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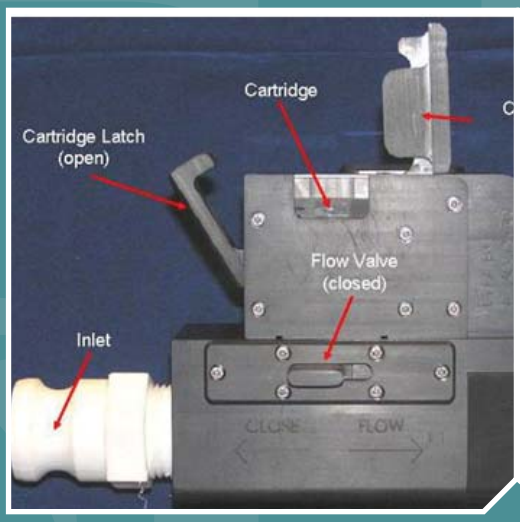
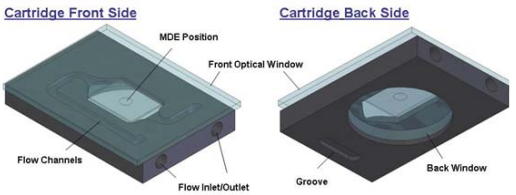
United States
Environmental Protection
Agency

Sporian Inline Biosensor System (IBS) Evaluation Summary





Office of Research and Development
National Homeland Security Research Center

Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development's National Homeland Security Research Center, funded and directed the research described herein under Contracts EP- C-09-041 with Shaw Environmental Incorporated. The EPA performed the work in collaboration with the US Army Engineering Research and Development Center (ERDC) Fluorescence Spectrocopy Lab. It has been reviewed by the Agency but does not necessarily reflect the Agency's views. No official endorsement should be inferred. EPA does not endorse the purchase or sale of any commercial products or services.

For questions about this report, please contact John Hall of the U.S. Environmental Protection Agency, National Homeland Security Research Center, 26 West Martin Luther King Drive, Cincinnati, Ohio, 513-487-2814, hall.john@epa.gov.

Foreword

Following the events of September 11, 2001, EPA's mission was expanded to address critical needs related to homeland security. Presidential Directives identify EPA as the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological attack.

As part of this expanded mission, the National Homeland Security Research Center (NHSRC) was established to conduct research and deliver products that improve the capability of EPA to carry out its homeland security responsibilities. One focus area of this research is the detection of potential contaminatnts within water systems. The Sporian inline biosensor system is designed for remote sensing of potable water supplies relevant to civilian and military communities. This research was performed in collaboaration with the United States Army Corps of Engineers.

NHSRC has made this publication available to assist water system utilities and operators by providing test data relating for a novel dection technology. This information is intended to move EPA one step closer to achieving its homeland security goals and its overall mission of protecting human health and the environment while providing sustainable solutions to our environmental problems.

Jonathan Herrmann, Director
National Homeland Security Research Center

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Acronyms and Abbreviations

CFU	colony forming unit
CSV	comma separated variable
EPA	U.S. Environmental Protection Agency
IBS	Inline biosensor system
GPH	Gallons per Hour
L	liter
MDE	Molecular detection element
mg	milligram
min	minute
mL	milliliter
mm	millimeter
NHSRC	National Homeland Security Research Center
PC	Personal Computer
S/N	Serial Number
Sig/etime	Flurosence signal over elapsed time
T&E	Test and Evaluation
USACE	United States Army Coprs of engineers

Objective

To evaluate Inline Biosensor System's (IBS) capability to detect biological contamination (e.g., *B. subtilis,* and *E. coli*) of drinking water in real time and ease of deployment in the field as a contamination warning device. IBS was designed and manufactured by Sporian Microsystems, Inc. (Sporian) for the US Army Corps of Engineers (USACE).

Instrument Overview

The IBS is a flow-through device that is equipped with a proprietary sensor cartridge designed to detect biological contamination. The IBS sensor cartridge contains a proprietary molecular detection element (MDE) which is also a fluorescing (emits light) media. When biological contamination is present, the fluorescing media is released from the MDE and bound to the contaminant. The rate of change of MDE media is roughly proportional to target concentration within the environment (how much target interacts with the MDE), and as such, high target concentrations may result in a very fast response (in the order of minutes) and depletion of the cartridge. A positive signal response is indicated by a decrease in fluorescing signal over elapsed time (in microseconds) as the MDE is exposed to the target biological contaminant within the environment. Due to processing variations, the initial value will differ from cartridge to cartridge. Over a period of time, the MDE becomes completely exhausted and needs to be replaced. Figure 1 shows the side view of the Sporian IBS device.

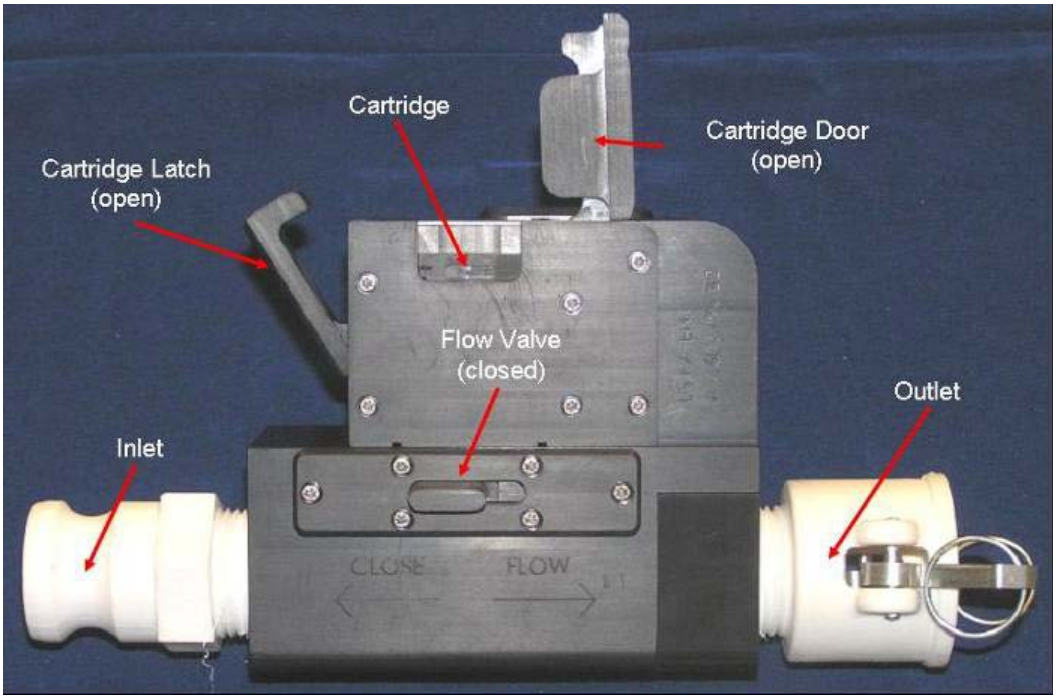

Figure 1. IBS side view with cartridge chamber door and latch open

Instrument Operation

As shown in Figure 1, the IBS includes a flow valve slide that routes a portion of the total flow through the inlet and outlet ports through an installed disposable MDE sensor cartridge. This valve does not affect the main flow from the inlet to the outlet port, but only routes a portion of the flow through the MDE cartridge. Moving the valve slide to the "Close" position blocks the flow to and from the cartridge ports. Moving the slide to the "Flow" position allows water to flow through the cartridge. This allows the user to replace a cartridge without stopping the main system flow.

