30
Mock - Known Amount >1µg	100.0%	85.9%	100.0%	30
Mock - Unknown Amount	97.8%	87.0%	99.9%	46

Saliva

The table below shows the performance of saliva identification in 170 extractions of known human saliva samples and 521 extractions of known non-saliva or non-human saliva samples. Non-saliva samples include blood, menstrual blood, semen, and vaginal fluid, and negative controls. Non-human saliva samples include those from domestic pets.

Due to variability of protein concentrations in saliva, a positive result for identification of saliva in a sample requires detection of a minimum of seven of the eight saliva peptide markers. All peptides from amylase (AMY1) and cystatin (CYTT) proteins must be present (6 peptides, 3 per protein) and the 7th peptide may be either the histatin (HIS1) marker protein or the LEG1 (LEG1H) marker.

		95% CI Lower	95% CI Upper	N
Sensitivity	88.2%	82.2%	92.49%	170
False Discovery Rate	0.0%	0.0%	3.11%	150
Accuracy	97.1%	95.5%	98.18%	691
Injection Repeatability	96.7%	95.0%	97.83%	691

The sensitivity reported above includes all known saliva samples, even those with very small known amounts of protein (to determine limits of detection, described above). Sensitivity is therefore also reported below for different types of known saliva samples.

Sensitivity of saliva Detection in Different Types of Known saliva Samples

		95% CI Lower	95% CI Upper	N
Neat Single	100.0%	93.9%	100.0%	74
Neat Mixture	100.0%	84.0%	100.0%	26
Mock - Known Amount 1µg and less	36.7%	20.5%	56.1%	30
Mock - Known Amount >1µg	96.7%	80.9%	99.8%	30
Mock - Unknown Amount	100.0%	65.5%	100.0%	10

Semen

The table below shows the performance of semen identification in 196 extractions of known human semen samples and 495 extractions of known non-semen or non-human semen samples. Non-semen samples include saliva, blood, menstrual blood, and vaginal fluid, and negative controls. Non-human semen samples include those from livestock.

Due to variability of protein concentrations in semen, a positive result for identification of semen in a sample requires detection of a minimum of seven of the nine semen peptide markers. All three peptides from prostate specific antigen (KLK3) must be present and at least 2 out of 3 peptide markers from both semenogelin 1 and 2 (SEMG1 and SEMG2) must be identified.

		95% CI Lower	95% CI Upper	N
Sensitivity	94.4%	89.9%	97.02%	196
False Discovery Rate	0.0%	0.0%	2.54%	185
Accuracy	98.4%	97.1%	99.16%	691
Injection Repeatability	99.0%	97.8%	99.56%	691

The sensitivity reported above includes all known semen samples, even those with very small known amounts of protein (to determine limits of detection, described above). Sensitivity is therefore also reported below for different types of known semen samples.

Sensitivity of Semen Detection in Different Types of Known Semen Samples

Sample		95% CI Lower	95% CI Upper	N
Neat Single	97.8%	91.4%	99.6%	90
Neat Mixture	100.0%	84.0%	100.0%	26
Mock - Known Amount 1µg and less	76.7%	57.3%	89.4%	30
Mock - Known Amount >1µg	100.0%	85.9%	100.0%	30
Mock - Unknown Amount	90.0%	66.9%	98.2%	20

“N” indicates the denominator of the calculated performance metric. For sensitivity, N is the total number of known positive samples. For false discovery rate N is the total number samples with a positive result, and for accuracy N is the total samples tested. A 95% percent confidence interval is calculated for each performance metric using the Wilson score interval[2] with Yates continuity correction[3] for improved interval estimation for values near 0 and 100%.

Detailed Results

Inconclusive and Not Detected Results

An inconclusive result for body fluid identification is assigned when the number of peptides identified in a sample do not meet the requirements for positive identification of a body fluid (see above for requirements for each body fluid), but more than 3 peptide markers for the body fluid are identified in the sample.

For duplicate injections of the same sample, if at least 1 injection meets full peptide requirements for identification of a body fluid (Detected), then the sample is considered positive for that body fluid (Detected). If at least 1 injection is Inconclusive for a body fluid and no injections meet requirements for detection, then the sample is considered Inconclusive. If all injections of a sample fail to identify more than 3 peptides from a body fluid, the sample result is Not Detected.

Blood: The table below shows the number of sample extractions assigned a result of Detected, Inconclusive, and Not Detected for blood. Target samples are all samples containing human blood, including menstrual blood and mixtures. Non-target samples are samples that do not contain human blood, which includes animal blood samples.

Call	Non-Target Samples	Target Samples
Detected	0	186
Inconclusive	3	11
Not Detected	476	15

Inconclusive results for blood included non-human blood samples. Non-human samples may share some peptide sequences with humans, and detection of more than 3 shared peptides will result in an inconclusive, but a full peptide marker profile will not be obtained. In all cases the inconclusive result was consistent in duplicate injections.

Blood inconclusive results in non-human blood samples

Sample Type		Total Injections	# of Blood Marker Peptides Identified
Non-Human Blood	Gorilla	2	8
Non-Human Blood	Cat	2	4
Non-Human Blood	Gorilla	2	8

The majority of Inconclusive results and all Not Detected results for blood in known blood samples were in mock sample swabs with very small amounts of protein deposited, described above in the Limits of Detection section. The table below details the number of samples with Inconclusive or Not Detected Results at each amount of blood protein.

Protein Amount (µg)	Inconclusive Samples	Not Detected Samples
0.05	0	6
0.10	0	6
0.25	3	3
0.50	5	0
1.00	1	0

Two mixture samples containing blood also had inconclusive results for detection of blood: a 1:1 Blood Saliva mixture and a Blood and Semen stain on fabric containing unknown amounts of protein for each body fluid.

Blood inconclusive results in samples containing human blood

Sample Type		Protein Ratio	# Inconclusive Samples
Blood Saliva	Neat	1:1	1
Blood Semen	Fabric Stain	unknown	1

Saliva: The table below shows the number of sample extractions assigned a result of Detected, Inconclusive, and Not Detected for saliva. Target samples are all samples containing human saliva, including mixtures. Non-target samples are samples that do not contain human saliva, which includes animal saliva samples.

Call	Non-Target Samples	Target Samples
Detected	0	150
Inconclusive	1	13
Not Detected	520	7

Inconclusive results for saliva in non-target samples included 1 known semen sample: a mock-type swab with unknown amount of protein. This inconclusive result in semen is due in part to the relatively high rate of detection of cystatin (CYTT) peptides in semen (see Saliva Peptide Details below).

Saliva inconclusive in non-saliva semen mock swab with unknown protein

Sample Type		# Samples Inconclusive for Saliva
Semen	Swab	1

All Inconclusive and Not Detected results for saliva in known saliva samples were in mock sample swabs with very small amounts of protein deposited, described above in the Limits of Detection section. The table below details the number of samples with Inconclusive or Not Detected Results at each amount of blood protein.

Protein Amount (µg)	Inconclusive Samples	Not Detected Samples
0.05	1	5
0.10	4	2
0.25	4	0
0.50	2	0
1.00	1	0
10.00	1	0

Semen: The table below shows the number of sample extractions assigned a result of Detected, Inconclusive, and Not Detected for semen. Target samples are all samples containing human semen, including mixtures. Non-target samples are samples that do not contain human semen, which include non-human semen.

