FINAL REPORT

EFFICACY & EFFECTIVENESS STUDIES OF LIVING JUNGLE® HAND SANITIZER AND DISINFECTANT SPRAY

PREPARED BY

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1.0 OBJECTIVES

To assess the efficacy & effectiveness of Living Jungle® hand sanitizer and disinfectant spray provided by MBH Cosmeceuticals Sdn Bhd against Gram positive, Gram negative, yeast and fungus that can be commonly found on hand and bench surface.

2.0 PROJECT METHODOLOGY

2.1 EFFICACY STUDY OF LIVING JUNGLE® HAND SANITIZER AND DISINFECTANT SPRAY

The standard culture of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Candida albican* and *common fungus found on solid surface* were sub-cultured on nutrient agar. The mentioned test organisms were inoculated in nutrient broth and incubated at 37°C for 18-20 hours and was used for their resistant / susceptibility test against tested disinfectant and sanitizer. The evaluation of the hand sanitizer was done by Kirby-Bauer technique which can be summarized as follows. The Whatman filter paper of standard size was punched out and sterilized separately in an autoclave (disk diffusion method). Each hand sanitizer/disinfectant spray was taken in a sterilized petri dish and the above filter paper was soaked in it and kept on the surface of the lawn culture (Figure 1). The plates were then kept at room temperature for 30 minutes before incubating it 37°C for 72 hours (bacteria) and at 25°C for 120 hours (yeast & mold) respectively. The results were observed and zone of inhibition was measured for each test using a ruler/calliper. The experiment was conducted in triplicate and the zone of inhibition is recorded as average of three readings. The negative control was put as filter disc soaked with sterilized distilled water.

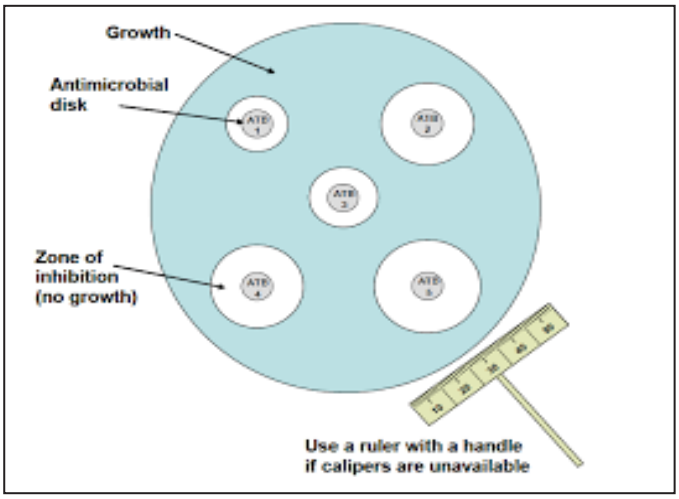

Figure 1. Diagram shows the disk diffusion method using a punched Whatman's filter paper soaked with test product.

2.2 EFFECTIVENESS STUDY OF LIVING JUNGLE® HAND SANITIZER AND DISINFECTANT SPRAY

The effectiveness of antiseptics was tested by making comparison of microorganisms found on subject's hands before and after antiseptic treatment. Since the bacterial reduction is noted on an individual basis, differences in hand hygiene is not a factor. Participants had no limitations regarding

race, gender, age, or social status. All participants acquired for this experiment were staff of faculty of Pharmacy Universiti Teknologi MARA Puncak Alam Campus. There were a total of 3 participant's/bench areas. To test both sanitiser and disinfectant effectiveness, Sabouraund Detrose and Tryptic Soy Agar plate were used to grow bacteria obtained from the subject's hands and work bench. Experiments were conducted in the laboratory environment.

A sterile cotton swab was used to swab the ventral surface of participant's hands to obtain initial bacteria. All areas of the hands were swabbed the same way, starting with the distal end of the fingers and moving towards the thumb. In between the fingers and the palms were also swabbed to obtain an accurate sample of bacteria on the hand at that time (Figure 2).