The cartridge attached to the IBS includes a glass window coated with the appropriate MDE, such that when inserted into the IBS, the MDE is positioned in front of the detection optics. When inserted, the cartridge also forms a channel for flow to the sensing area. Each cartridge is a one-time use device, meaning that once the positive detection has occurred, the cartridge has been irreversibly consumed, and needs to be replaced with a fresh cartridge. Figure 2 shows a schematic rendering of the MDE cartridge.

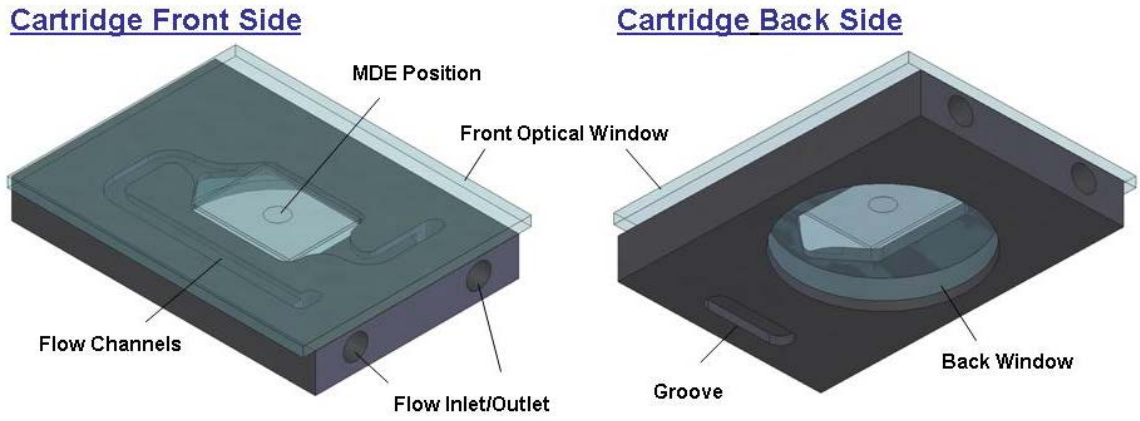

Figure 2. Schematic rendering of the MDE Sensor Cartridge

The IBS was designed for a nominal flow rate of 1,500 Gallons per Hour (GPH) for USACE, but will function in the as-shipped configuration at flow rates down to approximately 480 GPH. For flow rates below 480 GPH, inserts are provided (Figure 3) to ensure suitable flow through the MDE cartridge.

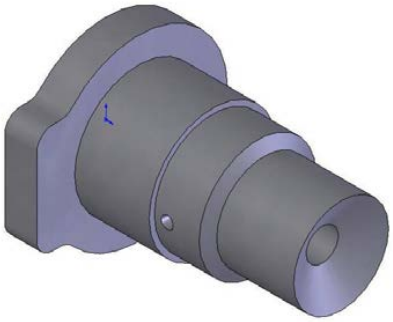

Figure 3. Low Flow Insert

The low flow inserts were used during the tests conducted at the EPA Test & Evaluation (T&E) Facility.

Test Protocol

The EPA/Shaw Environmental protocol for contaminant injection for real-time monitoring typically involves running the instrument for a certain period of time (at least 1-2 hours) to collect baseline data (representing normal field conditions) and then conducting the injection event followed by a post injection period to evaluate if the instrument recovers (i.e., the data are back to the normal baseline levels). This approach is desired by EPA for all online continuous monitoring equipment from a long-term equipment deployment perspective and it allows for automated contamination "event detection" using algorithms that are designed to detect statistically significant changes in water quality from the baseline or "normal" conditions.

Between August 15, 2011, and September 1, 2011, a total of eight injection events (excluding overnight and reagent blank runs) were performed for the instrument evaluation. During each test run, a designated amount of contaminant was mixed in 10-L dechlorinated tap water and injected into the single-pass pipe loop using a flow-controlled injection pump. Each test run consisted of the biological contaminant with the growth media and the dechlorinating agent (sodium thiosulfate) except for the blank runs which contained only the dechlorinating agent. The water flow rate of the single-pass pipe system was controlled at 23 gallons per minute (gpm) through the whole experiment. This flow rate is equivalent to one foot per second in the single- pass pipe. The 10-L contaminant solution was continuously injected into the pipe for 20 minutes (each event) at the injection port. The travel time from the injection port to the Sporian IBS is estimated to be roughly 4 minutes. Table 1 is the summary of events performed for the study. During the biological contaminant injection events, the average chlorine concentration varied between 1.1 and 1.2 mg/L. Varying amounts of sodium thiosulfate were injected to neutralize the chlorine: ~13 grams for Tests 1, 2, and 3, and 19 grams for the remainder of the test runs. Volumetrically, it is estimated that 4.8 mg/L and 6.95 mg/L of unreacted thiosulfate was injected into the system. Two Sporian units (Serial Numbers 005 and 007) were tested side by side during this testing.

