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RESEARCH COMMUNICATION

Comparing the bactericidal effect of ultraviolet germicidal irradiation between two time exposures against *Bacillus subtilis* on personal protective equipment

Ray Angelo B. Monje[1], Mariel Patricia P. Capuno[2], Manolo V. Hernal III[1], Maria Margarita M. Lota[3]*

*Corresponding author's email address: mmlota@up.edu.ph

[1]*College of Medicine, University of the Philippines Manila, Manila, Philippines*
[2]*Ateneo School of Medicine and Public Health, Pasig, Philippines*
[3]*Department of Medical Microbiology, College of Public Health, University of The Philippines Manila, Manila, Philippines*

ABSTRACT

Background: In the time of the COVID-19 pandemic, the use of personal protective equipment (PPE) as an infection control measure is recommended for healthcare settings to prevent the spread of the virus. However, given the increased demand for PPEs worldwide and the limited resources available in the country, supply shortages are experienced by many healthcare facilities and workers. Furthermore, most PPEs should be discarded after use and may only be reused under emergency situations. Thus, the study sought to determine how to maximize PPE usage.

Objective: The study aimed to compare the effect of Ultraviolet Germicidal Irradiation (UVGI) against Bacillus subtilis between 30 and 40-minute periods. Specifically, the study targetted to establish if there is a difference in the mean percent reduction in CFUs of *B. subtilis* between that of the 40-minute and 30-minute treatment group of N95 FFRs and coverall suits.

Methodology: The study is a secondary research which builds upon the experimental results of the commissioned study conducted by the Department of Medical Microbiology (DMM), College of Public Health, University of the Philippines Manila researchers to conduct data analysis. Furthermore, the PPE evaluated were coverall suits and N95 filtering facepiece respirators. They inoculated a total of 30 samples with B. subtilis for each type of PPE and subjected them to UVGI treatment in 30 and 40-minute periods. This study then employed inferential statistics, particularly a two-sample independent t-test, to further analyze the data from DMM and assess the difference between the two UVGI exposure durations on PPE decontamination.

Results: After calculating and analyzing the data using OpenEpi, the mean percent reduction was statistically greater (p-value = <0.0000001, $\alpha = 0.05$) in the 40-minute exposure than the 30-minute exposure. The mean percent reductions of the N95 respirators were 98.68% and 99.41% for the 30 and 40-minute exposures, respectively. On the other hand, the mean percent reductions of the coverall suits were 80.40% and 99.71% for the 30 and 40-minute exposures.

Conclusion: Based on the significant difference in bacterial reduction between the two time exposures, the longer UVGI time exposure was more effective as a disinfectant. The results of the study contributed to knowledge on infection control using UVGI and its possibility as a method of decontaminating PPEs.

Keywords: *UVGI, B. subtilis, N95 FFRs, coverall suits*

Introduction

During the COVID-19 pandemic, infection control measures are of utmost importance. Hence, the use of personal protective equipment such as wearing N95 respirators and coverall suits has been the norm among healthcare workers to contain the spread of the virus. The use of PPE is involved in infection control by acting as a barrier between the wearer and infectious agents [1]. Through this, the transmission of contaminants and infections among individuals is reduced, and the spread of infection in healthcare settings is minimized [1].

Due to the pandemic, PPE decontamination has been an emerging topic, with attempts to reuse single-use PPEs. Standards for time exposure that would eliminate all the microorganisms are yet to be set. Ultraviolet-C radiation is used as a bactericidal agent because of its short wavelength and high-frequency characteristics that produce a great amount of energy to destroy microbes. Not only does this disable DNA replication, but it also disrupts viral RNA and DNA [2,3]. Furthermore, it contains the maximum bactericidal wavelength in the range of 240 to 280 nm [1]. Several studies have demonstrated that UVGI can significantly lower the amount of viable viral pathogens on surfaces including N95 respirators without altering its filtering efficiency, fit, color, and odor [4-7]. Furthermore, studies revealed that UVGI is effective in killing bacteria and its spores [8-10]. In the experiment conducted by the DMM, the organism that was subjected to UVGI exposure was B. subtilis since it is a non-pathogenic and common laboratory contaminant [11,12]. It should be noted that none of the reviewed related literature of the researchers examined the effect of UVGI treatment on the reduction of *B. subtilis* at 30 minutes and 40 minutes of exposure.

