

Electronic Supplementary Information

Importance of specimen pretreatment for the low-level detection of mycobacterial lipoarabinomannan in human serum

Lars B. Laurentius,^a Alexis C. Crawford,^{a,b} Timothy S. Mulvihill,^b Jennifer H. Granger,^a Ryan Robinson,^{a,d} John S. Spencer,^g Delphi Chatterjee,^g Kimberly E. Hanson,^{e,f} and Marc D. Porter^{*a,b,c,d,f}

^aThe Nano Institute of Utah, ^bDepartments of Chemistry, ^cChemical Engineering, ^dBioengineering, ^eInternal Medicine, and ^fPathology, University of Utah, Salt Lake City, UT 84112, USA. ^gDepartment of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80523, USA.

*Corresponding author: marc.porter@utah.edu

Table of Contents

1. Reagents and materials	SI-1
2. Monoclonal antibody (mAb) selection	SI-2
3. Capture surface preparation	SI-2
4. Detection label preparation	SI-2
5. Figure S1: Representative SERS spectra from calibrant series for PILAM	SI-3
6. Literature Cited	SI-4

1. Reagents and materials

The following reagents were used as received: acetonitrile (Sigma-Aldrich), ethanol ($\geq 99.5\%$, Pharmco-AAPER), $\text{CF}_3\text{CO}_2\text{H}$ (99%, Alfa Aesar), HNO_3 ($\sim 70\%$, BDH Aristar), bovine serum albumin (BSA, Sigma-Aldrich), H_2SO_4 ($\sim 98\%$, Sigma-Aldrich), octadecanethiol (Fluka), HClO_4 (70%, Sigma-Aldrich), pooled AB human serum (Mediatech, Inc. and Innovative Research), and phosphoinositol-capped lipoarabinomannan (PILAM, Colorado State University). The following reagents were obtained from Thermo Fisher Scientific and used as received: methanol, sodium chloride, potassium carbonate, HCl (37%), dithiobis(succinimidyl propionate) (DSP), Tween 20, pH 8.5 borate buffer (BB) packs, BB containing 0.1% Tween 20 (BBT), pH 7.4 modified Dulbecco's phosphate-buffered saline (PBS) packs, PBS containing 0.1% Tween 20 (PBST), and StartingBlockTM blocking buffer. Other materials used include: optical adhesive 61 (Norland Products, Inc.), p-type silicon wafers (University Wafers), polydimethylsiloxane (Dow Corning Sylgard), 60 nm gold nanoparticles (AuNPs, Nanopartz), and Amicon Ultra 0.5 mL centrifugal filters (Millipore). The Raman reporter molecule [5,5'-dithiobis(succinimidyl-2-nitrobenzoate) (DSNB)] was synthesized as described previously.¹

2. Monoclonal antibody (mAb) selection

Three anti-lipoarabinomannan (LAM) monoclonal antibodies (mAbs, IgG₃ subclass), which were raised against LAM from *M. leprae*, were screened for use in the assay described in **Figure 1** of the full manuscript. These mAbs, designated CS906.1, CS906.7, and CS907.41, were first prepared and characterized for reactivity at Colorado State University in 1987.² Each of the nine possible combinations were tested for the capture and/or extrinsic Raman label (ERL) tagging of PILAM by measuring the SERS response of PILAM-spiked PBST (5.0 µg/mL). The levels of nonspecific ERL adsorption were also measured for PBST devoid of PILAM. The results of the tests with PILAM indicated that the signal found using the CS906.7 mAb for both capturing and labeling of PILAM was >2x that of the other combinations. The responses for the nine mAb combinations for the PBST blanks were, in contrast, statistically indistinguishable from each other. Based on these results, the CS906.7 mAb was used as the capture and labeling mAb in all subsequent experiments. We note that the epitope structure of PILAM that is recognized by CS906.7 has not been characterized. However, the structure of PILAM consists of multiple similar antigenic determinants that could react with CS906.7.²

