

Supplementary information for the manuscript:

The multivariate detection limit for *Mycoplasma pneumoniae* as determined by Nanorod Array-Surface Enhanced Raman Spectroscopy and comparison with limit of detection by qPCR

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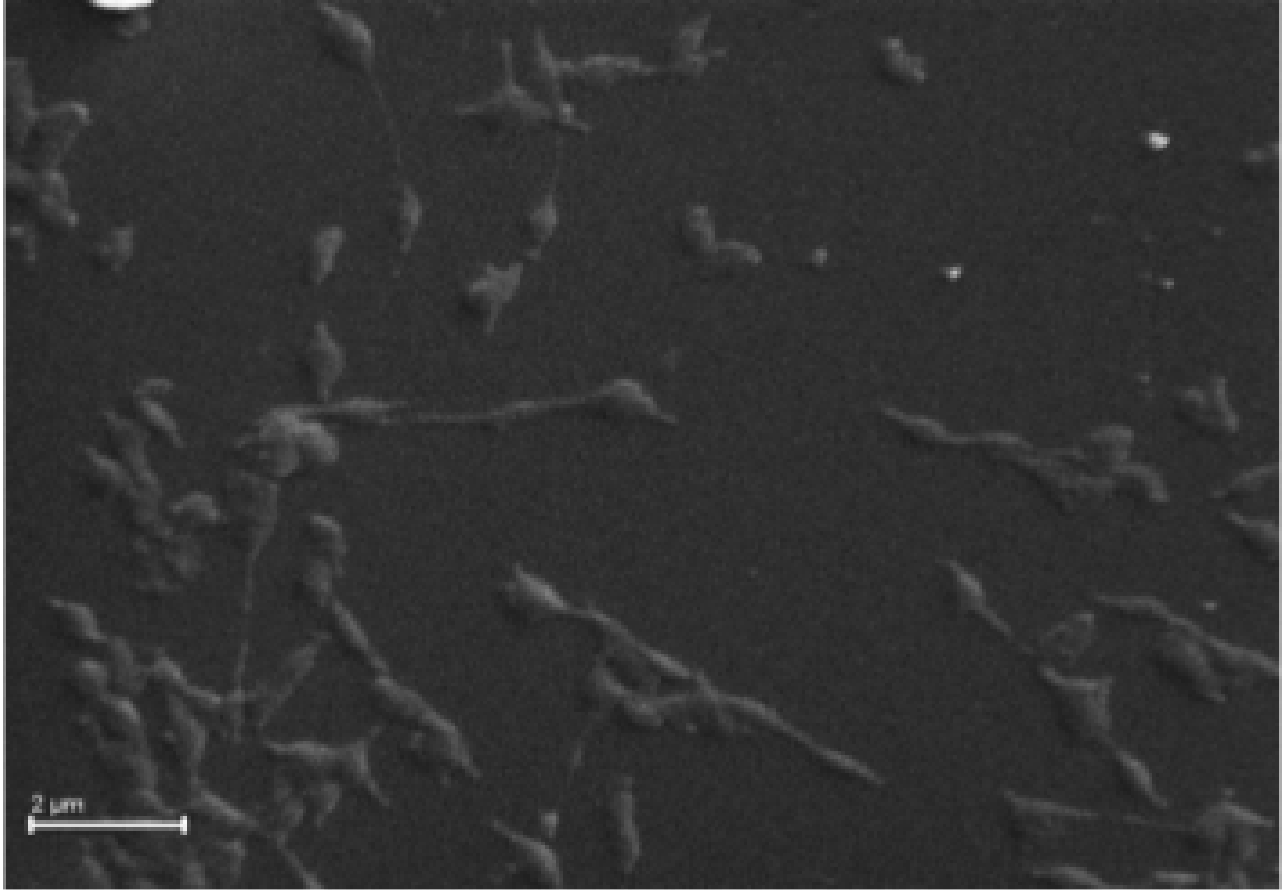
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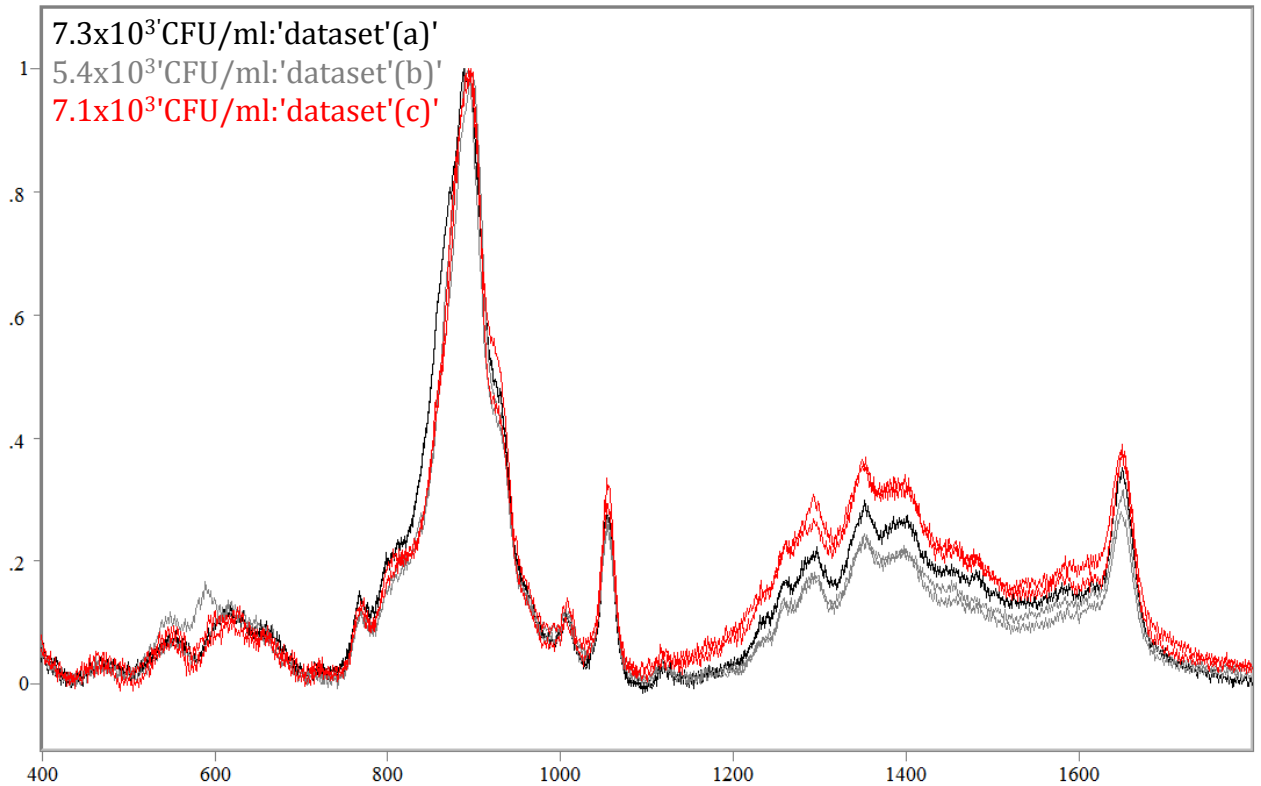
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