Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Direct Differentiation of Whole Blood for Forensic Serology Analysis by Thread Spray Mass Spectrometry

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Supporting information is summarized in the table below

1. **The Measurement of Blood Stain on 100% Cotton Fabric.** The 10 µL aliquot of fresh blood was applied to a cotton fabric and allowed to dry. The area of the blood stain was measured using the area of an ellipse, A = πab. The surface area of the oval-like dried blood spot was measured to be 43.4 mm2. A single thread was pulled from the bloodstained fabric. On average, the surface area of the bloodstain on the thread fiber was measured to ~4.4 mm2, confirming that only 10% of the initial stain is utilized for thread spray analysis.

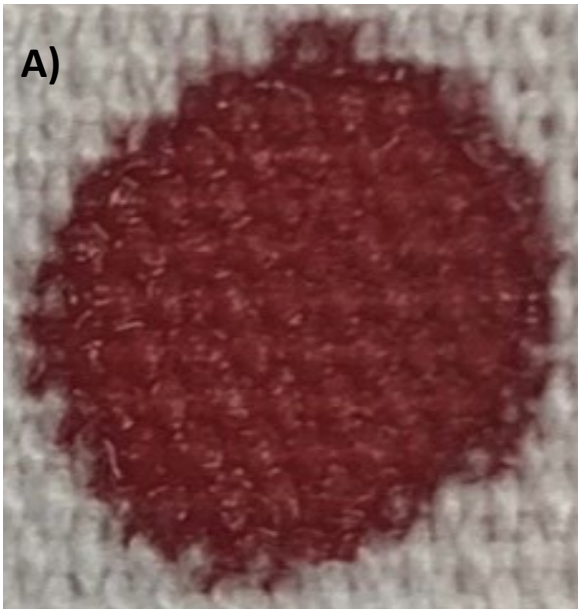

Figure S1. (a) Initial bloodstain on cotton thread with diameters 6.58 mm and 8.42 mm.

2. Aqueous Ammonium Acetate Protein Solution Analysis. Aqueous ammonium acetate solutions containing 100 µM of cytochrome c and lysozyme each were analyzed using thread spray mass spectrometry.

