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Dear Adam, Anthony, and Jason,

## RE: Summary of Transit phase 2 project results

We first want to thank you for contributing your products for use in the Copper in Transit (CIT) trial on Vancouver and Toronto transit vehicles. During the study, Trimco's products were randomly installed on stanchions on 3 buses and 4 SkyTrain cars in Vancouver and 3 buses, 2 subway cars, and 2 streetcars in Toronto. All transit vehicles were assigned to high ridership routes. Each copper stanchion had a mirror control directly opposite. These surfaces were audited every month for durability and every two months for microbial analysis by Westech Inc. - an independent auditing firm. Audits occurred 1-3 hours after peak morning rush hour use. Durability audits consisted of visual damage reports, RGB colour readings, magenta % readings, and copper concentration measurements. Microbial audits were performed prior to vehicle cleaning and disinfection and consisted of bacterial collection and enumeration using Petrifilm<sup>™</sup> aerobic count plates (in triplicate), and measurement of residual organic material with ATP Bioluminescence (ATPB) (single sample). At six and twelve months, select stanchions containing Trimco products were removed and underwent ex-situ microscopy to further assess durability.

The study also performed in-lab testing at the VGH Medical Microbiology Laboratory to assess the antimicrobial effects after 200 rounds of simulated cleaning and disinfection using a Wiperator<sup>™</sup>. Circular coupons (25mm diameter) were cut from your provided Trimco solid Cu covers product and wiped to simulate daily cleaning/disinfection with either an accelerated hydrogen peroxide (ES65H) or quaternary ammonium (Buckeye E23) cleaner. Additionally, a selection of coupons were wiped with artificial sweat to emulate daily use in the absence of cleaners, and a group of untreated coupons were also assessed. Coupons were then evaluated for antibacterial activity (in triplicate) using *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) following a modified Environmental Protection Agency protocol Antiviral activity was assessed using the median Tissue Culture Infectious Dose 50 (TCID<sub>50</sub>) assay for strains EPA approved surrogate strains for SARS-CoV2 (229E) and norovirus (MNV-1) (inducer of gastroenteritis).

## Here are the results for the Trimco product compared to Control surfaces:

# A. In-field studies (Bacterial colony forming units and ATPB results)

After 12 months of in-field use Trimco's Cu covers exhibited a 39.7% reduction in mean CFU counts and 85.6% reduction in ATP RLU. CFU differences between SS controls and Trimco were higher in Toronto compared to Vancouver, with the highest volume observed on subway cars. This can be attributed to the higher population density in Toronto, with subways having the highest turnover of users. In comparison, the ATP RLU reading were similar between Toronto and Vancouver. As ATP measures all cellular matter (fungi, viruses, skin cells, pollen, etc.) this could be attributed to Vancouver having a more temperate climate which is more optimal for growth and survival of organic material, overcoming the population differences.





Overall, the microbial efficacy of the 3 different Cu products combined (compared to SS) exhibited a significant 42.7% reduction in mean CFU and an 87.1% reduction in ATP RLU.

## At a glance summary of microbial results:

Table 1: Summary of the mean and median of the average CFU for all vehicles and cities at 12 months

	Control		Trimco Covers					
No. Stanchions	Mean (CFU)	Median (CFU)	No. Stanchions	Median (CFU)				
38	28.5	26.9 (2,249)	38	17.2	16.1 (1.4,163.7)			

<u>Table 2:</u> Summary of the mean and median of the average ATP RLU for all vehicles and cities at 12 months

	Control		Trimco Covers					
No.	Mean	Median	No.	Mean	Median			
Stanchions	(RLU)	(RLU)	Stanchions	(RLU)	(RLU)			
38	1187.8	895.5 (51,5397)	38	170.7	80 (0,1330)			

## Breakdown of results by vehicle & city

Table 3: Mean and median of the average CFU by city and vehicle type at 12 months

			Conti	rol	Trimco Cu Covers					
		No.	Mean	Median (CFU)	No.	Mean	Median (CFU)			
City	Vehicle	stanchions	(CFU)		stanchions	(CFU)				
Toronto	Bus	9	31.2	29.3(15.6,74.5)	9	33.7	24.5(11,163.7)			
Toronto	Streetcar	2	44.9	44.9(33.3,60.7)	2	42.7	42.7(42.6,42.8)			
Toronto	Subway	6	158.6	149.9(104.9,248.9)	6	31.2	35.6(13.4,46.1)			
Vancouver	Bus	9	10.3	10.7(2,73.1)	9	10.8	12.2(1.4,130.3)			
Vancouver	Skytrain	12	22.5	22.3(3.2,122.7)	12	9.3	10.8(3.5,27.3)			

