Biolennia LABORATORIES

StreetCar Transit Vehicle Follow-up Study – Aegis / Bombardier 2009-10

A. INTRODUCTION:

This study was designed to test the ability of Aegis Microbe Shield Technology to reduce the number of total bacteria found on the inside surfaces of public transportation vehicles under regular in use conditions. The first tests were done in May 2009 with a follow-up in June 2009. The present updated data are from March 2010.

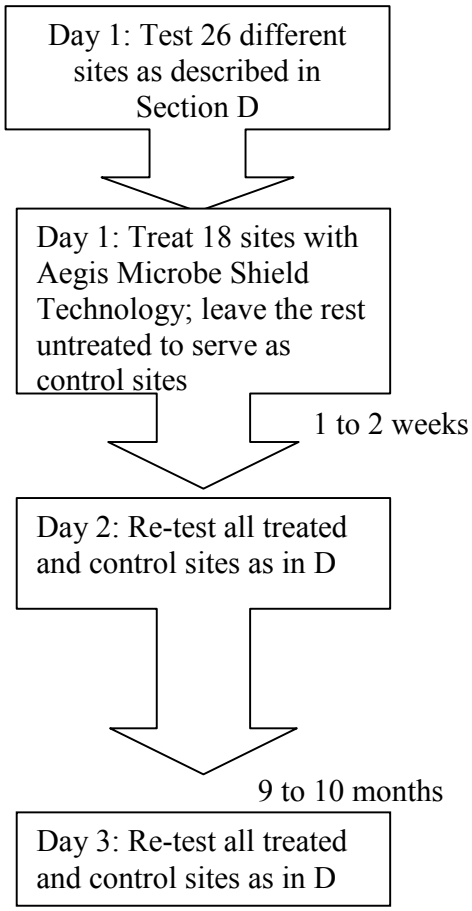
B. OBJECTIVE:

To measure the number of Total Heterotrophic Bacteria on various surfaces of a streetcar before and after the application of Aegis Microbe Shield Technology with a 10 month follow up.

C. EXPERIMENTAL DESIGN:

The tests include twenty-six different interior surfaces in each public transit vehicle.
The study flow diagram is summarized in Figure 1, details are described below.

Figure1: Study Flow diagram



Areas sampled and sites selection:

The sites sampled were randomly selected. Areas that are prone to direct contact with users were included and different types of materials were chosen (e.g. plastic, stainless steel, textiles, leather, carpets, etc). Treatment was applied to enough number of sites in order to assess the action of the sanitizing agent.

Sterile 100 cm^2 templates were used in an effort to standardize the areas tested. In some cases where irregular surfaces were swabbed (e.g. handles, buttons) the object was placed in the center of the template and all visible areas inside the template were swabbed. Special care was taken to identify the each surface swabbed so that approximately the same areas could be covered before and after application of the Aegis agent.

Treatment:

Immediately after carrying out the initial sampling, Aegis Treatment was applied to several sampled surfaces in the vehicle (treated sites), other surfaces were left untreated to serve as controls (untreated sites). Description of each site tested including identification of treated and untreated sites can be found in the Tables.

Testing intervals:

Testing was performed 13 days after treatment (Day 2), on all selected sites using the same method as on Day 1. A third set of tests was performed 10 months after treatment (Day 3). The vehicles were subject to regular service during the testing period.

D. MATERIALS AND METHODS

The methodology involved in the performance of this study follows the principles outlined by the United States Pharmacopeia and the American Public Health Association for Microbiological Monitoring of Surfaces. Details are described below:

Swab contact method

Sampling procedure:

The swab contact method was used to sample each site: A sterile swab was taken out of its pouch aseptically by grasping the end of the stick with sterile gloves. After placing the template in the selected area, a vial containing 5 ml of Letheen Broth (PH= 7.0 +/- 0.2 at 25^{o}C) was opened to moisten the sterile swab head removing the excess moisture by pressing against the walls of the tube. The swab was rubbed against the selected area thoroughly 3 times, reversing directions between strokes. This was repeated with a **second** sterile swab. After swabbing the area, the swab heads were positioned inside the liquid and the vial shaken by striking the palm of the hand for 10 secs. All samples were then placed in a refrigerated container and analyzed within 3 hours.