3. Capture surface preparation

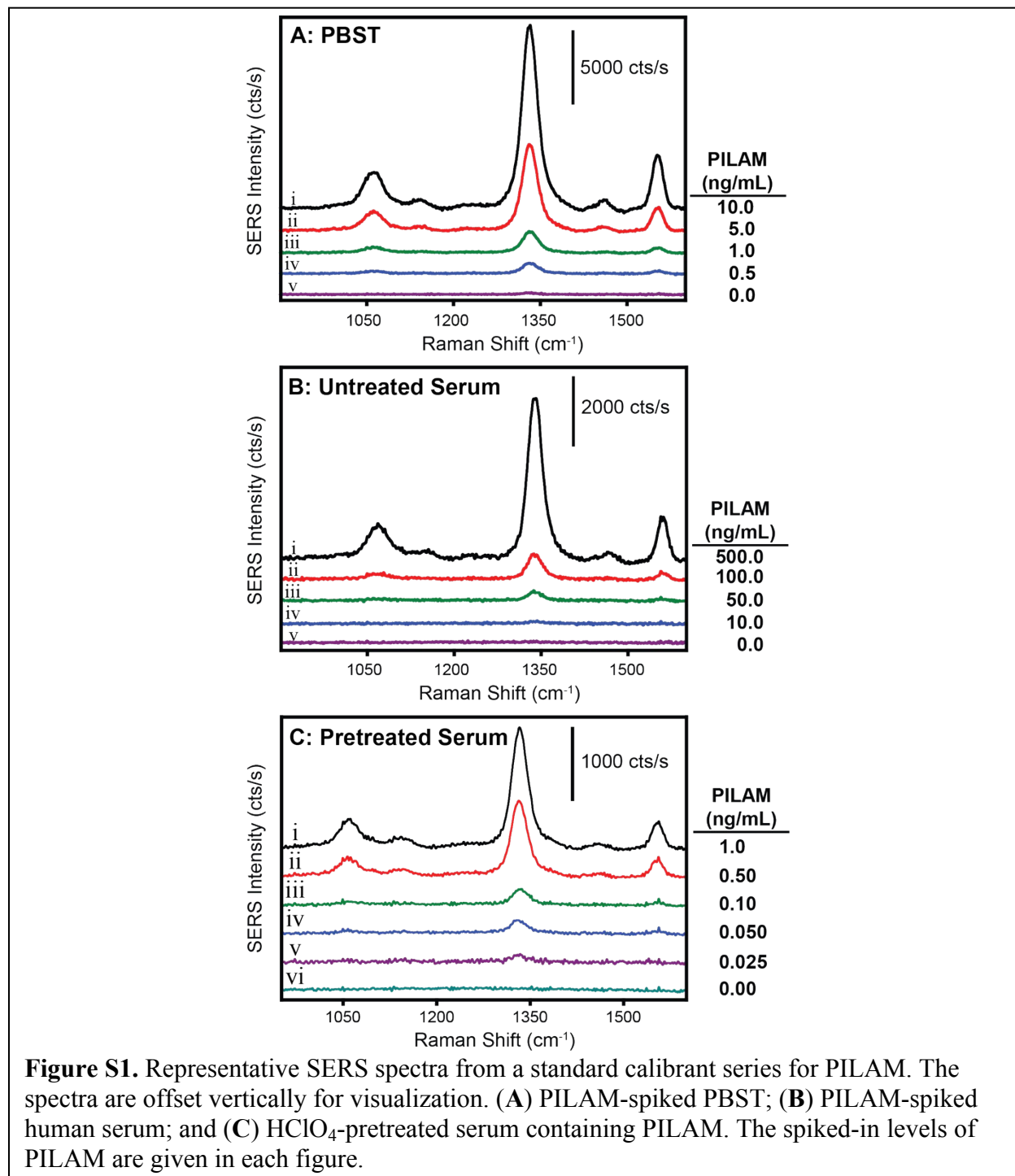
Gold-coated capture surfaces were prepared by template stripping.³ The process starts by thermally evaporating ~200 nm of gold on a silicon wafer, followed by bonding glass squares (1 × 1 cm) to the gold-coated silicon wafer with optical adhesive, which was cured by exposure to ultraviolet radiation for 2 h. Careful detachment of the glass sections from the silicon wafer exposes the template-stripped gold surface. These substrates were addressed with a polydimethylsiloxane stamp that has a centered, 2 mm diameter relief structure. The stamp was inked in a saturated ethanolic solution of octadecanethiol. This creates an uncoated 2 mm addressable pattern on the gold substrate that is surrounded by a hydrophobic boundary. The formation of the mAb layer on the gold address is shown in **Figure 1A** of the full manuscript, which includes the modification of the gold surface by submerging the entire substrate in a 1.0 mM solution of DSP for 1 h. Next, a capture mAb layer was adsorbed on the surface by pipetting 20.0 µL of 2.5 µg/mL of anti-LAM mAb onto the DSP-activated address for 1 h.⁴ Finally, the capture surface was rinsed with PBST, treated with StartingBlock™ buffer for 1 h, and rinsed again with PBST.