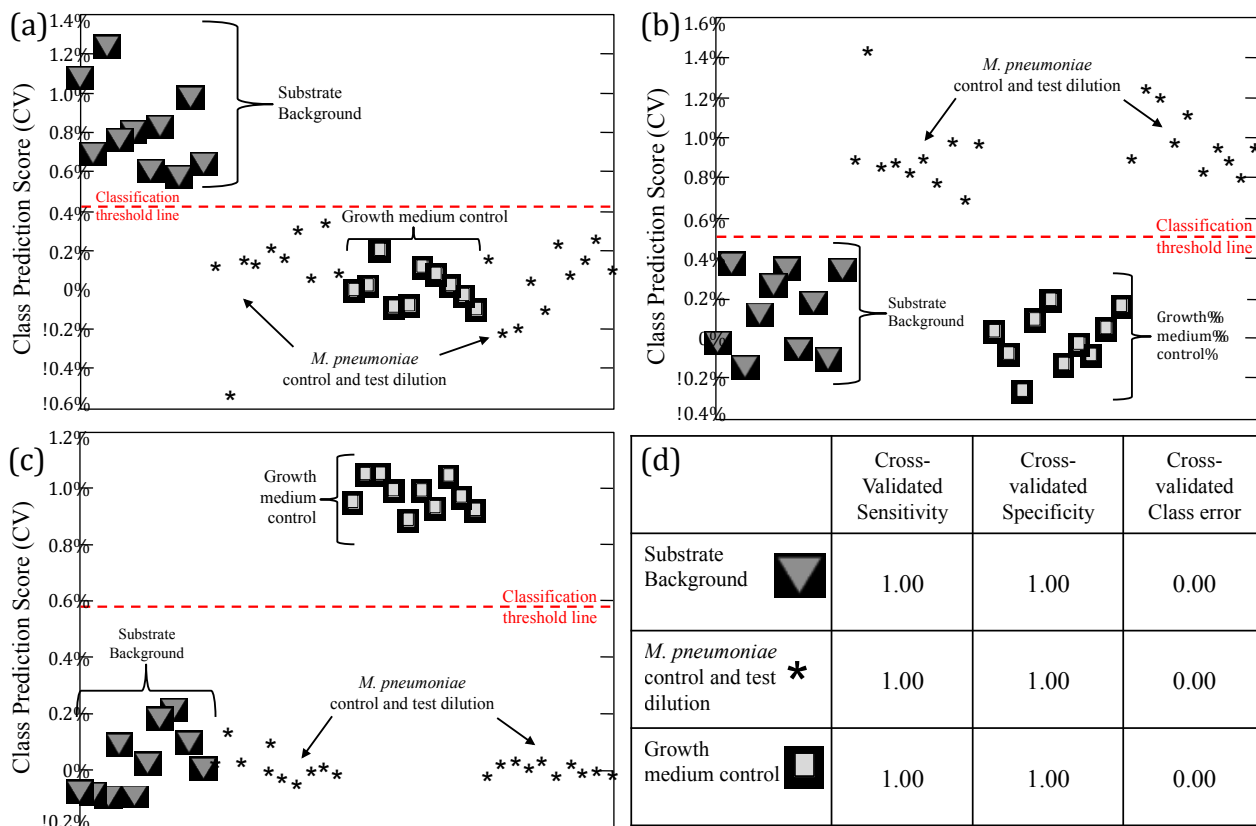
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Supplementary Fig. 1. SEM image of intact *M. pneumoniae* cells grown on and attached to glass coverslips.