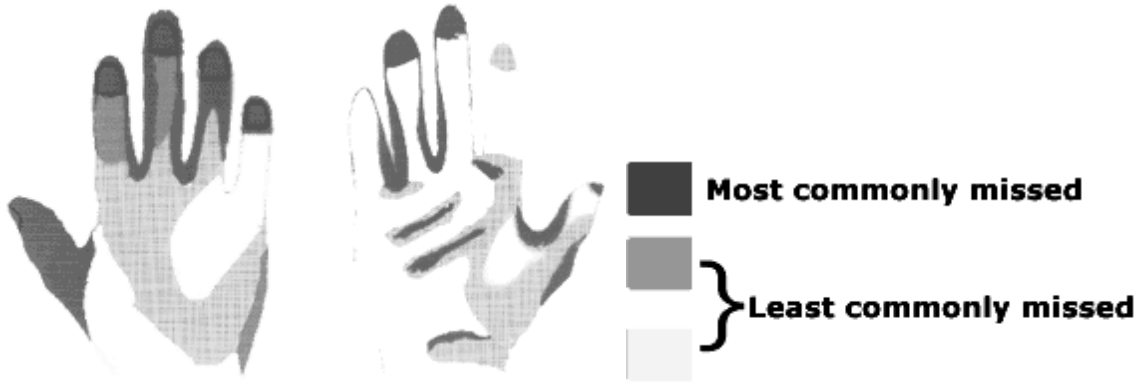

Figure 2. Diagram of commonly missed areas in hand washing given to participants before experimental

The swabs were then placed into a tube containing sterile nutrient broth. The tube was quickly capped to prevent contamination. This sample was a positive control, revealing how much bacteria had been acquired before the use of sanitisers. In case of disinfectant spray a work bench size 2 x 2 feet was swab. After the controls were obtained, sanitizer and disinfectant treatment was initiated. For hand sanitiser gels, the hands were completely moistened with the gel (1-2 ml), and all areas of the hands were rubbed until the gel evaporated. For disinfectant spray, tested workbench area was wiped with 1-2 ml disinfectant. After sanitiser/disinfectant treatment, swabbing procedure was repeated at defined time duration. (0,1, 5,10,20,30,40,60 minutes). Samples were then incubated at 37°C for 72 hours (bacteria) and at 25°C for 120 hours (yeast and mold) respectively. After the incubation period, the amount of bacteria, yeast and mold from the initial sample was compared to the sample taken after sanitizer/ disinfectant treatment completed. SPSS will be used to analyze the data. All data were calculated with basic descriptive statistics (mean and standard deviation).

3.0 RESULTS

3.1 EFFICACY STUDY OF LIVING JUNGLE® HAND SANITIZER AND DISINFECTANT SPRAY

The new hand sanitizer manufactured under the brand Living Jungle was compared with a market leader brand available in the market . The Living Jungle products exhibited inhibitory activity against the test isolates (Table 1), with zones of inhibition ranging from 0.5 mm to 2.5 mm. The results demonstrated that Living Jungle was as effective or even better compared to market leader hand

sanitizer brand against eliminating *B. subtilis, S. aureus, E. coli, C. albican* and fungus (Table 1 and Figure 3). These are the common bacteria, yeast and mold that can be detected on skin surface. The results shows that the Living Jungle hand sanitizer is able to eliminate Gram positive, Gram negative, yeast and fungus.

Table 1: Inhibitory effect of Living Jungle hand sanitizer, Market leader brand hand sanitizer, Living Jungle disinfectant spray and 70% IPA against selected Gram negative and positive bacteria, yeast and mold using as detected by disk diffusion technique.

Antibacterial agent	Zone of inhibition (mm)				
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Candida albican*	Fungus
LJ HS	0.5	1.33 ± 0.58	1.33 ± 0.58	1	2.5 ± 0.5
Market Leader Brand HS	0.5	1	0.67 ± 0.29	0.5	1.83 ± 0.76
LJ Spray	14 ± 1.73	27 ± 2.64	15.67 ± 5.13	2	2.33 ± 0.58
70% IPA	1.67 ± 1.15	1.5 ± 0.87	1.67 ± 0.58	1	2.16 ± 0.76

* Abbreviations: LJ: Living Jungle, IPA: isopropyl alcohol; HS: Hand sanitizer
* Data expressed in mean ± standard deviation

