BIOLENNIA LABORATORIES

Divine Mercy School Study – Aegis 2010

A. INTRODUCTION:

This study was designed to test the ability of Aegis Microbe Shield Technology to reduce the number of total bacteria found on selected surfaces in an elementary school under regular conditions.

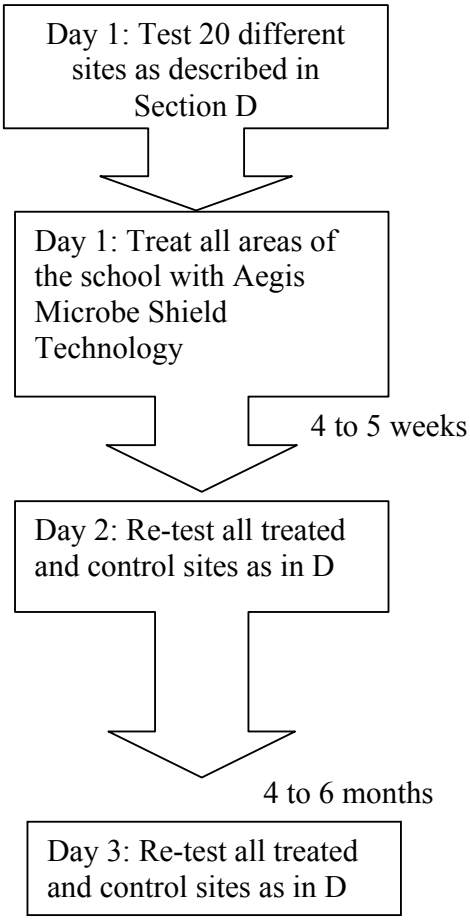
B. OBJECTIVE:

To measure the number of Total Heterotrophic Bacteria on various surfaces before and after the application of Aegis Microbe Shield Technology.

C. EXPERIMENTAL DESIGN:

The tests include twenty different surfaces in Divine Mercy Elementary School
The study flow diagram is summarized in Figure 1, details are described below.

Figure 1: 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, wood and cement block). Treatment was applied to a number of sites in order to assess the action of the sanitizing agent.

Areas of 100 cm^2 (measured by a template) were identified 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 centre of the template and all visible areas inside the template were swabbed. Special care was taken to identify each surface swabbed so that approximately the same areas could be covered before and after application of the Aegis agent.

Treatment:

Within a few days of carrying out the initial sampling, Aegis Treatment was applied to several surfaces in the school. Description of each site tested is attached with photos.

Testing intervals:

Testing was performed 30 days after treatment (Recall Day 2), on all selected sites using the same method as on Day 1. The school was subject to regular custodial cleaning during this 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 swab head removing the excess moisture by pressing against the walls of the tube. The swabs were rubbed against the selected area thoroughly 3 times, reversing directions between strokes. After swabbing the area, the swab head was positioned inside the liquid and the vial shaken by striking the palm of the hand for 10 seconds. All samples were then placed in a refrigerated container and analyzed within 3 hours.

Plating swab rinse solutions:

Upon arrival at the laboratory samples were assigned a unique number. Each sample was vortexed for 10 seconds, 5 ml (all) of the rinsing solution were dispensed into 100 mL of Sterile water, filtered through 0.22 um filters and incubated in sterile petri dishes containing Standard Plate Count Agar. All plates were incubated for 72 +/- 2hrs at 30 - 35 ^{o}C. The number of total heterotrophic bacteria on each sample was determined by the number of colony forming units on each plate.