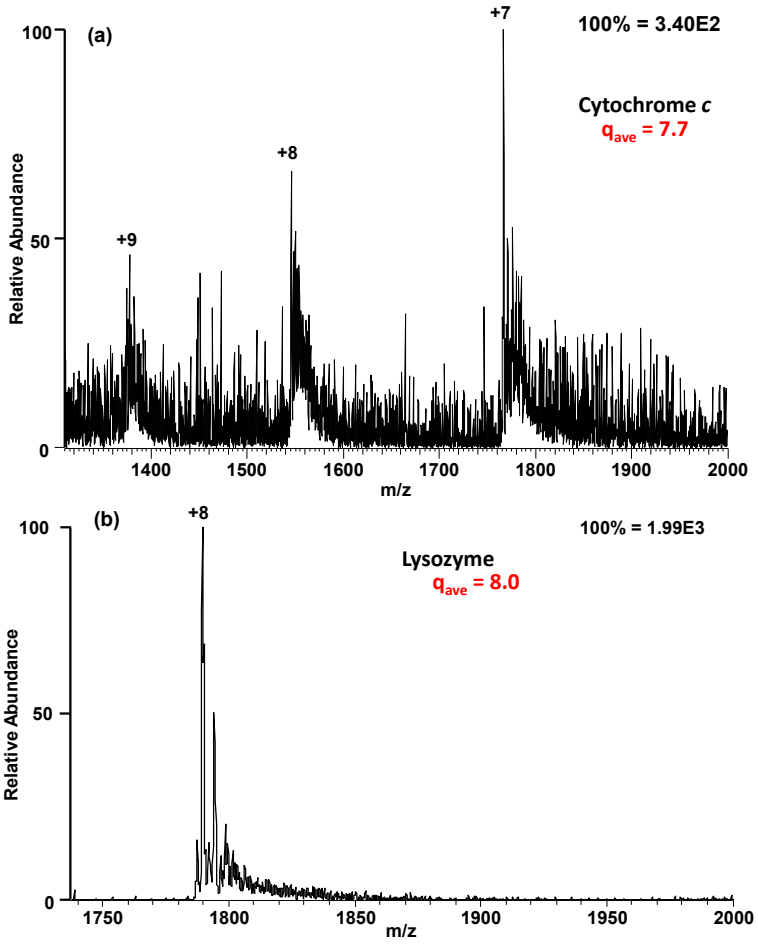

Figure S2. Optimization of untreated thread spray analysis of aqueous ammonium acetate protein solution on 100 % cotton of (a) cytochrome *c* (b) lysozyme. Spray voltage was 4kV for all thread spray experiments.

3. Whole Blood Lipid Identification by MS/MS. Lipid identification by thread spray mass spectrometry of whole blood derived from human, horse, and canine sources, in positive mode.

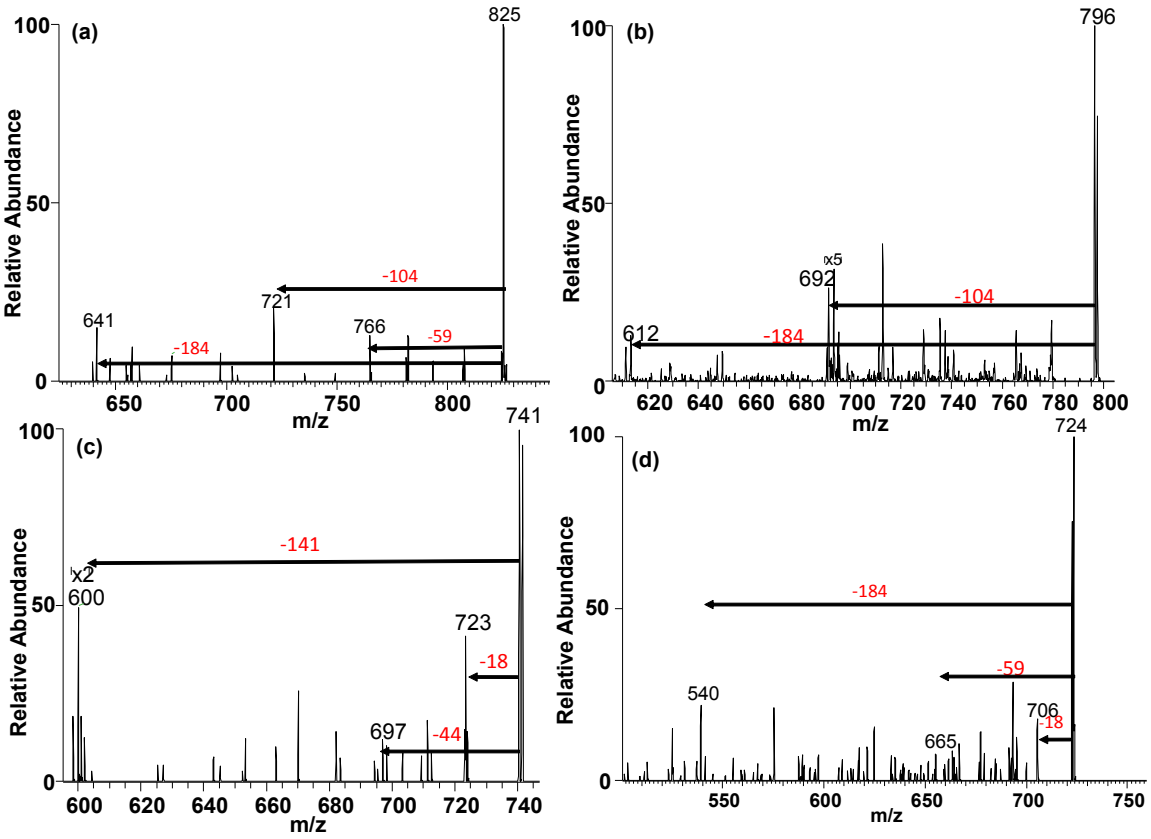

Figure S3. Tandem mass spectrometry analysis of phospholipids in whole human blood on cotton thread: phosphatidylcholines (PC) at m/z 825 (a) and m/z 796 (b); phosphatidylethanolamine (PE) at m/z 741 (c), and sphingomyelin at m/z 724 (d) in the positive ion mode.