Table 4: Mean and Median of the average ATP by city and vehicle type at 12 months

			Contro	bl	Trimco Cu Covers						
City	Vehicle	No. stanchions	Mean (RLU)	Median (RLU)	No. stanchions	Mean (RLU)	Median (RLU)				
Toronto	Bus	9	869.2	821(258,1524)	9	140.3	90(24,332)				
Toronto	Street car	2	1519	1519(1487,1551)	2	559.5	559.5(515,604)				
Toronto	Subway	6	1762.3	1103(106,5397)	6	335.8	182.5(16,1330)				
Vancouver	Bus	9	1498.8	1065(322,4873)	9	147.1	30(0,839)				
Vancouver	Skytrain	12	851.1	704.5(51,1605)	12	63.8	63(1,116)				





Results after 12-months in situ use for A) ATPB and B) log10 CFU counts





## Aggregate of all Cu products results after 12 months of use in field for A) ATP and B) log10 CFU counts

## B. In vitro (Microbial) results

## Wiperator:

Wiperator surfaces were inoculated with PA, SA, 229E, or MNV-1 in separate experiments. The bacterial inoculums were allowed to remain on surfaces for 0.5 and 1 hour for PA and 1 and 2 hours for SA, following which surfaces were sampled for remaining bacteria using sonication in Letheen broth as per the modified EPA protocol and plating on blood agar plates (BAPs) and Petrifilm. The viral inocula remained on the surfaces for 1 and 2 hours for both 229E and MNV-1 following which viruses were collected from the surfaces with cell media, and applied to cell lines to measure remaining viable virions using the TCID<sub>50</sub> assay.

## - Bacterial Results

For bactericidal analysis, SS and Trimco Surfaces were inoculated with PA and SA in a range of 3.8-4.3 x 10<sup>7</sup> CFU/mL in a simulated soil. Results are based on triplicate samples and demonstrate that Trimco met sanitizer claims for PA at 1 hour for all treatments compared to SS controls. Trimco did not met any sanitizer standards for SA at 1 or 2 hours with the CMBC cleaner, TTC cleaner, mechanical sweat, or untreated coupons. This supports previous results in our laboratory regarding the importance of evaluating copper products against various disinfectants. Please note that the value of the self-sanitizing products likely lies in the ability to continuously kill microorganisms rather than an initial kill at high inoculums.





**Table 5:** Antibacterial efficacy of Wiperator treated coupons after 200-rounds of cleaning with select cleaners or artificial sweat compared to untreated controls

	CMBC Cleaner					TTC Cle	eaner		Mechanical Sweat				Untreated			
	Mean cfu/mL	SD	% red	Log10 diff	Mean cfu/mL	SD	% red	Log10 diff	Mean cfu/mL	SD	% red	Log10 diff	Mean cfu/mL	SD	% red	Log10 diff
Pseudomonas aeruginosa, 0.5 hours																
Stainless Steel	4.91E+05	1.23E+05			1.15E+05	7.18E+04			3.66E+05	2.56E+05			5.43E+05	3.65E+05		
Trimco solid copper covers	7.01E+03	4.25E+03	98.69	1.88	2.67E+03	1.89E+03	98.12	1.73	1.13E+03	1.19E+03	99.97	3.47	1.71E+03	1.90E+03	99.97	3.50
Pseudomonas aeruginosa, 1 hour																
Stainless Steel	8.25E+04	4.77E+04			6.75E+04	4.30E+04			1.15E+05	3.38E+04			1.53E+05	1.09E+05		
Trimco solid copper covers	0.00E+00	0.00E+00	99.99*	5.15*	1.67E+02	1.44E+02	99.94*	3.27*	3.33E+02	2.89E+02	99.95*	3.35*	0.00E+00	0.00E+00	99.99*	5.42*
					Staph	ylococcus	aureus, 1	1 hour								
Stainless Steel	7.68E+06	6.96E+06			4.72E+06	8.44E+05			3.65E+06	1.72E+06			2.28E+06	1.05E+06		
Trimco solid copper covers	1.47E+06	7.31E+05	76.93	0.64	2.03E+05	1.25E+05	96.40	1.44	9.86E+05	3.08E+05	71.92	0.55	4.41E+05	5.85E+04	78.87	0.68
Staphylococcus aureus, 2 hours																
Stainless Steel	5.84E+06	1.81E+06			5.10E+06	1.80E+06			3.99E+06	4.09E+05			3.79E+06	3.54E+06		
Trimco solid copper covers	3.27E+05	1.81E+05	94.94	1.30	2.20E+05	2.27E+04	95.49	1.35	2.60E+05	1.10E+05	93.84	1.21	2.69E+05	7.92E+04	90.86	1.04

