

TITLE

A Disinfectant

FIELD OF INVENTION

5 This invention relates to a long acting human skin disinfectant, for example for the hands.

BACKGROUND

10 It is known to apply a disinfectant liquid to the human skin to kill germs, for example bacteria and virus'. A problem with many known disinfectants is that they are only effective for a short period and have to be reapplied frequently otherwise the person using them is relatively unprotected from infection if, for example, they touch a surface on which germs are located.

OBJECT OF THE INVENTION

15 It is an object of preferred embodiments of the invention to go at least some way towards addressing the above problem. While this applies to preferred embodiments, it should not be seen as a limitation on claims expressed more broadly. The object of the invention in its broadest form is simply to provide the public with a useful choice.

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DEFINITIONS

The term "comprises" or "has", if and when used in this document in relation to a combination of features, should not be seen as excluding the option of additional features in the combination
25 that are not mentioned.

SUMMARY OF THE INVENTION

30 According to one aspect the invention comprises the use of Organosilane and Benzalkonium in the manufacture of a disinfectant comprising:

- a) 0.25% - 0.35 % wt 3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium salt; and
- b) 0.09% – 0.11% wt Benzalkonium;

wherein the disinfectant is for providing, and provides, effective sanitising protection* on human skin for about 24 hours or more.

* The term “effective sanitising protection” means that the disinfectant kills $\geq 99\%$ (preferably $\geq 99.99\%$) of germs on human skin.

5 Optionally the disinfectant comprises:

- a) 0.25% - 0.35 % wt 3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium chloride;
and
- b) 0.09% – 0.11% wt Benzalkonium.

10 Optionally the disinfectant comprises:

- a) about 0.3 % wt 3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium chloride; and
- b) about 0.1% wt Benzalkonium.

Optionally the Benzalkonium is in salt form.

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Optionally the salt form comprises Benzalkonium Chloride.

Optionally the sanitising protection is against Coronavirus.

20 Optionally the sanitising protection is against SARS-CoV-1 and/or SARS-CoV-2 (eg CoVid-19 causing Coronavirus).

Optionally the disinfectant comprises Phenoxyethanol which functions in the disinfectant as a preservative.

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Optionally the Phenoxyethanol is present in the disinfectant at 0.7% - 1.3% wt.

Optionally the Phenoxyethanol is present in the disinfectant at 0.8% - 1.2% wt.

30 Optionally the Phenoxyethanol is present in the disinfectant at 0.9% - 1.1% wt.

Optionally the Phenoxyethanol is present in the disinfectant at about 1% wt.

Optionally the disinfectant comprises Ethylhexylglycerin which functions in the disinfectant as a preservative.

Optionally the Ethylhexylglycerin is present in the disinfectant at 0.07% - 0.13% wt.

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Optionally the Ethylhexylglycerin is present in the disinfectant at 0.08% - 0.12% wt.

Optionally the Ethylhexylglycerin is present in the disinfectant at 0.09% - 0.11% wt.

10 Optionally the Ethylhexylglycerin is present in the disinfectant at about 0.1% wt.

Optionally the sanitising protection is against one or more of *E.hirae*, *P.aeruginosa*, *S.aureus*, *E coli*, Norovirus, Candida, MRSA, Salmonella, Listeria, Flu Virus and MHV1 virus.

15 Optionally the sanitising protection is against one or more of *Enterococcus hirae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*.

Optionally the disinfectant is for providing, and provides, effective sanitising protection on human hands for about 24 or more hours.

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Preferably the disinfectant is for application as a liquid to the skin liberally to provide the effective sanitising protection. For example an amount of 0.5 ml – 1 ml, and preferably about 0.75ml, was found to be sufficient for two hands of an adult male of average size.

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DETAILED DESCRIPTION

Three aqueous foaming disinfectant formulations were prepared to have the make-up shown in the tables below.