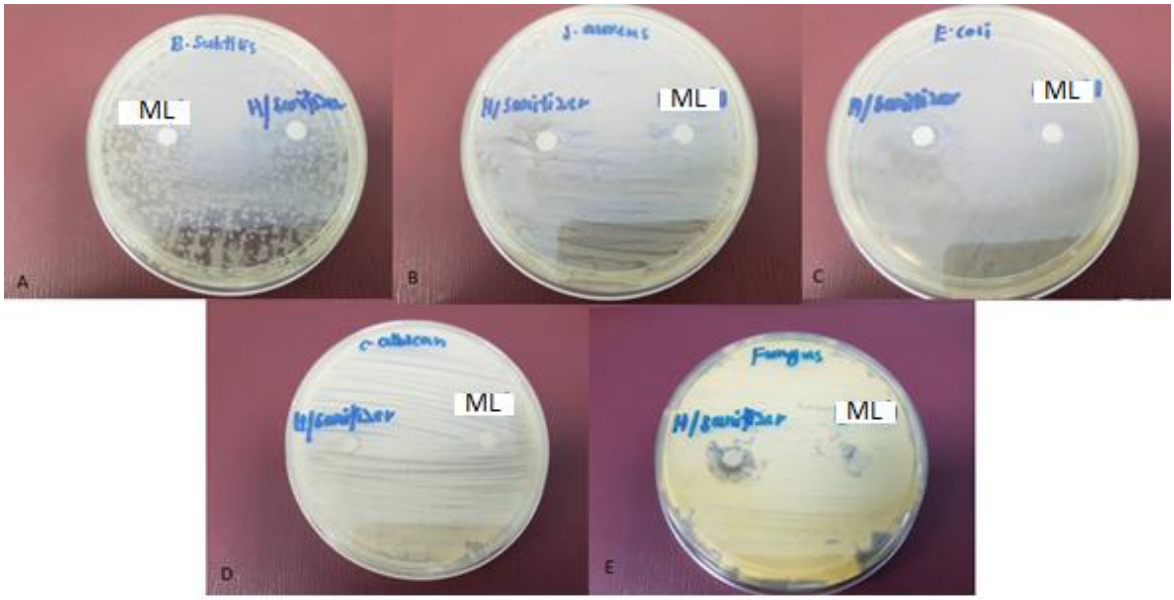

Figure 3: Agar well difussion test showing inhibition of (A) *B. Subtilis*, (B) *S. aureus* (C) *E. coli* (D) *C.albican* and (E) fungus by Living Jungle spray against Market leader brand hand sanitizer (ML).

The new disinfectant spray under the brand Living Jungle was compared with 70% isopropyl alcohol (IPA). The Living Jungle spray exhibited inhibitory activity against the test isolates (Table 1), with zones of inhibition ranging from 2 mm to 27 mm. The results demonstrated high zone of inhibition by Living Jungle spray compared to 70% IPA for *S. aureus, B. subtilis and E. coli* (Table 1; Figure 4). Meanwhile for yeast and mold, the Living Jungle was comparable to 70% IPA (Table 1; Figure 5). The result shows that the Living Jungle disinfectant spray is able to eliminate Gram positive, Gram negative, yeast and fungus.

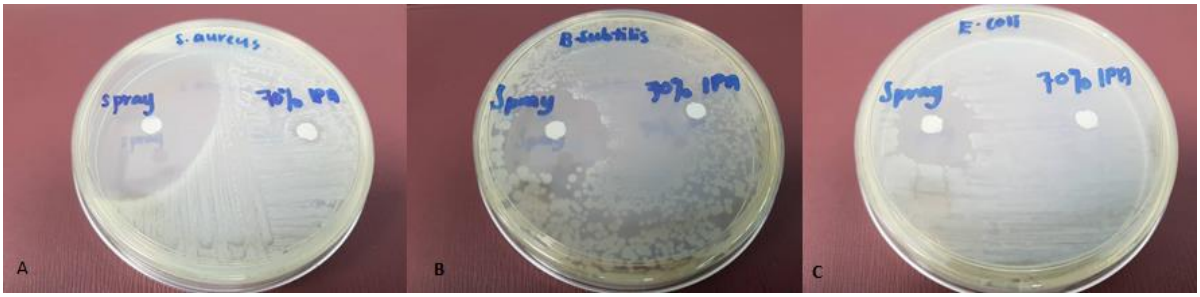

Figure 4: Agar well difussion test showing inhibition of (A) *S. aureus*, (B) *B. Subtilis* and (C) *E. coli* by Living Jungle spray against 70% IPA.

