

Supplementary Materials

Rapid detection and identification of bacterial meningitis pathogens in *ex vivo* clinical samples by SERS method and principal component analysis

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1. SERS substrates preparation

We obtained the nuclepore track-etched polycarbonate membranes (pore size 0.3 and 3 μm) from Whatman commercial company and used them successfully as a SERS platform. To prepare SERS platform we have cut these membranes and covered them with 50 nm of Au-Ag via PVD sputter coater (Leica, EM MED020). Afterwards, they were placed into a Petri dish to avoid contamination from the air.

Procedure of gold sputtering: to sputter a layer of gold we used PVD equipment from Leica, model EM MED020. The gold target was obtained from Mennica Metale Szlachetne, Warsaw, Poland. The size of the gold target was 54 mm in diameter, thickness of 0.5 mm, and gold purity was 3N. The vacuum during the gold sputtering was on the level of 10⁻² mbar. The

current of sputtering was 25 mA. No adhesive layer (chromium or titanium) was sputtered on the polymer mat before sputtering gold.

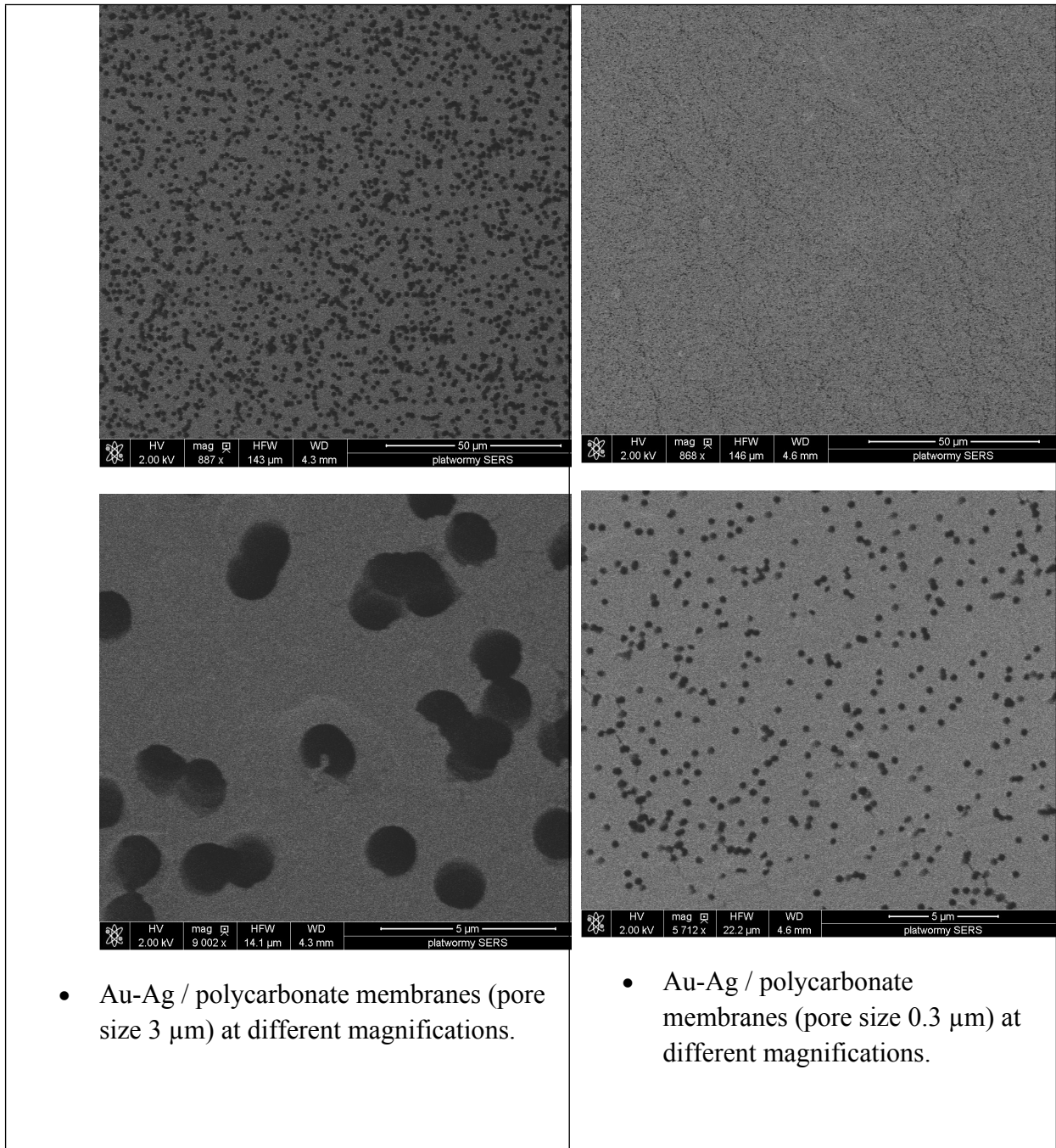


Fig. S1. SEM images of Au-Ag / polycarbonate membranes of pore sizes 0.3 and 3 μm covered with 50 nm of silver-gold via PVD process, (b) polycarbonate membrane covered with 50 nm of Au-Ag via PVD process at different magnifications, working as SERS-active platforms.

2. SERS analysis of control and infected cerebrospinal fluids samples.

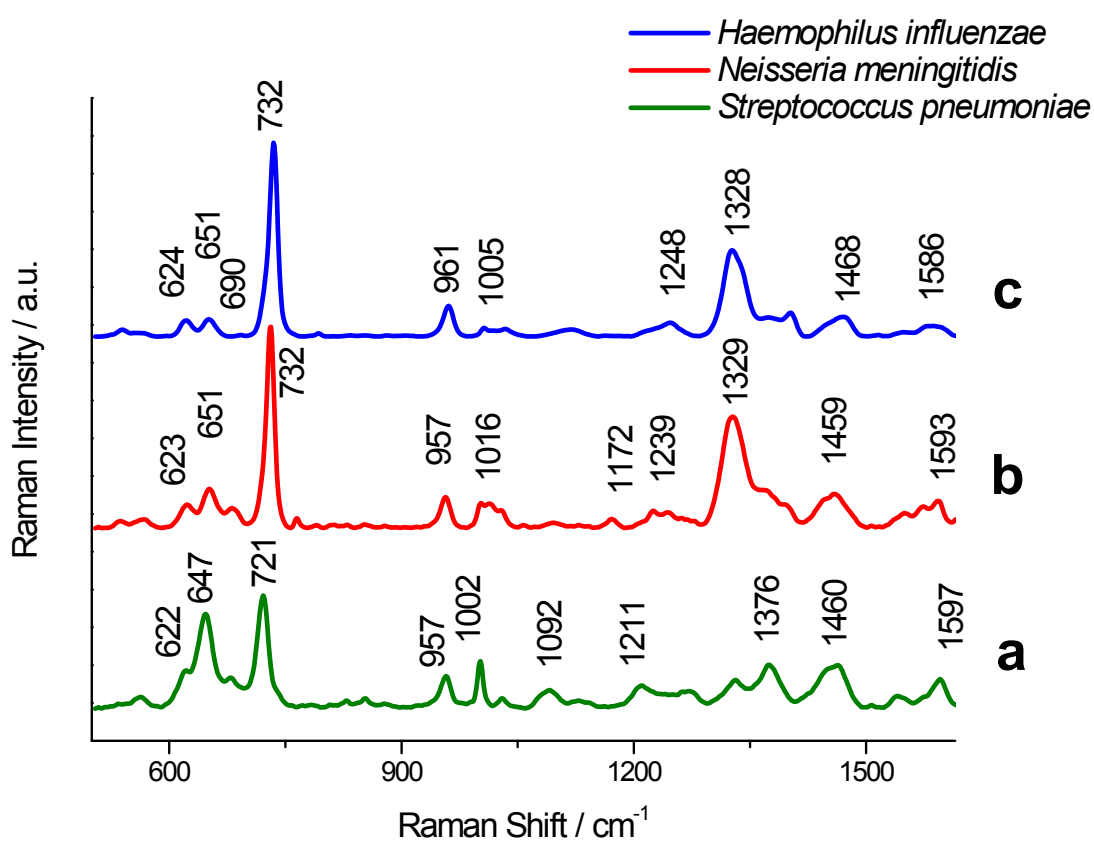
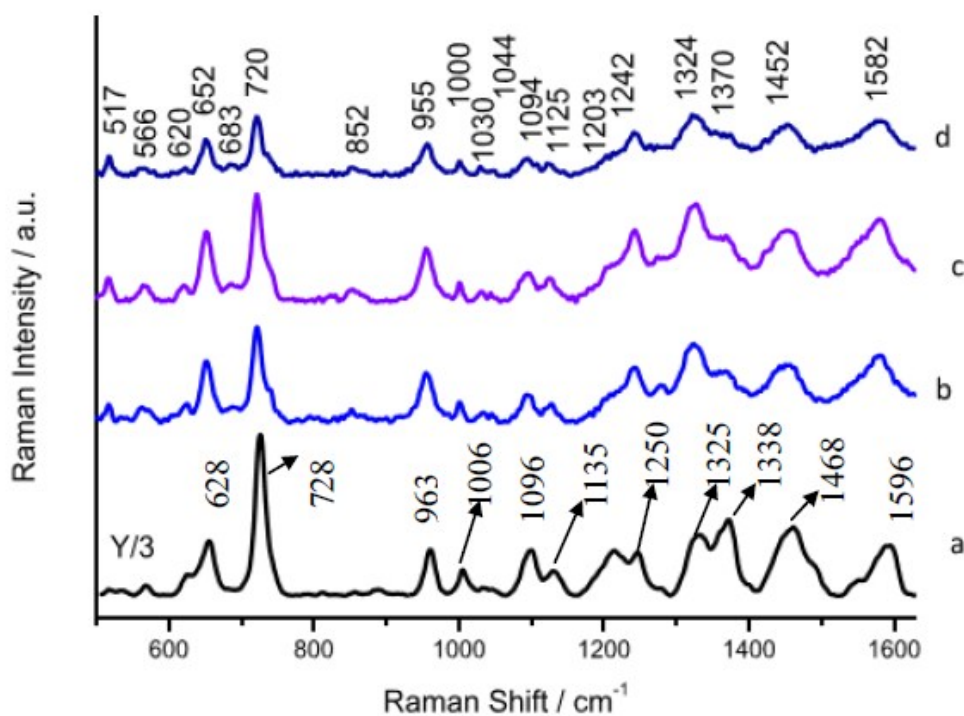


