Detection of *Bacillus anthracis* spores from non-porous surfaces using ‘bioluminescent’ reporter bacteriophage

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Abstract

*Bacillus anthracis* is a pathogenic spore-former and etiological agent of anthrax. Spores are naturally found in the environment where they can persist and remain infectious for more than 200 years. A contaminated area has potential to cause extensive disruption as it is uninhabitable until successful remediation. To ensure public health and preparedness for such an event, an efficient and rapid environmental detection system for spores is essential. To address this need, we previously generated a ‘light-tagged’ *B. anthracis*-specific reporter phage (Wβ::luxAB) which can rapidly and sensitively detect pure cultures from germinating spores by conferring a bioluminescent response.

The efficacy of Wβ::luxAB to detect *B. anthracis* ΔSterne spores from 3 non-porous contaminated surfaces was assessed. 2x2 inch coupons of stainless steel, glass and polycarbonate were used to represent the various surfaces. Coupons were inoculated with spores (101 to 104 CFU/coupon) suspended in 95% ethanol (EtOH), then left overnight for EtOH to evaporate, leaving ‘dried’ spores on the coupon surfaces. To sample, macrofoam swabs moistened with phosphate-buffered saline with 0.02% Tween 80 were used to methodically wipe the coupon surface to ‘collect’ spores, which had an estimated processing time of 1 min per coupon. Extraction efficiency was assessed by plating samples and controls for CFU onto brain heart infusion (BHI) agar plates. Swabs were submersed in media containing reporter phage (106 PFU/mL), vortexed vigorously for 2 min, incubated at 35°C with continuous shaking (250rpm) to allow for germination and phage infection, and then analyzed for bioluminescence after 4-8h. To emulate ‘real life’ environmental samples, swabs were also deliberately ‘dried’ by moistening in PBST harboring either Arizona test dust (10mg/mL), *Bacillus subtilis* spores, *Staphylococcus epidermidis* (104 CFU/mL) or all three contaminants combined before sampling.

Swab sampling extraction efficiency was similar from all 3 surfaces, consistently yielding 50-70% recovery of spores from coupons. *B. anthracis* was detectable from ‘clean’ coupons deliberately inoculated with spores, yielding a limit of detection of 101 CFU/coupon within 6 h or 8 h for polycarbonate, stainless steel and glass surfaces, respectively. Wβ::luxAB was able to detect 104 CFU within 8h from ‘dirty’ stainless steel, glass and polycarbonate coupons. As the methodology is simple with minimal hands-on time, the technology displays potential for rapid detection of viable spores from various non-porous surfaces under fieldable or laboratory conditions.

**Detection System:**

**Fig. 1.** *B. anthracis* ΔSterne Wβ::luxAB phage assay

**Fig. 2.** Bioluminescent signal response of ΔSterne spores

**Table 1.** Persistence of biowarfare bacteria

**Table 2.** National planning scenario for aerosol anthrax

**Table 3.** Steel coupon extraction efficiency

**Methods/Results:**

**Fig. 3.** Coupon spore inoculation

**Fig. 4.** Spore extraction and detection

**Fig. 5.** Spore detection from ‘clean’ steel coupons

**Fig. 6.** Detection from ‘clean’ glass & plastic coupons

**Fig. 7.** Detection of 10¹ CFU from ‘dirty’ coupons

**Fig. 8.** Bioluminescent signal response of ΔSterne spores

**References**

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