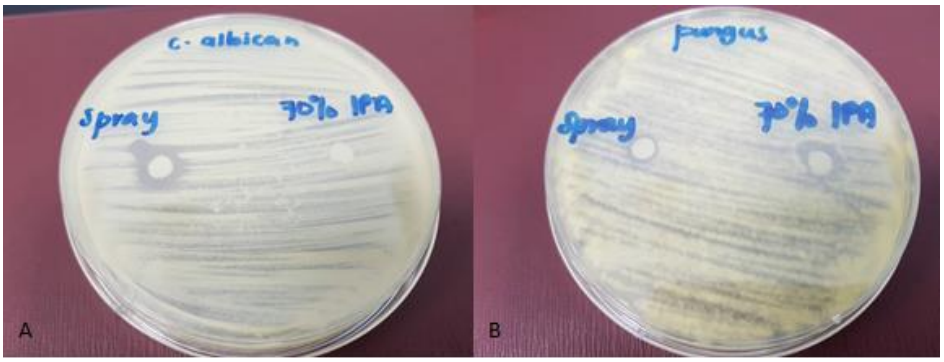

Figure 5: Agar well difussion test showing inhibition of (A) *C.albican*, and (B) fungus by Living Jungle spray against 70% IPA.

3.2 EFFECTIVENESS STUDY OF LIVING JUNGLE® HAND SANITIZER AND DISINFECTANT SPRAY

For the hand surface study, the experiment demonstrated that the effect Living Jungle hand sanitizer was comparable to Market Leader hand sanitizer brand which was free from bacterial, yeast and fungal infection instantly after application. This effects even can last for 60-minute post-treatment with CFU levels below detectable levels (Table 2: Figure 6 & 7). Meanwhile, the table surface study showed that Living Jungle disinfectant spray was able to eliminate the presence of microorganism which was comparable to 70% IPA by remaining free of bacterial, yeast and fungal infection even at 60-minute post-treatment (Table 2: Figure 8 & 9). These findings indicate that the Living Jungle hand sanitizer and disinfectant spray was able to eliminate the presence of microorganism by 99.99% instantly and can last up to 1-hour post-treatment period.

Table 2: CFU per plate before and after treatment with Living Jungle hand sanitizer, Market leader hand sanitizer brand, Living Jungle disinfectant spray and 70% IPA studied on hand and table surface.

Surface	Treatment	Mean pre-treatment CFU		Mean post-treatment CFU		Mean change(%)
		Aerobic bacteria	Yeast and mold	Aerobic bacteria	Yeast and mold	
Hand	LJ HS	250	70	ND	ND	-100
	ML HS	850	1000	ND	ND	-100
Table	LJ Spray	1100	600	ND	ND	-100
	70% IPA	160	150	ND	ND	-100

* Abbreviations: CFU: Colony forming unit; LJ: Living Jungle, IPA: isopropyl alcohol; HS: Hand sanitizer; ML: Market Leader; ND: not detectable (<10 CFU)