Table 1. Event Summary

Date	Test No.	Start Time	Stop Time	Injected Stock Contaminant Concentration	Injected Diluted Concentration	Grab Sample Sporian Port
8/15/2011	1	11:55	12:15	Flask #6 *E. coli* 4.2 x 10^9 CFU/ml	5 ml = 12,061 CFU/ml	119 CFU/ml
8/15/2011	2	14:40	15:00	Flask #6 *E. coli* 4.2 x 10^9 CFU/ml	5 ml = 12,061 CFU/ml	110 CFU/ml
8/15/2011	3	15:40	16:00	Flask #6 *E. coli* 4.2 x 10^9 CFU/ml	0.5 ml = 1,206 CFU/ml	12 CFU/ml
8/18/2011	4	16:15	16:35	Flask #8 *E. coli* 1.0 x 10^{11} diluted to 1.0 x 10^9	20 ml = 11,487 CFU/ml	190 CFU/ml
8/19/2011	Reagent Blank	10:00	10:20	*Sodium thiosulfate blank*		
8/19/2011	5	11:01	11:21	Flask #8 *E. coli* 1.0 x 10^{11} diluted to 1.0 x 10^9	20 ml = 11,487 CFU/ml	194 CFU/ml
8/24/2011	6	15:45	16:05	Flask #8 *E. coli* 1.0 x 10^{11} undiluted	2 ml = 114,881 CFU/ml	11,000 CFU/ml
8/25/2011	7	14:00	14:20	*Bacillus subtilis spores* 1.0 x 10^9	2 ml = 1,149 CFU/ml	2,500 CFU/ml
9/1/2011	8	11:00	11:20	*Bacillus subtilis spores* 1.0 x 10^9	6 ml = 3,446 CFU/ml	6,500 CFU/ml

Table 2 Sporian response data for Table 1 injections

Test No.	Grab Sample Sporian Port	Unit 005 Signal/Etime Change Observed	Unit 005 Estimated Peak Concentration	Unit 007 Signal/Etime Change Observed	Unit 007 Estimated Peak Concentration
1	119 CFU/ml	Yes	100,000 CFU/ml	No	4000 CFU/ml
2	110 CFU/ml	Yes	100,000 CFU/ml	Yes	100,000 CFU/ml
3	12 CFU/ml	Yes	100,000 CFU/ml	Yes	100,000 CFU/ml
4	190 CFU/ml	No	100,000 CFU/ml	No	100,000 CFU/ml
Reagent Blank		Yes	100,000 CFU/ml	No	80,000 CFU/ml
5	194 CFU/ml	Yes	80,000 CFU/ml	No	0 CFU/ml
6	11,000 CFU/ml	No	30,000 CFU/ml	Yes	80,000 CFU/ml
7	2,500 CFU/ml	No	100,000 CFU/ml	Yes	100,000 CFU/ml
8	6,500 CFU/ml	Yes	100,000 CFU/ml	Yes	100,000 CFU/ml

Data Collection and Processing
The IBS instrument is connected to PC/Netbook and utilizes a program named IBIPC that captures the data and logs the data to files on the PC in a "data" subdirectory. The location of this directory, by default, is the installation directory of the IBIPC program. Within the data subdirectory, a file is created for each day of logs. The current day is appended to the file named "data", and prior days' logs are in files which contain the date, such as "data.2011-08-15." The log files need to be processed further manually using Sporian- provided software to extract data into comma separated variable (.CSV) format that can be processed using a spreadsheet program such as Microsoft Excel.

Subsequent to the completion of the testing, Sporian has also provided a serial cable interface for enabling direct logging to the NexSens data logger available at the T&E Facility.

Evaluation Summary and Conclusions
The data collected using IBS units 005 and 007 were submitted to Sporian and USACE for further evaluation. The key observations from the EPA and Shaw Environmental testing are summarized below:

1) The IBS unit appears to be capable of detecting both dead and live biological contaminants since the reported concentrations by the unit were higher than the live cells determined from plate and culture analysis of the grab sample port. The estimated concentrations by the IBS units were consistently much higher than the injected cell densities.
2) The Sporian IBS device is not currently suitable for long-term monitoring as the cartridge is consumed quickly in continuous service. The overnight tests indicated that the signal decreases relatively quickly (in about 30 minutes), showing a strong evidence that chlorine removes the fluorophores from the MDE surface relatively quickly. The "useful life" of the cartridges should be clearly understood by the end users in relation to the target concentration of the contaminant.
3) There were numerous test runs where there was no observable deflection in the signal graph, yet relatively high concentrations of contaminant were reported. This could be a scaling issue with the graphs, but the correlation between the signal and the reported concentrations should be better understood to prevent false positives.
4) The units reported significant estimated concentrations of bacteria in response to the blank test runs indicating that the sodium thiosulfate may be reacting with the material on the cartridge.
5) In general, the observed signal response to *E. coli* was stronger than for *B. subtilis*.
6) The unit is susceptible to condensation and bubbling. EPA/Shaw tested the units at a much lower flow rate than the design flow rate. Future improvements to the flow cell may be required for low flow applications. All manufacturer recommendations should be strictly followed.
7) Inter unit reproducibility was low for the EPA/Shaw testing.
8) All of the EPA approved enzyme based *E. coli* detection methods require relatively lengthy incubation periods. This MDE technology if perfected could provide a useful "real time" rapid screening method for *E. coli* prior to enzyme based detection.

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Appendix A

Sporian Data Plots

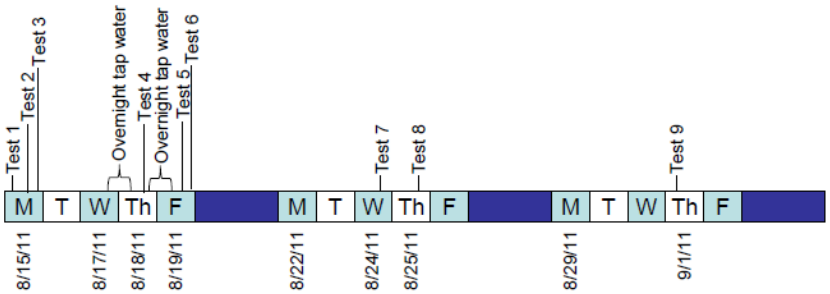


Figure 1. Timeline of various test events.

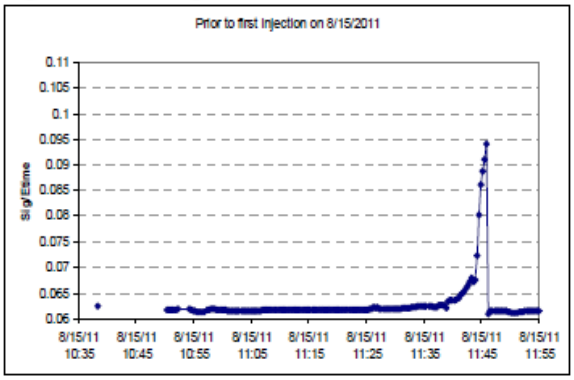


Figure 2. S/N 005 Sig/etime vs. time for period between set up and first test.