30 Formulation A

INCI Name	Function	CAS No.	% by Weight
Water	Solvent/Diluent	7732-18-5	to 100
Glycerin	Humectant	56-81-5	2.0
Phenoxyethanol	Preservative	122-99-6	0.9

Benzalkonium Chloride	Antimicrobial	68424-85-1	0.1
Ethylhexylglycerin	Preservative	70445-33-9	0.1
3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium chloride	Antimicrobial and Surfactant	27668-52-6	0.1

Formulation B

INCI Name	Function	CAS No.	% by Weight
Water	Solvent/Diluent	7732-18-5	to 100%
Glycerin	Humectant	56-81-5	2.0%
Phenoxyethanol	Preservative	122-99-6	0.9%
Benzalkonium Chloride	Antimicrobial	68424-85-1	0.1%
Ethylhexylglycerin	Preservative	70445-33-9	0.1%
3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium chloride	Antimicrobial and Surfactant	27668-52-6	0.3%

Formulation C

INCI Name	Function	CAS No.	% by Weight
Water	Solvent/Diluent	7732-18-5	to 100%
Glycerin	Humectant	56-81-5	2.0%
Phenoxyethanol	Preservative	122-99-6	0.9%
Benzalkonium Chloride	Antimicrobial	68424-85-1	0.1%
Ethylhexylglycerin	Preservative	70445-33-9	0.1%
3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium chloride	Antimicrobial and Surfactant	27668-52-6	0.5%

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In each case the Formulation was prepared as follows-

Step 1

10 Dissolve all the 3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium chloride in a sufficient portion of water so it makes up a pre-mix totaling about 6% weight of final product.

Step 2

Dissolve or suspend all the benzalkonium chloride in a sufficient portion of the water to provide a pre-mix solution or suspension totaling 0.2% weight of final product.

5 Step 3

In a separate mixing vessel, add water totaling 90.8% weight of the final product.

Step 4

10 Separately add each of the premixes (described in steps 1 and 2) to the water of step 3, and blend.

Step 5

15 Add all the phenoxyethanol and ethylhexylglycerin to the contents of the mixing vessel, and blend.

Step 6

Add all the glycerin to the components in the mixing vessel, and blend.

Step 7

20 Mix well (for ≥ 10 minutes) until all the ingredients are homogenized.

Tests for Disinfectant Performance

Each formulation was tested to see how effective it was in killing germs. This included a quantitative suspension test to evaluate bactericidal activity according to the well-known
25 European test standard EN 13727. The formulations were also tested for virucidal activity by the surface carrier technique of ASTM 1053. Additionally, each formulation was subjected to tests to determine its effectiveness at 24 hours after being applied to human skin (hands). The tests were carried out as follows.

30 Step 1 (Culture Growth)

Two test organisms were selected and evaluated in the trial:

- *Staphylococcus aureus* NZRM 147 (ATCC 6538)
- *Escherichia coli* NZRM 2577 (ATCC 8739)

Culture suspensions were prepared for the organisms using overnight culture plates. The culture growth was harvested from each plate and inoculated to 10mL Tryptone Soy Broth (TSB) to achieve an approximate organism count of 10^9 cfu/mL.

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Step 2 (Chemical Inactivation)

A combination of neutraliser and dilution was used to achieve an effective neutralising system. In particular, Tryptone soy broth with 0.5% Lecithin and 4% Tween 20 (TLT) was used as a neutralising solution. Hand Sanitizer Formulation at 'ready to use' (RTU) concentration was diluted at a ratio of 1:1000 (10µL in 10mL TLT, the same volume and dilution ratio as treated Vitro-skin 2cm²). 10mL of the diluted formulation was then individually spiked to give a cell level sufficient to render a test organism concentration within the countable range. In the presence of TLT the antimicrobial property of the test products was neutralized in order to recover any viable micro-organisms. The recovery of the test organism in the presence of test product was not less than 70% of that recovered from the inoculum control.

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Step 3

In a Biological Safety Cabinet, two 2cm² synthetic skin swatches were each pre-treated with 10µL of the test product at the RTU concentration. Each pair of skin swatches was rubbed together to distribute the test product, and dried under HEPA laminar air. The formulation remained on the swatches for sufficient time to demonstrate the residual efficacy of each test product. Duplicate tests were performed at each time point for all test products (including the reference product) and the water control.

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Step 4 – (Water control - Baseline Control)

The synthetic skin swatches were also pre-treated with sterile water in the same manner as the test product, and tested at the same time points along-side the test product.

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Step 5

At various time points, each treated skin swatch was inoculated with 10µL of culture suspension at an approximate concentration of 10^9 cfu/mL, and evenly distributed with a sterile 1µL loop. An exposure time of 5 minutes was allowed prior to transferring the skin swatch into neutralising broth (TLT).

Step 6

The inoculated neutralising broth was mixed and serially diluted to aid in determining the number of viable bacteria remaining.

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Step 7

Step 5 and 6 were followed to test the water control in the same manner as the test product.

