Data/Metadata

**Manuscript: “Development of a high-throughput method for processing sponge-stick samples to detect viable *Bacillus anthracis* spores”**

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**Glossary/Dictionary for Terms, Variables, and Acronyms Used to Present the DATA/Metadata**

***Bacillus anthracis*:** The bacterium that causes anthrax infection.

**Spores:** Spores are hardy dormant forms of bacteria that can survive harsh environmental conditions.

**Sponge-stick:** A sampling device/tool to collect samples from non-porous surfaces.

**SS: Sponge-stick**

**Aliquot:** A small volume of liquid withdrawn/taken out from the total volume for analysis.

**mL:** Milliliter.

**µL:** Microliter.

**CFU:** Colony Forming Unit.

**ATD:** Arizona Test Dust.

**MicroFunnel Filter:** MicroFunnel Filter is a plastic assembly for membrane filter-based filtration of bacterial suspension. The membrane filter is then transferred onto a growth medium plate and incubated at an optimum growth temperature. After incubation, the colonies formed of bacterial growth are counted to determine the number of bacteria present in a sample.

**RV:** Rapid Viability.

**T0:** Time Zero: Taking out an aliquot of culture suspension for DNA extraction at Zero hour incubation time for bacterial growth – no incubation, i.e., immediately after mixing the sample in a growth medium and shaking but before incubating at an optimum growth temperature.

**T9:** Time 9 hours: Taking out an aliquot of culture suspension for DNA extraction after 9 hours of incubation time for bacterial growth at an optimum growth temperature.

**Polymerase:** This catalyst (in this work, DNA polymerase enzyme) synthesizes DNA and amplifies (makes copies of) the DNA.

**PCR:** Polymerase Chain Reaction. This is an assay used to detect presence of deoxyribo nucleic acid (DNA) of any biological organism or entity, including disease causing microorganisms/germs (including bioterrorism agents), from a sample. DNA polymerase generates copies of the original DNA. In this PCR assay, the DNA polymerase enzyme uses nucleotides (building blocks of DNA), and forward and reverse primers (short nucleotide sequence pieces within the same part of the gene that is amplified). The primers prime PCR to amplify the original DNA and continue a chain of 45 DNA amplification (PCR) cycles to make millions of copies of this small gene part. A fluorescently labeled probe (a short nucleotide sequence piece of the same gene part) is also included in the assay. The probe binds in between the forward and the reverse primers on the gene part DNA to be amplified. As the part of the target gene (DNA) undergoes amplification, this probe gets degraded/chewed up and emits fluorescence as a result of enzyme action, and it is measured by a specially designed instrument. When the fluorescence amount crosses a set threshold value at a particular PCR cycle number (Cycle Threshold, CT), it indicates the amplification of the specific target gene part, which, in turn, indicates presence of the specific biological organism or entity in a sample. In this work, it indicates the presence of *Bacillus anthracis* spores in a sample.

**CT(T0):** PCR cycle threshold value for the T0 aliquot PCR analysis.

**CT(T9):** PCR cycle threshold value for the T9 aliquot PCR analysis.

**∆CT:** A change (∆) in the PCR cycle threshold (CT) number between two conditions. In the RV-PCR method, PCR assay is performed before and after incubation for bacterial growth, and the CT values are measured. The CT value of the PCR assay performed after incubation/growth (Time 9 hours, T9) is deducted from the CT value of the assay performed before incubation/growth (Time Zero, T0). This difference in the CT values is called ∆CT. For an assay positive result, the ∆CT value (number) is set to indicate a certain increase in the amount of the nucleic acid (DNA) and therefore, it has to be equal to or greater than (≥) the set value. Accordingly, the ∆CT value indicates presence of viable/live biological organism or entity in a sample. In this work, it indicates the presence of viable/live *Bacillus anthracis* spores in a sample.

**Avg:** Average.

**SD:** Standard Deviation.

**ND:** Not Detected.

**Avg (SD):** Average (Standard Deviation) calculated from triplicate aliquots from each sample timepoint (e.g., T0, T9) DNA extract.