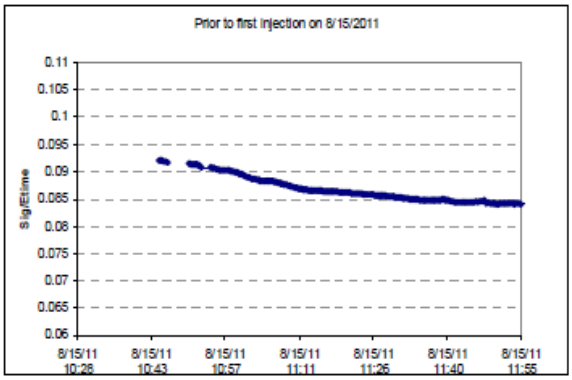


Figure 3. S/N 007 Sig/etime vs. time for period between set up and first test.

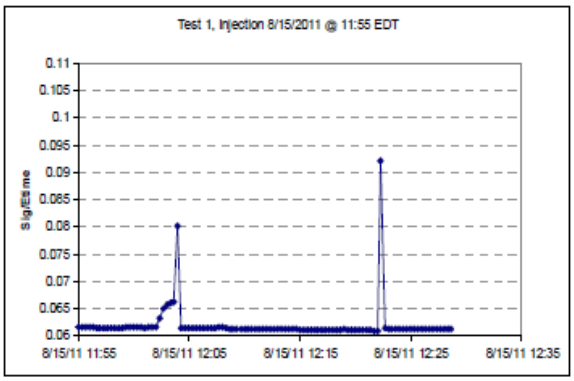


Figure 4. S/N 005 Sig/etime vs. time for injection #1 – E. coli.

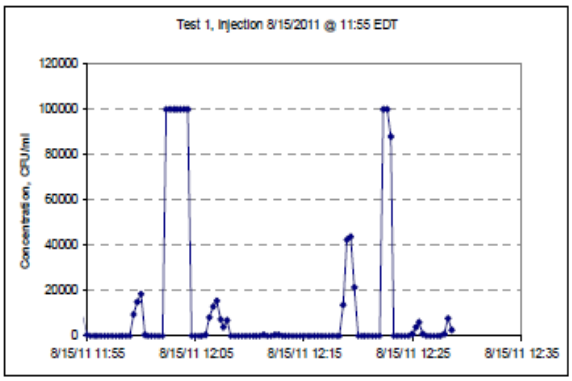


Figure 5. S/N 005 Estimated concentration vs. time for injection #1 – E. coli.

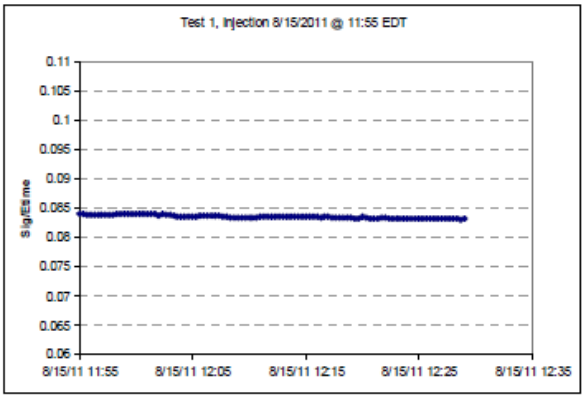


Figure 6. S/N 007 Sig/etime vs. time for injection #1 – E. coli.

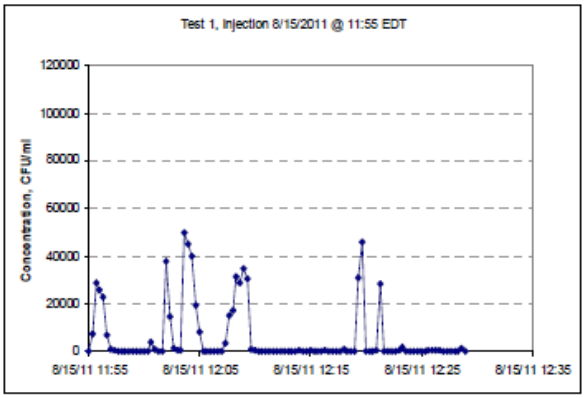


Figure 7. S/N 007 Estimated concentration vs. time for injection #1 – E. coli.

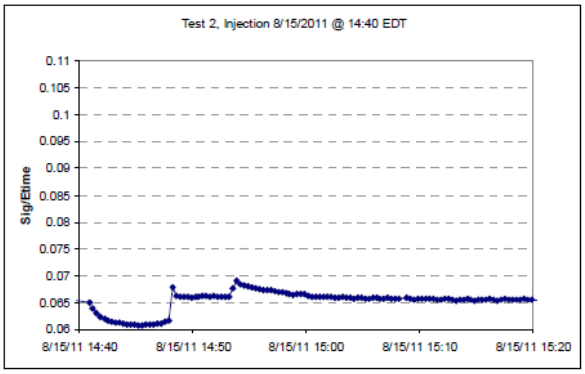


Figure 8. S/N 005 Sig/etime vs. time for injection #2 – E. coli.

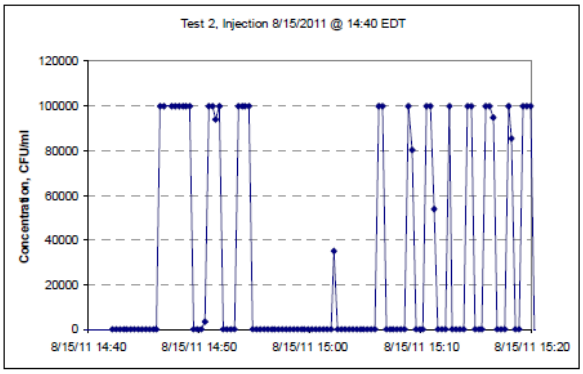


Figure 9. S/N 005 Estimated concentration vs. time for injection #2 – E. coli.

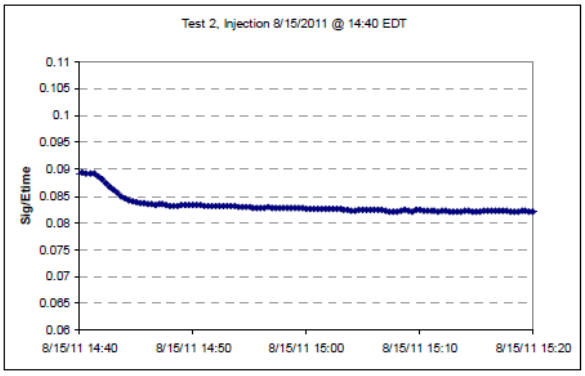


Figure 10. S/N 007 Sig/etime vs. time for injection #2 – E. coli.