Step 8

10 The neutralised solutions for the test products and the water control were serially diluted using 0.1% Peptone, and each dilution was plated in duplicate using Standard Plate Count Agar.

Step 9

The plates were incubated at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 48 hours.

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The tests gave the results detailed below.

Results

Quantitative Suspension test for evaluating bactericidal activity

20 **Methodology: EN13727**

Test Conditions (per EN13727)	
Test Concentration	Neat
Contact Time	1 Minute
Neutralizer/Dilution	Formulation A: T6 1:10 (all organisms) Formulation B: T6 1:10 (all organisms) Formulation C: T6 1:10 - E.coli, P.aeruginosa and E.hirae; T5 1:100 - S.aureus
Test Conditions	Clean (0.03% BSA)
Test Temperature	Room Temperature

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Surviving Organisms after Exposure to each Formulation – 1 Minute Contact Time (per EN13727)				
Formulation	Organism	Inoculum Control Count	Surviving Test Organisms	
		CFU/mL (Log ₁₀)	CFU/mL (Log ₁₀)	Log reduction
Formulation A	<i>S.aureus</i> ATCC 6538	6.20 x 10 ⁷ (7.79)	<10 (<1.00)	>6.79
	<i>E.coli</i> NCTC 10538	2.00 x 10 ⁷ (7.30)	<10 (<1.00)	>6.30
	<i>P.aeruginosa</i> ATCC 15442	4.10 x 10 ⁷ (7.61)	<10 (<1.00)	>6.61
	<i>E.hirae</i> ATCC 10541	3.90 x 10 ⁷ (7.59)	<10 (<1.00)	>6.59
Formulation B	<i>S.aureus</i> ATCC 6538	2.35x 10 ⁷ (7.37)	<10 (<1.00)	>6.37
	<i>E.coli</i> NCTC 10538	3.10 x 10 ⁷ (7.49)	<10 (<1.00)	>6.49
	<i>P.aeruginosa</i> ATCC 15442	4.20 x 10 ⁷ (7.62)	<10 (<1.00)	>6.62
	<i>E.hirae</i> ATCC 10541	1.55 x 10 ⁷ (7.19)	<10 (<1.00)	>6.19
Formulation C	<i>S.aureus</i> ATCC 6538	4.85 x 10 ₇ (7.69)	<100 (<2.00)	>5.69
	<i>E.coli</i> NCTC 10538	3.65 x 10 ₇ (7.56)	<10 (<1.00)	>6.56
	<i>P.aeruginosa</i> ATCC 15442	2.60 x 10 ₇ (7.41)	<10 (<1.00)	>6.41
	<i>E.hirae</i> ATCC 10541	2.25 x 10 ₇ (7.35)	<10 (<1.00)	>6.35

CFU = Colony Forming Unit

Neutralisation Validation Results (per EN13727)						
Formulation	Organism	Validation Suspension, CFU/ml (Nv)	Experimental Condition, CFU/ml (A)	Neutralizer Control, CFU/ml (B)	Method Validation, CFU/ml (C)	Pass/Fail
Formulation A	<i>S.aureus</i> ATCC 6538	41	49	58	39	Pass
	<i>E.coli</i> NCTC 10538	42	32	30	35	Pass

	<i>P.aeruginosa</i> ATCC 15442	103	73	105	105	Pass
	<i>E.hirae</i> ATCC 10541	23	22	23	31	Pass
Formulation B	<i>S.aureus</i> ATCC 6538	24	16	44	13	Pass
	<i>E.coli</i> NCTC 10538	31	16	82	98	Pass
	<i>P.aeruginosa</i> ATCC 15442	42	27	74	56	Pass
	<i>E.hirae</i> ATCC 10541	16	16	90	83	Pass
Formulation C	<i>S.aureus</i> ATCC 6538	66	73	62	51	Pass
	<i>E.coli</i> NCTC 10538	42	32	30	43	Pass
	<i>P.aeruginosa</i> ATCC 15442	103	73	105	94	Pass
	<i>E.hirae</i> ATCC 10541	23	22	23	17	Pass

N_v is the number of cells per ml in the validation suspension.

- A, B and C are the numbers of surviving cells in the experimental conditions control (A),
5 neutralizer control (B) and method validation (C), at the end of the contact time
A, B & C should preferably be $\geq 50\%$ the value of N_v .