Plating swab rinse solutions:

Upon arrival to the laboratory samples were assigned a unique number. Each sample was vortexed for 10 seconds, 0.1 ml and 1 ml aliquots of the rinsing solution were dispensed in sterile petri dishes. Approx. 20 ml of Tryptic Soy Agar (PH 7.3 +/- 0.2 at 25 ^{o}C) was then poured in each petri dish. All plates were incubated for 48 +/- 2hs at 30 - 35 ^{o}C. The number of total heterotrophic bacteria on each sample was calculated by multiplying the number of colony forming units on each plate by the dilution plated and total volume of the vial.

Controls:

-Letheen broth (in vials) sterility control: One un-inoculated vial containing 5 ml of rinsing solution was processed with each set of samples.
-Swabs sterility control: One unused sterile swab was processed with each set of samples.
-Tryptone Soya Broth: One un-inoculated plate was incubated along with the inoculated plates to ensure sterility of the media.
-Media viability Controls: The ability of Letheen agar and Tryptone Soya Broth to recuperate aerobic bacteria was tested after preparing each media lot as per Biolennia's Standard Operating Procedures.

Good Laboratory Procedures:

Good laboratory procedures inherent to the performance of this study (e.g media preparation) are described in a detailed series of SOPs maintained at Biolennia Laboratories.

Personnel and Testing Facilities:

The study director for this project was Dean Swift BSc. Maria Calimano B.Sc. was responsible for sampling. Resumes for technical personnel are maintained and available upon request. The Microbiology study was conducted at Biolennia Laboratories, 5000 M Dufferin Street, Toronto, ON, M3H5T5

E. RESULTS:

Table 1: Results for the enumeration of Total Heterotrophic Bacteria on various surfaces of Street Car # 4154 **before** (May 27, 2009), **after** (June 9, 2009) treatment with Aegis Microbe Shield Technology and **Follow-up** on March 26, 2010.

Site Number	Description	Total Heterotrophic Bacteria (cfu/sample)		
		27 May 2009 No Treatment	9 June 2009 Post-Treatment	26 March 2010
1	Air vent middle vent, window freeze, second seat	135	25	8
2	Stanchion door at stairs	165	5	15
3	Rubber stanchion at door entrance	190	15	12
4	Fare box slot, operator's button	1995	175	21
5	Conductor hand rail, left side in front of conductor seat	35	< 1	24
6	Control buttons (interior, cab, panel)	960	165	9
7	Conductor key pad "trump"	955	135	26
8	Conductor phone handle, both sides, button	165	10	86
9	Conductor seat cushion at front	705	165	62
10	Head rest Conductor seat	2200	1150	180
11	Second seat, left side	825	25	240
12	Stanchion at second seat, upright	20	5	28
13	Top of first seat	65	< 1	620
14	Upper stanchion beside second seat	105	10	2
15	Window handle at third seat	50	15	21
16	Air vent back corner behind double seat	125	60	640
17	Air vent back left behind seats	255	15	240
18	Seat left side behind windscreen opposite of middle door	990	255	760

