Electronic Supplementary information:

Raman spectroscopic screening of High and Low molecular weight fractions of human serum

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1. No correlation was found between the concentrations of globulin and albumin in patient samples

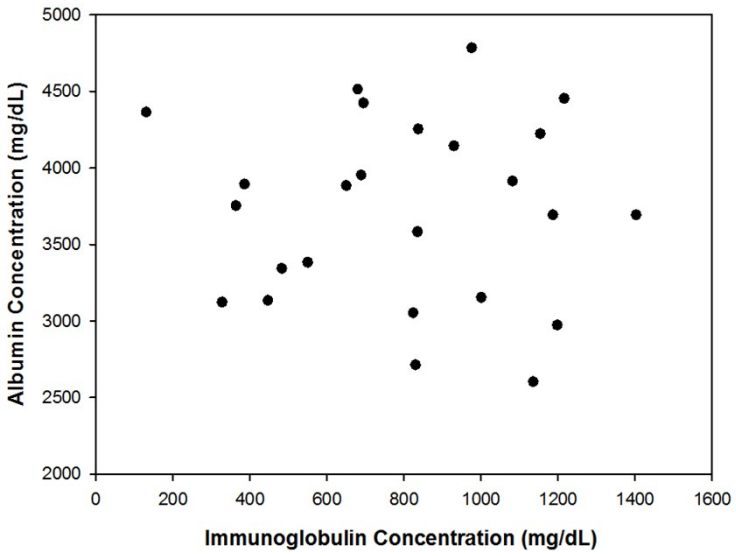

Figure S1. Plot of the concentrations of albumin and immunoglobulin for each patient