4. Whole Blood Lipid Identification. Lipid identification by mass spectrometry of whole blood of human, horse, and canine blood in positive mode.

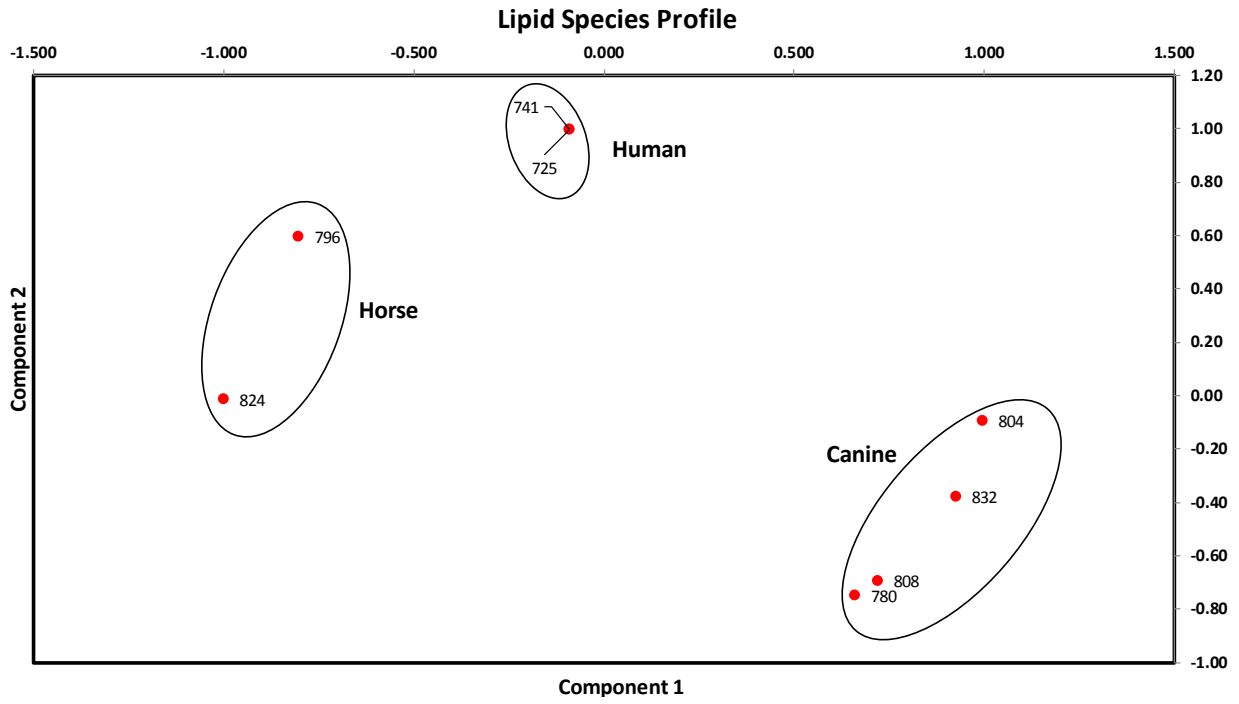

Figure S4. Analysis of lipids. Two-dimensional principal-component analysis was applied on spectral data acquired by thread spray from human, horse, and canine blood (n=5).

5. SEM Images of Cotton Thread: SEM Images of 100% cotton thread, cotton thread with blood applied, and cotton thread that contains blood which has been held under room temperature conditions.