Fig.S2. SERS spectra of (a) *S. pneumoniae*; (b) *N. meningitidis*, and (c) *H. influenzae* bacteria multiplied before SERS experiment by cultivation in BWP growth medium and then deposited onto an Ag-Au coated polycarbonate membrane. Experimental conditions: 0.5 mW,

785 nm excitation, 30 seconds acquisition time (mapping mode). The SERS spectra have been baseline corrected, normalized and shifted vertically for better visualization. Each SERS spectrum was averaged from 20 measurements in different places of SERS platform, and then spectra have been baseline-corrected, normalized, and shifted vertically for better visualization.

Fig.S3. SERS spectra of a control CSF sample (a) and of CSF infected by *N. meningitidis* (b),



S. pneumoniae (c), *H. influenzae* (d) recorded onto polymer-based SERS-surfaces. Experimental conditions: 0.5 mW, 785 nm excitation, 30 seconds acquisition time (mapping mode). The SERS spectra have been baseline corrected, normalized and shifted vertically for better visualization.

For the control CSF sample (Fig. S3a) the intense bands at 728, 1096, 1135, 1377, 1596 cm^{-1} were assigned to vibrations of the nucleic bases of DNA and lipids ¹. The features at 1469, 1250, 1006, and 964 cm^{-1} are associated with CH_2 deformation, the amide III, the symmetric ring breathing bands of phenylalanine and protein, and C-C stretching, respectively ². Aromatic amino acid residues, phenylalanine, tyrosine, and tryptophan were expected to have bands at 628, 659, 866, 1173 and 1213 cm^{-1} . The prominent SERS peaks located at 659, 728, 963, 1006, 1096, 1134 and 1468 cm^{-1} can be consistently observed also in infected samples (Fig.S3b-d).