2. No correlation was found between the concentrations of urea and glucose in patient samples

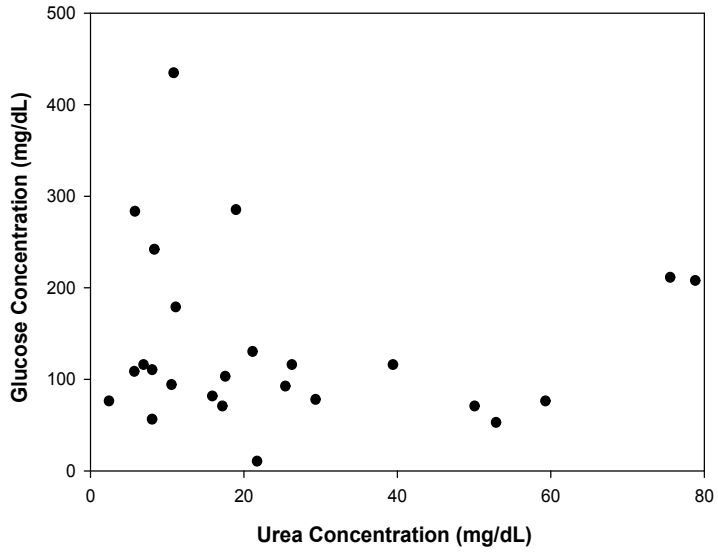

Figure S2: Plot of the concentrations of urea and glucose for each patient

3. Reference spectrum of beta-carotene

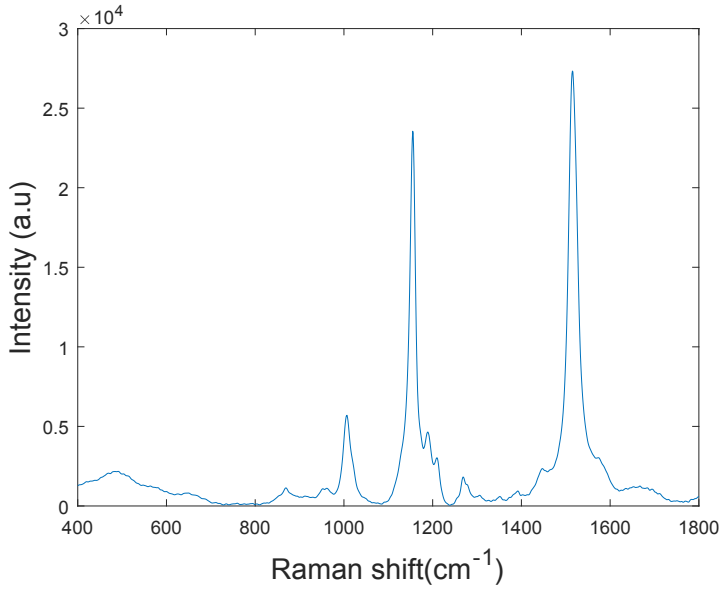

Figure S3: Raman spectrum of beta-carotene used for EMSC correction of human serum from patient samples

4. Reference spectrum of human serum

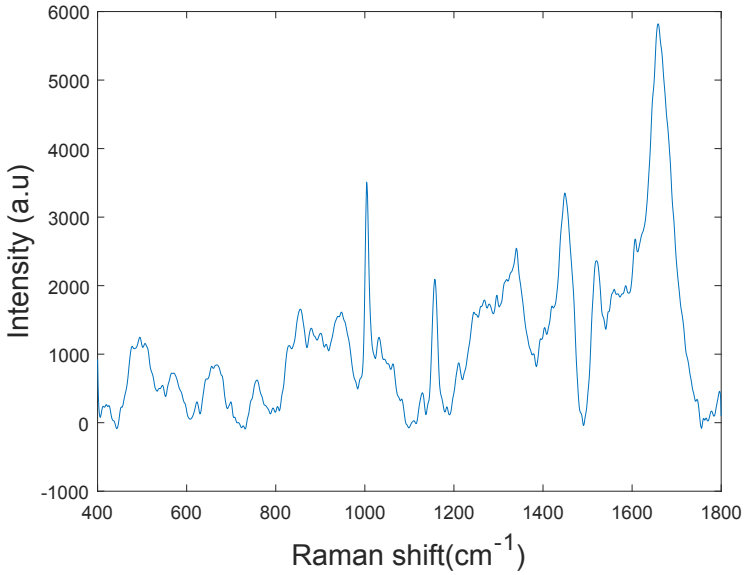

Figure S4: Raman spectrum of human serum used for EMSC correction of total protein and globulin from patient samples