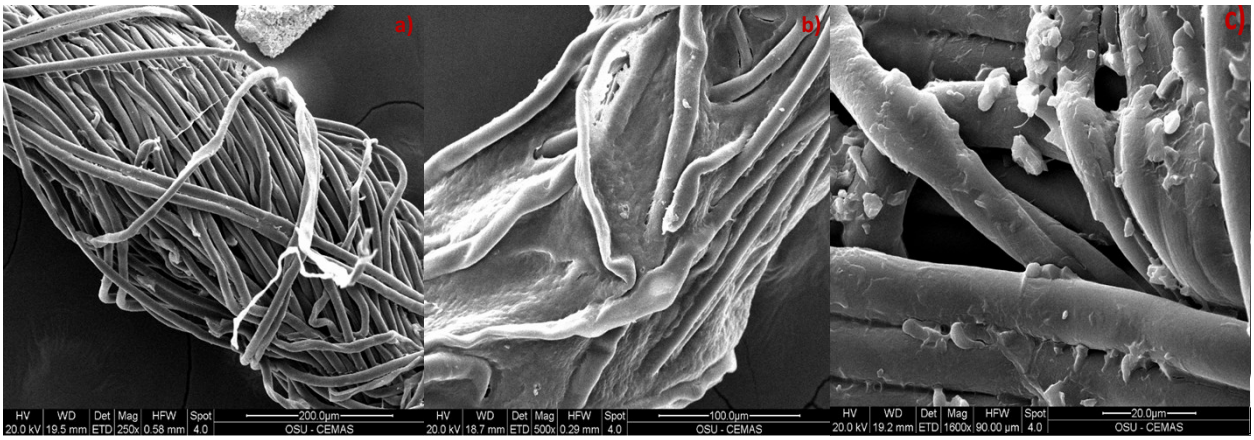

Figure S5. SEM images of cotton thread with magnifications: a) of untreated thread (250X) b) of thread with blood applied (500X) and c) thread stored under room temperature condition for one week (1600X).

6. Detection of Iron (Fe) by Energy Dispersive x-ray Spectroscopy (EDS) analysis.

EDS analysis of cotton fabric that had no blood (blank), fabric with blood applied, fabric with blood applied a week prior (stability).

Table S1. Energy dispersive x-ray spectroscopy (EDS) measurement of elemental composition (weight %) on cotton thread on blank thread and thread with blood applied

Blank		Blood Applied	
Element (k)	Weight %	Element (k)	Weight %
C	58.74	C	66.2
O	33.91	O	24.03
Na	0	Na	0.16
Mg	0	Mg	0
Al	0	Al	0.07
Si	0	Si	0.02
Ca	0.08	K	0.14
Ti	0.1	Ti	0.05
Fe	0.07	Fe	0.15
Ni	0.09	Ni	0.12
Au	7.01	Au	9.06

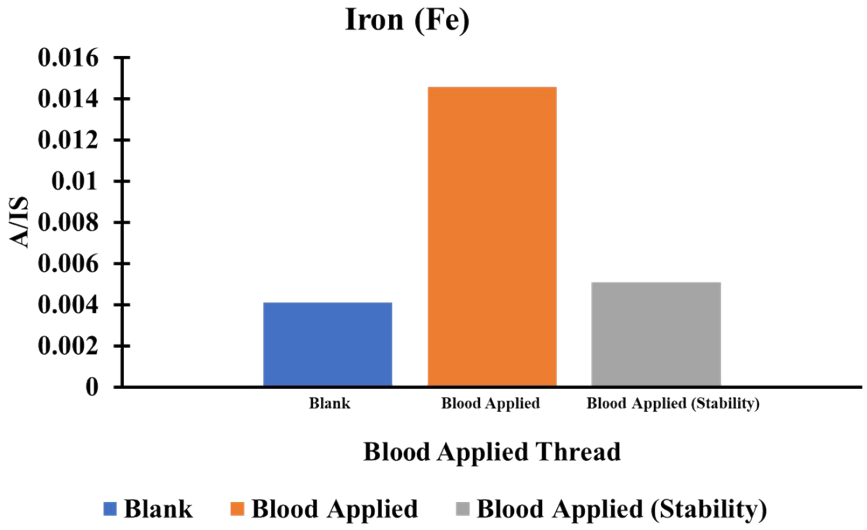

Figure S6. EDS measurement of iron content in cotton thread under different conditions with oxygen (common to cellulose thread) as an internal standard.