The Department of Medical Microbiology of the College of Public Health, University of the Philippines Manila, conducted a project in 2020 that involved the decontamination of PPEs [13]. One of the primary objectives was to determine the bactericidal effect of ultraviolet germicidal irradiation (UVGI) on personal protective equipment (PPE) using prototype equipment. Using data from the said study, this study further analyzed the bactericidal effect of UVGI on PPEs in two time groups: 30 minutes and 40 minutes. The findings of the study will help in the decontamination efforts of health facilities, particularly in places where PPE shortage exists. The results of the study may also be shared with the scientific community to contribute knowledge on bacterial susceptibility to UVC decontamination, PPE decontamination, and the potential reuse of PPEs.

Research Objective

This study aimed to compare the effect of ultraviolet germicidal irradiation against *B. subtilis* between the 40-minute exposure and the 30-minute exposure. Specifically, it aimed to determine if there is a difference in the mean percent reduction in CFUs of *B. subtilis* between that of the 40-minute and 30-minute treatment group of N95 FFRs and coverall suits.

Methodology

The study sourced the figures used for its data analysis from the experiment conducted by the DMM [12]. Moreover, it was exempted from ethical review (2021-014-EX) by the University of the Philippines Manila Ethics Review Board. The experiment of the DMM consisted of 4 trials, with 2 trials each for N95 respirators and coverall suits (30 and 40-minute groups). Each trial consisted of a control group and a treatment group with 30 samples. The areas of inoculation are shown in Figures 1 and 2. Each sample in the trial consisted of a disk inoculated with vegetative *B. subtilis*, using dilutions of 0.5 McFarland standard. The disks of the treatment groups were subjected to UVGI, cultured, and incubated for 24-48 hours. The results of the experiment, which consisted of the number of CFUs in each sample, served as the secondary data for this study. With each PPE having 30 samples per time trial, a total of 120 CFU counts were obtained. The data consisted of the number of CFUs (30 samples per PPE), totaling to 120 CFU counts for the treatment groups.

The study used the experimental results of the DMM. The data was further analyzed by calculating the percent reduction of B. subtilis through log reduction. This allowed the researchers to compare the relative number of microbes after UVGI exposure treatment (Figure 3). Afterward, the percent reduction, which is defined as the average percent reduction of all samples per PPE, was computed from the log reduction for both time exposures (Figure 4).

Independent t-test, an inferential statistical test, was conducted using the statistical program OpenEpi. This was done to determine whether there was a significant difference in the mean percent reduction between the 30-minute UVGI treatment and 40-minute UVGI treatments. The confidence level was set at 95% (p=0.05). Moreover, the standard deviation, p-value, mean difference, and interval estimate were determined (Table 1).

Results

To determine if there is a difference in the mean percent reduction in CFUs of *B. subtilis* between that of the 40-minute and 30-minute treatment group of N95 FFRs and coverall suits, the results tabulated in Table 1 were assessed. The computed p-value for both N95 FFRs and coverall suits was <0.0000001 which is less than the set significant value of 0.05. Therefore, the null hypothesis is rejected, and it is concluded that the mean percent reduction in CFUs of B. subtilis in the 40-minute treatment group is significantly greater than the mean percent reduction in the 30-minute treatment group, in both N95 FFRs and coverall suits. This conclusion is supported by the mean difference of -0.73529 for the N95 FFRs and the mean difference of -19.3083 for the