5. PLSR was performed on shorter spectral region of γ globulin from patient serum samples

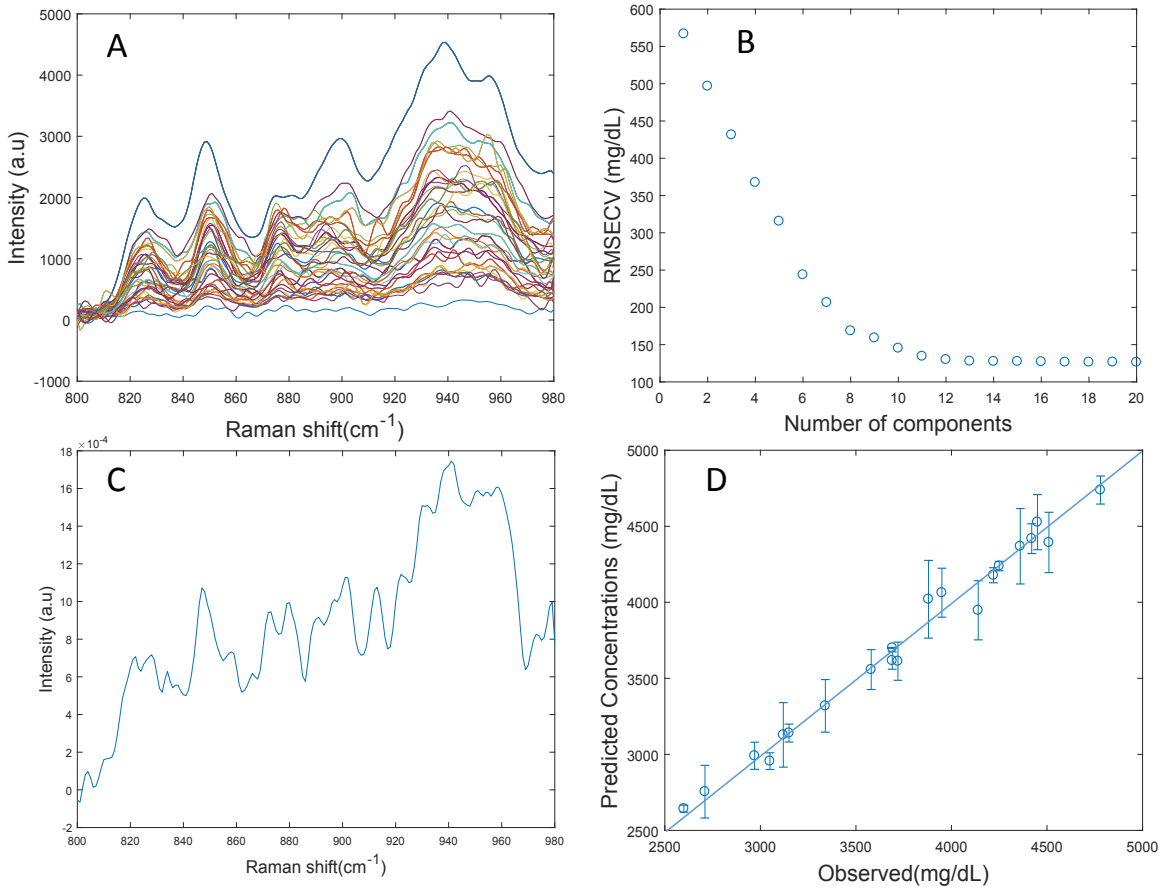

Figure S5. (A) EMSC corrected Raman spectra of γ globulin of patient serum samples from 800cm^{-1} to 980cm^{-1} (329 mg/dL, 690 mg/dL, 836 mg/dL and 1404 mg/dL), the spectra has been offset for clarity. (B): Evolution of RMSECV of the data set (C): plot of PLSR coefficient for regression against γ globulin concentration shows the peaks of γ globulin, (D): Linear predictive model for γ globulin concentration built from the PLSR analysis.