Call	Non-Target Samples	Target Samples
Detected	0	185
Inconclusive	1	11
Not Detected	494	0

Inconclusive results for semen in non-semen samples included one non-probative mock swab blood sample. This non-probative mock swab was one of eight non-probative mock blood samples prepared during a serial dilution (individual 1747). Other samples from this same serial dilution did not show any semen peptides. A replicate serial dilution of this same sample (individual 1747) did not reveal any semen peptides. It was determined that the original mock sample was contaminated at the bench as all mock body fluid samples (blood, saliva and semen) were prepared at the same time. Moving forward, all non-probative mock body fluid samples will be prepared at different times to ensure contamination does not occur.

Semen inconclusive in non-semen blood mock swab

Sample Type		Blood Protein Amount (µg)	# Samples Inconclusive for Semen
Blood	Swab	10	1

Inconclusive results for semen in known semen samples included mock swabs with unknown amounts of semen protein, neat semen samples, and mock swabs with known low amounts of semen protein. Both neat semen samples inconclusive for semen were repeat extractions of the same individual donor. No known human semen samples obtained a Not Detected result for semen.

Semen inconclusive results in known semen samples

Sample Type		Protein Amount (µg)	# Samples Inconclusive for Semen
Semen	Neat		2
Semen	Swab	unknown	2
Semen	Swab	0.05	6
Semen	Swab	0.1	1

Blood Peptide Details

The table below shows the number of blood marker peptides (out of 9 total blood markers assayed) that were detected in all LCMS injection runs included in the validation, listed by sample type.

Number of blood marker peptides identified in all validation LCMS runs, by sample type

Body Fluid	0	1	2	3	4	5	6	7	8	9
Blood	12	15	3	1	2	2	6	3	14	300
Blood Saliva	0	0	0	0	0	0	1	1	1	19
Blood Saliva Semen	0	0	0	0	0	0	0	0	2	10
Blood Semen	0	0	0	0	0	0	0	0	3	33
Menstrual Blood	0	0	0	0	0	0	0	0	0	8
Negative Control	337	0	0	0	0	0	0	0	0	0
Non-Human Blood	5	5	4	2	2	0	0	0	4	0
Non-Human Saliva	12	0	0	0	0	0	0	0	0	0
Non-Human Semen	20	0	0	0	0	0	0	0	0	0
Saliva	280	7	6	0	0	0	0	0	0	0
Saliva Semen	24	0	0	0	0	0	0	0	0	0
Semen	328	12	1	0	0	0	0	0	0	0
Vaginal Fluid	8	0	0	0	0	0	0	0	0	0

Band 3 Anion Protein (B3AT)

False Discovery Rates for B3AT peptides. Non-human blood samples are not included as shared sequences cause correct detection of some peptides in non-human samples.

Peptide		95% CI Lower	95% CI Upper	N
ADFLEQPVLGFVR	1.24%	0.457%	3.03%	404
ASTPGAAAQIQEVK	2.30%	1.127%	4.48%	391
IPPDSEATLVLVGR	1.24%	0.458%	3.04%	403

"N" indicates the denominator of the peptide false discovery rate, which is the total number of LCMS injections with a positive result for the peptide. A 95% percent confidence interval is calculated for each metric using the Wilson score interval with Yates continuity correction [2-3] for improved interval estimation for values near 0 and 100%. N.B. while, a few peptides in the Molecular Serology Assay may occasionally be detected outside of their specific body fluid, because of the required redundancy of detecting multiple different peptides for each body fluid's identification, no body fluids were falsely identified. Samples with a false peptide identified were "Not Detected" (less than three peptides identified), or "Inconclusive" (more than four peptides identified, but less than the required number for detections). All inconclusive results are detailed in the above section.

Number of passing sample injections for B3AT peptides in non-target samples by sample type, including non-human blood

Sample	ADFLEQPVLGFVR	ASTPGAAAQIQEVK	IPPDSEATLVLVGR
Negative Control	0	0	0
Non-Human Blood	4	4	10
Non-Human Saliva	0	0	0
Non-Human Semen	0	0	0

Sample	ADFLEQPVLGFVR	ASTPGAAAQIQEVK	IPPDSEATLVLVGR
Saliva	3	1	1
Saliva Semen	0	0	0
Semen	2	8	4
Vaginal Fluid	0	0	0

Hemoglobin Beta (HBB)

False Discovery Rates for HBB peptides. Non-human blood samples are not included as shared sequences cause correct detection of some peptides in non-human samples.

Peptide		95% CI Lower	95% CI Upper	N
LLVVYPWTQR	0%	0%	1.17%	405
SAVTALWGK	0%	0%	1.17%	406
VNVDEVGGEALGR	0%	0%	1.16%	409

Number of passing sample injections for HBB peptides in non-target samples by sample type, including non-human

Sample	LLVVYPWTQR	SAVTALWGK	VNVDEVGGEALGR
Negative Control	0	0	0
Non-Human Blood	9	4	14
Non-Human Saliva	0	0	0
Non-Human Semen	0	0	0
Saliva	0	0	0
Saliva Semen	0	0	0
Semen	0	0	0
Vaginal Fluid	0	0	0

Hemoglobin Alpha (HBA)

False Discovery Rates for HBA peptides. Non-human blood samples are not included as shared sequences cause correct detection of some peptides in non-human samples.

Peptide		95% CI Lower	95% CI Upper	N
FLASVSTVLTSK	1.02%	0.327%	2.77%	393
TYFPHFDLSHGSAQVK	2.33%	1.189%	4.39%	429
VGAHAGEYGAEALER	0.00%	0.000%	1.20%	395

N.B. while, a few peptides in the Molecular Serology Assay may occasionally be detected outside of their specific body fluid, because of the required redundancy of detecting multiple different peptides for each body fluid's identification, no body fluids were falsely identified. Samples with a false peptide identified were "Not Detected" (less than three peptides identified), or "Inconclusive" (more than four peptides identified, but less than the required number for detections). All inconclusive results are detailed in the above section.

Number of passing sample injections for HBA peptides in non-target samples by sample type, including non-human

Sample	FLASVSTVLTSK	TYFPHFDLSHGSAQVK	VGAHAGEYGAEALER
Negative Control	0	0	0
Non-Human Blood	4	10	0
Non-Human Saliva	0	0	0
Non-Human Semen	0	0	0
Saliva	4	10	0
Saliva Semen	0	0	0
Semen	0	0	0
Vaginal Fluid	0	0	0

Saliva Peptide Details

The table below shows the number of saliva marker peptides (out of 8 total saliva markers assayed) that were detected in all LCMS injection runs included in the validation, listed by sample type.