3. The calculation of the reproducibility of SERS spectra.

The reproducibility analysis of recorded the SERS spectra for each ABO system with A and B antibody respectively, were processed with a Savitzky-Golay second derivative method. Correlation coefficients between all non-identical spectral pairs ($i \neq j$) in the same date set were determined from the data according to the formula:

$$P_{i,j} = \frac{\sum_{k=1}^W (I_i(k) - \bar{I}_i)(I_j(k) - \bar{I}_j)}{\sigma_i \sigma_j} \quad [1]$$

where i, j were the indexes of the spectra in the data matrix, k was the wave number index of the individual spectra, I was the spectral intensity, W was the spectral range, and σ_i was the standard deviation of the spectrum³. Once the correlation coefficients $P_{i,j}$ were calculated, Γ , the average of the off-diagonal correlation coefficients, was determined:

$$\Gamma \equiv \frac{2 \sum_{i=1}^N \sum_{j=i+1}^N P_{ij}}{N(N-1)}$$

[2]

Γ varied between 0 and 1, where 1 was the case of identical spectra and 0 the case of completely uncorrelated spectra. Γ , as defined in Equation 2, was used to evaluate the reproducibility of the recorded spectra of meningitis pathogens. In Fig. S4 are gathered the obtained results for *H. Influenza*. The calculated average spectral correlation coefficients for all studied meningitis pathogens: *S. pneumoniae*, *H. influenza*, and *N. Meningitidis* are gathered in the Table 1S.