Supplementary Fig. 2. Baseline-corrected and normalized SERS spectra of intact-cell *M. pneumoniae* at the 10^3 CFU/ml dilutions from the three independent substrates. Two spectra are shown from each dataset representing spectra from duplicate wells of the 10^3 CFU/ml dilutions. Spectra from dataset (a) are shown in black, spectra from dataset (b) in dark gray, and spectra from dataset (c) in red.



Supplementary Fig. 3. Example of PLS-DA modeling scheme used to determine NA-SERS sensitivities and specificities for intact-cell *M. pneumoniae* samples and to generate the statistics given in Supplementary Tables 4-6. Each individual shape represents a single pre-processed NA-SERS spectrum. Each panel represents a cross-validated class prediction score for (a) class 1, substrate background spectra; (b) class 2, *M. pneumoniae* control and test dilution spectra; and (c) class 3, growth medium control spectra. For all panels, substrate background spectra are represented by upside-down dark-gray triangles, *M. pneumoniae* control and test dilution spectra by black asterisks, and growth medium control spectra by light gray squares. The red-dotted line indicates the classification threshold line for positive class prediction. Cross-validated statistics for the model (d) were obtained using Venetian blinds with 6 data splits and represent the prediction performance of the PLS-DA plots shown in a-c.

Supplementary Table 1. Ct values for qPCR dataset (a).

| Dilution (cells/ml) | Rep 1 | Rep 2 | Rep 3 |
|---------------------|-------|-------|-------|
| 2.3×10^7 | 24.04 | 24.20 | 24.24 |
| 2.3×10^6 | 26.30 | 26.52 | 26.60 |
| 2.3×10^5 | 29.32 | 29.26 | 29.49 |
| 2.3×10^4 | 32.23 | 32.43 | 32.61 |
| 2.3×10^3 | 36.50 | 35.16 | 37.09 |
| 2.3×10^2 | - | - | - |
| 2.3×10^1 | - | - | - |
| 2.3×10^0 | - | - | - |

- Indicates detectable amplification was not observed for this replicate

Supplementary Table 2. Ct values for qPCR dataset (b).

| Dilution (cells/ml) | Rep 1 | Rep 2 | Rep 3 |
|---------------------|-------|-------|-------|
| 2.8×10^7 | 23.58 | 23.38 | 23.50 |
| 2.8×10^6 | 28.00 | 26.11 | 26.71 |
| 2.8×10^5 | 30.16 | 30.06 | 29.86 |
| 2.8×10^4 | 34.57 | 34.14 | 33.30 |
| 2.8×10^3 | - | - | 39.26 |
| 2.8×10^2 | - | - | - |
| 2.8×10^1 | - | - | - |
| 2.8×10^0 | - | - | - |

- Indicates detectable amplification was not observed for this replicate

Supplementary Table 3. Ct values for qPCR dataset (c).

| Dilution (cells/ml) | Rep 1 | Rep 2 | Rep 3 |
|---------------------|-------|-------|-------|
| 2.5×10^7 | 23.58 | 23.49 | 23.53 |
| 2.5×10^6 | 26.76 | 26.84 | 26.71 |
| 2.5×10^5 | 29.98 | 29.85 | 30.20 |
| 2.5×10^4 | 33.27 | 32.65 | 33.03 |
| 2.5×10^3 | 35.6 | - | 35.45 |
| 2.5×10^2 | - | - | - |
| 2.5×10^1 | - | - | - |
| 2.5×10^0 | - | - | - |

- Indicates detectable amplification was not observed for this replicate

Supplementary Table 4. PLS-DA modeling statistics for NA-SERS analysis of intact-cell *M. pneumoniae* dataset (a)

| Dilution by Genome equivalents (cells/ml) | PLS-DA Sensitivity After CV | PLS-DA Specificity After CV | PLS-DA Class Error After CV | PLS-DA RMSECV |
|---|-----------------------------|-----------------------------|-----------------------------|---------------|
| [1] Mpn 7.3x10 ⁸ | 0.9 | 0.95 | 0.075 | 0.24 |
| [2] SP4 10 ⁻² | 0.9 | 0.95 | 0.075 | 0.27 |
| [3] Subs. bkg | 1 | 0.97 | 0.02 | 0.14 |
| [1] Mpn 7.3x10 ⁶ | 0.9 | 1 | 0.05 | 0.25 |
| [2] SP4 10 ⁻⁴ | 0.9 | 1 | 0.05 | 0.24 |
| [3] Subs. bkg | 1 | 1 | 0 | 0.13 |
| [1] Mpn 7.3x10 ⁵ | 1 | 1 | 0 | 0.22 |
| [2] SP4 10 ⁻⁵ | 1 | 1 | 0 | 0.16 |
| [3] Subs. bkg | 1 | 1 | 0 | 0.21 |
| [1] Mpn 7.3x10 ⁴ | 0.9 | 0.9 | 0.1 | 0.32 |
| [2] SP4 10 ⁻⁶ | 1 | 0.93 | 0.03 | 0.28 |
| [3] Subs. bkg | 1 | 0.97 | 0.02 | 0.24 |
| [1] Mpn 7.3x10 ³ | 0.95 | 0.95 | 0.05 | 0.24 |
| [2] SP4 10 ⁻⁷ | 0.9 | 0.9 | 0.1 | 0.29 |
| [3] Subs. Bkg | 1 | 0.97 | 0.02 | 0.18 |
| [1] Mpn 7.3x10 ² | 0.95 | 0.9 | 0.075 | 0.30 |
| [2] SP4 10 ⁻⁸ | 1 | 0.97 | 0.02 | 0.26 |
| [3] Subs. bkg | 1 | 1 | 0 | 0.23 |
| [1] Mpn 7.3x10 ¹ | 0.95 | 1 | 0.025 | 0.23 |
| [2] SP4 10 ⁻⁹ | 0.7 | 0.97 | 0.17 | 0.32 |
| [3] Subs. Bkg | 1 | 0.93 | 0.03 | 0.26 |
| [1] Mpn 7.3x10 ⁰ | 0.9 | 0.89 | 0.10 | 0.35 |
| [2] SP4 10 ⁻¹⁰ | 0.56 | 0.93 | 0.25 | 0.33 |
| [3] Subs. Bkg | 0.9 | 1 | 0.05 | 0.17 |
| [1] Mpn 7.3x10 ⁻¹ | 0.95 | 0.9 | 0.075 | 0.31 |
| [2] SP4 10 ⁻¹¹ | 0.9 | 0.97 | 0.06 | 0.34 |
| [3] Subs. bkg | 0.9 | 0.86 | 0.11 | 0.31 |