Number of saliva marker peptides identified in all validation LCMS runs, by sample type

Sample	0	1	2	3	4	5	6	7	8
Saliva	1	3	6	4	5	4	28	106	136
Blood Saliva	0	0	0	0	0	0	2	17	3
Saliva Semen	0	0	0	0	0	1	2	14	7
Blood Saliva Semen	0	0	0	0	0	0	2	10	0
Semen	112	53	93	72	9	1	1	0	0
Blood Semen	12	0	14	10	0	0	0	0	0
Blood	352	6	0	0	0	0	0	0	0
Negative Control	330	7	0	0	0	0	0	0	0
Non-Human Saliva	12	0	0	0	0	0	0	0	0
Non-Human Blood	21	1	0	0	0	0	0	0	0
Vaginal Fluid	8	0	0	0	0	0	0	0	0
Menstrual Blood	8	0	0	0	0	0	0	0	0
Non-Human Semen	20	0	0	0	0	0	0	0	0

Salivary Amylase (AMY1)

False Discovery Rates for AMY1 peptides

Peptide		95% CI Lower	95% CI Upper	N
ALVFVDNHDNQR	0.597%	0.103%	2.38%	335
IYVSDDGK	0.608%	0.105%	2.42%	329
LSGLLDLALGK	0.920%	0.238%	2.89%	326

N.B. while, a few peptides in the Molecular Serology Assay may occasionally be detected outside of their specific body fluid, because of the required redundancy of detecting multiple different peptides for each body fluid's identification, no body fluids were falsely identified. Samples with a false peptide identified were "Not Detected" (less than three peptides identified), or "Inconclusive" (more than four peptides identified, but less than the required number for detections). All inconclusive results are detailed in the above section.

Number of passing sample injections for AMY1 peptides in non-target samples by sample type, including non-human

Sample	ALVFVDNHDNQR	IYVSDDGK	LSGLLDLALGK
Blood	0	0	0
Blood Semen	0	0	0
Menstrual Blood	0	0	0
Negative Control	0	0	1
Non-Human Blood	0	0	0
Non-Human Saliva	0	0	0
Non-Human Semen	0	0	0
Semen	2	2	2
Vaginal Fluid	0	0	0

Cystatin (CYTT)

False Discovery Rates for CYTT peptides

Peptide		95% CI Lower	95% CI Upper	N
ALHFVISEYNK	23.4%	19.6%	27.6%	445
ATEDEYYR	39.9%	35.9%	44.1%	569
SQPNLDTCAFHEQPELQK	37.2%	33.1%	41.4%	549

N.B. while, a few peptides in the Molecular Serology Assay may occasionally be detected outside of their specific body fluid, because of the required redundancy of detecting multiple different peptides for each body fluid's identification, no body fluids were falsely identified. Samples with a false peptide identified were "Not Detected" (less than three peptides identified), or "Inconclusive" (more than four peptides identified, but less than the required number for detections). All inconclusive results are detailed in the above section.

Number of passing sample injections for CYTT peptides in non-target samples by sample type, including non-human

Sample	ALHFVISEYNK	ATEDEYYR	SQPNLDTCAFHEQPELQK
Blood	0	0	0
Blood Semen	11	24	23
Menstrual Blood	0	0	0
Negative Control	0	2	0

Sample	ALHFVISEYNK	ATEDEYYR	SQPNLDTCAFHEQPELQK
Non-Human Blood	0	0	0
Non-Human Saliva	0	0	0
Non-Human Semen	0	0	0
Semen	93	201	181
Vaginal Fluid	0	0	0

Both LEG1 and histatin are short proteins, HIS has only 1 peptide, and LEG1 has only 1 peptide suitable for detection by the LCMS method (Multiple Reaction Monitoring) used in this assay.

LEG1

False Discovery Rates for LEG1 peptide

Peptide		95% CI Lower	95% CI Upper	N
ESPGQLSDYR	6.77%	4.34%	10.3%	310

N.B. while, a few peptides in the Molecular Serology Assay may occasionally be detected outside of their specific body fluid, because of the required redundancy of detecting multiple different peptides for each body fluid's identification, no body fluids were falsely identified. Samples with a false peptide identified were "Not Detected" (less than three peptides identified), or "Inconclusive" (more than four peptides identified, but less than the required number for detections). All inconclusive results are detailed in the above section.

Number of passing sample injections for LEG1 peptide in non-target samples by sample type, including non-human

Sample	ESPGQLSDYR
Blood	2
Blood Semen	0
Menstrual Blood	0
Negative Control	3
Non-Human Blood	1
Non-Human Saliva	0
Non-Human Semen	0
Semen	15
Vaginal Fluid	0

Histatin (HIS1)

False Discovery Rates for HIS1 peptide

Peptide	95% CI Lower	95% CI Upper	N

Peptide		95% CI Lower	95% CI Upper	N
EFPFYGDYGSNYLYDN	5.98%	3.17%	10.7%	184

N.B. while, a few peptides in the Molecular Serology Assay may occasionally be detected outside of their specific body fluid, because of the required redundancy of detecting multiple different peptides for each body fluid's identification, no body fluids were falsely identified. Samples with a false peptide identified were "Not Detected" (less than three peptides identified), or "Inconclusive" (more than four peptides identified, but less than the required number for detections). All inconclusive results are detailed in the above section.

Number of passing sample injections for HIS1 peptide in non-target samples by sample type, including non-human

Sample	EFPFYGDYGSNYLYDN
Blood	4
Blood Semen	0
Menstrual Blood	0
Negative Control	1
Non-Human Blood	0
Non-Human Saliva	0
Non-Human Semen	0
Semen	6
Vaginal Fluid	0

Semen Peptide Details

The table below shows the number of semen marker peptides (out of 9 total blood markers assayed) that were detected in all LCMS injection runs included in the validation, listed by sample type.

Number of semen marker peptides identified in all validation LCMS runs, by sample type

Sample	0	1	2	4	5	6	7	8	9
Semen	0	0	0	1	3	19	28	58	232
Blood Semen	0	0	0	0	0	1	6	5	24
Saliva Semen	0	0	0	0	0	0	9	3	12
Blood Saliva Semen	0	0	0	0	0	0	6	2	4
Saliva	240	50	3	0	0	0	0	0	0
Blood	331	24	1	1	1	0	0	0	0
Negative Control	337	0	0	0	0	0	0	0	0
Non-Human Saliva	12	0	0	0	0	0	0	0	0
Non-Human Blood	21	1	0	0	0	0	0	0	0
Vaginal Fluid	4	4	0	0	0	0	0	0	0

Sample	0	1	2	4	5	6	7	8	9
Menstrual Blood	7	1	0	0	0	0	0	0	0
Blood Saliva	20	2	0	0	0	0	0	0	0
Non-Human Semen	13	7	0	0	0	0	0	0	0

Semenogelin 1 (SEMG1)

False Discovery Rates for SEMG1 peptides

Peptide		95% CI Lower	95% CI Upper	N
EQDLLSHEQK	2.71%	1.43%	4.94%	406
HLGGSQQLLHNK	14.29%	10.84%	18.54%	343
SQIQAPNPK	0.50%	0.08%	2.00%	400

N.B. while, a few peptides in the Molecular Serology Assay may occasionally be detected outside of their specific body fluid, because of the required redundancy of detecting multiple different peptides for each body fluid's identification, no body fluids were falsely identified. Samples with a false peptide identified were "Not Detected" (less than three peptides identified), or "Inconclusive" (more than four peptides identified, but less than the required number for detections). All inconclusive results are detailed in the above section.