5. PLSR was performed on varying cocnentration of albumin in water (5-50mg/dL)

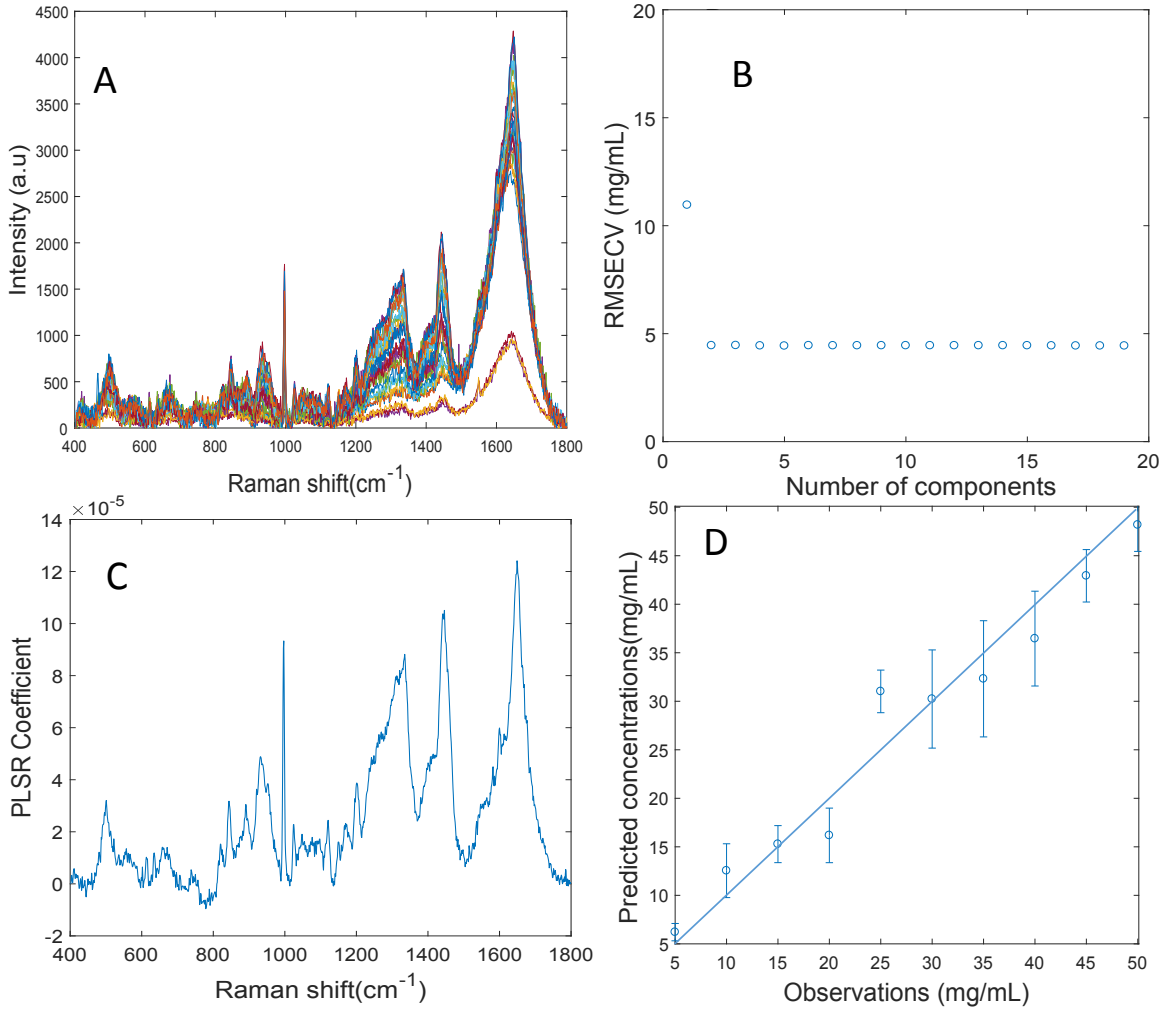

Figure S6. (A): Rubberband corrected Raman spectra of varying concentrations of Albumin from 5mg/mL to 50mg/mL (500mg/dL to 5000mg/dL) in distilled water, (B): Evolution of RMSECV on the validation model, (C): plot of PLSR coefficient with Albumin features, (D): Linear predictive model built from the PLSR analysis. The RMSECV is calculated as 1.58mg/mL (158mg/dL)