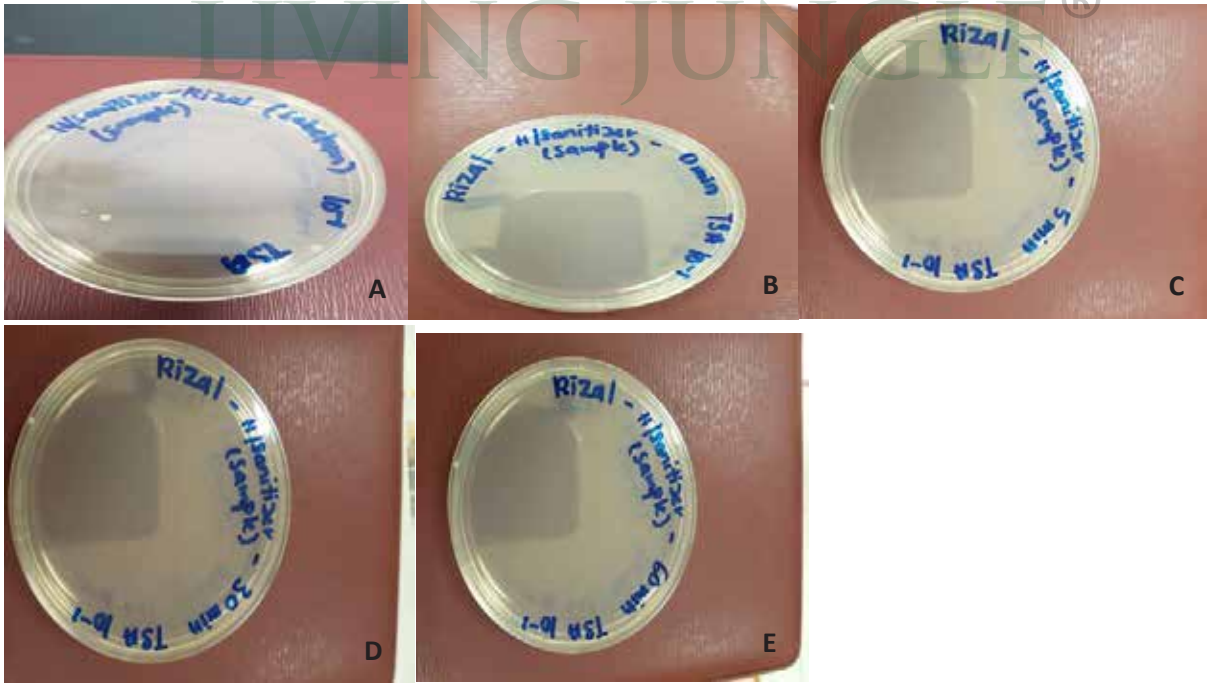

Figure 6: Microorganism presence determination before treatment (A), immediately (B), after 5 minutes (C), after 30 minutes (D) and 1 hour (E) treatment with Living Jungle hand sanitizer.