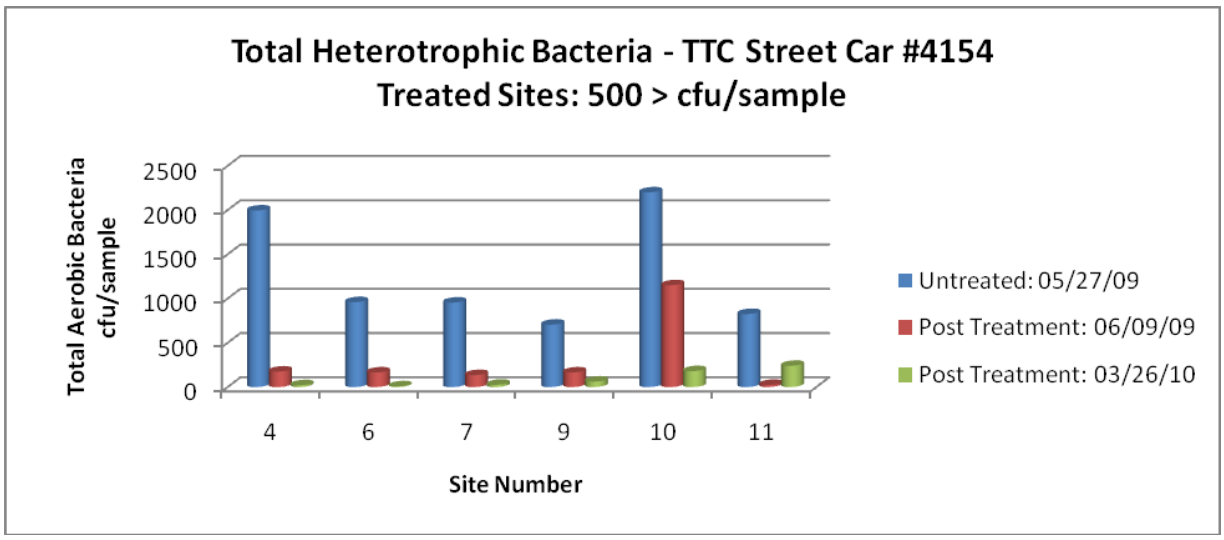


Figure 1: Results for the enumeration of Total Heterotrophic Bacteria on various surfaces of the Street Car #4154 before (May 27, 2009) and after (June 9, 2009) treatment with Aegis Microbe Shield Technology. Follow up March 26, 2010 Sites showing > 500 cfu initially.

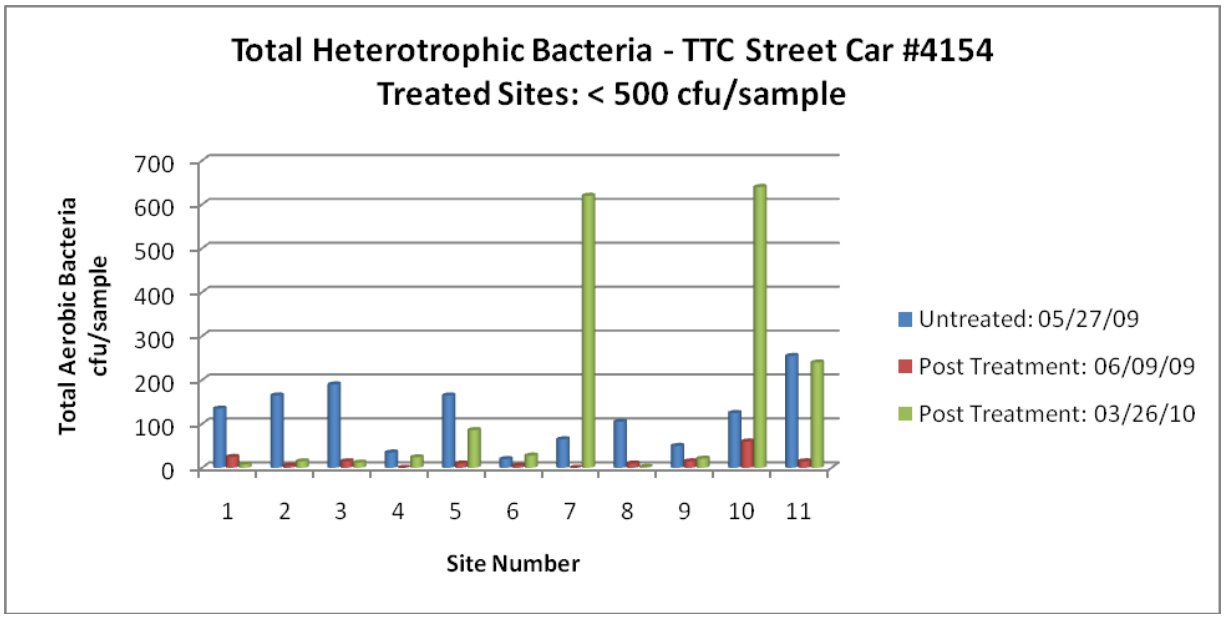


Figure 2: Results for the enumeration of Total Heterotrophic Bacteria (on various surfaces of the Street Car #4154 before (May 27, 2009) and after (June 9, 2009) treatment with Aegis Microbe Shield Technology. Follow up March 26, 2010 Sites showing < 500 cfu initially.