7. PLSR result of varying concentrations of glucose in distilled water (100-1000mg/dL) (2)

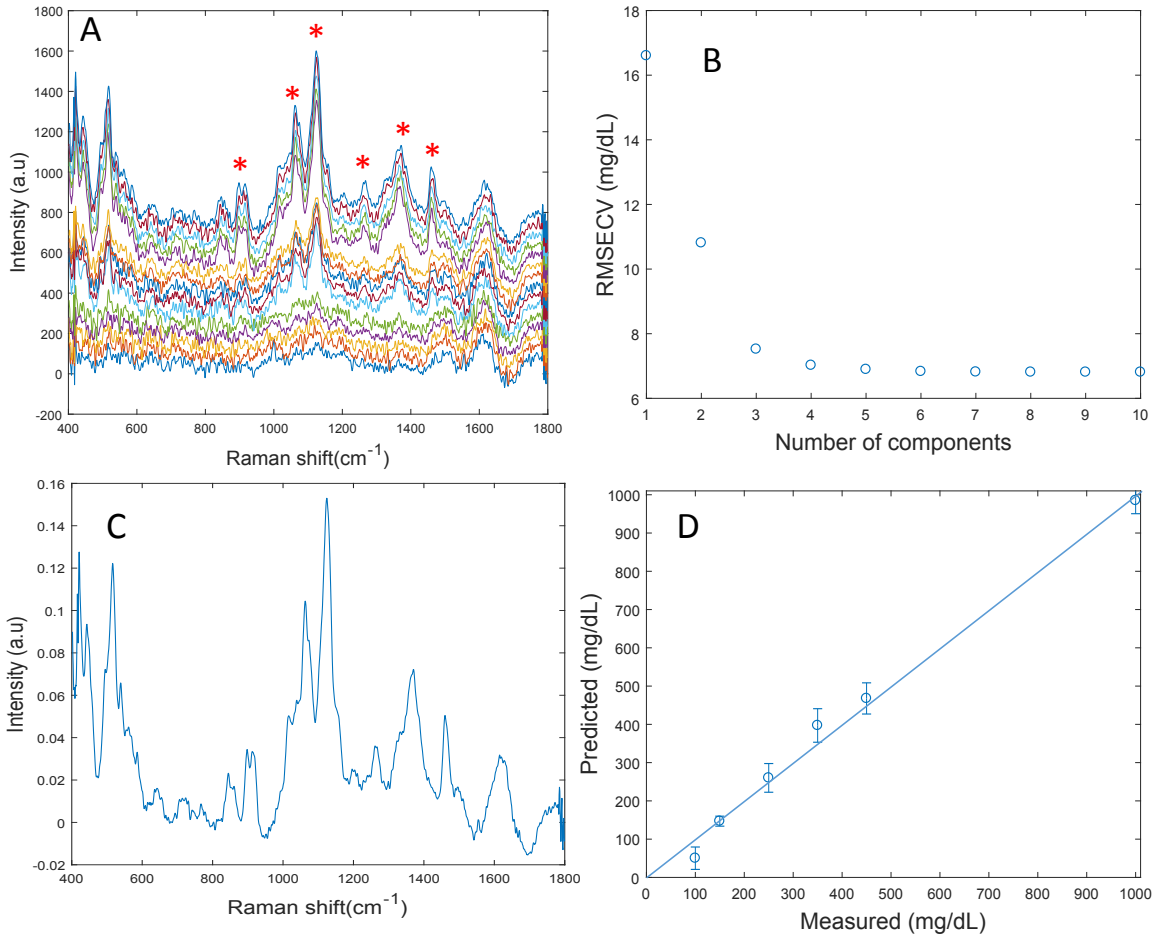

Figure S7. (A): EMSC corrected Raman spectra of filtrate obtained after centrifugal filtration with 10kDa filters of varying concentrations of glucose (5 x100mg/dL, 5 x 450mg/dL and 5 x 1000mg/dL, spectra offset for clarity), in distilled water and signature peaks of glucose are highlighted with asterisks, (B): Evolution of the RMSECV on the validation model, (C): plot of PLSR coefficient with glucose features, (D): Predictive model built from the PLSR analysis. The value displayed in the PLSR model is an average of the concentration predicted with the corresponding standard deviation calculated from the 20 iterations of the cross validation. The RMSECV and R^2 values were calculated as 10.93mg/dL and 0.9705 respectively.

8. PLSR analysis performed on the filtrate collected after centrifugal filtration of glucose spiked in serum samples using 10kDa filters (2)