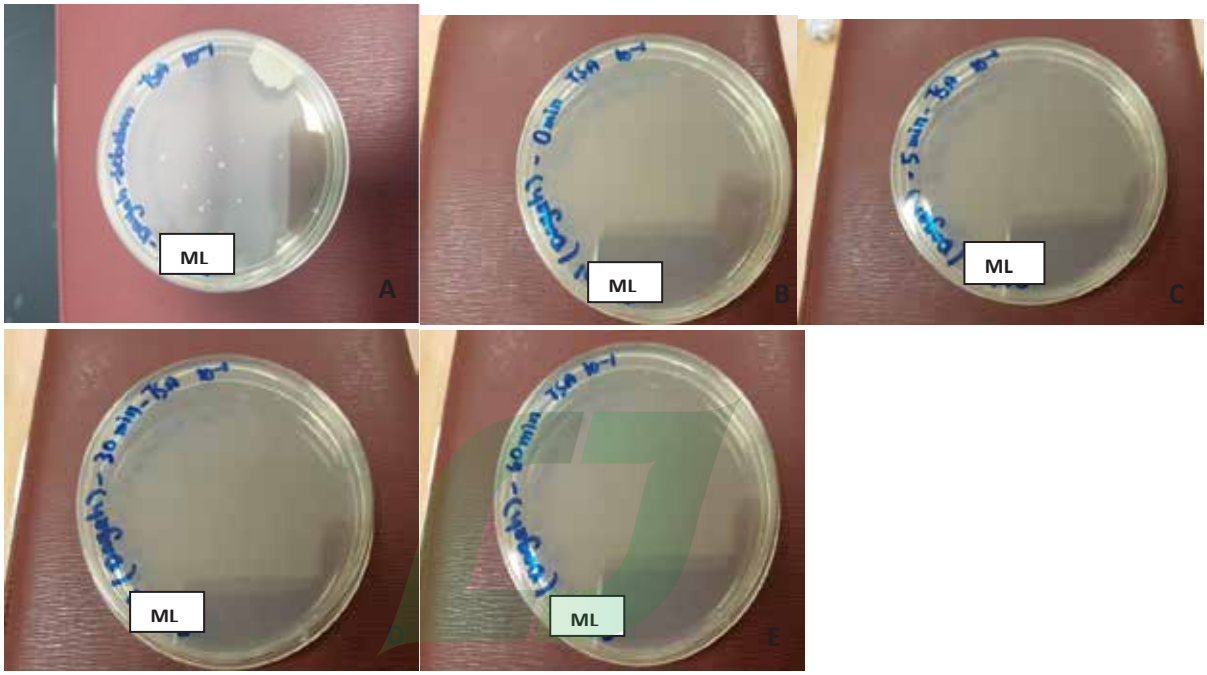

Figure 7: Microorganism presence determination before treatment (A), immediately (B), after 5 minutes (C), after 30 minutes (D) and 1 hour (E) treatment with Market Leader hand sanitizer brand (ML).

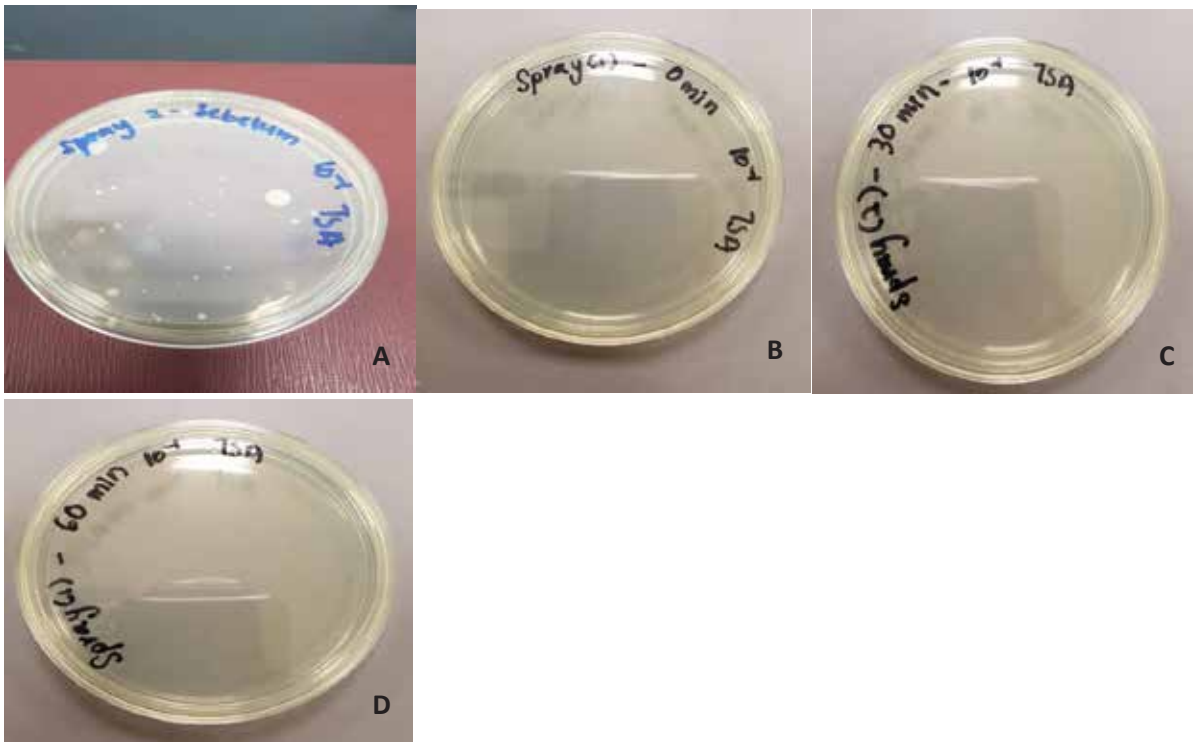

Figure 8: Microorganism presence determination before treatment (A), immediately (B) after 30 minutes (C) and 1 hour (D) treatment with Living Jungle disinfectant spray.