**BC3 PCR Assay:**  This assay targets and amplifies a specific segment of nucleic acid on the chromosome of *Bacillus anthracis*.

**EPA1 PCR Assay:** This assay targets and amplifies a specific segment of nucleic acid on the pXO1 plasmid of *Bacillus anthracis*.

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**Data/Metadata**

**Graphical Abstract**

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***Graphical Abstract Figure.*** *Schematic of Major Steps Involved in the Traditional Stomacher-Based and High-Throughput Method-Based Processing of Sponge-Sticks Samples*

The sponge-sticks samples processed using the stomacher-based method is shown at the top. The sponge-stick’s sponge part that is used for sample collection from a surface is detached and placed in a stomacher plastic bag containing a buffer. The stomacher bag with the sponge is sealed and placed in the stomacher for shaking and releasing bacteria. The resultant suspension is then collected and plated on a growth medium. The bacterial colonies formed on the growth medium are counted to determine number of bacteria present in the sponge-stick sample representing the surface of sample collection.

The sponge-sticks samples processed using the high-throughput method is shown at the bottom. The sponge-stick’s sponge part that is used for sample collection from a surface is detached and placed in a 50 mL conical tube containing a buffer. Multiple sponge-sticks and corresponding 50 mL tubes in a rack are shown for the high-throughput method. The tubes are processed, and the resultant suspension is then collected and plated on a growth medium. The bacterial colonies formed on the growth medium are counted to determine number of bacteria present in the sponge-stick sample representing the surface of sample collection.

**Table 1.** Recovery of *B. anthracis* Sterne spores from inoculated sponges in two rinse steps.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Condition |  | First Rinse |  | Second Rinse |
|  | Volume Recovered | Colony Counts a | Total CFUb |  | Volume Recovered | Colony Counts a | Total CFUb |
|  | Plate 1 | Plate 2 | Plate 3 |  | Plate 1 | Plate 2 | Plate 3 |
| Initial Experiment | Positive control(inoculated buffer without sponge) |  | 24.4 mL | 82 | 97 | 84 | 21391 |  | NA | NA | NA | NA | NA |
|  | 24.4 mL | 84 | 88 | 89 | 21228 |  | NA | NA | NA | NA | NA |
|  | 24.4 mL | 87 | 81 | 83 | 20415 |  | NA | NA | NA | NA | NA |
| Negative Control (uninoculated sponge) |  | 28.6 mL | 0 | 0 | 0 | 0 |  | 10.6 mL | 0 | 0 | 0 | 0 |
|  | 29.6 mL | 0 | 0 | 0 | 0 |  | 9.0 mL | 0 | 0 | 0 | 0 |
|  | 28.5 mL | 0 | 0 | 0 | 0 |  | 9.1 mL | 0 | 0 | 0 | 0 |
| Recovery From Sponge |  | 28.5 mL | 64 | 62 | 55 | 17195 |  | 8.8 mL | 19 | 27 | 15 | 1789 |
|  | 30.3 mL | 59 | 73 | 61 | 19493 |  | 9.6 mL | 33 | 27 | 28 | 2816 |
|  | 30.2 mL | 51 | 50 | 57 | 15905 |  | 9.4 mL | 21 | 16 | 18 | 1723 |
| Replicate Experiment | Positive control(inoculated buffer without sponge) |  | 24.4 mL | 97 | 101 | 103 | 24481 |  | NA | NA | NA | NA | NA |
|  | 24.0 mL | 107 | 113 | 105 | 26000 |  | NA | NA | NA | NA | NA |
|  | 24.4 mL | 105 | 98 | 121 | 26352 |  | NA | NA | NA | NA | NA |
| Negative Control (uninoculated sponge) |  | 28.9 mL | 0 | 0 | 0 | 0 |  | 9.8 mL | 0 | 0 | 0 | 0 |
|  | 29.6 mL | 0 | 0 | 0 | 0 |  | 9.4 mL | 0 | 0 | 0 | 0 |
|  | 27.8 mL | 0 | 0 | 0 | 0 |  | 10.0 mL | 0 | 0 | 0 | 0 |
| Recovery From Sponge |  | 29.2 mL | 58 | 82 | 83 | 21705 |  | 9.2 mL | 24 | 23 | 28 | 2300 |
|  | 29.2 mL | 54 | 73 | 70 | 19175 |  | 10.0 mL | 26 | 26 | 33 | 2833 |
|  | 26.6 mL | 43 | 55 | 53 | 13389 |  | 9.0 mL | 43 | 32 | 28 | 3090 |