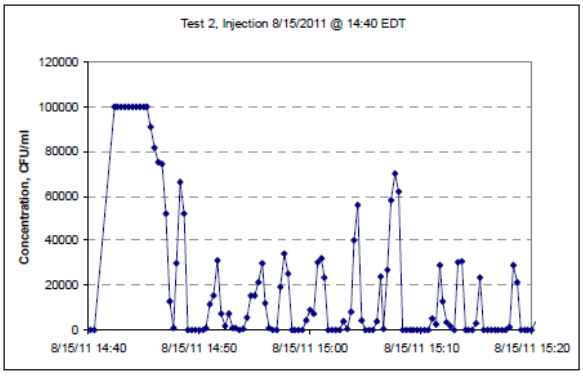


Figure 11. S/N 007 Estimated concentration vs. time for injection #2 – E. coli.

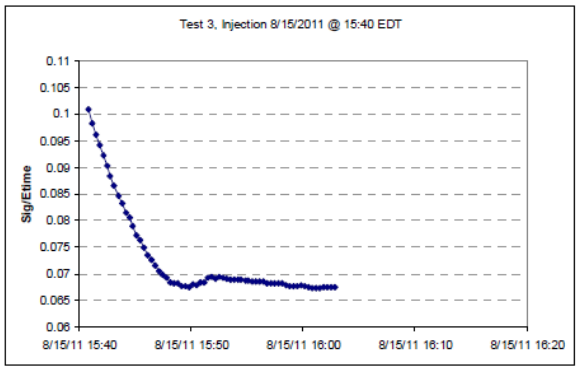


Figure 12. S/N 005 Sig/etime vs. time for injection #3 – E. coli.

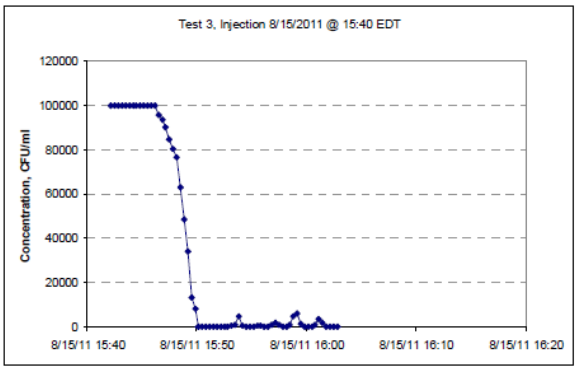


Figure 13. S/N 005 Estimated concentration vs. time for injection #3 – E. coli.

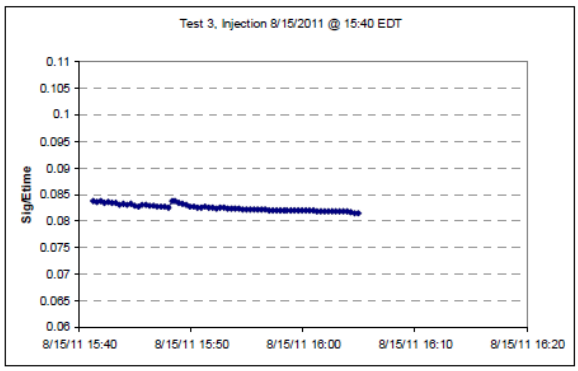


Figure 14. S/N 007 Sig/etime vs. time for injection #3 – E. coli.

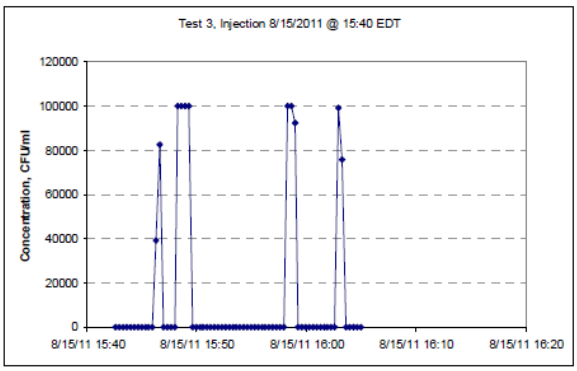


Figure 15. S/N 007 Estimated concentration vs. time for injection #3 – E. coli.

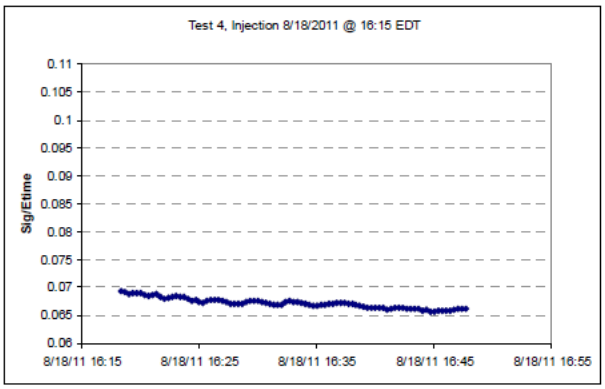


Figure 16. S/N 005 Sig/etime vs. time for injection #4 – E. coli.

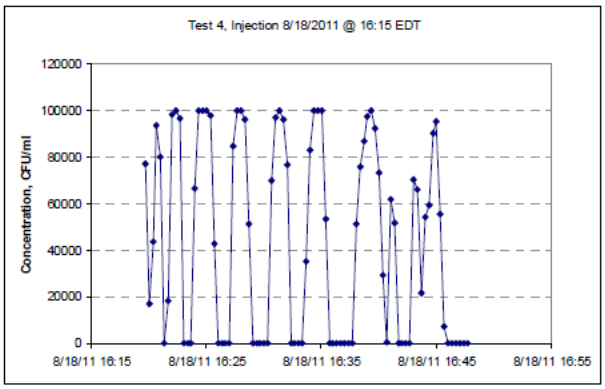


Figure 17. S/N 005 Estimated concentration vs. time for injection #4 – E. coli.

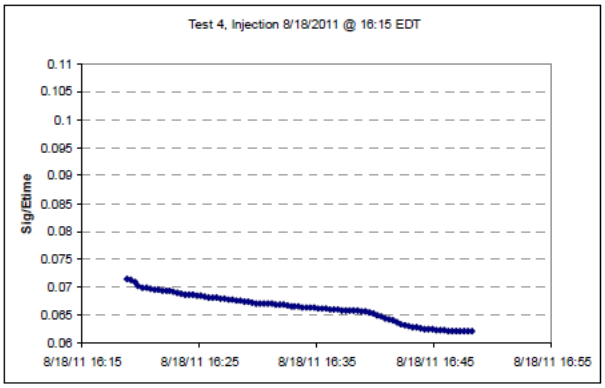


Figure 18. S/N 007 Sig/etime vs. time for injection #4 – E. coli.