% Reduction = (SS-copper)/SS \* 100

\* Considered a sanitizer (≥99.9% reduction, ≥3 log10 reduction, after 60 minutes

PA inoculum CFU mean: 3.8x10<sup>7</sup>

SA inoculum CFU mean: 4.3x10<sup>7</sup>

#### - Viral Results

For virucidal analysis, SS and Trimco surfaces were inoculated with MNV-1 or 229E with a range of 1-5 x10<sup>5</sup> TCID<sub>50</sub>/mL in artificial sweat-cell media mixture for 1 and 2 hours. Results are based on triplicate samples and demonstrate that rubbing with artificial sweat for 200 rounds significantly enhanced the antiviral capabilities of Trimco for 229E but not MNV-1. For 229E, Trimco coupons showed an average percent reduction in virus of 98.3% and 96.4% at 1 and 2 hours, respectively. After 1 hour with MNV-1, viral load was reduced by an average of 94.8% which increased to 99.4% after 2 hours.



Antiviral efficacy after 200 rounds of cleaning with disinfectants or artificial sweat for A) SARS CoV-2 analog and B) norovirus analog

# C. Durability Testing

The colorimetry measurements revealed that on average, the Trimco products became darker after installation and use , likely due to oxidation or carbon-contamination of the stanchion resulting from daily usage.

After 12 months of installation, the EDS elemental cross-sectional analysis did not indicate any signs of dealloying. Top-down EDS maps of all three surfaces used for 12 months revealed the presence of a considerable amount of carbon-containing substance (carbon-contamination). This contamination reduced the effective copper surface area, potentially decreasing copper release. The carbon-contamination was observed to be more concentrated in surface crevices and grooves.







Trimco stanchions: (a) just after installation, (b) after 12 months of installation, where stanchion is getting darker, possibly due to oxidation or carbon-contamination of the stanchion resulting from daily usage.



The average changes in the surface color of the stanchions were analyzed using colorimetry. The surface appearance was described using the three coordinates, a\* (red/magenta-green), b\* (yellowblue) and L\* (lightness-black to white) in the CIELab color reference space. The a\* value represents the degree of redness/magentaness (-a\*) or greenness (+a\*) of a color, the b\* value represents the degree of yellowness (+b\*) or blueness (-b\*), and the L\* value represents the lightness of a color, ranging from black (0) to white (100). Decreasing values for a\*, b\* or L\* toward 0 indicate that the surface of copper is getting darker, possibly due to oxidation or carbon-contamination of the stanchion resulting from daily usage.



SEM-EDS cross-sectional analysis of as-received Trimco product.



SEM-EDS cross-sectional analysis of Trimco product after 6 months of installation in SkyTrain. No significant damage to the material was observed.



SEM-EDS cross-sectional analysis of Trimco product after 12 months of installation in SkyTrain. No significant damage to the material was observed.



Top-down SEM images of the Trimco products: (a) as-received, (b) after 12 months of installation in CMBC Bus, (c) after 12 months of installation in SkyTrain, (d) after 12 months of installation in TTC Bus, (e) after 6 months of installation in TTC Car, and (f) after 6 months of installation in TTC Sub. Please note that the scratches visible in the SEM images are a result of the production process and are oriented perpendicular to the long axis of the stanchion. Black areas are carbon contamination.





# D. Summary

After evaluation of your product after 12 months of in-field use, we observed a 39.7% reduction in the mean bacterial colony counts and 85.6% reduction in the ATP RLU. Colorimetry analysis indicated that your product became darker after installation, likely due to oxidation or carbon-contamination of the copper surface. EDS elemental cross-sectional analysis did not show any sign of dealloying. Top-down EDS maps indicated a considerable amount of carbon-contaminating substances particularly in surface crevices and grooves, which potentially decreased copper release.

In-vitro evaluation of your product revealed consistent bactericidal activity against gram negative bacteria after 200 rounds of wiping with cleaners or artificial against gram negative bacteria after 1 hour of exposure. Your product did not meet sanitizer claims for a gram positive after 2 hours. Trimco's product showed great antiviral activity against a SARS-CoV2 and norovirus surrogate virus strain with an average of 96.6% reduction after 1 hour and 97.9% after 2 hours.

Once again, thank you for your participation in this innovation evaluation of self-sanitizing surfaces. We are happy to answer any additional questions you might have pertaining to your results.

Sincerely,

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