Number of passing sample injections for SEMG1 peptides in non-target samples by sample type, including non-human

Sample	EQDLLSHEQK	HLGGSQQLLHNK	SQIQAPNPK
Blood	2	12	2
Blood Saliva	1	0	0
Menstrual Blood	0	0	0
Negative Control	0	0	0
Non-Human Blood	0	1	0
Non-Human Saliva	0	0	0
Non-Human Semen	0	7	0
Saliva	8	36	0
Vaginal Fluid	0	0	0

Semenogelin 2 (SEMG2)

False Discovery Rates for SEMG2 peptides

Peptide		95% CI Lower	95% CI Upper	N
GQLPSGSSQFPHGQK	7.08%	4.72%	10.41%	353
GSISIQTEEK	0.48%	0.08%	1.93%	413
LWVHGLSK	0.24%	0.01%	1.55%	414

N.B. while, a few peptides in the Molecular Serology Assay may occasionally be detected outside of their specific body fluid, because of the required redundancy of detecting multiple different peptides for each body fluid's identification, no body fluids were falsely identified. Samples with a false peptide identified were "Not Detected" (less than three peptides identified), or "Inconclusive" (more than four peptides identified, but less than the required number for detections). All inconclusive results are detailed in the above section.

Number of passing sample injections for SEMG2 peptides in non-target samples by sample type, including non-human

Sample	GQLPSGSSQFPHGQK	GSISIQTEEK	LWVHGLSK
Blood	15	2	0
Blood Saliva	0	0	1
Menstrual Blood	0	0	0
Negative Control	0	0	0
Non-Human Blood	0	0	0
Non-Human Saliva	0	0	0
Non-Human Semen	0	0	0
Saliva	6	0	0
Vaginal Fluid	4	0	0

Prostate-Specific Antigen (KLK3)

False Discovery Rates for KLK3 peptides

Peptide		95% CI Lower	95% CI Upper	N
IVGGWECEK	1.19%	0.44%	2.94%	417
LSEPAELTDAVK	0.72%	0.18%	2.28%	414
SVILLGR	0.24%	0.01%	1.56%	413

N.B. while, a few peptides in the Molecular Serology Assay may occasionally be detected outside of their specific body fluid, because of the required redundancy of detecting multiple different peptides for each body fluid's identification, no body fluids were falsely identified. Samples with a false peptide identified were "Not Detected" (less than three peptides identified), or "Inconclusive" (more than four peptides identified, but less than the required number for detections). All inconclusive results are detailed in the above section.

Number of passing sample injections for KLK3 peptides in non-target samples by sample type, including non-human

Sample	IVGGWECEK	LSEPAELTDAVK	SVILLGR
Blood	0	1	1
Blood Saliva	0	0	0
Menstrual Blood	1	0	0
Negative Control	0	0	0

Sample	IVGGWECEK	LSEPAELTDAVK	SVILLGR
Non-Human Blood	0	0	0
Non-Human Saliva	0	0	0
Non-Human Semen	0	0	0
Saliva	4	2	0
Vaginal Fluid	0	0	0

Negative Controls

Only 7 negative control injections had any body fluid marker peptide identifications. These marker peptide false positives only occurred as a single peptide per injection, therefore an Inconclusive or Detected result was not assigned to any negative control injections.

Peptides Found	Number of NC Injections
0	330
1	7

Percent of Negative Control LCMS injections with at least 1 body fluid marker peptide detected

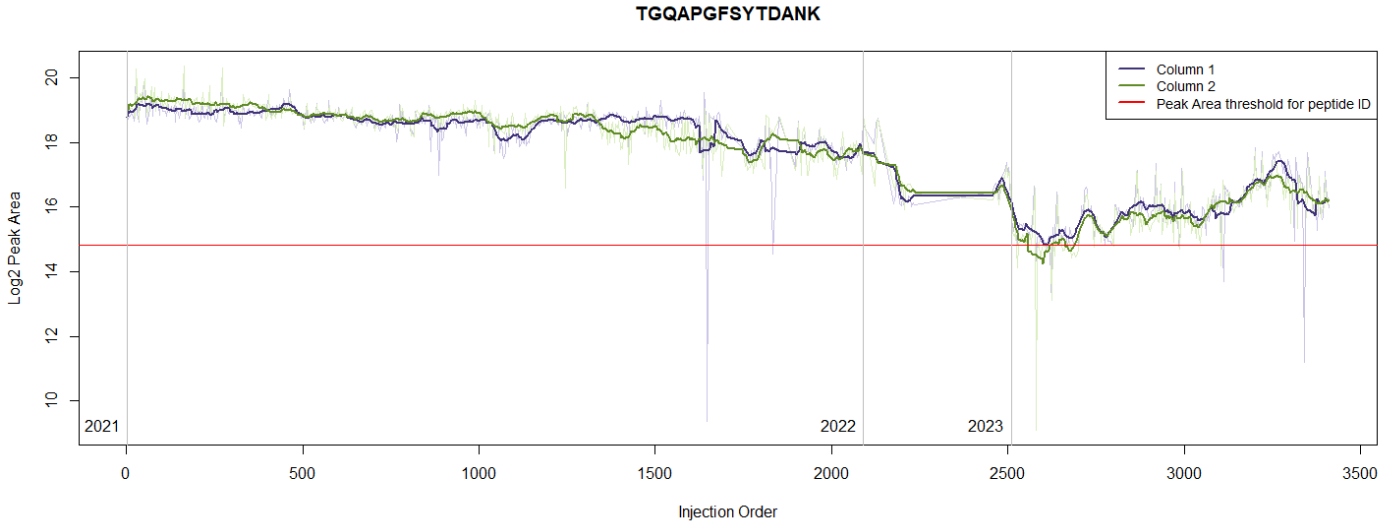
Peptide False Positive Rate	95% CI Lower	95% CI Upper	N
2.08%	0.913%	4.42%	337

