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**Summary Report**

**to**

**20/20 GeneSystems Incorporated**

**on**

**Bio-Identification Kit Testing: Tier I and II**

**September 13, 2002**

**By**

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## **20/20 GeneSystems Bio-Identification Kit Testing Summary Report: Tier I and II**

### **Objective:**

To provide a summary of Tier I and II results that evaluated whether the FIRSTCHECK kit can detect the proteins in a preparation of dried bacterial spores, similar to spores of *B. anthracis*, contaminated onto a hard surface.

### **Procedures:**

The test was divided into two tiers. Tier I testing involved preparing samples of freeze-dried *Bacillus thuringiensis* (Bt) spores from both the "Clean" and "Dirty" spore stock preparation. A duplicate set of freeze-dried spores was enumerated to verify the concentration of spores added to the freeze-dried tubes. The objective of this test was to demonstrate that the FIRSTCHECK detection kit could detect the protein of the freeze-dried spores.

Tier II testing encompassed determining the level of detection of the FIRSTCHECK kit using a liquid spore suspension. During this test, a series of 10 ml spore suspensions ranging from  $1 \times 10^1$  to  $1 \times 10^8$  spores per mL were prepared. These levels of concentrations were aliquoted (100  $\mu$ L) directly into the bottom of the FIRSTCHECK kit protein detection tube along with the swab.

An additional test was conducted in order to demonstrate the limit of detection using freeze-dried spore samples at concentration ranges from  $10^4$  to  $10^6$ .

### **Data Analysis:**

Results from all testing was recorded as a positive or negative result. A positive result was indicated by a developing purple color within ten minutes of adding the protein detection swab to the detection tube containing the spores. A negative result was indicated by an absence of purple color within ten minutes from the time the protein swab was added. When a negative result was encountered, a negative control swab was added to the detection tube in order to verify the detection kit was working properly (i.e, a purple color should be observed; if not, assay inhibition may have occurred). Refer to the result tables in attachments A (Tier I), B (Tier II), and C (additional Tier II).

**ATTACHMENT A**  
**Tier I Testing Results**

**1. FIRSTCHECK Kit Testing:**

<b>Sample Type*</b>	<b>Kit Results</b>	<b>Document Appearance of Result</b>
Clean Spores Iteration #1	Positive	A bright purple color appeared as soon as the indicator swab was inserted into test tube.
Clean Spores Iteration #2	Positive	A bright purple color appeared as soon as the indicator swab was inserted into test tube.
Clean Spores Iteration #3	Positive	A bright purple color appeared as soon as the indicator swab was inserted into test tube.
Dirty Spores Iteration #1	Positive	A bright purple color appeared as soon as the indicator swab was inserted into test tube.
Dirty Spores Iteration #2	Positive	A bright purple color appeared as soon as the indicator swab was inserted into test tube.
Dirty Spores Iteration #3	Positive	A bright purple color appeared as soon as the indicator swab was inserted into test tube.
Control Kit	Positive	A light purple color appeared as soon as both the protein indicator swab and the control swab was inserted into the protein tube.

\*Freeze-dried 100 µl spores (i.e., approx.  $1 \times 10^7$  spores) in a glass test tube

Note: A visual examination of the freeze-dried spores was conducted before testing. The "Clean" spores were a slightly whiter color than the "Dirty" spores.

**2. Verification of Inoculum Levels (Enumeration of freeze-dried spores):**

Sample Type	Dilutions Plated (CFU/plate)			Average (CFU/plate)	CFU/ml	Average CFU/mL
	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>			
Clean #1	33, 37, 41	7, 4, 5	0, 1, 3	39	*3.9x10 <sup>6</sup>	5.8x10 <sup>6</sup>
Clean #2	82, 75, 71	18, 13, 11	9, 3, 4	76	*7.6x10 <sup>6</sup>	
Dirty #1	129, 107, 136	12, 9, 7	3, 1, 0	124	1.2x10 <sup>7</sup>	1.3x10 <sup>7</sup>
Dirty #2	128, 157, 140	15, 13, 11	2, 1, 2	142	1.4x10 <sup>7</sup>	

\* The average CFU/ml of the Clean spore preparation was slightly less than the target inoculum level stated in the Test Plan. As a result, samples Clean #1 and Clean #2 were enumerated again using a 0.05% Triton X + PBS solution in the 10<sup>-1</sup> and 10<sup>-2</sup> dilution test tubes. The use of a Triton X solution was to break up clumping that can occur in clean spore preparations.