7. Tandem Mass Spectrometry Analysis of the Heme Cofactor. Heme analysis on common household textiles using MeOH/H_2O (1:1, v/v pH 3 using acetic acid) as a spray solvent.

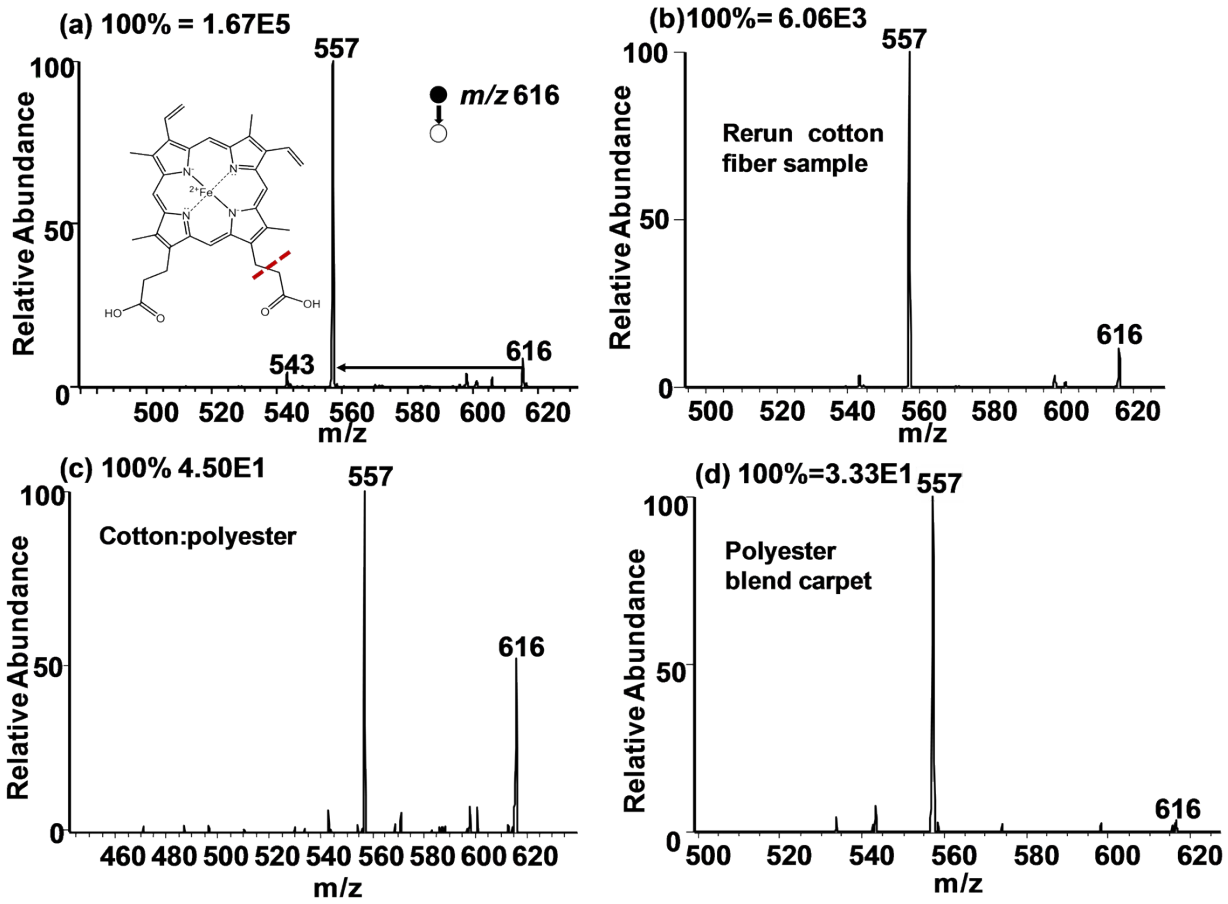

Figure S7. Tandem MS-MS of heme group (m/z 616) of (a) cotton (b) re-analysis of cotton (c) cotton polyester, (d) polyester blend carpet.