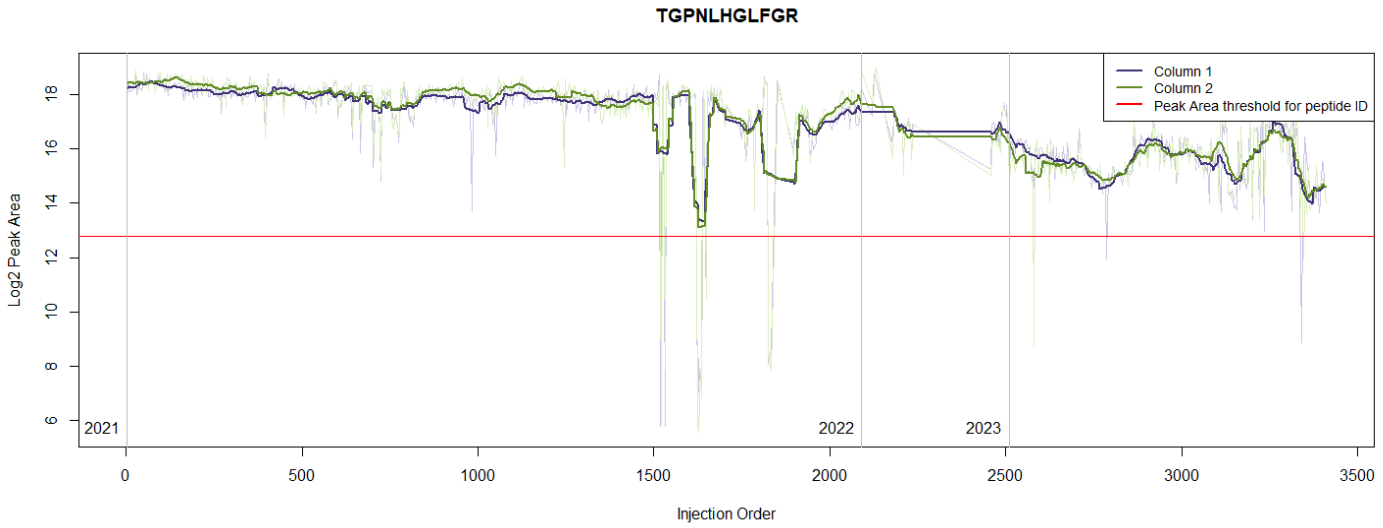
“N” indicates the denominator of the peptide false positive rate, which is the total number of negative control LCMS injections. A 95% percent confidence interval is calculated for each metric using the Wilson score interval with Yates continuity correction [2-3] for improved interval estimation for values near 0 and 100%.

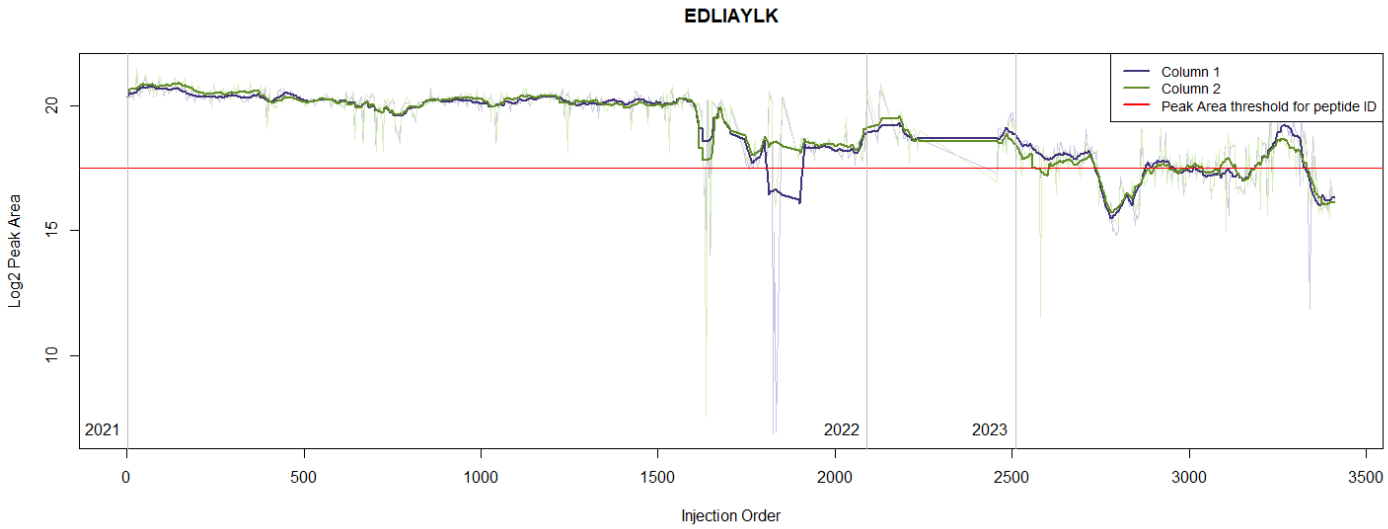
Cytochrome C Control

Cytochrome C (Cyto-C) is a predigested protein used to measure the volume of sample that is injected in the high-performance liquid chromatography instrument. It is added to each sample immediately prior to HPLC analysis. In the initial validation, only one Cyto-C peptide (TGQAPGFSYTDANK) was evaluated. However, over time, analysis showed that detection of this Cyto-C peptide declined at a faster rate than other Cyto-C peptides (see figure below). Consequently, detection of any one of three Cyto-C peptides (TGQAPGFSYTDANK, EDLIAYLK, or TGPNLHGLFGR) is sufficient for demonstrating its presence in a sample.

The plots below show the peak area of each of three Cyto-C control peptides over time for all validation LCMS runs. The heavy lines are a five-day rolling average of the peak area, and the fine lines are the unaveraged peak areas.


TGQAPGFSYTDANK
Column 1
Column 2
Peak Area threshold for peptide ID
Log2 Peak Area
Injection Order
2021
2022
2023


TGPNLHGLFGR
Column 1
Column 2
Peak Area threshold for peptide ID
Log2 Peak Area
Injection Order
2021
2022
2023


EDLIAYLK
Column 1
Column 2
Peak Area threshold for peptide ID
Log2 Peak Area
Injection Order
2021
2022
2023

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Based upon the validation work performed, the molecular serology assay is suitable to detect body fluids (blood, saliva, and semen) on casework samples.

Approved by: Approval Date: 04-14-23

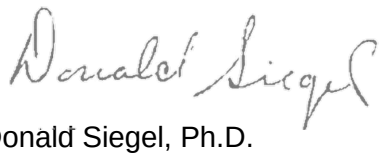

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Table 1 Summary of Experiments & Sample Numbers

Negative controls and instrument calibration standards were included in all runs.

BCA Assay Validation Part 1

BSA Quant

Sample Type	Dilutions[1]	Batches	Replicates	Analysts	Total BCA Wells
BSA	13	6	3	2	468

[1] 11 dilutions plus neg control and water

BCA Assay Validation Part 2

Body Fluids Quant

Sample Type	Donors	Dilutions	Batches	Replicates	Analysts	Total BCA Wells
Blood	2	4	6	3	2	288
Semen	2	4	6	3	2	288
Saliva	3	4	6	3	2	432
						1008

Body Fluid Classification Assay Validation

Body Fluid Samples - Classification Validation

Sample Type	Donors	Extractions* per Donor	Analysts	Total Prepared	LC Injections (per prepared sample)	Total MS Runs
Blood	13	1	2	26	2	52
Blood	1	3	3	9	2	18
Blood	2	3	2	12	2	24
Blood	2	4	2	16	2	32
Blood	2	7	1	14	2	28
Semen	7	1	1	7	2	14
Semen	5	1	2	10	2	20
Semen	4	1	2	8	3	24
Semen	4	1	3	12	2	24
Semen	3	3	2	18	2	36
Semen	3	3	3	27	2	54
Semen	1	6	2	12	2	24
Saliva	5	1	1	5	2	10
Saliva	15	1	2	30	2	60
Saliva	3	3	2	18	2	36
Saliva	2	5	2	20	2	40
*Extractions/batches occured on different days						496

Body Fluid Non-Probative Mock Samples Assay Validation

Body Fluid Non-Probative Mock Samples Known Protein Amount[2] Classification Validation