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 Micrylium'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:

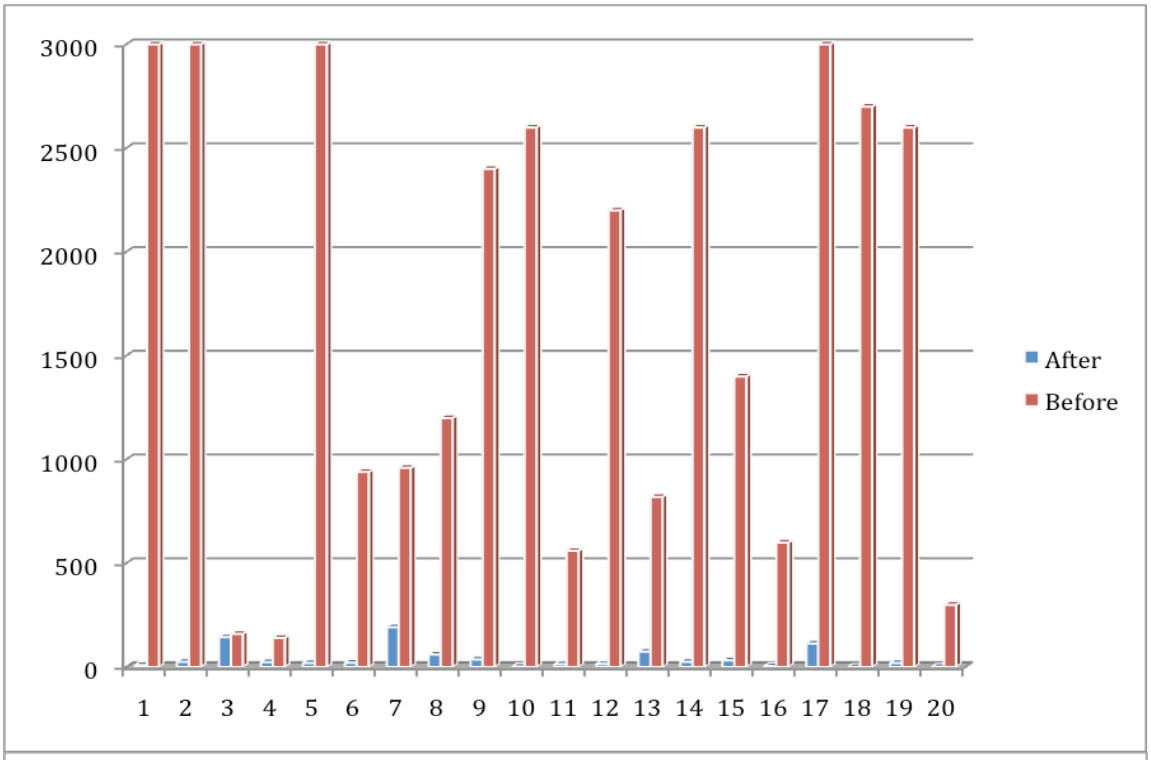
The study director for this project was Dean Swift BSc. B.Ed. Cert. Tox. Resumes for technical personnel are maintained and available upon request. The Microbiology study was conducted at Biolennia Laboratories, 5000 M, Dufferin Street, Toronto, ON, M3H5T5