8. Forensic Bluestar®/Thread Spray Application on 100% Cotton. A crime scene bloodstain analysis scenario on a bloodstained cotton fabric was performed using Forensic Bluestar and thread spray mass spectrometry. This scenario first analyzed forensic Bluestar on to the fabric for visual assessment then threads were pulled from the fabric for thread spray mass spectrometry and vice versa. The blank (control) Bluestar analysis was carried out using chlorine bleach as a positive indicator for Bluestar.

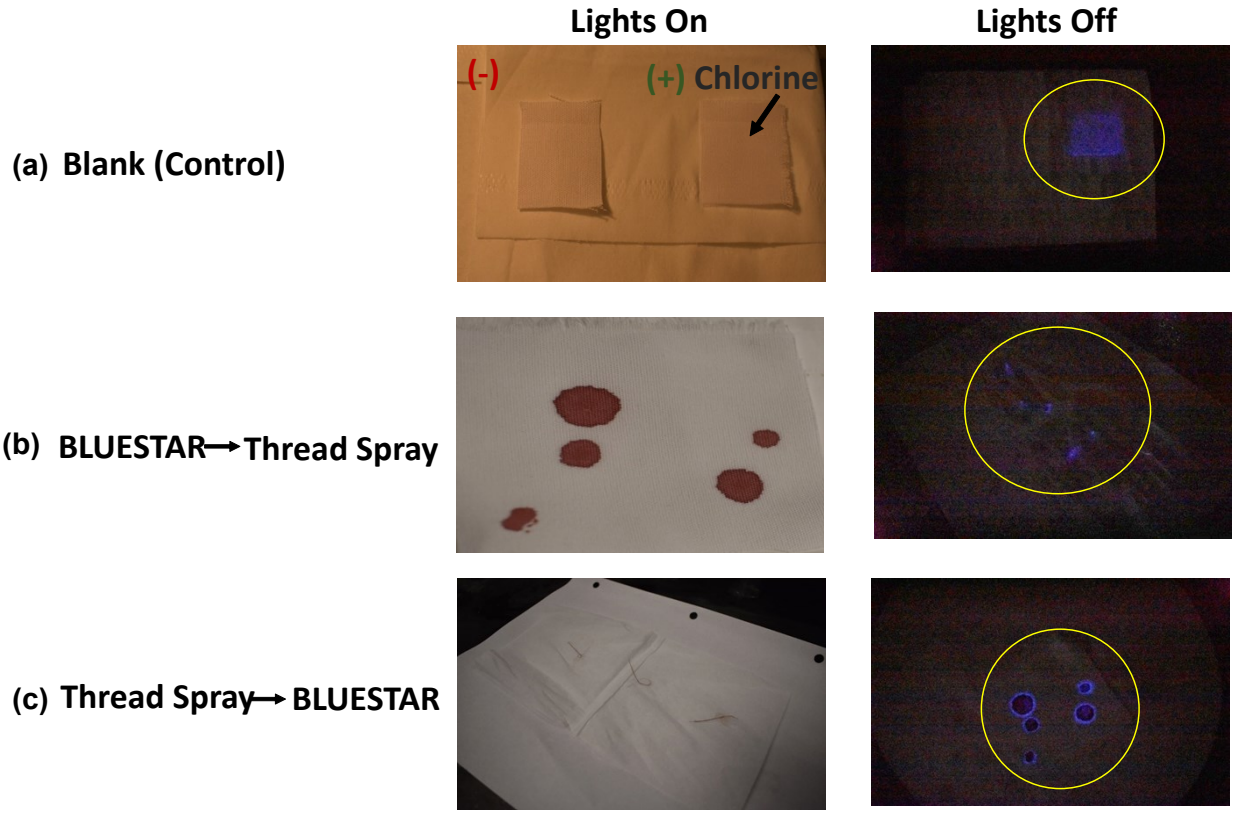

Figure S8. Visual assessment of forensic Bluestar application: (a) controlled bleach analysis indicating negative and positive results, (b) the use of Bluestar test prior to thread spray analysis and (c) Bluestar analysis after thread spray application. Accompanying ion intensities for thread spray analysis, before and after Bluestar test are provided in Table S2 below.