**2.a. Second Enumeration of Clean Spore Samples**

Sample Type	Dilutions Plated (cfu/plate)			Average CFU/plate	CFU/ml	Average CFU/ml
	10 <sup>-5</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>			
Clean #1	118, 87, 108	22, 18, 20	2, 0, 4	104	1.0x10 <sup>7</sup>	1.1x10 <sup>7</sup>
Clean #2	110, 115, 101	19, 23, 25	0, 3, 1	109	1.1x10 <sup>7</sup>	

**3. Conclusions**

- a. Both samples, clean and dirty spore preparations, tested positive for the presence of the protein using the FIRSTCHECK test kit. Samples contained approximately 1x10<sup>7</sup> spores.
- b. The verification of the spore inoculum level indicated that the dirty spore freeze-dry preparation was within the target concentration level of 10<sup>7</sup> - 10<sup>8</sup>. The clean spore freeze-dry preparation was slightly lower than the target concentration. To verify the colony counts, the clean spore freeze-dried preparation was enumerated a second time using a 0.05% Triton X + PBS solution. The results from the second enumeration of the clean spore freeze-dry preparations indicate that the initial concentration of the spores was within the target concentration of 10<sup>7</sup> - 10<sup>8</sup>.

**ATTACHMENT B  
Tier II Testing**

**1. Preparation of  $1 \times 10^8$  spore/ml Suspension**

Sample Type	Dilutions Plated (CFU/plate)			Average CFU/plate	CFU/ml
	$10^{-5}$	$10^{-6}$	$10^{-7}$		
Clean	All 3 TNTC	160, 98, 96	4, 7, 9	118	$1.2 \times 10^8$
Dirty	All 3 TNTC	164, 201, 198	11, 7, 10	188	$1.9 \times 10^8$
Hood Air Control Plate	No Growth				
Negative Media Control Plate	No Growth				

**2. Inoculation of FIRSTCHECK Kit Testing Tubes**

➤ Test Run used an inoculation volume of 10  $\mu$ l.

Sample Type	Dilution	Kit Results	Appearance of Result
Clean #1-3	$10^6$	Positive	A distinct purple color appeared after approximately 5 minutes.
Clean #1-3	$10^5$	Positive ?	A light purple color appeared after approximately 8 minutes.
Clean #1-3	$10^4$	Negative	No color change was observed after the 10 minute time period.
Dirty #1-3	$10^6$	Positive	A distinct purple color appeared after approximately 5 minutes.
Dirty #1-3	$10^5$	Positive ?	A light purple color appeared after approximately 8 minutes.
Dirty #1-3	$10^4$	Negative	No color change was observed after the 10 minute time period.

### 3. Tier II Conclusions

- a. Based on Tier II – Run #2 test results, the detection level of the kit for both the Clean and Dirty Bt spore preparations was in the range of  $10^{5-6}$  spores. Note: Positive results (presence of faint purple color) for the  $10^5$  spore samples did not occur until 8 minutes into the reaction.
- b. Adding 100  $\mu\text{l}$  inoculum (spore suspension) directly to the bottom of the kit test tube diluted the protein detection solution resulting in a false negative reaction. A 10  $\mu\text{l}$  dose volume did not appear to affect the functionality of the test.