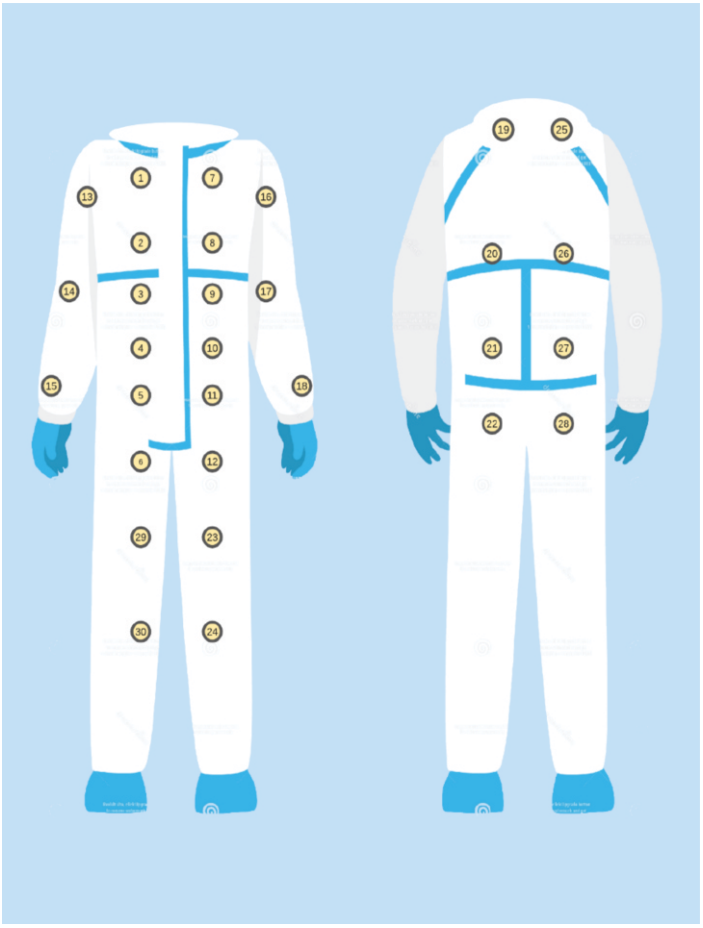

Figure 1. *Coverall Suit Model with Samples of DMM Experiment; Front (L), Back (R)*

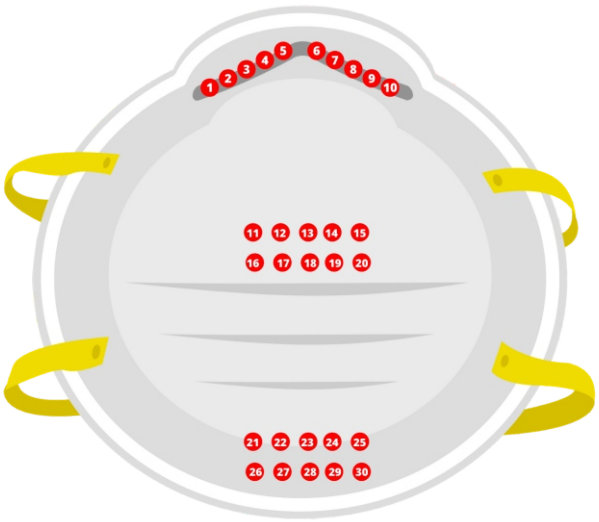

Figure 2. *N95 FFR Model with Samples of DMM Experiment*

$$Log\ Reduction = log_{10}(CFU_{control}) - log_{10}(CFU_{treatment})$$

Figure 3. *Formula for Log Reduction*

$$\%\ Reduction = (1 - 10^{-LR})\ x\ 100\%$$

Figure 4. *Formula for Conversion of Log Reduction into Percent Reduction*

Table 1. *OpenEpi Independent T-Test Results*

Sample		Sample Size (n)	Mean Percent Reduction of CFUs (x)	Standard deviation (s)	p-value (=0.05)	Mean Difference	Interval estimate	
							Lower Limit	Upper Limit
N95 FFR	30 mins	30	98.68%	0.03	<0.0000001	-0.74	-0.75	-0.72
	40 mins	30	99.41%	0.02				
Coverall suits	30 mins	30	80.40%	0.25	<0.0000001	-19.31	-19.40	-19.21
	40 mins	29	99.71%	0.01				

coverall suits. These negative values indicate that the mean percent reduction in the 40-minute group is statistically greater as compared to the mean percent reduction in the 30-minute group. Furthermore, the same conclusion can be made when the interval estimate is evaluated. The null value of zero is not found within the range of -0.748505 to -0.722074 for the N95 FFRs. Likewise, the interval estimate of -19.4017 to -19.2149 for the coverall suits does not contain the null value of zero. Hence, the null hypothesis is rejected.

Discussion

Comparing the effect of UVGI against *B. subtilis* between the 30-minute and the 40-minute exposures, results showed that the 40-minute treatment group had a significantly greater mean percent reduction in CFUs of *B. subtilis* than the 30-minute treatment group of N95 FFRs and coverall suits.