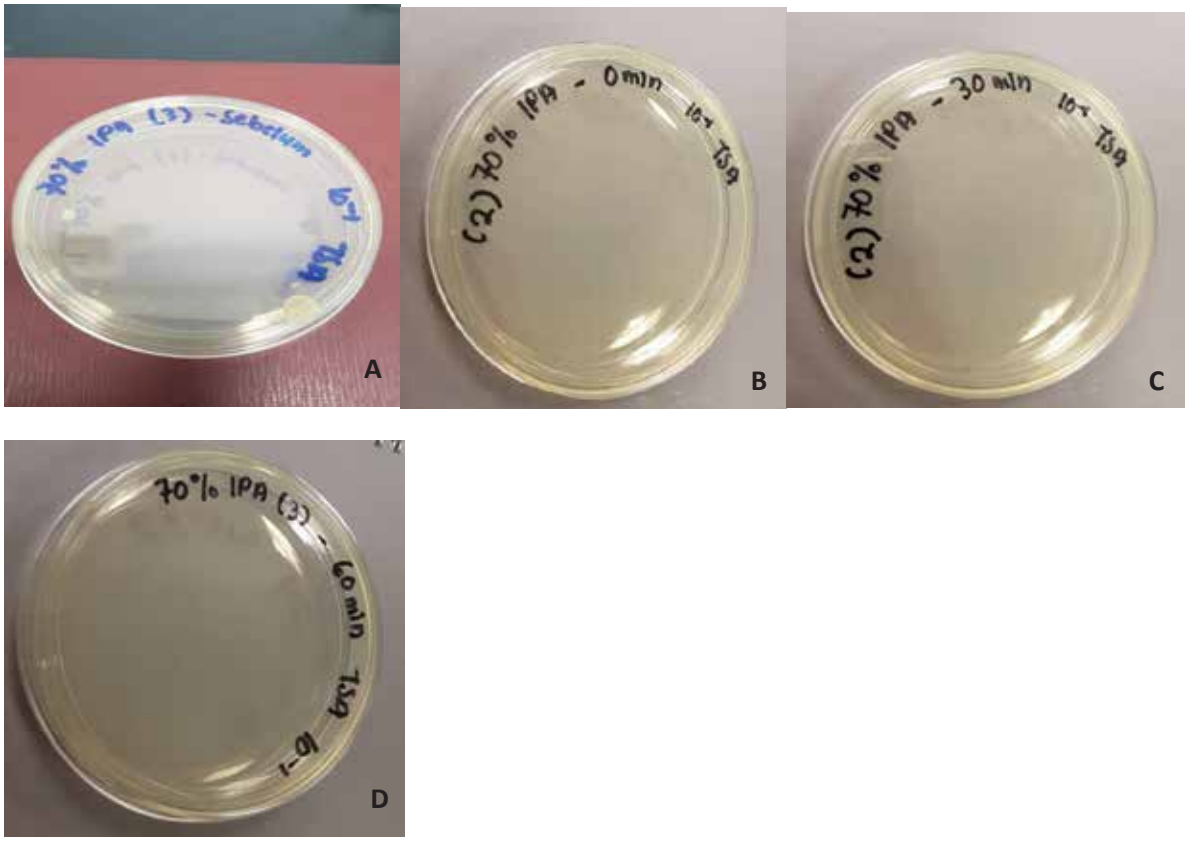

Figure 9: Microorganism presence determination before treatment (A), immediately (B) after 30 minutes (C) and 1 hour (D) treatment with IPA 70 %.

4.0 CONCLUSION

The results showed that the Living Jungle hand sanitizer and disinfectant spray were able to eliminate Gram positive, Gram negative, yeast and fungus that can be commonly found on hand and bench surface. It is interesting to note that Living Jungle disinfectant spray demonstrated high zone of inhibition compared to 70% IPA for S. *aureus*, B. *subtilis* and *E. coli*. Meanwhile for yeast and mold, it was comparable to 70% IPA. The Living Jungle hand sanitizer shows comparable effect of microbial reduction when compared with a market leader brand in hand sanitizer. As a conclusion, the result indicated that the Living Jungle hand sanitizer and disinfectant spray was able to eliminate the presence of microorganism on hand and bench surface by 99.99% instantly and can last for an hour post-treatment period.