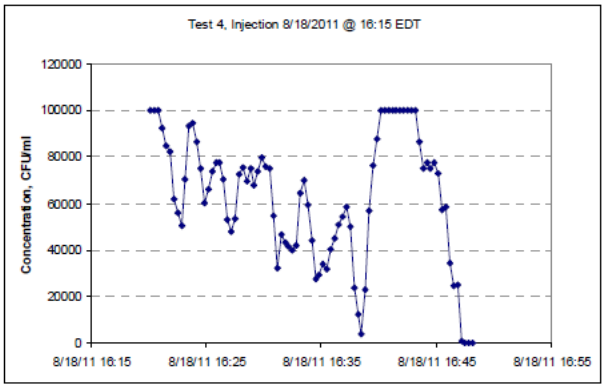


Figure 19. S/N 007 Estimated concentration vs. time for injection #4 – E. coli.

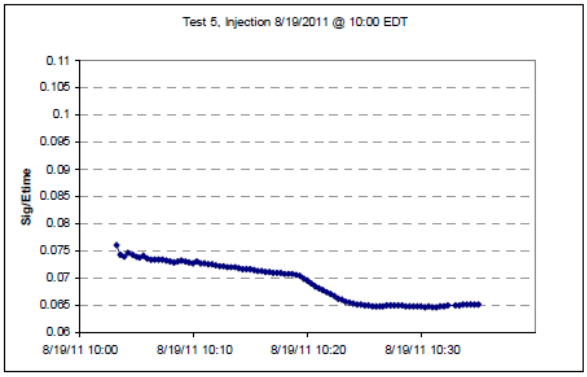


Figure 20. S/N 005 Sig/etime vs. time for injection #5 – sodium thiosulfate.

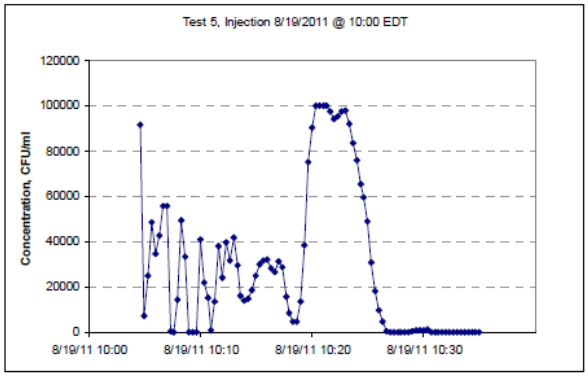


Figure 21. S/N 005 Estimated concentration vs. time for injection #5 – sodium thiosulfate.

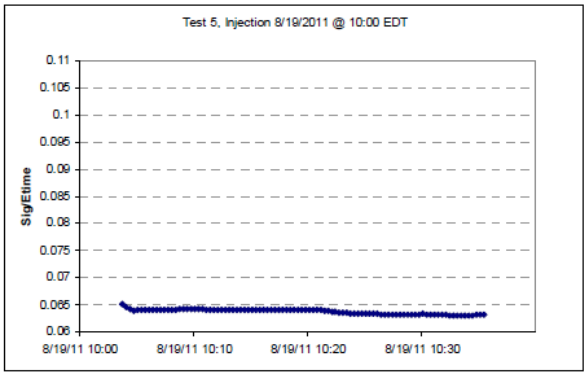


Figure 22. S/N 007 Sig/etime vs. time for injection #5 – sodium thiosulfate.

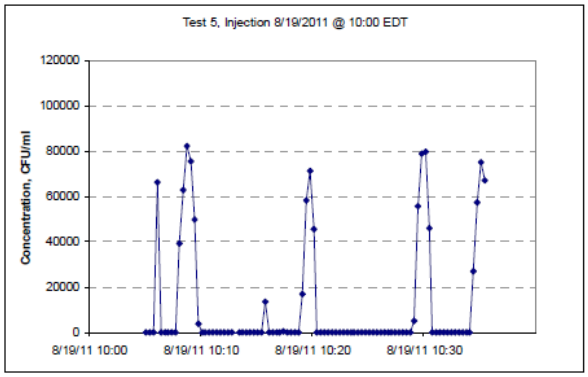
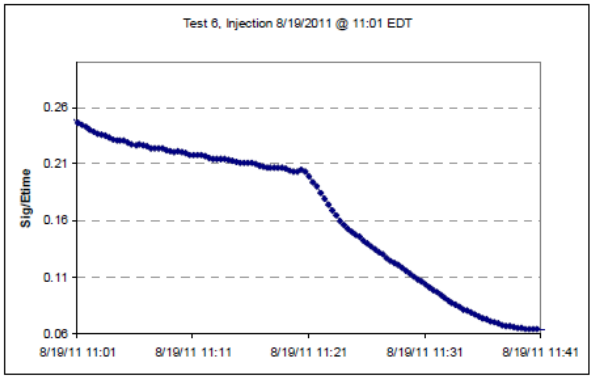


Figure 23. S/N 007 Estimated concentration vs. time for injection #5 – sodium thiosulfate.



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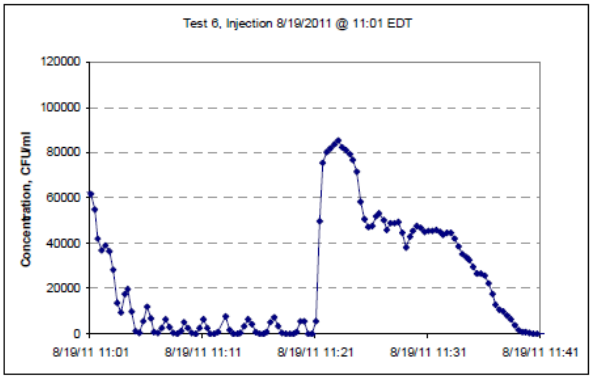

Figure 25. S/N 005 Estimated concentration vs. time for injection #6 – E. coli.

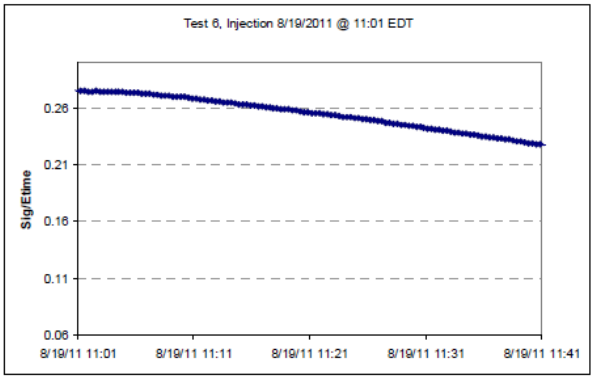

Figure 26. S/N 007 Sig/etime vs. time for injection #6 – E. coli.