The results are consistent with the study conducted by Lin

et al., where the relative survival of B. subtilis spore colonies exponentially decayed as the exposure time increased [10]. Furthermore, in a similar study conducted by Fischer et al., a greater germicidal effect of UVC on N95 FFR contaminated with SARS-CoV-2 was observed as the exposure time was increased [5]. According to the hierarchy of resistance to sterilization and disinfection published by the CDC, the effect of sterilization and chemical germicides on microorganisms differ depending on their levels of resistance [14]. Vegetative bacteria like B. subtilis are more resistant than medium-in-size viruses or lipid viruses like SARS-CoV-2 [14]. Hence, successfully eliminating B. subtilis through sterilization may potentially eliminate coronaviruses like SARS-CoV-2.

The results of the study showed that UVGI is more effective on coverall suits in a 40-minute exposure. The observed variation of percent reductions in the 30-minute time exposure found on the coverall suits may be explained by surface aberrations, such as the presence of folds and the positioning in the UVGI prototype, which may have affected UV exposure and absorption. The WHO mentioned the low penetrative power of UV light may not adequately penetrate through pleats or folds which are found in coverall suits [15]. Furthermore, bacterial elimination improved in the 40-minute exposure treatment, despite the presence of folds. This is consistent with the findings of Yang et al. which found that greater bacterial and fungal reduction can be achieved in longer time exposures of UVC [8].

The study only utilized secondary data. Hence, certain factors that may potentially have an impact on the results were not discussed. These factors include the distance between the PPE and UVGI, model and material of PPEs, specifications of the UVGI device, and number of trials conducted.

Conclusion and Recommendations

The UVGI decontamination method of N95 FFRs and coverall suits achieved greater bacterial elimination of B. subtilis in the 40-minute exposure than the 30-minute exposure. Thus, the longer time exposure was more effective as a disinfectant than the shorter time exposure given the significant difference in their bacterial reduction.

In times of scarce resources, it is recommended that hospital administrations expose the used N95 FFRs and coverall suits to UVGI to reduce contamination. Government institutions, such as the Department of Health and other concerned stakeholders regarding infection control, are encouraged to invest in studies and experiments on UVGI as a decontamination method. The increased knowledge on UVGI may contribute to the possibility of establishing it as a method that could allow the safe reuse of PPEs. Future researchers are suggested to look into other factors that were not in the scope of the study such as the distance of the PPE from UVGI, model and material of PPEs, and UVGI treatment conditions. Moreover, other microorganisms are recommended to be tested for UVGI treatment.

Acknowledgements

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APPENDIX

Table 2. *N95 FFR Results*

	Treatment Group (30 minutes)		Treatment Group (40 minutes)	
Sample	**CFU/mL**	**Percent Reduction (%)**	**CFU/mL**	**Percent Reduction (%)**
Control	$6.80x10^{7}$	–	$1.70x10^{7}$	–
1	0	99.9999985%	0	99.9999941%
2	0	99.9999985%	0	99.9999941%
3	0	99.9999985%	0	99.9999941%
4	0	99.9999985%	0	99.9999941%
5	0	99.9999985%	0	99.9999941%
6	0	99.9999985%	0	99.9999941%
7	$4.00x10^{6}$	94.1176471%	0	99.9999941%
8	0	99.9999985%	0	99.9999941%
9	0	99.9999985%	$2.00x10^{6}$	99.9999941%
10	0	99.9999985%	0	99.9999941%
11	0	99.9999985%	0	99.9999941%
12	0	99.9999985%	0	99.9999941%
13	0	99.9999985%	0	99.9999941%
14	0	99.9999985%	0	99.9999941%
15	0	99.9999985%	0	99.9999941%
16	$5.00x10^{6}$	92.6470588%	0	99.9999941%
17	0	99.9999985%	0	99.9999941%
18	$1.00x10^{6}$	98.5294118%	0	99.9999941%
19	$5.00x10^{6}$	92.6470588%	0	99.9999941%
20	$1.00x10^{7}$	85.2941176%	0	99.9999941%
21	0	99.9999985%	0	99.9999941%
22	0	99.9999985%	0	99.9999941%
23	0	99.9999985%	0	99.9999941%
24	0	99.9999985%	0	99.9999941%
25	0	99.9999985%	$1.00x10^{6}$	99.9999941%
26	0	99.9999985%	0	99.9999941%
27	$2.00x10^{6}$	97.0588235%	0	99.9999941%
28	0	99.9999985%	0	99.9999941%
29	0	99.9999985%	0	99.9999941%
30	0	99.9999985%	0	99.9999941%