a Plate colony forming units (CFU) are from 100 µL of the final suspension.

b Total CFU recovered was calculated based on the volume recovered and the plate colony counts.

**Table 2.** Recovery of *B. anthracis* Sterne spores from inoculated sponges using different shaking methods.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Condition | MicroFunnel Filter Colony Counts | Total CFU Recovered a |
| Filter 1 | Filter 2 | Filter 3 |
| Initial Experiment | Negative Control (uninoculated sponge) | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 |
| Orbital Shaker | 73 | 100 | 26 | 199 |
| 100 | 96 | 19 | 215 |
| 92 | 81 | 25 | 198 |
| Multi-tube Vortexer | 64 | 103 | 23 | 190 |
| 83 | 88 | 27 | 198 |
| 83 | 98 | 16 | 197 |
| Reciprocating Shaker | 54 | 74 | 32 | 160 |
| 89 | 67 | 28 | 184 |
| 68 | 80 | 26 | 174 |
| Replicate Experiment | Negative Control (uninoculated sponge) | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 |
| Orbital Shaker | 91 | 73 | 7 | 171 |
| 105 | 83 | 20 | 208 |
| 85 | 95 | 11 | 191 |
| Multi-tube Vortexer | 73 | 81 | 16 | 170 |
| 63 | 63 | 36 | 162 |
| 79 | 75 | 28 | 182 |
| Reciprocating Shaker | 84 | 63 | 16 | 163 |
| 87 | 79 | 14 | 180 |
| 86 | 66 | 21 | 173 |

a Total CFU recovered was calculated as the sum of the CFU recovered on all MicroFunnel filters for a sample.

**Table 3.** Comparison of recoveries of *B. anthracis* Sterne spores using the high-throughput method described here and the traditional stomacher-based method from sponges inoculated at a 104-spore inoculum level (25,800 ± 600, 25,800 ± 800, and 25,900 ± 1,100 CFU per sponge for the first, second, and third experiments, respectively).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Condition | Volume Recovered | CFUa | Total CFUb |
| Plate 1 | Plate 2 | Plate 3 | Plate 4 |
| Experiment #1 | High-throughput | 4.70 mL | 84 | 91 | 102 | NA | 21698 |
| 3.95 mL | 98 | 86 | 93 | NA | 18236 |
| 4.10 mL | 100 | 89 | 98 | NA | 19612 |
| 4.00 mL | 91 | 74 | NA | NA | 16500 |
| Stomacher | 5.20 mL | 79 | 73 | 81 | NA | 20193 |
| 6.10 mL | 63 | 81 | 55 | NA | 20232 |
| 4.45 mL | 84 | 89 | NA | NA | 19246 |
| Experiment #2 | High-throughput | 2.10 mL | 167 | 152 | 172 | NA | 17185 |
| 3.60 mL | 84 | 93 |  | NA | 15930 |
| 3.20 mL | 112 | 106 | 105 | NA | 17227 |
| 3.70 mL | 109 | 102 | 83 | NA | 18130 |
| Stomacher | 5.20 mL | 71 | 65 |  | NA | 17680 |
| -------------------------------Sample Lost----------------------------- |
| 4.45 mL | 45 | 69 |  | NA | 12683 |
| Experiment #3 | High-throughput | 3.50 mL | 74 | 90 | 104 | NA | 15633 |
| 3.95 mL | 133 | 125 | 110 | NA | 24227 |
| 2.90 mL | 89 | 87 | 89 | NA | 12808 |
| 2.80 mL | 134 | 141 | 131 | NA | 18947 |
| Stomacher | 4.75 mL | 92 | 80 | 76 | 90 | 20069 |
| 5.55 mL | 64 | 72 | 62 | NA | 18315 |
| 5.35 mL | 92 | 79 | 78 | 92 | 22804 |

a Plate colony forming units (CFU) are from 100 µL of a 1:5 dilution of the final sample.