Class [1]: Mpn = *M. pneumoniae* control and test dilution; Class [2]: SP4 = growth medium control dilution; Class [3]: Subs. bkg = nanorod substrate background; CV = cross-validation; RMSECV = root mean square error after cross-validation

Supplementary Table 5. PLS-DA modeling statistics for NA-SERS analysis of intact-cell *M. pneumoniae* dataset (b)

| Dilution by Genome equivalents (cells/ml) | PLS-DA Sensitivity After CV | PLS-DA Specificity After CV | PLS-DA Class Error After CV | PLS-DA RMSECV |
|---|-----------------------------|-----------------------------|-----------------------------|---------------|
| [1] Mpn 5.4x10 ¹⁰ | 0.9 | 0.84 | 0.13 | 0.34 |
| [2] SP4 10 ⁰ | 0.67 | 0.95 | 0.19 | 0.34 |
| [3] Subs. bkg | 1 | 1 | 0 | 0.07 |
| [1] Mpn 5.4x10 ⁸ | 1 | 1 | 0 | 0.16 |
| [2] SP4 10 ⁻² | 1 | 1 | 0 | 0.10 |
| [3] Subs. bkg | 1 | 1 | 0 | 0.11 |
| [1] Mpn 5.4x10 ⁶ | 1 | 0.95 | 0.025 | 0.23 |
| [2] SP4 10 ⁻⁴ | 0.9 | 1 | 0.05 | 0.25 |
| [3] Subs. Bkg | 1 | 1 | 0 | 0.11 |
| [1] Mpn 5.4x10 ⁴ | 1 | 0.9 | 0.05 | 0.20 |
| [2] SP4 10 ⁻⁶ | 0.9 | 0.95 | 0.075 | 0.29 |
| [3] Subs. bkg | 1 | 0.95 | 0.025 | 0.19 |
| [1] Mpn 5.4x10 ³ | 1 | 1 | 0 | 0.21 |
| [2] SP4 10 ⁻⁷ | 0.9 | 0.95 | 0.075 | 0.26 |
| [3] Subs. Bkg | 1 | 1 | 0 | 0.20 |
| [1] Mpn 5.4x10 ² | 0.9 | 0.95 | 0.075 | 0.25 |
| [2] SP4 10 ⁻⁸ | 0.9 | 0.95 | 0.075 | 0.27 |
| [3] Subs. Bkg | 1 | 1 | 0 | 0.14 |
| [1] Mpn 5.4x10 ¹ | 0.7 | 0.8 | 0.25 | 0.41 |
| [2] SP4 10 ⁻⁹ | 0.9 | 0.9 | 0.1 | 0.31 |
| [3] Subs. Bkg | 1 | 1 | 0 | 0.22 |
| [1] Mpn 5.4x10 ⁰ | 0.7 | 0.8 | 0.25 | 0.52 |
| [2] SP4 10 ⁻¹⁰ | 0.7 | 0.85 | 0.225 | 0.49 |
| [3] Subs. Bkg | 1 | 1 | 0 | 0.16 |
| [1] Mpn 5.4x10 ⁻¹ | 0.9 | 0.86 | 0.11 | 0.27 |
| [2] SP4 10 ⁻¹¹ | 0.9 | 0.95 | 0.075 | 0.27 |
| [3] Subs. bkg | 1 | 0.95 | 0.025 | 0.25 |