- By way of summary, (TMD 110, EN13727), Formulations A and B showed greater than 6 log
reduction against *S.aureus*, *E.coli*, *P.aeruginosa* and *E.hirae* at 1 minute contact time.
10 Formulation C showed greater than 6 log reduction against *E.coli*, *P.aeruginosa* and *E.hirae*
and greater than 5 log reduction against *S.aureus* at 1 Minute contact time. All three formulas
resulted in a >99.99% reduction in microorganisms after 1 minute contact.

Results of Virucidal Test by Carrier Method

Methodology: ASTM E1053

Test Conditions (ASTM E1053)	
Virus Strain	Murine hepatitis virus (MHV1) ATCC/VR-261
Cell Substrate	A9 cells ATCC/CCL- 1.4
Test Concentration	Neat
Contact Time	1 minute
Test Temperature	Room temperature
Test Condition	Clean
Neutraliser	3 cc Sephadex Gel in PBS (Phosphate Buffer Saline)

Formulation A: MHV1 test/control results (1 minute contact)					
Virus Dilution	Number of Inoculated Wells	Virus Control	Cytotoxicity	Neutralisation	Test Sample
10 ⁻¹	4	4 ⁺ /4	C	C	C
10 ⁻²	4	4 ⁺ /4	0 ⁺ /4	4 ⁺ /4	3 ⁺ /4
10 ⁻³	4	4 ⁺ /4	0 ⁺ /4	4 ⁺ /4	3 ⁺ /4
10 ⁻⁴	4	4 ⁺ /4	N/A	N/A	1 ⁺ /4
10 ⁻⁵	4	4 ⁺ /4	N/A	N/A	0 ⁺ /4
10 ⁻⁶	4	3 ⁺ /4	N/A	N/A	0 ⁺ /4
10 ⁻⁷	4	2 ⁺ /4	N/A	N/A	N/A
10 ⁻⁸	4	0 ⁺ /4	N/A	N/A	N/A
Log ₁₀	-	6.77	1.5	1.5	3.33
Log₁₀ Reduction of Virus after Treatment				3.44	

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Formulation B: MHV1 Test/Control Results (1 minute contact)					
Virus Dilution	Number of Inoculated Wells	Virus Control	Cytotoxicity	Neutralisation	Test Sample
10 ⁻¹	4	4 ⁺ /4	C	C	C
10 ⁻²	4	4 ⁺ /4	0 ⁺ /4	4 ⁺ /4	4 ⁺ /4
10 ⁻³	4	4 ⁺ /4	0 ⁺ /4	4 ⁺ /4	3 ⁺ /4
10 ⁻⁴	4	4 ⁺ /4	N/A	N/A	0 ⁺ /4
10 ⁻⁵	4	4 ⁺ /4	N/A	N/A	0 ⁺ /4
10 ⁻⁶	4	3 ⁺ /4	N/A	N/A	0 ⁺ /4
10 ⁻⁷	4	2 ⁺ /4	N/A	N/A	N/A
10 ⁻⁸	4	0 ⁺ /4	N/A	N/A	N/A
Log ₁₀	-	6.77	1.5	1.5	3.33
Log₁₀ Reduction of Virus after Treatment				3.44	

Formulation C: MHV1 Test/Control Results (1 minute contact)					
Virus Dilution	Number of Inoculated Wells	Virus Control	Cytotoxicity	Neutralisation	Test Sample
10 ⁻¹	4	4 ⁺ /4	C	C	C
10 ⁻²	4	4 ⁺ /4	0 ⁺ /4	4 ⁺ /4	4 ⁺ /4
10 ⁻³	4	4 ⁺ /4	0 ⁺ /4	4 ⁺ /4	4 ⁺ /4
10 ⁻⁴	4	4 ⁺ /4	N/A	N/A	1 ⁺ /4
10 ⁻⁵	4	4 ⁺ /4	N/A	N/A	0 ⁺ /4
10 ⁻⁶	4	3 ⁺ /4	N/A	N/A	0 ⁺ /4
10 ⁻⁷	4	2 ⁺ /4	N/A	N/A	N/A
10 ⁻⁸	4	0 ⁺ /4	N/A	N/A	N/A
Log ₁₀	-	6.77	1.5	1.5	3.67
Log₁₀ Reduction of Virus after Treatment				3.10	

Note:

- 5 Presence of virus is presented in a X⁺ of 4 format i.e. X⁺/4:

X⁺ – number of wells (X) with presence of virus (+)

4 – total number of inoculated wells.