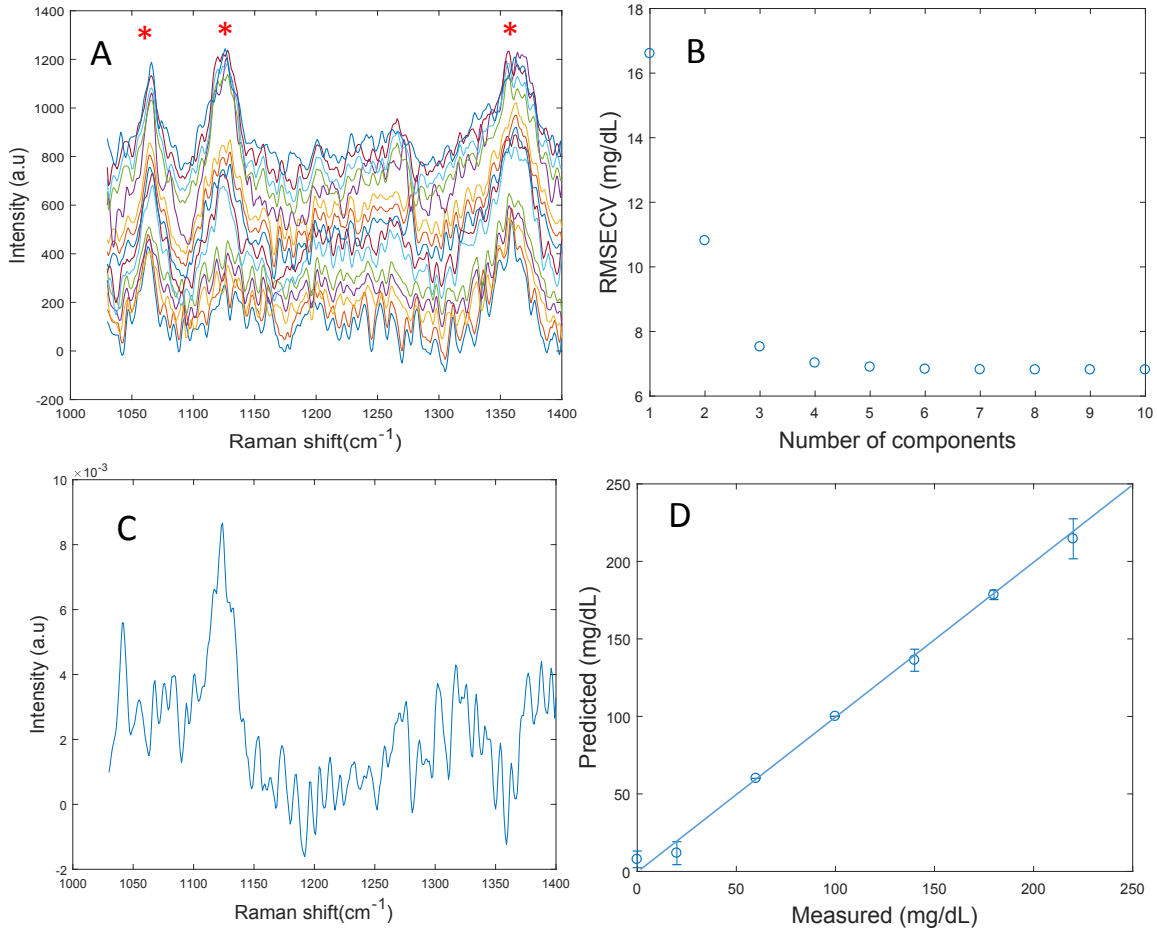

Figure S8. (A): EMSC corrected Raman spectra of filtrate obtained after centrifugal filtration with 10kDa filters of glucose spiked in serum (spiked concentrations 5 x 0mg/dL, 5 x 120mg/dL and 5 x 220mg/dL, offset for clarity) and the signature peaks of glucose are highlighted by asterisks, (B): Evolution of RMSECV of the data set (C): plot of PLSR coefficient with glucose features, (D): Predictive model built from the PLSR analysis. The value displayed in the PLSR model is an average of the concentration predicted with the corresponding standard deviation calculated from the 20 iterations of the cross validation. The RMSECV and R^2 values were calculated as 1.66mg/dL and 0.9914

9. PLSR analysis performed on the filtrate collected after centrifugal filtration of patient samples using 10kDa filters (2)