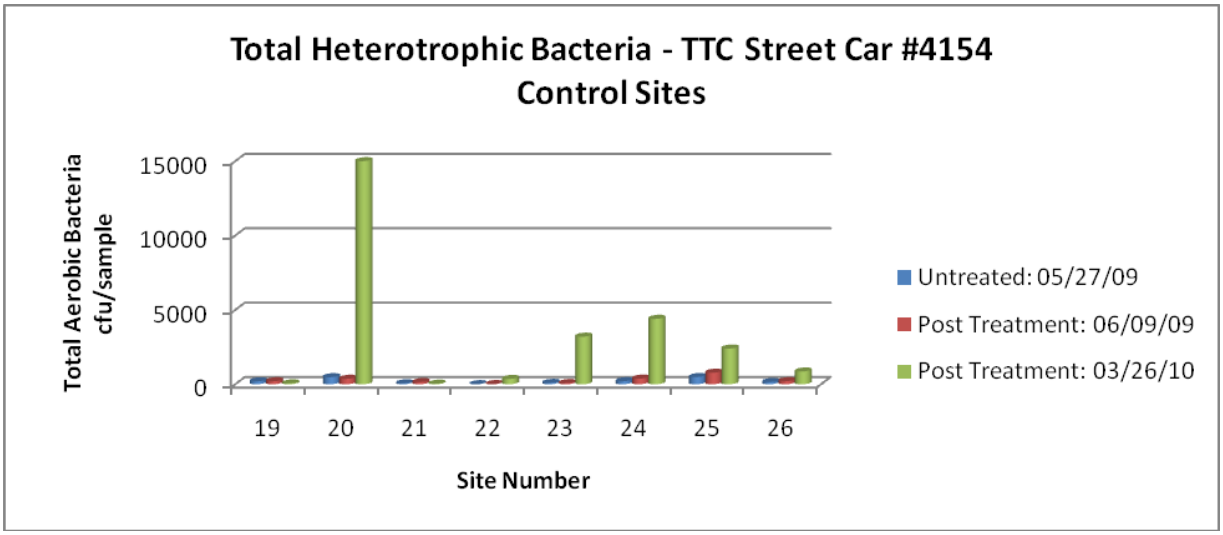
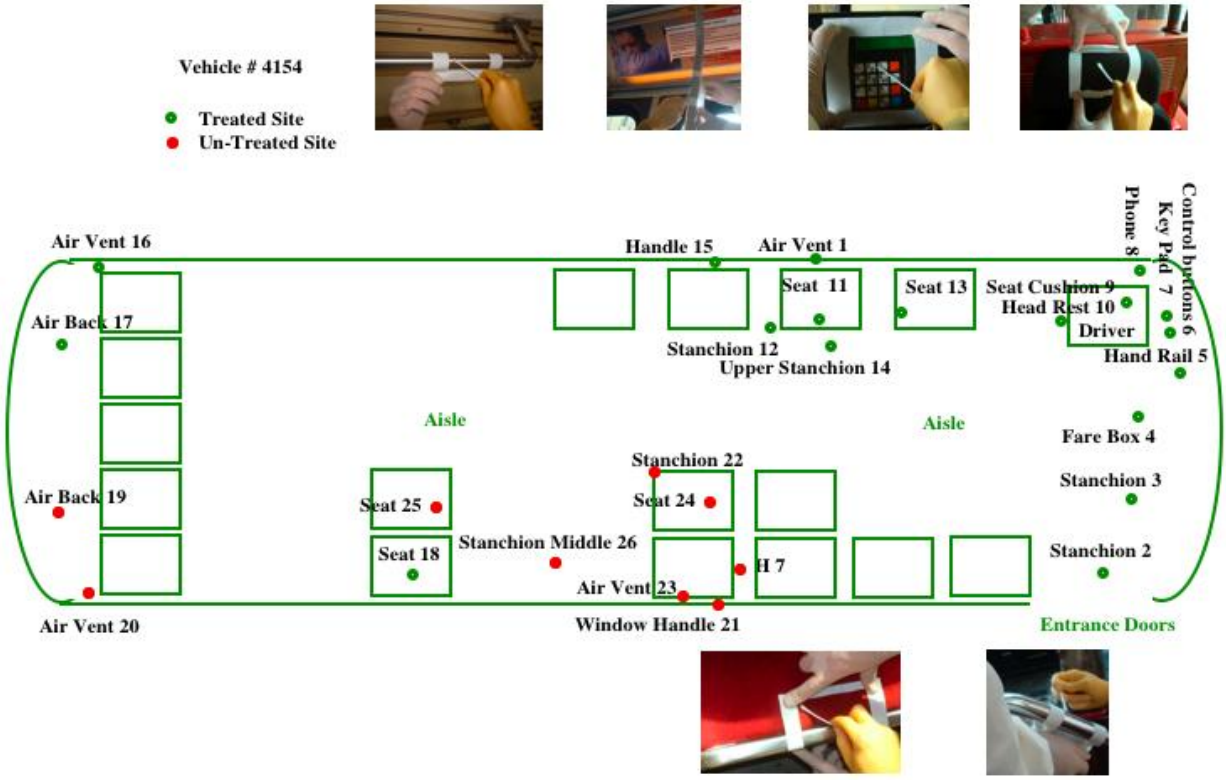