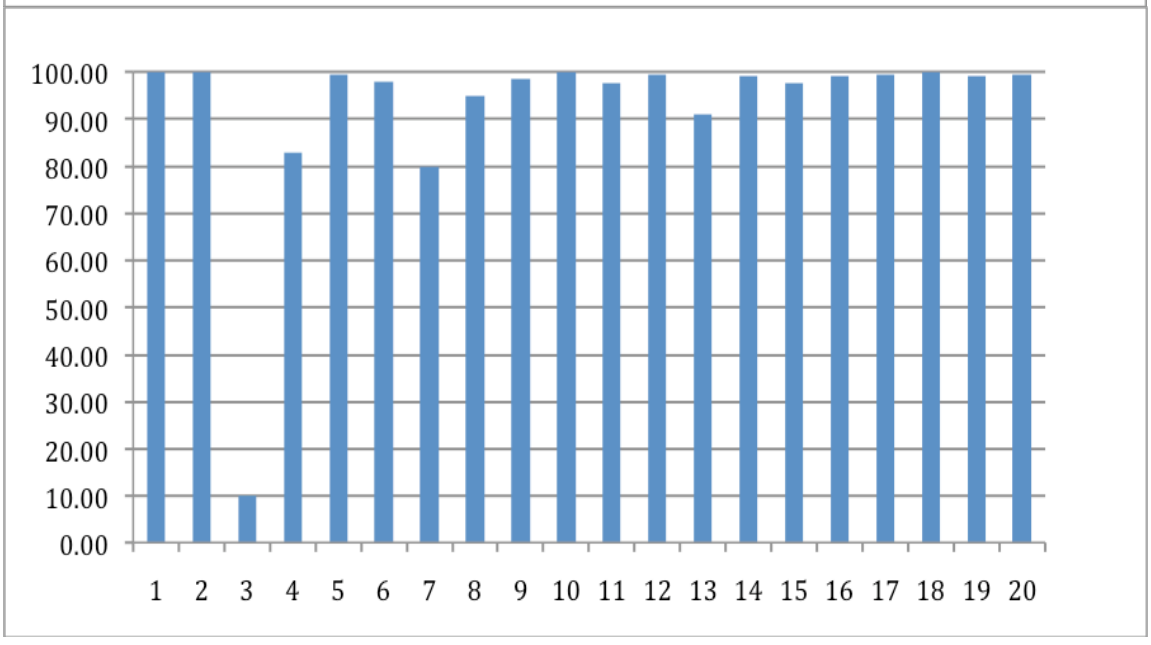
E. RESULTS:

The following tables and graphs summarize the results obtained for the enumeration of Total Heterotrophic Bacteria on various surfaces around the school before and after treatment with Aegis Microbe Shield Technology.

Table 1: Results for the enumeration of Total Heterotrophic Bacteria on various school surfaces

Site Number	Description	Total Heterotrophic Bacteria (cfu/sample)	
		1/2/2010 Prior to Treatment	3/3/10 Post-Treatment
1	Front Entrance Handicap	14400	10
2	Principal's Door	19000	25
3	Exit A	160	144
4	Soap handle Boys' Washroom	140	24
5	Room 218 Keyboard	3600	20
6	Room 218 Desk 1	940	21
7	Room 218 Desk 2	960	192
8	Room 217 Mat	1200	61
9	Phones - common	2400	37
10	Phone - office	2600	3
11	Kindergarten desk	560	14
12	Kindergarten Play Stove	2200	15
13	Gym Bench	820	74
14	Stair C Handrail	2600	25
15	Stair C Door	1400	33
16	Computer Lab Keyboard	600	5
17	Science Counter	15600	114
18	Boys' Toilet Seat	2700	<1
19	Drinking Fountain	2600	20
20	Wall outside Room 105	300	2





Graph 1. Before/After representation, excluding large inital counts to preserve scale.
Graph 2. Percentage Reduction Basis Presentation
Note: Site 3 had only 160 colonies at time of initial testing and these counts were reduced by 10%.
The same site was retested 2 weeks later at 11 AM after recess.
Only 9 colonies were identified when retested, yielding 94.37% reduction.

F. CONCLUSION:

In this study we determined that the treatment of school surfaces with Aegis Microbe Shield Technology resulted in generally a 2 to 3 $\log_{10}$ reduction in the number of recoverable Heterotrophic Bacteria.

Of special note, the two highest areas of contamination were the Front Entrance Handicap button and the principal's office door. These were colonized to a higher than expected level and treatment reduced the numbers dramatically.

The reduction on Exit A was not great, but given that the initial contamination was only 160 colonies it is still significant. We returned to the site last week and tested the first three sites again and counted 16, 3 and 9 colonies respectively, this illustrating continuous antimicrobial effect.

The most interesting observation was the variety of both bacterial and fungal species found in the initial sampling. On retest, the numbers were significantly lower, but fungal species were almost totally eliminated and the variety of bacterial species found was limited.

Given that disease often requires as few as 50 organisms to initiate, reductions of 10x to 1000x as found in this study indicate a benefit to the children and staff.

Dean Swift
Research Director
March 24, 2010