b Total CFU recovered were calculated based on the volume recovered and the plate colony counts, accounting for the 1:5 dilution ratio

**Table 4.** Comparison of recoveries of *B. anthracis* Sterne spores using the high-throughput method described here, and the traditional stomacher-based method from sponges inoculated at a low inoculum level (227 ± 25, 265 ± 11, and 227 ± 17 CFU per sponge for the first, second, and third replicate experiments, respectively).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Condition | Volume Recovered | MicroFunnel Filter Colony Counts | Total Sporesc |
| Filter 1a | Filter 2b |
| Experiment #1 | High-throughput | 3.65 mL | 48 | 80 | 128 |
| 3.05 mL | 59 | 100 | 159 |
| 2.9 mL | 60 | 71 | 131 |
| 4.1 mL | 49 | 88 | 137 |
| Stomacher | 4.5 mL | 22 | 79 | 101 |
| 5.8 mL | 22 | 108 | 130 |
| 5.3 mL | 21 | 139 | 160 |
| Experiment #2 | High-throughput | 3.8 mL | 55 | 91 | 146 |
| 3.05 mL | 82 | 77 | 159 |
| 4.2 mL | 47 | 130 | 177 |
| 1.8 mL | 83 | 35 | 118 |
| Stomacher | 4.5 mL | 31 | 115 | 146 |
| 5.8 mL | 5 | 143 | 148 |
| 5.3 mL | 24 | 128 | 152 |
| Experiment #3 | High-throughput | 3.7 mL | 32 | 127 | 159 |
| 1.7 mL | 78 | 57 | 135 |
| 2.2 mL | 69 | 87 | 156 |
| 4 mL | 49 | 91 | 140 |
| Stomacher | ----------------------------Sample Lost------------------------------- |
| 5.2 mL | 3 | 96 | 99 |
| 4.7 mL | 42 | 135 | 177 |

a Filter 1 was from 1 mL of sample filtered onto a MicroFunnel Filter.

b Filter 2 was from the remainder of the sample, after removal of 1 mL for Filter 1.

c Total CFU recovered was calculated as the sum of the CFU recovered on all MicroFunnel filters for a sample.

**Table 5.** Comparison of recoveries of *B. anthracis* Sterne spores with and without addition of ATD.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Condition | Volume Recovered | MicroFunnel Filter Colony Counts | Total CFUc |
| Filter 1a | Filter 2a | Filter 3b |
| Initial Experiment | Without ATD | 4.2 mL | 65 | 43 | 98 | 206 |
| 3.3 mL | 49 | 58 | 72 | 179 |
| 4.0 mL | 65 | 51 | 102 | 218 |
| With ATD | 3.8 mL | 55 | 52 | 91 | 198 |
| 4.3 mL | 50 | 53 | 103 | 206 |
| 3.3 mL | 66 | 52 | 79 | 197 |
| Replicate Experiment | Without ATD | 3.8 mL | 50 | 64 | 93 | 207 |
| 4.0 mL | 54 | 58 | 88 | 224 |
| 4.1 mL | 44 | 40 | 87 | 170 |
| With ATD | 3.1 mL | 64 | 59 | 58 | 191 |
| 2.4 mL | 77 | 64 | 36 | 169 |
| 3.1 mL | 65 | 68 | 48 | 206 |

a Filters 1 and 2 were from 1 mL aliquots of sample filtered onto MicroFunnel Filters.

b Filter 3 was from the remainder of the sample, after removal of 1 mL each for Filters 1 and 2.

c Total CFU recovered was calculated as the sum of the CFU recovered on all MicroFunnel filters for a sample.

