1. Materials and methods

1.1 Materials and patient samples

A total of 34 blood plasma samples were obtained and pooled from two patient groups (HIT positive N=11, HIT negative N =23) from Johns Hopkins University Hospital. The study protocol was approved by the Institutional Review Board (IRB) of Johns Hopkins Hospital (IRB protocol: IRB00097630). Hollow square capillary tubes (ID=0.7mm, wall=0.14mm) were purchased from Vitrocom (New Jersey, USA). Rhodamine 6G (R6G) and 4-Nitrothiophenol (4-NTP) powders were purchased from Sigma-Aldrich. The Ag-ink precursor was obtained from Kunshan Hisense Electronics Co., Ltd (SC-100, 20 wt%, China).

1.2 Preparation of silver nanoparticle-coated capillary tube

The Ag-ink coating on the capillary tube was performed by a precursor-driven method, as described in our earlier work.[1]Specific concentrations of the Ag-ink solution were created by diluting the stock solution with the required volumes of isopropanol. The capillary tubes were soaked with Ag-ink for 5 min, and a lint-free tissue was used to remove the excess ink. The ink-coated tubes were finally placed onto a preheated hot plate (at 135℃) for 10 min. The resultant Ag nanoparticle coating was characterized by a field-emission scanning electron microscope (JEOL, 6700F SEM) at an accelerating voltage of 5kV (Figure S1). A PerkinElmer Lambda 950 spectrometer was used to obtain the UV-vis spectra and a KeyenceVK-X1003D laser scanning confocal microscope was used for the surface profile measurements.

1.3 Acquisition of SERS spectra from the patient serum

SERS spectra were obtained using a home-built, fiber probe-based portable Raman system equipped with 830 nm laser excitation, which has been previously described.[2] Multiple SERS

spectra (~4-6 spectra) were collected at each sampling site. A total of 166 averaged spectra (65 from sera of HIT-positive patients and 101 from sera of HIT-negative patients) were obtained from 34 post-operative patients. Briefly, the excitation beam was delivered at the sample through the central fiber of the lensed fiber-optic Raman bundled contact probe. The back-scattered Raman signal was collected by the annular ring of collection fibers surrounding the central delivery fiber in the probe that is coupled to a spectrograph. The probe design was identical to that described by Motz et al.[3]Each SERS spectrum was averaged for five consecutive measurements. For evaluation of plasmonic enhancement of the capillary substrate, R6G and 4-NTP were used as Raman reporters. The analyte solutions were directly sucked into the Ag-ink capillary tube, and subsequently interrogated with the portable Raman system. For estimating the SERS enhancement factor, a Horiba Xplora Plus Raman microscope was used with a 532 nm laser excitation and 50x microscope objective.

1.4 Data analysis

Raw SERS data composed of intrinsic Raman signals of various biomarkers, fluorescence background, and other noise components were background subtracted using a fifth-order polynomial fitting algorithm as detailed in Zhao *et al.*[4] SERS spectra in the region of 600 to 1800 cm^{-1} was chosen to avoid scattering from the fiber background in the lower wavenumber region. Three multivariate analysis methods (PC-LDA, PLS-DA, and Lasso-DA) were tested for evaluation of diagnostic power in the SERS measurements. Principal component analysis, an unsupervised dimensionality reduction method, was employed along with linear discriminant analysis (PC-LDA).[5]

PLS-DA, which is a versatile algorithm that can be used for predictive and descriptive modeling as well as for discriminative variable selection, was also used.[6]Discriminant analysis based on

Lasso (least absolute shrinkage and selection operator) was employed to create another decision model. Lasso is a regression analysis method that performs both variable selection and regularization in order to simultaneously enhance the prediction accuracy and interpretability of the statistical model it produces.[7,8]The receiver operating characteristic (ROC) curves were generated to compare the performance of the three models for differentiating HIT-positive and HIT-negative samples.

2. Performance and optimization of SERS capillary tube

The capillary tube treated with higher concentration of Ag-ink (1:1) forms a thick and bright layer on the surface, where clusters of AgNPs were observed to coalesce into a large size by showing a shiny color compared with 1:2 and 1:4 Ag-ink to isopropanol ratio. For the latter two, densely packed individual nanoparticles were clearly observed, where 1: 4 concentration ratio results in a less dense coverage of AgNPs with a dark hue pattern. However, no essential differences in size of single AgNP (average 23.7±3.7nm) were observed among all these three groups.