Class [1]: Mpn = *M. pneumoniae* control and test dilution; Class [2]: SP4 = growth medium control dilution; Class [3]: Subs. bkg = nanorod substrate background; CV = cross-validation; RMSECV = root mean square error after cross-validation

Supplementary Table 6. PLS-DA modeling for NA-SERS analysis of intact-cell *M. pneumoniae* dataset (c)

| Dilution by Genome equivalents (cells/ml) | PLS-DA Sensitivity After CV | PLS-DA Specificity After CV | PLS-DA Class Error After CV | PLS-DA RMSECV |
|---|-----------------------------|-----------------------------|-----------------------------|---------------|
| [1] Mpn 7.1x10 ⁹ | 0.9 | 0.5 | 0.25 | 0.45 |
| [2] SP4 10 ⁻¹ | 0.9 | 0.6 | 0.17 | 0.44 |
| [3] Subs. Bkg | 1 | 1 | 0 | 0.12 |
| [1] Mpn 7.1x10 ⁷ | 0.9 | 0.95 | 0.075 | 0.26 |
| [2] SP4 10 ⁻³ | 1 | 0.95 | 0.025 | 0.25 |
| [3] Subs. Bkg | 1 | 1 | 0 | 0.12 |
| [1] Mpn 7.1x10 ⁵ | 1 | 1 | 0 | 0.09 |
| [2] SP4 10 ⁻⁵ | 1 | 1 | 0 | 0.11 |
| [3] Subs. Bkg | 1 | 1 | 0 | 0.12 |
| [1] Mpn 7.1x10 ⁴ | 1 | 1 | 0 | 0.17 |
| [2] SP4 10 ⁻⁶ | 1 | 1 | 0 | 0.15 |
| [3] Subs. bkg | 1 | 1 | 0 | 0.18 |
| [1] Mpn 7.1x10 ³ | 1 | 0.95 | 0.025 | 0.18 |
| [2] SP4 10 ⁻⁷ | 1 | 1 | 0 | 0.08 |
| [3] Subs. Bkg | 1 | 1 | 0 | 0.16 |
| [1] Mpn 7.1x10 ² | 1 | 0.95 | 0.025 | 0.24 |
| [2] SP4 10 ⁻⁸ | 0.9 | 0.95 | 0.075 | 0.27 |
| [3] Subs. Bkg | 0.9 | 1 | 0.05 | 0.25 |
| [1] Mpn 7.1x10 ¹ | 1 | 0.95 | 0.025 | 0.27 |
| [2] SP4 10 ⁻⁹ | 1 | 0.85 | 0.075 | 0.27 |
| [3] Subs. Bkg | 1 | 1 | 0 | 0.20 |
| [1] Mpn 7.1x10 ⁰ | 0.8 | 0.9 | 0.15 | 0.24 |
| [2] SP4 10 ⁻¹⁰ | 1 | 0.95 | 0.025 | 0.19 |
| [3] Subs. Bkg | 1 | 0.95 | 0.025 | 0.14 |

Class [1]: Mpn = *M. pneumoniae* control and test dilution; Class [2]: SP4 = growth medium control dilution; Class [3]: Subs. bkg = nanorod substrate background; CV = cross-validation; RMSECV = root mean square error after cross-validation