Table 2: Results for the enumeration of Total Heterotrophic Bacteria on various surfaces of the Street Car #4154 for untreated (control) sites tested on May 27, 2009, June 9, 2009 and March 26, 2010.

Site Number	Description	Total Heterotrophic Bacteria (cfu/sample)		
		27 May 2009	9 June 2009	26 March 2010
19	Air vent, back, right side	195	205	60
20	Air vent, right side, back corner (behind seat)	475	370	15000
21	Window handle second double seat, right side	65	145	38
22	Stanchion seat, second double seat	10	15	360
23	Air vent second double seat, first vent	95	80	3200
24	Seats, second double seat at aisle	205	378	4400
25	Seat behind windscreen right side, behind middle doors	485	775	2400
26	Middle doors stanchion going downstairs	145	205	860

Figure 3: Results for the enumeration of Total Heterotrophic Bacteria on various surfaces of Street car #4154 for untreated (control) sites tested on May 27, 2009 and June 9, 2009. Follow-up was March 26, 2010.



F. CONCLUSION:

In this study we determined that the treatment of transit car surfaces with Aegis Microbe Shield Technology resulted in generally a 1 $\log_{10}$ to 2 $\log_{10}$ reduction in the number of recoverable Heterotrophic Bacteria. In contrast, the untreated sites showed a general increase between samplings. Of the untreated sites 19, 21 & 22 were the cleanest sites on follow-up. The follow-up was done with a two swab recovery technique increasing the number of viable organisms collected.

Of special note, the three highest areas of contamination were the surrounding the Operator. They were the Fare Box button and the Telephone. On these surfaces, the Aegis Treatment resulted in a 100x reduction in the number of recoverable bacteria at the fare box and greater than 10x at the headrest. The headrest improved with the treatment about 2x, but one must be aware that the scalp harbours the highest numbers of bacteria of all external body areas.

The only results that seemed out of pattern were 13, 16 and 17. Perhaps TTC background information will help with this. The vent @ site 16 was visibly dirty with several mm of dirt.

Given that disease often requires as few as 50 organisms to initiate, reductions of 2x to 100x as found in this study indicate a benefit to the transit users and especially the drivers.

Dean Swift
Research Director
April 1, 2010