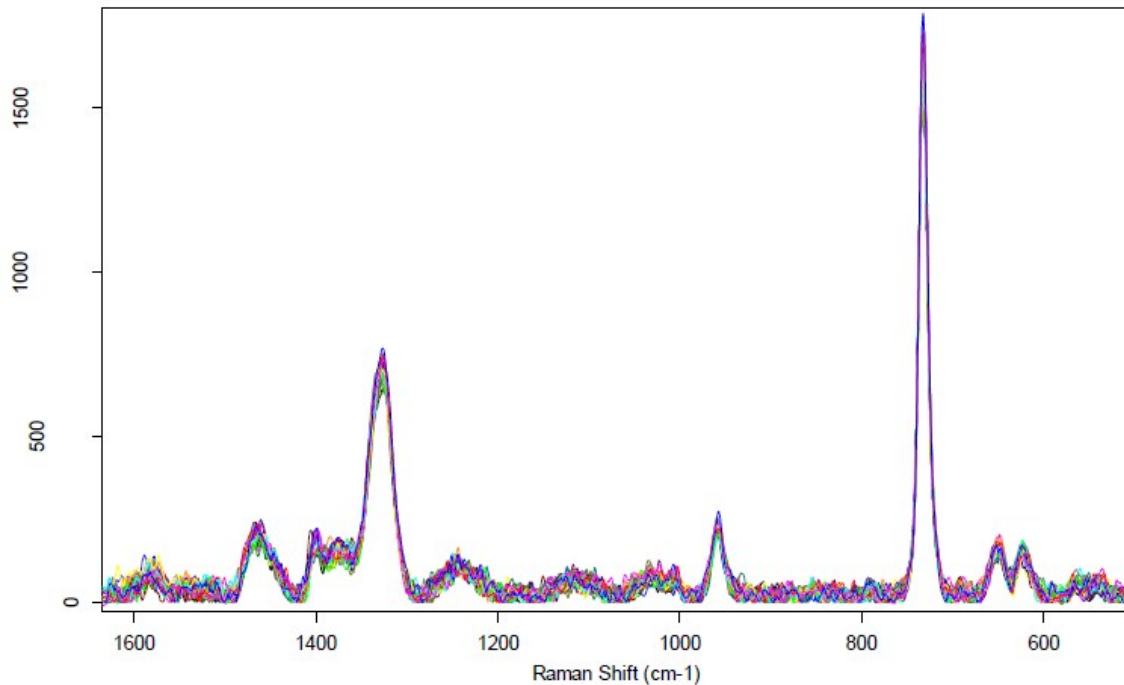
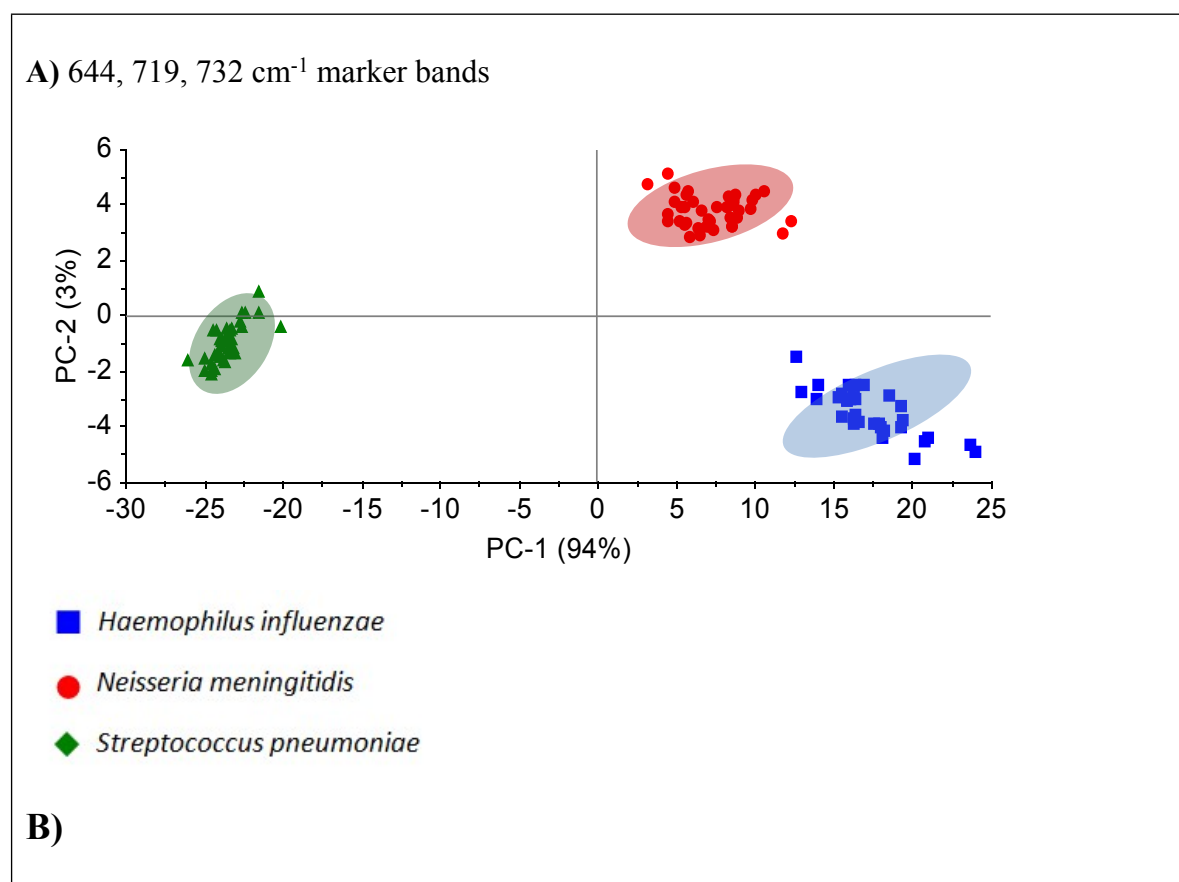


Figure S4. The example of reproducibility of 20 SERS spectra for *H. Influenza* recorded from different points at one polymer-based SERS-surfaces, (using mapping mode).

Table 1S. The calculated average spectral correlation coefficients for all studied meningitis pathogens: *S. pneumoniae*, *H. influenza*, and *N. Meningitidis*.

Meningitis pathogens	Γ (correlation coefficient)
<i>S. pneumoniae</i>	0.94
<i>H. influenza</i>	0.95
<i>N. Meningitidis</i>	0.92