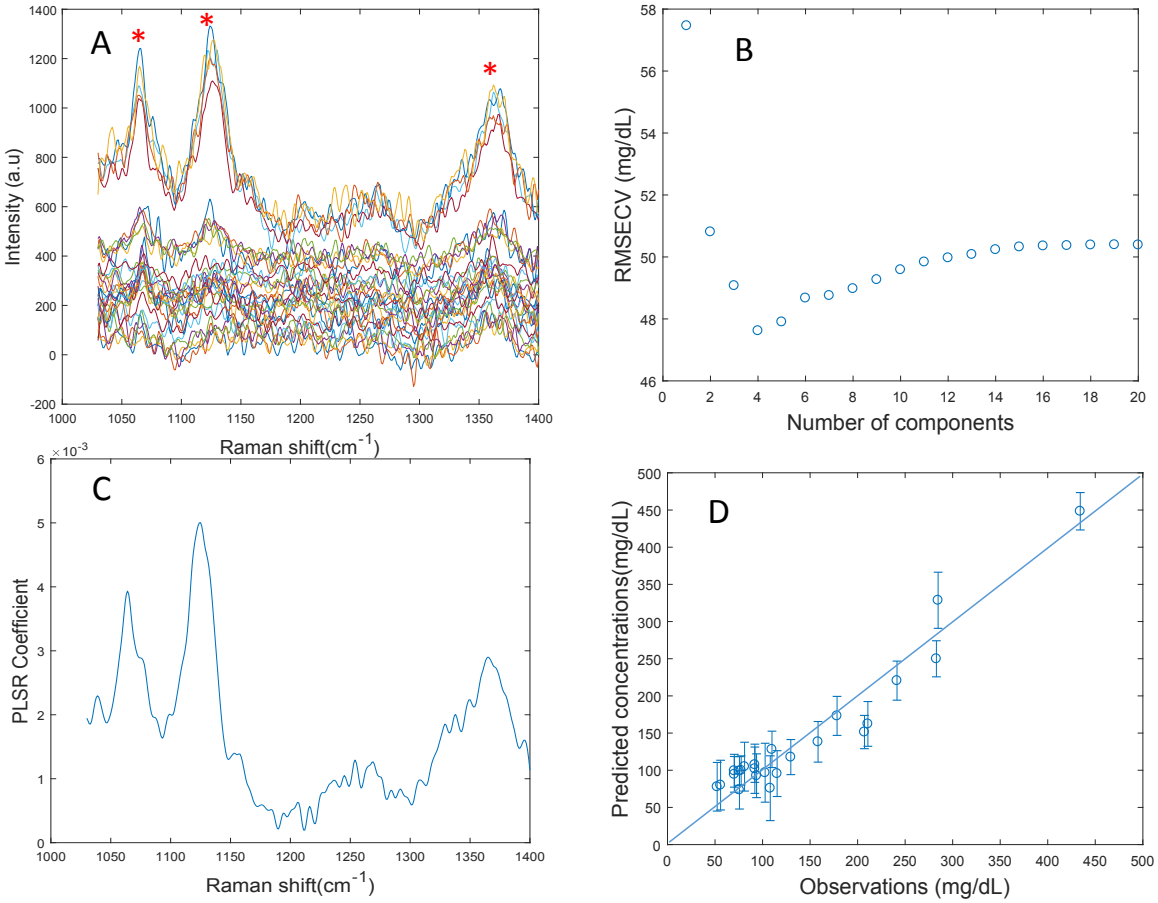

Figure S9. (A): EMSC corrected Raman spectra of filtrate obtained after centrifugal filtration with 10kDa filters of patient samples (5 x 52.25mg/dL, 5 x 75.67mg/dL, 5 x 93.69mg/dL, 5 x 210.81mg/dL and 5 x 434.35mg, offset for clarity) and the signature peaks are marked by asterisks, (B): Evolution of RMSECV of the data set, (C): plot of PLSR coefficient with glucose features, (D): Predictive model built from the PLSR analysis. The value displayed in the PLSR model is an average of the concentration predicted with the corresponding standard deviation calculated from the 20 iterations of the cross validation The RMSECV and R^2 values were calculated as 1.84mg/dL and 0.84 respectively.