Sample Type	Donors*	Evidence Type	Samples[2]	Analysts	LC Injections (per prepared sample)	Total MS Runs
Blood	5	Swab	60	2	2	120
Semen	6	Swab	60	2	2	120
Saliva	6	Swab	60	2	2	122
[2] 20 µg, 10 µg, 2 µg, 2 µg, 1 µg, 0.5 µg, 0.25 µg, 0.1 µg, 0.05 µg						362
*Body fluid donors also used for semen and saliva						

Body Fluid Mock Samples Assay Validation

Body Fluid Mock Samples (QA PT Test Mock Samples (Bode Cellmark Forensics LabCorp IQAS) Classification Validation

Sample Type	Donors*	Evidence Type	Samples	Analysts	Age (mo.)	LC Injections (per prepared sample)	Total MS Runs
Blood		Card	31	2	12-132	2	68
Blood		Fabric	8	2	24-132	2	16
Blood/Semen		Fabric	7	2	24-132	2	14
Semen		Swab	13	2	12-60	2	26
Saliva	6	Swab	10	2	0	2	22
							146
*Body fluid donors also used for semen and saliva							

Body Fluid Mixtures Assay Validation

Body Fluid Mixtures Classification Assay Validation

Mixture Type	Mixture preparations	Ratios	Extractions per preparation	Analysts	Total Prepared for LCMS	LC Injections (per prepared sample)	Total MS Runs
Blood/Semen	2	1:1	1	2	4	2	8
Blood/Saliva	2	1:1	1	2	4	2	8
Semen/Saliva	2	1:1	1	2	4	2	8
Blood/Semen	7	1:12,1:8,1:4,1:1,4:1,8:1,12:1	1	1	7	2	14
Blood/Saliva	7	1:12,1:8,1:4,1:1,4:1,8:1,12:1	1	1	7	2	14
Semen/Saliva	7	1:12,1:8,1:4,1:1,4:1,8:1,12:1	1	1	7	2	14
Blood/Semen/Saliva	4	1:1:1,1:1:10,1:10:1,10:1:1	1	1	4	2	8
							74

Analysis of Non-Targeted Samples

Non-Target Samples - Classification Assay Validation

Sample Type	Donors	Extractions* per donor	Analysts	Total Prepared for LCMS	LC Injections (per prepared sample)	Total MS Runs
Human Vaginal Fluid	2	1	2	4	2	8
Human Menstrual BL	2	1	2	4	2	8
Non-Human Blood	6	1	2	12	2	24
Non-Human Saliva	3	1	2	6	2	12
Non-Human Semen	8	1	1	8	2	16
						52

QA PT Kits Used	
Bode	**CTS**
910	09-571
949	14-572
448	16-572
711	17-5705
658	18-5704
992	18-5702
182	09-575
899	10-575
181	08-572
458	07-572
393	07-574
176	08-575
502	20-5781
401	
851	
1037	
1258	
1476	
1737	
192	
423	
663	
924	
1123	
1717	
1888	

Mixture Samples (Lab Made)	
Blood/Semen	B1396/SE2164; B1747/SE2051; B1777/SE2369
Blood/Saliva	B1396/SA1468; B1747/SA1324; B1777/SA1312
Saliva/Semen	SA1468/SE2164; SA1324/SE2051; SA1312/SE2369
Blood/Saliva/Semen	B1777/SA1312/SE2369

Non-target Samples	
Vaginal Fluid	VF B994 D13, VFB333 D13
Menstrual Blood	MB 277, MB 513
Non-Human Blood	Canis lupus (dog), Felis catus (cat), Bos taurus (cow), Sus scrofa (pig), and Gallus gallus (chicken)
Non-Human Semen	Sus scrofa (boar) and Bos taurus (oxen)
Non-Human Saliva	Canis lupus (dog) and Felis catus (cat)

Table 2 Volunteer Samples (anonymized but including demographic data)

Semen Donors

Donors	Age	Race	Gender
2020	36	White	Male
2046	28	Asian	Male
2038	48	Asian	Male
2135	39	White	Male
2369	40	White	Male
2164	31	White	Male
2031	27	Hispanic	Male
2033	23	Hispanic	Male
2043	26	Hispanic	Male
2051	25	White	Male
2063	35	White	Male
2069	32	White	Male
2071	20	Hispanic	Male
2076	26	Black	Male
2007	38	White	Male
2009	30	White	Male
1008	32	Black	Male
1000	24	South Asian	Male
1012	26	Hispanic	Male
2014	29	White	Male
246	45	Asian	Male vasectomized
265	49	Hispanic	Male vasectomized
T5629	65	White	Male vasectomized
T5633	58	White	Male vasectomized
T5634	54	White	Male vasectomized
T5640	47	White	Male vasectomized
T6330	39	White	Male vasectomized

Saliva Donors

Donors	Age	Race	Gender
1345	30	White	Female
1532	26	White	Male
1740	27	White	Male
1347	30	Black	Male
1295	33	White	Female
1494	42	Female	Black
1569	30	White	Female
1344	48	Black	Female
1524	48	Asian	Male
1409	31	Asian	Male
1487	26	Asian	Female
1468	35	White	Female
1327	32	White Asian	Female
1324	40	Asian	Male
1317	26	White	Female
1432	28	White	Female
1312	23	White	Female
1313	39	White	Male
1309	41	Black	Female
1433	37	White	Male
1747	23	Hispanic	Female
DS	69	White	Male
HYY	45	Asian	Male
2031	27	Hispanic	Male

Blood Donors

Donors	Age	Race	Gender
1396	46	Asian	Male
1298	26	White	Female
1516	34	Hispanic	Male
1294	23	White	Female
1235	29	White	Female
1492	34	Black	Female
1293	55	White	Male
1469	38	White	Female
1777	35	Mix	Female
1632	40	Asian	Male
1810	51	Male	White
1843	42	Asian	Male
1712	41	Asian	Female
1723	32	Black	Male
1836	37	Asian	Female
1910	33	Hispanic	Female
1917	30	Hispanic	Female
1963	52	White	Male
1845	43	White	Female
1747	23	Hispanic	Female