4. PCA analysis



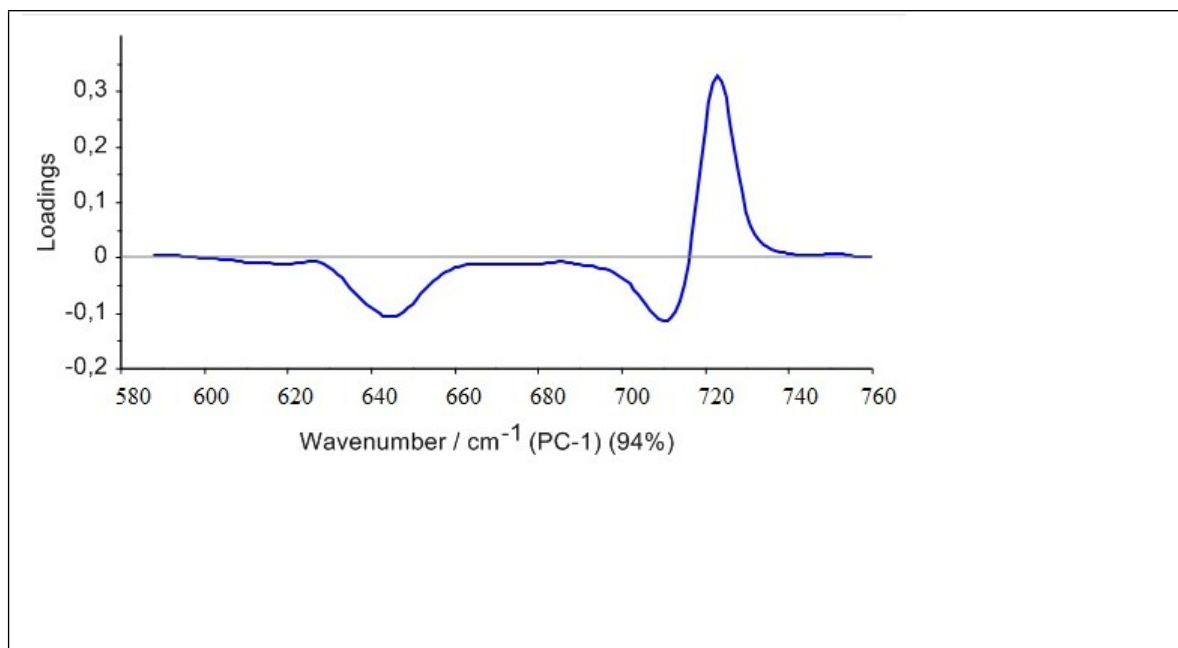


Fig.S5. The comparison of the calculated PCA analysis for three studied bacteria (A) PC-1 versus PC-2 scores with (B) PC-1 loadings plot for the regions of band at 644, 719, 732 cm^{-1} .

5. **Detection of Neopterin level in CSF samples as a marker of meningitis bacterial infections.**

The SERS spectrum of neopterin (Figure S6) is dominated by bands at 695, 1292, 1521 and 1689 cm^{-1} , due to C-C vibration and ring modes, N-H bending modes, NH_2 symmetric deformation, and C=O stretching vibrations, respectively ⁴.

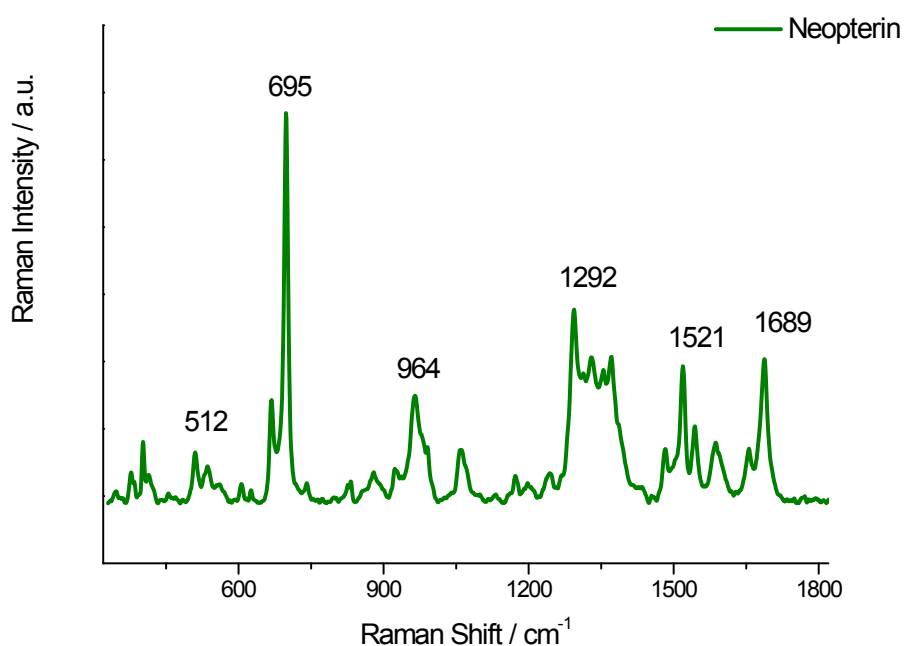


Figure S6. SERS spectrum of neopterin adsorbed onto membrane - based SERS platform from 35.0 nmol/L neopterin solution in a PBS buffer.