Table S2. Total heme ion intensities from human blood on cotton thread after machine wash.

Human Blood Presumptive Analysis on Cotton	Avg MS Intensity
Bluestar followed by Thread Spray	$2.1\times10^3 \pm 103$
Thread Spray followed by Bluestar	$2.3\times10^3 \pm 115$

9. Ultra-selective Bluestar®/Thread Spray Application on heme. Whole human blood and canine blood were applied to an untreated cotton, then machine washed. After the blood containing fabric was thoroughly cleaned, it was then analyzed using both forensic Bluestar and thread spray mass spectrometry. Thread spray mass spectrometry detection of heme MS/MS (*m/z* 616) fragment ion peak *m/z* 557.

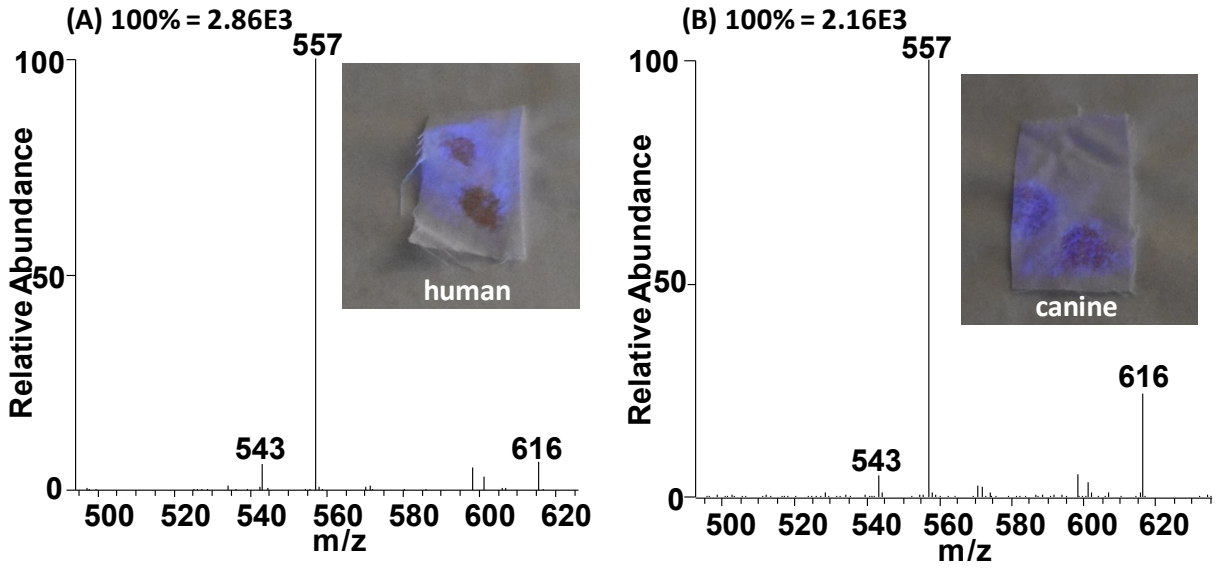

Figure S9. MS/MS analysis of heme on cotton thread for (a) human blood and (b) canine blood (insert) Bluestar analysis of machine washed fabric of human blood and canine blood.

Table S3. Total heme ion intensities from human blood on cotton thread after machine wash.

Human Blood Presumptive Analysis on Cotton (Post-Wash)	Avg MS Intensity
Bluestar followed by Thread Spray	$2.4\times10^3 \pm 815$
Thread Spray followed by Bluestar	$8.4\times10^3 \pm 662$

Table S4. Total heme ion intensities from canine blood on cotton thread after machine wash.

Canine Blood Presumptive Analysis on Cotton (Post-Wash)	Avg MS Intensity
Bluestar followed by Thread Spray	$2.2\times10^3 \pm 660$
Thread Spray followed by Bluestar	$1.7\times10^4 \pm 4650$