4. Detection label preparation

The assay relies on the plasmonic enhancement that develops from the close proximity of the DSNB label to the AuNP surface.^{5, 6} This forms the basis of an ERL, which is composed of a 60 nm AuNP that has been modified with DSNB and anti-LAM mAbs (**Figure 1B** of the full manuscript). ERLs were prepared by incubating a 1.0 mL suspension of AuNPs in 2.0 mM BB for 1 h at 4°C with 10 µL of 1.0 mM DSNB dissolved in acetonitrile. DSNB forms a thiolate monolayer at the AuNP surface and acts as the Raman reporter molecule (RRM). Next, 10 µL (100 µg/mL) of anti-LAM mAbs were added and reacted with the AuNP suspension at 4 °C for 1 h. To minimize nonspecific interactions and stabilize the suspension, 100 µL of a 10% (w/v) BSA solution in 2.0 mM BB was added. After 1 h, excess reactants were removed via centrifugation at ~2,000g for 10 min. The resulting clear supernatant was carefully removed, and the ERL pellet was resuspended in 1.0 mL of 1% BSA in 2.0 mM BB. This process was repeated two more times, with the last step resuspending the pellet in 0.25 mL of 2% BSA in 2.0 mM BB containing 150 mM NaCl. The final concentration of the ERLs was determined to be 8.0×10^{10} particles/mL, using the UV-Vis spectrophotometric method of Haiss and colleagues.⁷

5. Figure S1: Representative SERS spectra from calibrant series for PILAM

All of the spectral features in **Figure S1** are assignable to the vibrational modes of the functional groups of the DSNB-derived coating on the ERLs, which include a symmetric nitro stretch, $\nu_s(\text{NO}_2)$, at 1336 cm^{-1} and an aromatic ring mode at 1558 cm^{-1} .⁸ Modes from other components in the immunoassay (*e.g.*, antibodies, antigens, and blocking agents) are not detectably enhanced.¹



6. Literature cited

1. D. S. Grubisha, R. J. Lipert, H.-Y. Park, J. Driskell and M. D. Porter, *Anal. Chem.*, 2003, **75**, 5936-5943.
2. H. Gaylord, P. J. Brennan, D. B. Young and T. M. Buchanan, *Infect. Immun.*, 1987, **55**, 2860-2863.
3. M. Hegner, P. Wagner and G. Semenza, *Surf. Sci.*, 1993, **291**, 39-46.
4. C. Y. Lim, N. A. Owens, R. D. Wampler, Y. Ying, J. H. Granger, M. D. Porter, M. Takahashi and K. Shimazu, *Langmuir*, 2014, **30**, 12868-12878.
5. M. Moskovits, *Rev. Mod. Phys.*, 1985, **57**, 783-826.
6. R. L. McCreery, *Raman Spectroscopy for Chemical Analysis*, John Wiley & Sons, Inc., New York, 2000.
7. W. Haiss, N. T. K. Thanh, J. Aveyard and D. G. Fernig, *Anal. Chem.*, 2007, **79**, 4215-4221.
8. M. D. Porter, R. J. Lipert, L. M. Siperko, G. Wang and R. Narayanan, *Chem. Soc. Rev.*, 2008, **37**, 1001-1011.