### 4. Recommendations

**(note- the recommendations listed below were discussed with 20/20 GeneSystems, and subsequently conducted as additional Tier II testing; refer to Attachment C for the results)**

- a. Repeat Tier II testing using the dirty spore suspension only, and test six replicate freeze-dried samples at  $10^4$ ,  $10^5$ , and  $10^6$  spores. Each dilution tested will be enumerated in order to produce a more accurate detection limit.
- b. Test silica ( $\text{SiO}_2$ ), a non-protein filler, with the FIRSTCHECK kit.

**ATTACHMENT C**  
**Tier II Additional Testing**

**1. Preparation of  $1 \times 10^8$  spore/ml Suspension**

Sample Type	Dilutions Plated (CFU/plate)			Average CFU/plate	CFU/ml
	$10^{-5}$	$10^{-6}$	$10^{-7}$		
Dirty*	All 3 TNTC	All 3 TNTC	83, 89, 93	88	$8.8 \times 10^8$ ( $9 \times 10^8$ )
Hood Air Control Plate	No Growth				
Negative Media Control Plate	No Growth				
PBS + Triton X (0.05%) Control	No Growth				

\*Enumeration was performed using phosphate buffered saline (PBS) with Triton-X at a final concentration of 0.05% throughout the entire dilution sequence, in order to prevent clumping of the Bt spores.

**2. FIRSTCHECK kit Inoculation with Dried Spores**

- The Dirty Spore preparation was diluted to a concentration of  $10^5$ ,  $10^6$ , and  $10^7$  spores per mL. An aliquot of 100  $\mu$ l of each concentration was dispensed into glass test tubes and covered with a Kimwipe wipe and secured with a rubber band.
- Spore preparations were freeze-dried overnight in a LabConco freeze dryer.
- An aliquot of 300  $\mu$ l was removed from the bottom of the FIRSTCHECK detection tube and directly dispensed into the glass test tube containing the dried spores.
- The protein detection swab was inserted after the detection kit testing solution was dispensed.

**2. FIRSTCHECK kit Inoculation with Dried Spores, Cont'd**

Sample Type	Conc. Spores per Tube	Kit Results	Appearance of Result
Dirty #1-6	$9 \times 10^6$	Positive	A distinct purple color appeared after approximately 5 minutes.
Dirty #1-6	$9 \times 10^5$	Positive ?	A very light purple color appeared after approximately 10 minutes.
Dirty #1-6	$9 \times 10^4$	Negative	No color change was observed after the 10 minute time period.

**3. Conclusions**

- a. The detection limit of the spore preparation tested was in the range of  $10^6$  freeze-dried spores. There was a slight positive result with a concentration of  $10^5$ , however the purple color was only observed after 10 minutes.
- b. The spore concentrations were nearly 1 log higher than desired at  $9 \times 10^4$ ,  $9 \times 10^5$  and  $9 \times 10^6$  spores/mL, instead of the desired  $1 \times 10^4$ ,  $1 \times 10^5$ , and  $1 \times 10^6$  spore/mL.

**4. Evaluation of Silica**

Procedure: Aerosil®, a fumed hydrophobic silica, was tested. A small amount (tip of spatula; too small to weigh, but clearly visible) was added directly to the FIRSTCHECK kit protein tube, and to tubes containing  $10^6$  freeze-dried spores. The results are shown in the table below.

Sample Type	Conc. Spores per Tube	Kit Results	Appearance of Result
Aerosil® added directly to protein kit tube (one sample tested)	None	Negative	No color change was observed after the 10 minute time period. Control result: color appeared after adding control swab
Aerosil® and Freeze-Dried Spores (triplicates)	$10^6$	Positive ?	A very light grey-purple color appeared after 5 minutes.



## **Conclusions: Evaluation of Silica (Aerosil®)**

Aerosil® did not test positive for protein. After addition of the control coupon, a faint purple color was observed, demonstrating the silica material did not cause a false negative result.

The samples containing Aerosil® plus  $10^6$  spores did test positive for protein, however it should be clearly noted that the color observed within 5-10 minutes was faint and appeared gray-purple. Spore only samples were not tested.

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