*Control group – Baseline disks with B. subtilis that were not subjected to UV radiation

Table 3. *Coverall Suits Results*

	Treatment Group (30 minutes)		Treatment Group (40 minutes)	
Sample	**CFU/mL**	**Percent Reduction (%)**	**CFU/mL**	**Percent Reduction (%)**
Control	$2.21x10^{8}$		$3.60x10^{7}$	
1	$1.00x10^{6}$	99.5475113%	0	99.9999972%
2	$1.00x10^{6}$	99.5475113%	0	99.9999972%
3	$6.00x10^{6}$	97.2850679%	0	99.9999972%
4	$1.00x10^{6}$	99.5475113%	0	99.9999972%
5	$9.30x10^{7}$	57.9185520%	0	99.9999972%
6	$3.80x10^{7}$	82.8054299%	0	99.9999972%
7	$2.00x10^{6}$	99.0950226%	0	99.9999972%
8	$1.00x10^{6}$	99.5475113%	0	99.9999972%
9	0	99.9999995%	$2.00x10^{6}$	94.4444444%
10	$5.10x10^{7}$	76.9230769%	0	99.9999972%
11	$1.81x10^{8}$	18.0995475%	CONTAMINATED	
12	$4.70x10^{7}$	78.7330317%	0	99.9999972%
13	0	99.9999995%	0	99.9999972%
14	$2.70x10^{7}$	87.7828054%	$1.00x10^{4}$	99.9722222%
15	$9.10x10^{7}$	58.8235294%	0	99.9999972%
16	$3.70x10^{7}$	83.2579186%	0	99.9999972%
17	$3.20x10^{7}$	85.5203620%	0	99.9999972%
18	$1.19x10^{8}$	46.1538462%	0	99.9999972%
19	$1.31x10^{8}$	40.7239819%	0	99.9999972%
20	$7.00x10^{6}$	96.8325792%	0	99.9999972%
21	$2.00x10^{5}$	99.9095023%	0	99.9999972%
22	$4.10x10^{7}$	81.4479638%	$1.00x10^{6}$	97.2222222%
23	$1.87x10^{8}$	15.3846154%	$1.00x10^{4}$	99.9722222%
24	$5.00x10^{4}$	99.9773756%	0	99.9999972%
25	$2.80x10^{7}$	87.3303167%	0	99.9999972%
26	0	99.9999995%	0	99.9999972%
27	$1.25x10^{8}$	43.4389140%	0	99.9999972%
28	$5.00x10^{7}$	77.3755656%	0	99.9999972%
29	$2.00x10^{6}$	99.0950226%	0	99.9999972%
30	$7.00x10^{4}$	99.9683258%	0	99.9999972%

*Control group – Baseline disks with B. subtilis that were not subjected to UV radiation