SERS performance of silver-coated capillary tubes prepared with different Ag-ink to isopropanol ratios (1:1, 1:2 and 1:4) were investigated by recording the R6G spectra (as shown in Figure S2). Prominent characteristic R6G peaks were observed among all three substrates. The acquired spectra exhibit characteristic peaks at 1173 cm^{-1} (assigned to out-of-plane vibration and in-plane vibration of C-H bonds), 1302, 1355, 1510 and 1641 cm^{-1} (assigned to the aromatic C-C stretching vibration mode).[12] However, compared to the lower overall intensity obtained at 1:1 concentration and higher fluorescence background involved with 1:4 concentration under the same experimental conditions, the optimal performance of R6G SERS spectra was achieved at the 1:2 concentration. It is quite straightforward that density and distribution pattern of AgNPs presented on capillary tube surface influence their plasmonic activity, thus the final SERS performance. In other words,

excessive coating of AgNPs tends to result in a relatively thick silver-film, while inadequate AgNPs usually means insufficient numbers of “hot spots”, thus impeding the resultant electromagnetic enhancement to Raman signal.

A simple way to provide objective evaluation of SERS substrate is to quantitatively compare the performance of the SERS capillary tube based on corresponding Raman and SERS signals of Raman probes, and we employed the 4-NTP solution to calculate the enhancement factor (EF), which was found to be ~2.5 x 10^7 when capillary tube was treated with the optimal 1:2 concentration. The EF was calculated using methods reported elsewhere[9-11] and with the equation:

$$EF = (I_{SERS}/ N_{Surf})/(I_{Bulk}/N_{Bulk})$$

where I_{SERS} and I_{Bulk} are the Raman intensities from the capillaries and the powder 4-NTP respectively. The N_{Bulk} and N_{surf} are the number of 4-NTP molecules under the laser area in case of powder Raman and the SERS experiments respectively.

In addition, to evaluate the detection sensitivity for the optimized capillary tube (i.e. derived from 1:2 Ag-ink: isopropanol ratio), varying concentrations of 4-NTP (10^{-3} and 10^{-9} M) were measured (Figure S3). SERS signals could be clearly identified with 4-NTP concentration down to 10^{-9} M, and potentially better detection limits might be expected after further optimization, for example, shifting the laser excitation much closer to plasmonic peak of silver nanoparticle.

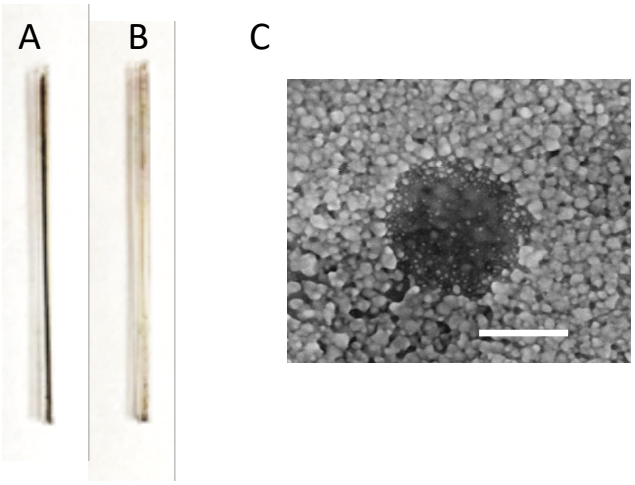

Figure S1. The Ag-film is formed on only one side of the square capillary tube. (A) The side of the square capillary coated with Ag-film (after heating) is seen on the top. (B) The capillary shown in (A) with the Ag-film coated side on the right side (when the tube is rotated clockwise from position A by 90^{o}). (C) FESEM image of the Ag-film and the morphology of the hole shows small nanoparticles coalesced together. The scale bar corresponds to 100 nm.

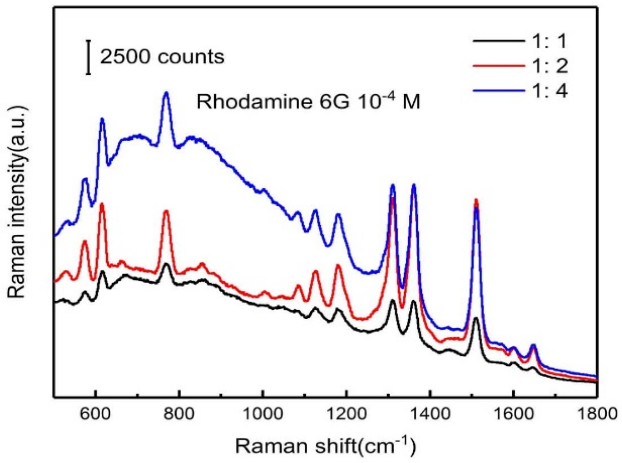

Figure S2. SERS spectra of Rhodamine 6G at concentration of 10^{-4} M from capillary tubes prepared at different Ag-ink to isopropanol ratios under the same measurement conditions. Spectra were shifted for better visualization.