10. PLSR performed on varying concentrations of urea in water

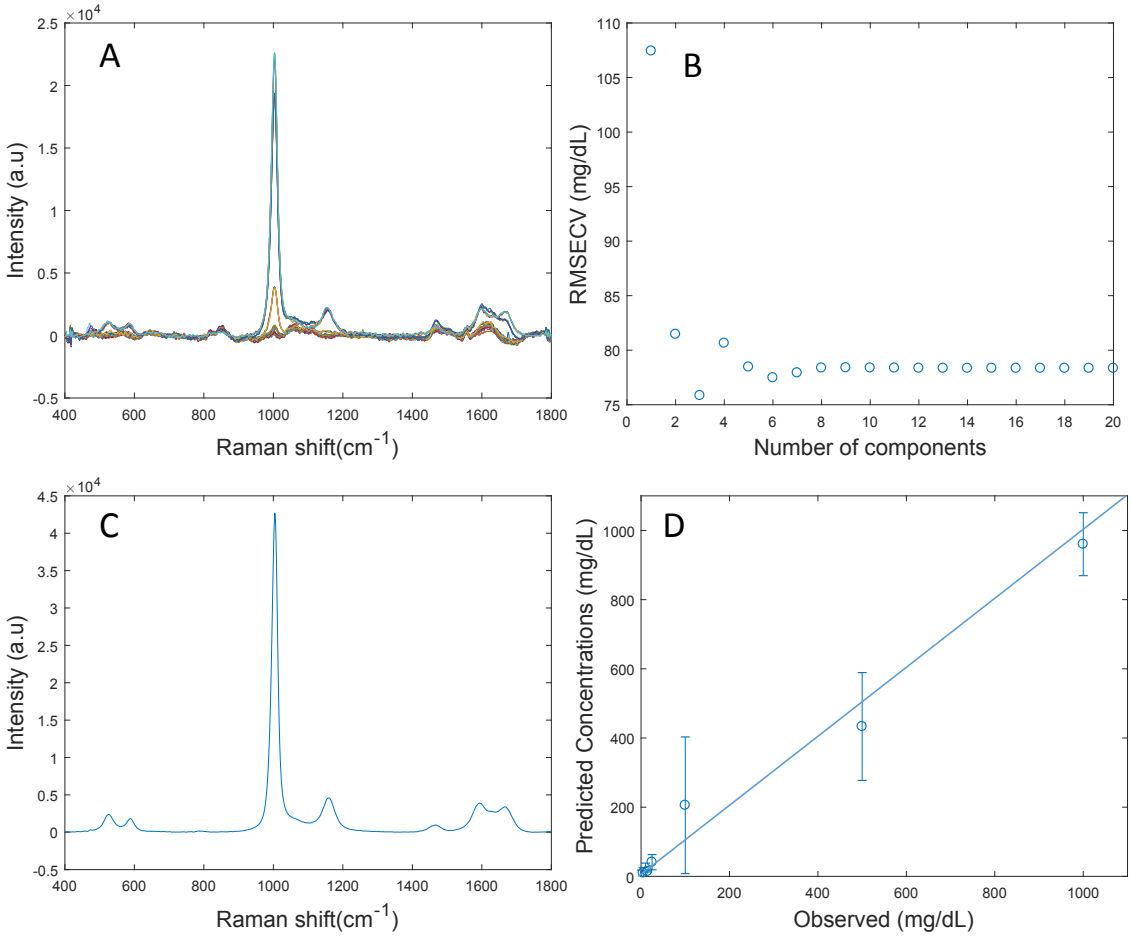

Figure S10. (A): EMSC corrected Raman spectra of filtrate obtained after centrifugal filtration with 10KDa filters of urea spiked in water (1mg/dL to 1000mg/dL), (B): Evolution of RMSECV of the data set (C): plot of PLSR coefficient with urea features, (D): Linear predictive model built from the PLSR analysis. The RMSECV, R^2 and overall standard deviation values values were calculated as 70.4044mg/dL, 0.9048 and 1.0975mg/dL.

References

1. Parachalil DR, Brankin B, McIntyre J, Byrne HJ. Raman spectroscopic analysis of high molecular weight proteins in solution – considerations for sample analysis and data pre-processing. Analyst. 2018;143(24):5987–98.

2. Parachalil DR, Brankin B, McIntyre J, Byrne HJ. Analysis of bodily fluids using Vibrational Spectroscopy : A direct comparison of Raman scattering and Infrared absorption techniques for the case of glucose in blood. Analyst (Accepted) 2019.