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technology that can
high speci city (no fals
10^4 spores for B. anthracis[1]).
decade, various techniques have be
biological agents. Notable techniques
[2], uorescence [3], luminescence [4], infra
spectroscopy [7], as well as the polymerase chain reac
While all of these techniques have been employed for biologic
detection to some extent (e.g. identi cation of anthrax on mail sorting
equipment by PCR), none of them satisfy all of the requirements of
speed, sensitivity, selectivity, and eld ruggedness, especially the latter
as required by military personnel.

e most successful eld techniques are based on immunoassays, such as enzyme-linked immunosorbent assays (ELISA) [9,10]. In an e ort to improve upon this technique, a number of other antigen-antibody based binding event reporter methods have been investigated. ese techniques include cantilever [11,12], electrochemical [13], magnetic [14], piezoelectric [15], and surface plasmon resonance (SPR) based devices [16]. While these techniques are relatively fast, inexpensive, and easy to use, they su er from high false-positive and false-negative rates due to a lack of speci city and sensitivity [17]. In the speci c case of B. anthracis, other Bacillus spores, such as B. cereus, are common in the environment, and they share the same surface antigens used for antibody binding [18], increasing the false-positive rate.

Recognizing the limitations of all the techniques mentioned above, we have been developing a surface-enhanced Raman spectroscopy (SERS) based assay for the detection of trace quantities of biological

easily synthesi
surfaces. Furthermore, the
which extends their usable lifetime making

Previously, we reported the ability of this approach to detect 10^9 B. anthracis-Sterne spores per mL in less than 20 minutes [27].

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Received January 20, 2016; Accepted February 27, 2016; Published March 03, 2016

Citation: Shende C, Huang H, Sperry J, Farquharson S (2016) Species Selective Measurement of 10 B. anthracis-Sterne Spores within 10 Minutes by Surface-Enhanced Raman Spectroscopy. J Anal Bioanal Tech 7: 302. doi:10.4172/2155-9872.1000302

Unfortunately, the addition of the peptide likely dampened the plasmon eld responsible for the SER e ect. Here we describe the addition of a second SER-active material to the assay in the form of a silver colloid to measure 10 B. anthracis Sterne spores in a 10^3 spores/mL sample within 10 minutes, representing an improvement in sensitivity of 6 orders-of-magnitude!

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All chemicals, reagents, and solvents, including those used to prepare the SER-active sol-gels, were purchased and used as received from Sigma-Aldrich (Milwaukee WI). All Bacillus samples were obtained from the American Type Culture Collection (Manassas, VA) and prepared by Professor Jay Sperry (University of Rhode Island) [28]. Stock solutions were serially diluted to produce the measured concentrations. Concentrations were veri ed by direct count of spores in 4 × 10^{-3} µL triplicate samples using a light microscope [25]. e concentrations of these samples were determined to be 5.7 × 10^9, 1.7 × 10^{10}, 5.5 × 10^9, and 6.1 × 10^9 spores/mL for B. anthracis Sterne, B. cereus, B. megaterium and B. subtilis, respectively. e ATYPLPIR peptide [26] used in this study was custom synthesized by New England Peptide (Gardner, MA). Glass capillaries, tubing, syringes and syringe ports were obtained from VWR Scienti c (Arlington Heights, IL).

SER-ac - ca a

SER-active capillaries (Simple SERS Sample Capillaries, REA) were prepared according to published procedures [28,29] by mixing a silver amine precursor and an alkoxide precursor at 1:1 v/v.

e silver amine precursor consisted of a 1:1:2 v/v/v ratio of 1N $AgNO_3$/28% NH_4OH/CH_3OH, while the alkoxide precursor consisted of methyltrimethoxysilane. e SERS capillaries were prepared by drawing 20 µL of the silver-doped sol-gels into 10 cm long, 0.8 mm inner diameter glass capillaries to produce ~1 cm long sol-gel segments.

e segments were allowed to gel and cure for 12 hours, a er which the incorporated silver ions were reduced with dilute $NaBH_4$. Silver 265.3434 340.444 Tm(4)TjETver 27n1 Tf0.374 Tw 9 12C 4 Tco.24idsilane. e

capillary. Measurements of 10^5 B. anthracis-Sterne spores/mL using this procedure produced intense DPA spectra for binding times of 30, 15, and 5 minutes (Figure 2). Since one of the goals was to establish the minimum time to perform the measurement, the 5 min binding time was used for the B. anthracis-Ames samples, as it produced a su ciently intense DPA spectrum.

Next, the other bacilli at 10^5 spores/mL were measured on the B. anthracis speci c peptide functionalized SERS capillaries. Again, measurements followed the above procedure, but in this case 15 min binding times were used to better represent the selectivity of the peptide. As shown in Figure 3A, the intensities of the 1007 cm^{-1} peak for B. cereus, B. megaterium, and B. subtilis are ~10%, 6%, and 5%, respectively, of that for B. anthracis-Sterne. e presence of DPA in of that for

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