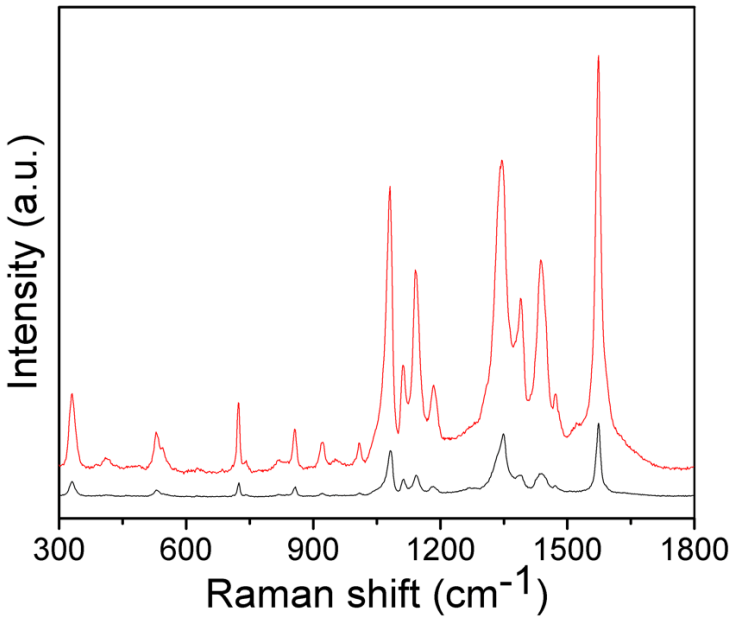

Figure S3. SERS spectra of 10^{-3} M (red) and 10^{-9} M (black) solutions of 4-NTP acquired from capillary tube that was prepared from 1:2 Ag-ink: isopropanol volume ratio. Spectra have been offset vertically for the sake of visual clarity.

Table S1: *Tentative band assignment of blood serum samples obtained from HIT positive and healthy patients.*[13,14]

Band position (cm^{-1})	Tentative band assignments
686	DNA bases, Phe, Tyr
855	Tyr, Pro, C-C str, CCH def
928	Pro, Val, C-C backbone, Lipids
1045	Gly, Phosphate, Carbohydrate
1131	C-C skeletal str, lipids
1239	Amide III, phosphate str, C-H bending in lipids
1332	C-C str of phenyl, CH_2 twist

1401	Bending modes of methyl
1450	CH_2 bending, CH_2 def
1604	Cytosine, Phe, Tyr
1647	Amide I

3. References:

[1] Z. Huang, S. Siddhanta, C. Zhang, T. Kickler, G. Zheng, I. Barman, *J. Raman Spectrosc.* **2017**, *48*, 1365-1374.

[2] S. K. Paidi, S. Siddhanta, R. Strouse, J. B. Mcgivney, C. Larkin, I. Barman, *Anal. Chem.* **2016**, *88*, 4361-4368.

[3] J. T. Motz, M. Hunter, L. H. Galindo, J. A. Gardecki, J. R. Kramer, R. R. Dasari, M. S. Feld, *Appl. Opt.* **2004**, *43*, 542-554.

[4] J. Zhao, H. Lui, D. I. McLean, H. Zeng, *Appl. Spectrosc.* **2007**, 61, 1225-1232.

[5] A. M. Martínez, A. C. Kak, *IEEE Transactions on Pattern Analysis & Machine Intelligence*, **2001**, 23, 228-233.

[6] S. Duraipandian, W. Zheng, J. Ng, J. J. H. Low, A. Ilancheran, Z. Huang, *Anal. Chem.* **2012**, *84*, 5913-5919.

[7] R. Tibshirani, *J. R. Stat. Soc. B* **1996**, *58*, 267-288.

[8] S. Reid, R. Tibshirani, *Biostatistics* **2016**, *17*, 364-376.

[9] W. B. Cai, B. Ren, X. Q. Li, C. X. She, F. M. Liu, X. W. Cai, Z. Q. Tian, *Surf. Sci.* **1998**, *406*, 9-22.

[10] E. C. Le Ru, E. Blackie, M. Meyer, P. G. Etchegoin, *J. Phys. Chem. C* **2007**, *111*, 13794-13803.

[11] V. S. Tiwari, O. Tovmachenko, G. K. Darbha, W. Hardy, J. P. Singh, P. C. Ray, *Chem. Phys. Lett.* **2007**, *446*, 77-82.

[12] X. N. He, Y. Gao, M. Mahjouri-Samani, P. N. Black, J. Allen, M. Mitchell, W. Xiong, Y. S. Zhou, L. Jiang, Y. F. Lu, *Nanotechnology* **2012**, *23*, 205702.

[13] Z. Movasaghi, S. Rehman, I. U. Rehman, *Appl. Spec. Rev.* **2007**, *42*, 493-541.

[14] Y. Tan, B. Yan, L. Xue, X. Luo, P. Ji, *Lipids in Health Dis*. **2017**, *16***,** 73(1-9).