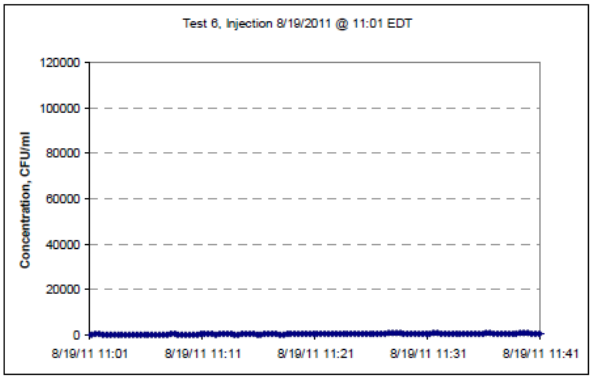

Figure 27. S/N 007 Estimated concentration vs. time for injection #6 – E. coli.

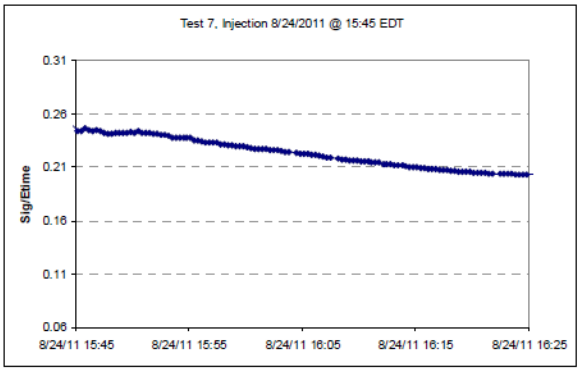

Figure 28. S/N 005 Sig/etime vs. time for injection #7 – E. coli.

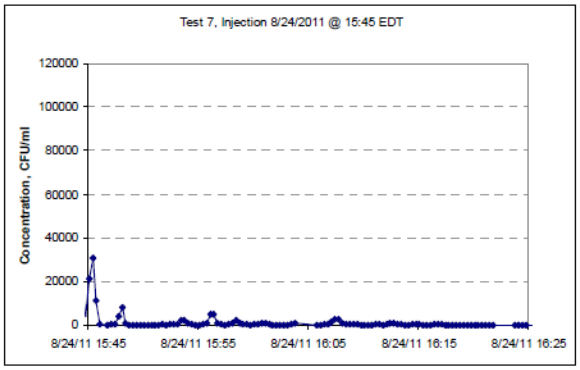

Figure 29. S/N 005 Estimated concentration vs. time for injection #7 – E. coli.

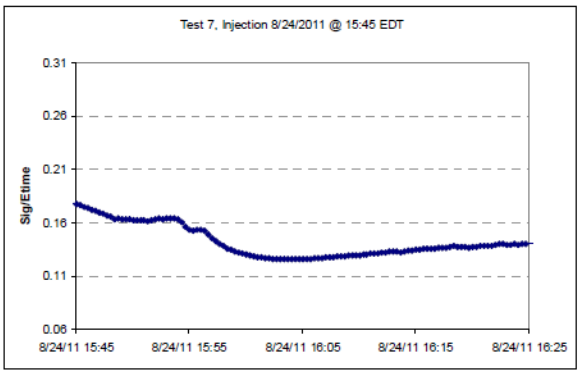

Figure 30. S/N 007 Sig/etime vs. time for injection #7 – E. coli.

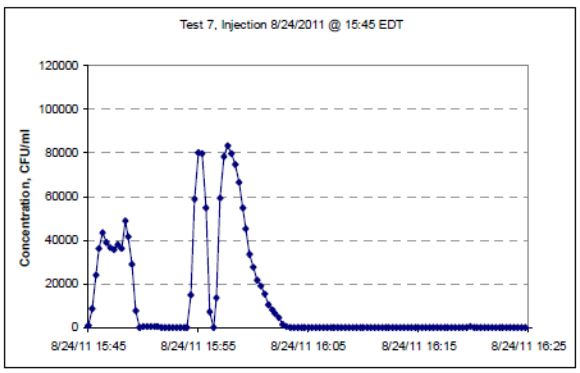

Figure 31. S/N 007 Estimated concentration vs. time for injection #7 – E. coli.

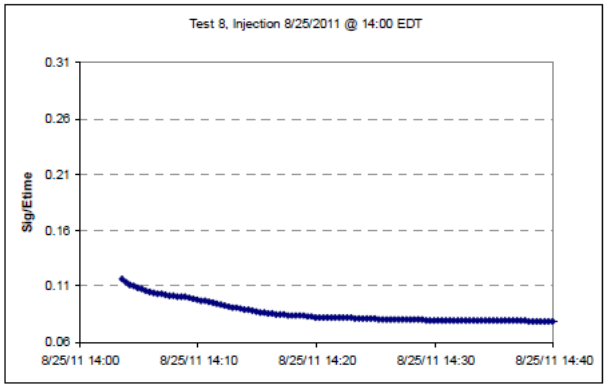

Figure 32. S/N 005 Sig/etime vs. time for injection #8 – B. subtilis.

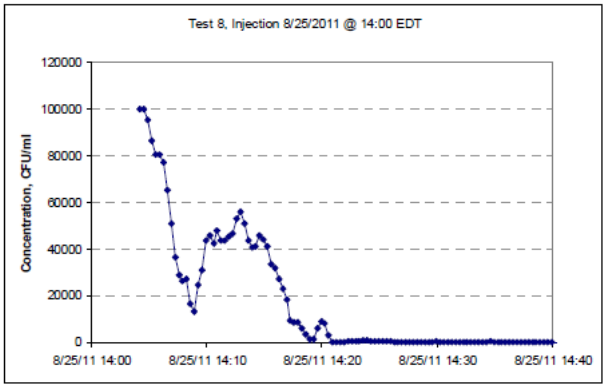

Figure 33. S/N 005 Estimated concentration vs. time for injection #8 – B. subtilis.

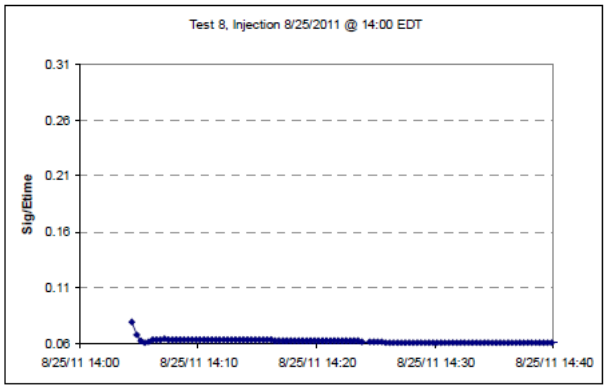

Figure 34. S/N 007 Sig/etime vs. time for injection #8 – B. subtilis.