**Table 6.** RV-PCR results for SS samples inoculated with 40 *B. anthracis* Sterne spores with and without Arizona Test Dust (ATD). “Avg.” indicates average, and “SD” indicates standard deviation. “ND” indicates not detected.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Condition | ReplicateNumber |  | BC3 Chromosomal Assay |  | EPA-1 Assay |
|  | CT | DCT |  | CT | DCT |
|  | T0 | T9 |  | T0 | T9 |
| Without ATD | 1 |  | ND | 23.0 | 22.0 |  | ND | 22.0 | 23.0 |
| 2 |  | ND | 23.6 | 21.4 |  | ND | 22.5 | 22.5 |
| 3 |  | ND | 23.7 | 21.3 |  | ND | 22.6 | 22.4 |
| Avg. |  | ND | 23.5 | 21.5 |  | ND | 22.4 | 22.6 |
| SD |  |  | 0.4 | 0.4 |  |  | 0.3 | 0.3 |
| 1 |  | ND | 24.8 | 20.2 |  | ND | 23.6 | 21.4 |
| 2 |  | ND | 24.6 | 20.4 |  | ND | 23.3 | 21.7 |
| 3 |  | ND | 24.4 | 20.6 |  | ND | 23.5 | 21.5 |
| Avg. |  | ND | 24.6 | 20.4 |  | ND | 23.5 | 21.5 |
| SD |  |  | 0.2 | 0.2 |  |  | 0.1 | 0.1 |
| 1 |  | ND | 22.7 | 22.3 |  | ND | 21.6 | 23.4 |
| 2 |  | ND | 22.1 | 22.9 |  | ND | 22.2 | 22.8 |
| 3 |  | ND | 22.2 | 22.8 |  | ND | ND | NA |
| Avg. |  | ND | 22.3 | 22.7 |  | ND | 21.9 | 23.1 |
| SD |  |  | 0.3 | 0.3 |  |  | 0.4 | 0.4 |
| **Overall Avg** |  | **ND** | **23.5** | **21.5** |  | **ND** | **22.6** | **22.4** |
| **Overall SD** |  |  | **1.0** | **1.0** |  |  | **0.8** | **0.8** |
| With ATD | 1 |  | ND | 24.5 | 20.5 |  | ND | 22.9 | 22.1 |
| 2 |  | ND | 24.2 | 20.8 |  | ND | 23.8 | 21.2 |
| 3 |  | ND | 24.3 | 20.7 |  | ND | 24.3 | 20.7 |
| Avg. |  | ND | 24.4 | 20.6 |  | ND | 23.7 | 21.3 |
| SD |  |  | 0.2 | 0.2 |  |  | 0.7 | 0.7 |
| 1 |  | ND | 26.7 | 18.3 |  | ND | 28.5 | 16.5 |
| 2 |  | ND | 25.9 | 19.1 |  | ND | 26.4 | 18.6 |
| 3 |  | ND | 26.2 | 18.8 |  | ND | 26.7 | 18.3 |
| Avg. |  | ND | 26.3 | 18.7 |  | ND | 27.2 | 17.8 |
| SD |  |  | 0.4 | 0.4 |  |  | 1.1 | 1.1 |
| 1 |  | ND | 23.4 | 21.6 |  | ND | 22.1 | 22.9 |
| 2 |  | ND | 24.2 | 20.8 |  | ND | 22.1 | 22.9 |
| 3 |  | ND | 22.9 | 22.1 |  | ND | -- | -- |
| Avg. |  | ND | 23.5 | 21.5 |  | ND | 22.1 | 22.9 |
| SD |  |  | 0.7 | 0.7 |  |  | 0.0 | 0.0 |
| **Overall Avg.** |  | **ND** | **24.7** | **20.3** |  | **ND** | **24.3** | **20.7** |
| **Overall SD** |  |  | **1.3** | **1.3** |  |  | **2.4** | **2.4** |

**Data Statement**

The data used in this manuscript will be available in the online published article.