In order to test the performance of our SERS surface in terms of sensitivity and low detection limit (LOD), the plot of SERS intensity of the marker band at 695 cm^{-1} versus the concentration of neopterin in CSF was constructed (Fig. S7). The samples of neopterin in CSF with different concentrations, reflecting clinically relevant neopterin titers, (3 – 100 nmol/L) were prepared. In the linear region the calibration curve was fitted as $y = 157.82x$ and the correlation coefficient (R^2) was 0.995. For the linear calibration curve, it was assumed that the SERS intensity at 695 cm^{-1} (y) is linearly related to the concentration of neopterin (x). The low detection limit (LOD) was estimated using the signal-to-noise method ⁵.

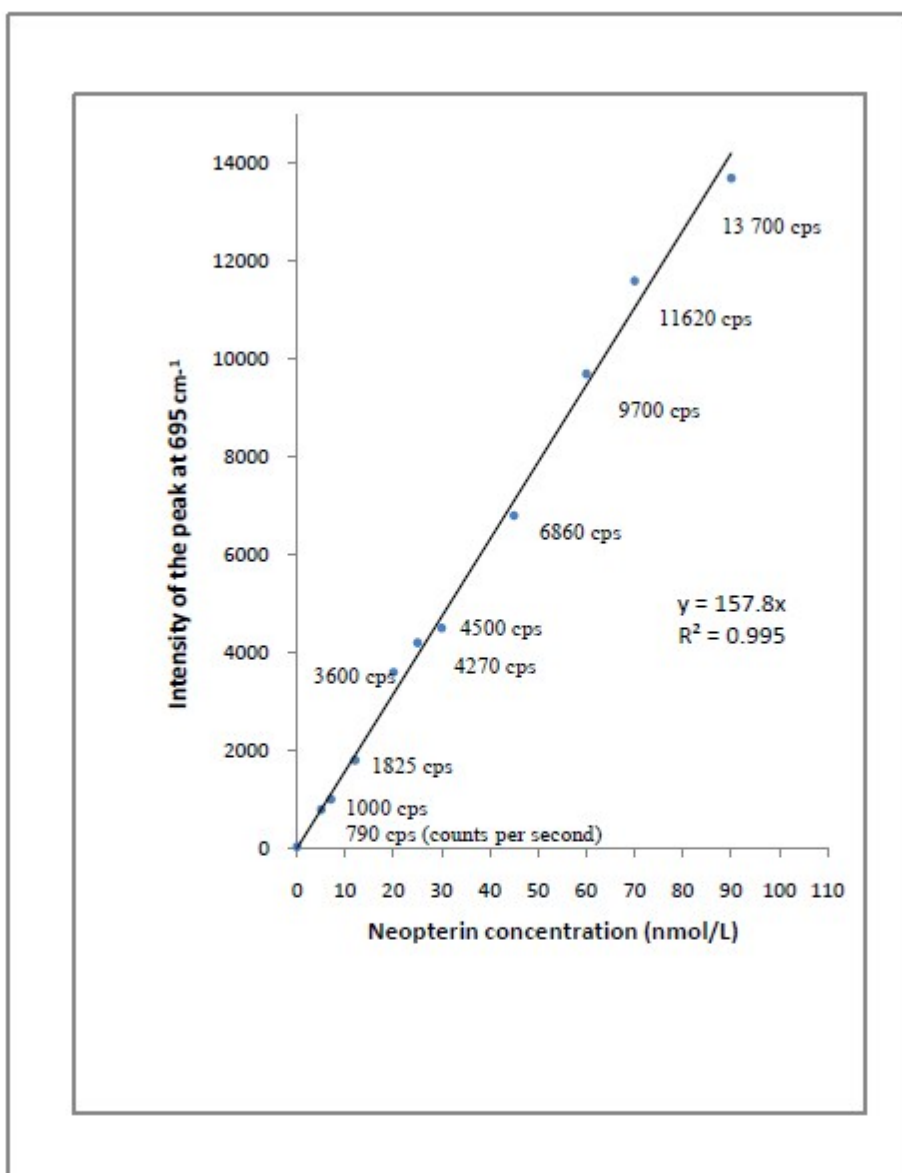