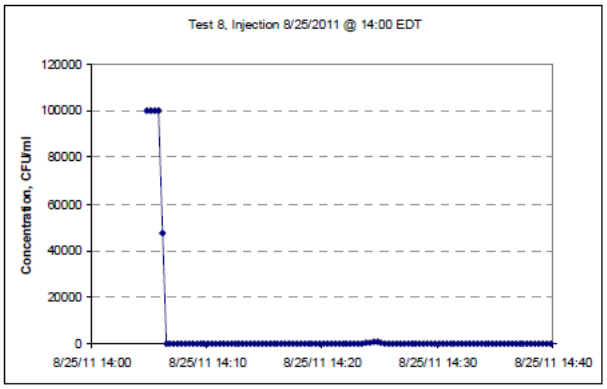

Figure 35. S/N 007 Estimated concentration vs. time for injection #8 – B. subtilis.

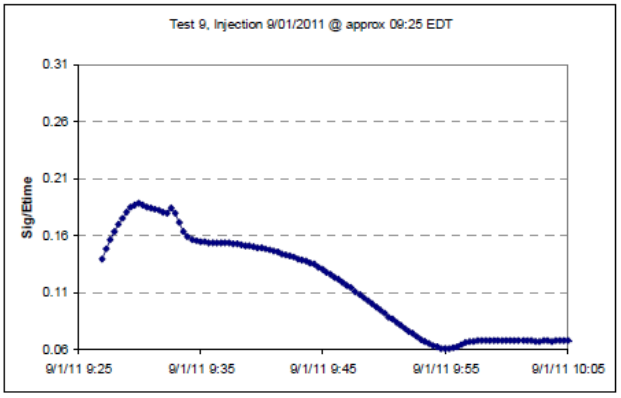

Figure 36. S/N 005 Sig/etime vs. time for injection #9 – B. subtilis.

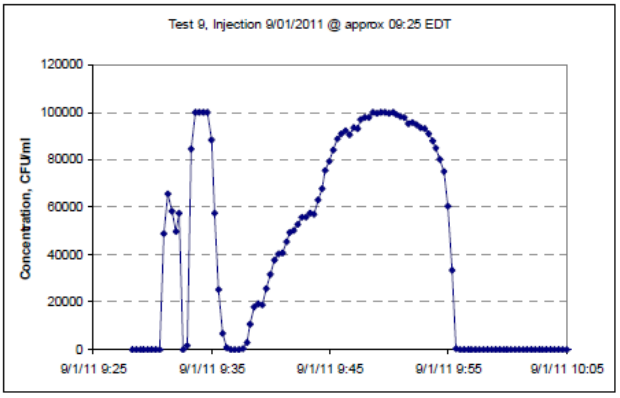

Figure 37. S/N 005 Estimated concentration vs. time for injection #9 – B. subtilis.

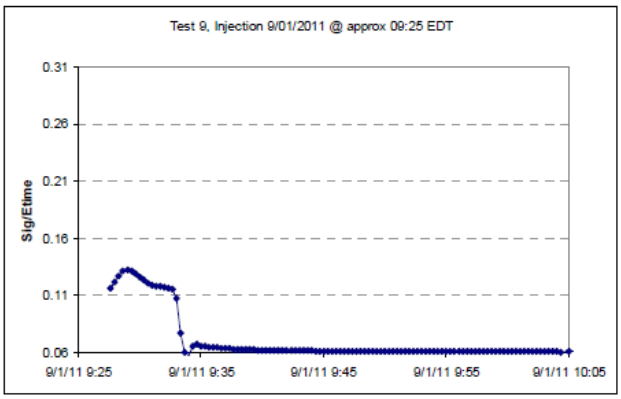

Figure 38. S/N 007 Sig/etime vs. time for injection #9 – B. subtilis.

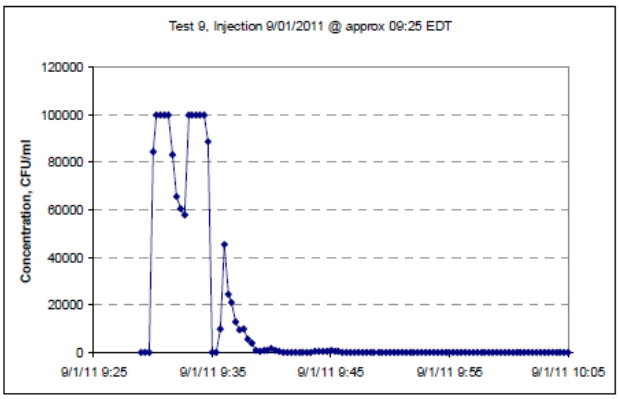

Figure 39. S/N 007 Estimated concentration vs. time for injection #9 – B. subtilis.

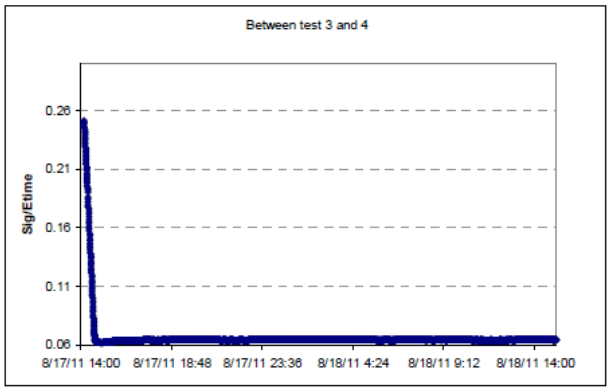

Figure 40. S/N 007 Sig/etime vs. time for "Overnight Matrix Background" test night of 8/17 to 8/18/11.

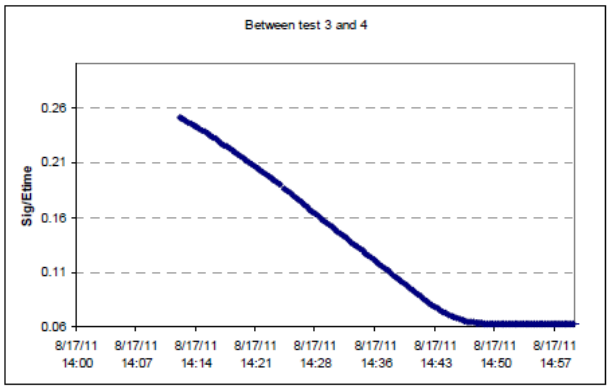

Figure 41. S/N 007 Sig/etime vs. time for "Overnight Matrix Background" test night of 8/17 to 8/18/11 focusing on signal change in first approx. 30 minutes.

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