All Mock Samples				
Sample Name	Sample Type	Body Fluid	Sample prepration	Aged (months)
TPP (2131)	Swab	SA	Homemade	0
TPP (2131)	Swab	SA	Homemade	0
TPP (2131)	Swab	SA	Homemade	0
TPP (2131)	Swab	SA	Homemade	0
TPP (2131)	Swab	SA	Homemade	0
TPP (2131)	Swab	SA	Homemade	0
LO (2132)	Swab	SA	Homemade	0
LO (2132)	Swab	SA	Homemade	0
LO (2132)	Swab	SA	Homemade	0
LO (2132)	Swab	SA	Homemade	0
NF (2133)	Swab	SA	Homemade	0
NF (2133)	Swab	SA	Homemade	0
NF (2133)	Swab	SA	Homemade	0
NF (2133)	Swab	SA	Homemade	0
JZ (2134)	Swab	SA	Homemade	0
JZ (2134)	Swab	SA	Homemade	0
JZ (2134)	Swab	SA	Homemade	0
JZ (2134)	Swab	SA	Homemade	0
JZ (2134)	Swab	SA	Homemade	0
JZ (2134)	Swab	SA	Homemade	0
IA (2135)	Swab	SA	Homemade	0
IA (2135)	Swab	SA	Homemade	0
IA (2135)	Swab	SA	Homemade	0
IA (2135)	Swab	SA	Homemade	0
IA (2135)	Swab	SA	Homemade	0
IA (2135)	Swab	SA	Homemade	0
Negative	Swab	NC	Homemade	0
Negative	Swab	NC	Homemade	0
Negative	Swab	NC	Homemade	0
Negative	Swab	NC	Homemade	0
Negative	Swab	NC	Homemade	0
Negative	Swab	NC	Homemade	0
Negative #7	Swab	NC	Homemade	0
Negative #7	Swab	NC	Homemade	0
Negative 7621	Swab	NC	Homemade	0
Negative 7621	Swab	NC	Homemade	0
Negative Control	Neat	NC	Homemade	0
Negative Control	Neat	NC	Homemade	0
Negative Control 1 Mock	Swab	NC	Homemade	0
Negative Control 1 Mock	Swab	NC	Homemade	0
Negative Control Mock 1	Swab	NC	Homemade	0
Negative Control Mock 1	Swab	NC	Homemade	0
1000	Swab	SE	Homemade	0
1000	Swab	SE	Homemade	0
1000	Swab	SE	Homemade	0

All Mock Samples				
Sample Name	Sample Type	Body Fluid	Sample prepration	Aged (months)
1000	Swab	SE	Homemade	0
1000	Swab	SE	Homemade	0
1000	Swab	SE	Homemade	0
1000	Swab	SE	Homemade	0
1000	Swab	SE	Homemade	0
1000	Swab	SE	Homemade	0
1000	Swab	SE	Homemade	0
1000	Swab	SE	Homemade	0
1000	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2046	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2135	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0
2164	Swab	SE	Homemade	0

All Mock Samples				
Sample Name	Sample Type	Body Fluid	Sample prepration	Aged (months)
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
2396	Swab	SE	Homemade	0
Negative Ctrl	Neat	NC	Homemade	0
Negative Ctrl	Neat	NC	Homemade	0
Negative Ctrl	Swab	NC	Homemade	0
Negative Ctrl	Swab	NC	Homemade	0
Negative 2	Swab	NC	Homemade	0
Negative 2	Swab	NC	Homemade	0
19390	Card	BL	QAQC	12
19390	Card	BL	QAQC	12
19389	Card	BL	QAQC	12
19389	Card	BL	QAQC	12
19383	Card	BL	QAQC	12
19383	Card	BL	QAQC	12
19388	Card	BL	QAQC	12
19388	Card	BL	QAQC	12
Negative 19	Card	NC	QAQC	12
Negative 19	Card	NC	QAQC	12
Negative 17	Card	NC	QAQC	36
Negative 17	Card	NC	QAQC	36
Negative 17	Card	NC	QAQC	36
Negative 17	Card	NC	QAQC	36
17323	Card	BL	QAQC	36
17323	Card	BL	QAQC	36
17323	Card	BL	QAQC	36
17323	Card	BL	QAQC	36
18347	Card	BL	QAQC	24
18347	Card	BL	QAQC	24
17330	Card	BL	QAQC	36
17330	Card	BL	QAQC	36
18364	Card	BL	QAQC	12
18364	Card	BL	QAQC	12
16-572-3 Mix	Fabric	BL	QAQC	48
16-572-3 Mix	Fabric	BL	QAQC	48
16-572-4	Fabric	BL	QAQC	48

All Mock Samples				
Sample Name	Sample Type	Body Fluid	Sample prepration	Aged (months)
16-572-4	Fabric	BL	QAQC	48
17-5705-3 Mix	Fabric	BL	QAQC	36
17-5705-3 Mix	Fabric	BL	QAQC	36
17-5705-4	Fabric	BL	QAQC	36
17-5705-4	Fabric	BL	QAQC	36
Negative 18	Card	NC	QAQC	24
Negative 18	Card	NC	QAQC	24
Negative 18	Card	NC	QAQC	24
Negative 18	Card	NC	QAQC	24
Negative 16-5	Fabric	NC	QAQC	48
Negative 16-5	Fabric	NC	QAQC	48
Negative 16-5 3	Fabric	NC	QAQC	48
Negative 16-5 3	Fabric	NC	QAQC	48
Negative 17-5 3	Fabric	NC	QAQC	36
Negative 17-5 3	Fabric	NC	QAQC	36
Negative 17-5 4	Fabric	NC	QAQC	36
Negative 17-5 4	Fabric	NC	QAQC	36
15238	Card	BL	QAQC	60
15238	Card	BL	QAQC	60
Negative 15	Card	NC	QAQC	60
Negative 15	Card	NC	QAQC	60
16287	Card	BL	QAQC	48
16287	Card	BL	QAQC	48
09-575:1	Card	BL	QAQC	132
09-575:1	Card	BL	QAQC	132
Negative 9-5	Card	NC	QAQC	132
Negative 9-5	Card	NC	QAQC	132
09-575:2	Card	BL	QAQC	132
09-575:2	Card	BL	QAQC	132
Negative 09-575:2	Card	NC	QAQC	132
Negative 09-575:2	Card	NC	QAQC	132
09-575:2	Card	BL	QAQC	132
09-575:2	Card	BL	QAQC	132
Negative 09-575:2	Card	NC	QAQC	132
Negative 09-575:2	Card	NC	QAQC	132
09-575:3 Mix	Fabric	BL	QAQC	132
09-575:3 Mix	Fabric	BL	QAQC	132
Negative 9-5 3	Fabric	NC	QAQC	132
Negative 9-5 3	Fabric	NC	QAQC	132
09-575:4	Fabric	BL	QAQC	132
09-575:4	Fabric	BL	QAQC	132
Negative 09-575:4	Fabric	NC	QAQC	132
Negative 09-575:4	Fabric	NC	QAQC	132
10-571:1	Card	BL	QAQC	120
10-571:1	Card	BL	QAQC	120

All Mock Samples				
Sample Name	Sample Type	Body Fluid	Sample prepration	Aged (months)
Negative 10-571:1	Card	NC	QAQC	120
Negative 10-571:1	Card	NC	QAQC	120
10-571:2	Card	BL	QAQC	120
10-571:2	Card	BL	QAQC	120
Negative 10-571:2	Card	NC	QAQC	120
Negative 10-571:2	Card	NC	QAQC	120
10-571:3	Fabric	BL	QAQC	120
10-571:3	Fabric	BL	QAQC	120
Negative 10-571:3	Fabric	NC	QAQC	120
Negative 10-571:3	Fabric	NC	QAQC	120
10-571:4	Fabric	BL	QAQC	120
10-571:4	Fabric	BL	QAQC	120
Negative 10-571:4	Fabric	NC	QAQC	120
Negative 10-571:4	Fabric	NC	QAQC	120
18338	Card	BL	QAQC	24
18338	Card	BL	QAQC	24
18343	Card	BL	QAQC	24
18343	Card	BL	QAQC	24
18344	Card	BL	QAQC	24
18344	Card	BL	QAQC	24
18345	Card	BL	QAQC	24
18345	Card	BL	QAQC	24
Negative	Card	NC	QAQC	24
Negative	Card	NC	QAQC	24
185704 #1	Card	BL	QAQC	24
185704 #1	Card	BL	QAQC	24
185704 #2	Card	BL	QAQC	24
185704 #2	Card	BL	QAQC	24
185704 #4	Fabric	BL	QAQC	24
185704 #4	Fabric	BL	QAQC	24
185704 #3 Mix	Fabric	BL	QAQC	24
185704 #3 Mix	Fabric	BL	QAQC	24
Negative#6	Fabric	NC	QAQC	24
Negative#6	Fabric	NC	QAQC	24
18354	Card	BL	QAQC	24
18354	Card	BL	QAQC	24
18359	Card	BL	QAQC	24
18359	Card	BL	QAQC	24
18360	Card	BL	QAQC	24
18360	Card	BL	QAQC	24
18361	Card	BL	QAQC	24
18361	Card	BL	QAQC	24
Negative#8	Card	NC	QAQC	24
Negative#8	Card	NC	QAQC	24
18376	Card	BL	QAQC	12