Fig. S7. The relationship between the intensity of the band at 695 cm⁻¹ versus the concentration of neopterin in CSF in the range from 0 to 90 nmol/L. Experimental conditions: 0.5 mW, 785 nm excitation, 4 x 40 seconds acquisition time. Each SERS spectrum was averaged from ten measurements in different places of SERS platform.

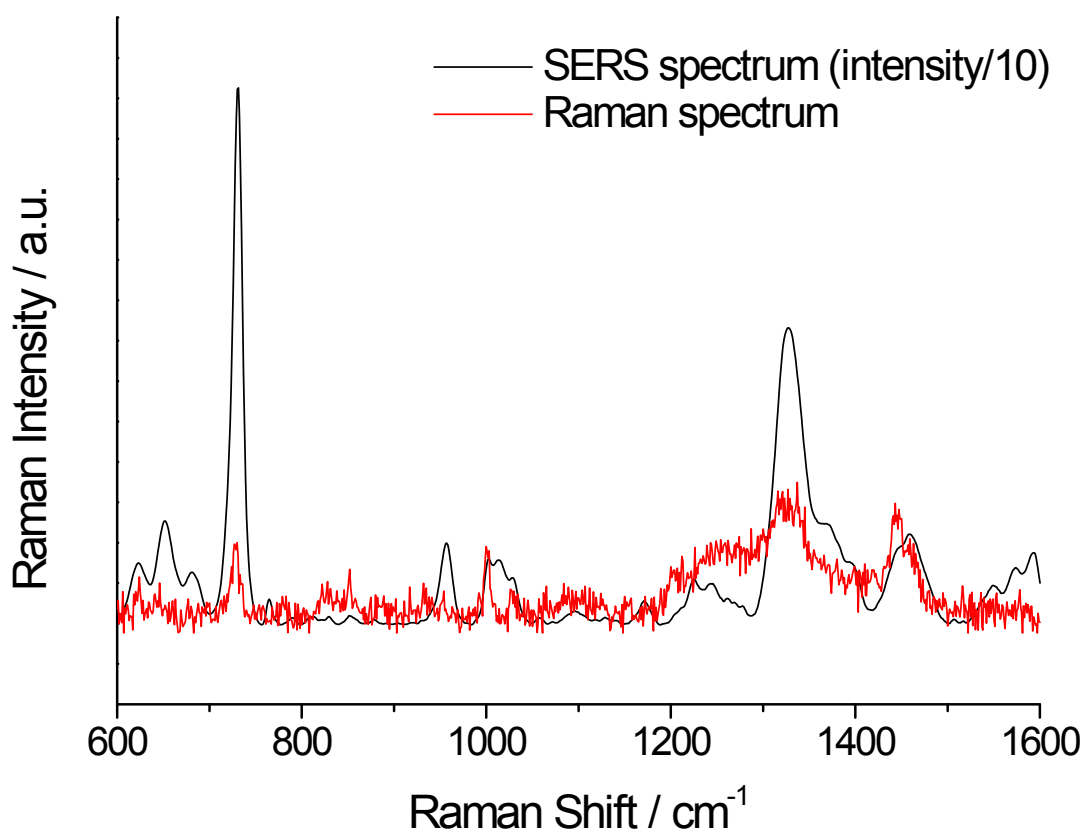


Figure S8. The comparison of normal Raman (red) and SERS (dark) spectra of *N. meningitides* bacteria multiplied before experiment by cultivation in BWP growth medium. The purified bacteria were dispersed in Millipore water to a concentration of 10^2 CFU/mL and then deposited onto glass slide (for Raman measurement) and onto Ag-Au coated polycarbonate membrane (for SERS measurement). Experimental conditions: 0.5 mW, 785 nm excitation, 30 seconds acquisition time. The SERS spectrum was divided by 10 for better visualization.

References

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