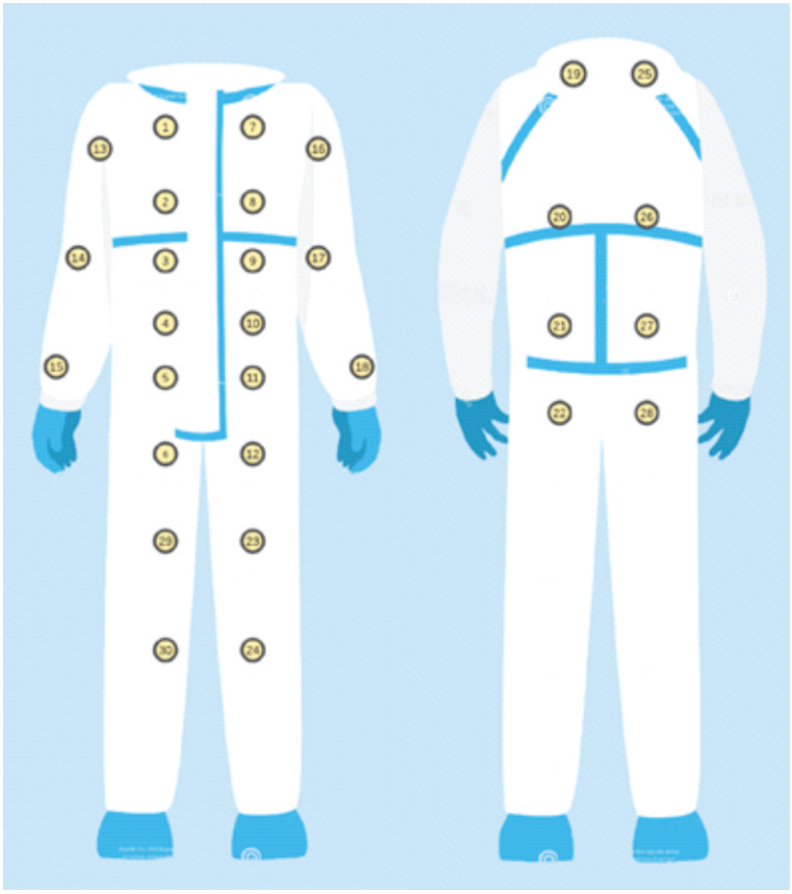

Figure 1. *Coverall Suit Model with Samples of DMM Experiment; Front (L), Back (R)*

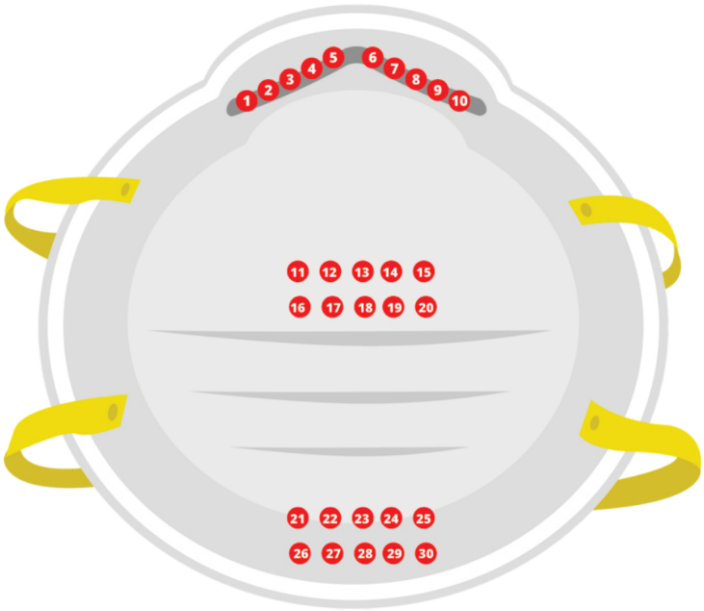

Figure 2. *N95 FFR Model with Samples of DMM Experiment*

Two-Sample Independent *t* Test

Input Data

Two-sided confidence interval 95%

	Sample size	Mean	Std. Dev.	Std. Error
Group-1	30	98.6764694	0.03	
Group-2	30	99.4117592	0.02	

Result	*t* statistics	*df*	p-value[1]	Mean Difference	Lower Limit	Upper Limit
Equal variance	-111.699	58	<0.0000001	-0.73529	-0.748467	-0.722113
Unequal variance	-111.699	51	<0.0000001	-0.73529	-0.748505	-0.722074

	F statistics	*df*(numerator,denominator)	p-value[1]
Test for equality of variance[2]	2.25	29,29	0.03265

[1] p-value (two-tailed)

[2] Hartley's *f* test for equality of variance

Results from OpenEpi, Version 3, open source calculator--t_testMean
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

Figure 3. *N95 OpenEpi Two-Sample Independent t-Test Results*

Two-Sample Independent *t* Test

Input Data

Two-sided confidence interval 95%

	Sample size	Mean	Std. Dev.	Std. Error
Group-1	30	80.4024132	0.25	
Group-2	29	99.7107256	0.01	

Result	*t* statistics	*df*	p-value[1]	Mean Difference	Lower Limit	Upper Limit
Equal variance	-415.471	57	<0.0000001	-19.3083	-19.4014	-19.2153
Unequal variance	-422.674	29	<0.0000001	-19.3083	-19.4017	-19.2149

	F statistics	*df*(numerator,denominator)	p-value[1]
Test for equality of variance[2]	625	29,28	<0.0000001

[1] p-value (two-tailed)

[2] Hartley's *f* test for equality of variance

Results from OpenEpi, Version 3, open source calculator--t_testMean
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

Figure 4. *Coverall Suit OpenEpi Two-Sample Independent t-Test Results*