All Mock Samples				
Sample Name	Sample Type	Body Fluid	Sample prepration	Aged (months)
18376	Card	BL	QAQC	12
Negative #9	Card	NC	QAQC	12
Negative #9	Card	NC	QAQC	12
185702 #3	Fabric	BL	QAQC	24
185702 #3	Fabric	BL	QAQC	24
185702 #4 Mix	Fabric	BL	QAQC	24
185702 #4 Mix	Fabric	BL	QAQC	24
Negative #10	Fabric	NC	QAQC	24
Negative #10	Fabric	NC	QAQC	24
19395	Card	BL	QAQC	12
19395	Card	BL	QAQC	12
Ngative #11	Card	NC	QAQC	12
Ngative #11	Card	NC	QAQC	12
15249	Card	BL	QAQC	60
15249	Card	BL	QAQC	60
15249 Negative	Card	BL	QAQC	60
15249 Negative	Card	BL	QAQC	60
15258	Card	BL	QAQC	48
15258	Card	BL	QAQC	48
15258 Negative	Card	BL	QAQC	48
15258 Negative	Card	BL	QAQC	48
14572 #1 #2 cont.	Card	NC	QAQC	72
14572 #1 #2 cont.	Card	NC	QAQC	72
14572 #1	Card	BL	QAQC	72
14572 #1	Card	BL	QAQC	72
14572#2	Card	BL	QAQC	72
14572#2	Card	BL	QAQC	72
14572 #3	Fabric	BL	QAQC	72
14572 #3	Fabric	BL	QAQC	72
14572 #3 cont.	Fabric	NC	QAQC	72
14572 #3 cont.	Fabric	NC	QAQC	72
14572 #4 cont.	Fabric	NC	QAQC	72
14572 #4 cont.	Fabric	NC	QAQC	72
14572 #4 Mix	Fabric	BL	QAQC	72
14572 #4 Mix	Fabric	BL	QAQC	72
10575 #1 #2 cont.	Card	NC	QAQC	120
10575 #1 #2 cont.	Card	NC	QAQC	120
10575 #3 cont.	Fabric	NC	QAQC	120
10575 #3 cont.	Fabric	NC	QAQC	120
10575 #1	Card	BL	QAQC	120
10575 #1	Card	BL	QAQC	120
10575#2	Card	BL	QAQC	120
10575#2	Card	BL	QAQC	120
10575 #3	Fabric	BL	QAQC	120
10575 #3	Fabric	BL	QAQC	120

All Mock Samples				
Sample Name	Sample Type	Body Fluid	Sample prepration	Aged (months)
10575 #4 Mix	Fabric	BL	QAQC	120
10575 #4 Mix	Fabric	BL	QAQC	120
10575 #4 cont.	Fabric	NC	QAQC	120
10575 #4 cont.	Fabric	NC	QAQC	120
16280	Swab	SE	QAQC	60
16280	Swab	SE	QAQC	60
17309	Swab	SE	QAQC	48
17309	Swab	SE	QAQC	48
17316	Swab	SE	QAQC	48
17316	Swab	SE	QAQC	48
20413	Swab	SE	QAQC	12
20413	Swab	SE	QAQC	12
20439	Swab	SE	QAQC	12
20439	Swab	SE	QAQC	12
20452	Swab	SE	QAQC	12
20452	Swab	SE	QAQC	12
19387	Swab	SE	QAQC	24
19387	Swab	SE	QAQC	24
19402	Swab	SE	QAQC	24
19402	Swab	SE	QAQC	24
18363	Swab	SE	QAQC	36
18363	Swab	SE	QAQC	36
18348	Swab	SE	QAQC	36
18348	Swab	SE	QAQC	36
17334	Swab	SE	QAQC	48
17334	Swab	SE	QAQC	48
19407	Swab	SE	QAQC	24
19407	Swab	SE	QAQC	24
NC	Swab	NC	Homemade	0
NC	Swab	NC	Homemade	0
1312	Swab	SA	Homemade	0
1312	Swab	SA	Homemade	0
1312	Swab	SA	Homemade	0
1312	Swab	SA	Homemade	0
1312	Swab	SA	Homemade	0
1312	Swab	SA	Homemade	0
1532	Swab	SA	Homemade	0
1532	Swab	SA	Homemade	0
1532	Swab	SA	Homemade	0
1532	Swab	SA	Homemade	0
1532	Swab	SA	Homemade	0
1532	Swab	SA	Homemade	0
1487	Swab	SA	Homemade	0
1487	Swab	SA	Homemade	0
1487	Swab	SA	Homemade	0

All Mock Samples				
Sample Name	Sample Type	Body Fluid	Sample prepration	Aged (months)
1487	Swab	SA	Homemade	0
1487	Swab	SA	Homemade	0
1487	Swab	SA	Homemade	0
1432	Swab	SA	Homemade	0
1432	Swab	SA	Homemade	0
1432	Swab	SA	Homemade	0
1432	Swab	SA	Homemade	0
1432	Swab	SA	Homemade	0
1432	Swab	SA	Homemade	0
1747	Swab	SA	Homemade	0
1747	Swab	SA	Homemade	0
1747	Swab	SA	Homemade	0
1747	Swab	SA	Homemade	0
1747	Swab	SA	Homemade	0
1747	Swab	SA	Homemade	0
Negative	Swab	NC	Homemade	0
Negative	Swab	NC	Homemade	0
NC1	Swab	NC	QAQC	0
NC1	Swab	NC	QAQC	0
20-5781 #1 -1	Swab	SA	QAQC	12
20-5781 #1 -1	Swab	SA	QAQC	12
20-5781 #1 -2	Swab	SA	QAQC	12
20-5781 #1 -2	Swab	SA	QAQC	12
20-5781 #1 -3	Swab	SA	QAQC	12
20-5781 #1 -3	Swab	SA	QAQC	12
20-5781 #1 -4	Swab	SA	QAQC	12
20-5781 #1 -4	Swab	SA	QAQC	12
Negative Control	Swab	NC	Homemade	0
Negative Control	Swab	NC	Homemade	0