Example: In the event of a 3⁺/4 result, this indicates that 3 of the total 4 wells show a positive virus response.

- 10 Absence of virus in each response is recorded as “0”

Cytotoxic response is recorded as “C”

Calculated virus titre = 106.77TCID₅₀/0.1ml (6.77 log₁₀)

Cell control - 4 wells with healthy cell monolayer

* The Reed & Muench LD₅₀ Method was used for determining the virus titre endpoint.

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To summarise the results (TMCV 006, ASTM E1053), regarding cytotoxicity and neutralisation, the Formulations A and B showed virucidal efficacy against MHV1 by achieving 3.44 log reduction in virus concentration after 1 minute’s exposure period at room temperature. The formulations C also showed virucidal efficacy against MHV1 by achieving 3.10 log reduction in virus concentration after 1 minute’s exposure period at room temperature. This indicates that each formulation results in >99.9% reduction in viral count after 1 minute contact time, with Formulas A and B exhibiting slightly better results than Formula C.

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Results

The 24-hour Residual Anti-Microbiological Study of the Hand Disinfectant Product

Methodology: The above Custom In-vitro Study Protocol

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Test Conditions	
Test Organism	- Staphylococcus aureus NZRM 147 (ATCC 6538) - Escherichia coli NZRM 2577 (ATCC 8739)
Test Concentration	Neat or Ready To Use
Conditioning Time Points	5 minutes, 30 minutes, 1hr, 2hr, 4hr, 6hr and 24hr
Contact Time	5 Minutes at each conditioning time point
Neutralizer/Dilution	Tryptone soy broth with 0.5% Lecithin and 4% Tween 20 (TLT)/1:1000 Dilution (10µL in 10mL TLT, same volume and dilution ratio as the treated 2cm ² synthetic skin substrate)
Test Conditions	In Biological Safety Cabinet, two 2cm ² synthetic skin swatches were each pre-treated with 10µL of the test product at the RTU concentration
Test Temperature	Ambient Temperature (defined as 20 ± 5°C)
References	- ASTM E3058 – 16: Standard Test Method for Determining the Residual Kill Activity of Hand Antiseptic Formulations. - PAS2424:2014 – Quantitative surface test for the evaluation of residual antimicrobial (bactericidal and/or yeasticidal) efficacy of liquid chemical disinfectants on hard non-porous surfaces – Test method. - TGA instructions for disinfectant testing Version 2.1, March 2020. - British Pharmacopoeia 2020 Appendix XVI C. Efficacy of Antimicrobial Preservation.

Neutraliser Validation 10µL in 10mL TLT (1 in 1,000 dilution)				
Formulation/Sample	Organism	Average	Recovery	Result
Formulation A	<i>E.coli</i>	70.6	103.5	Pass
	<i>S.aureus</i>	67.8	105.3	Pass
Formulation B	<i>E.coli</i>	69.4	101.8	Pass
	<i>S.aureus</i>	61.6	95.7	Pass
Formulation C	<i>E.coli</i>	68.6	100.6	Pass
	<i>S.aureus</i>	67.4	104.7	Pass
Reference Formulation	<i>E.coli</i>	71.6	105.0	Pass
	<i>S.aureus</i>	66.8	103.7	Pass

Inoculum Control	<i>E.coli</i>	68.2	N/A	N/A
	<i>S.aureus</i>	64.4	N/A	N/A

Residual Efficacy against Staphylococcus Aureus NZRM 147 (ATCC 6538)					
Conditioning Time Point	Sample	Result (CFU/Swatch)	Average result	Log 10	Log Reduction
5 minutes	Formula A	21500000	19600000	7.29	0.12
	Formula A duplicate	17700000			
	Formula B	15	15	1.18	6.24
	Formula B duplicate	<10			
	Formula C	16500000	16525000	7.22	0.20
	Formula C duplicate	16550000			
	Formula Ref	28000000	27250000	7.44	-0.02
	Formula Ref duplicate	26500000			
	Water control - Baseline	26000000	26000000	7.41	N/A
	Baseline duplicate	26000000			
30 minutes	Formula A	24000000	23000000	7.36	-0.11
	Formula A duplicate	22000000			
	Formula B	180	590	2.77	4.48
	Formula B duplicate	1000			
	Formula C	20000000	23250000	7.37	-0.12
	Formula C duplicate	26500000			
	Formula Ref	28000000	26750000	7.43	-0.18
	Formula Ref duplicate	25500000			
	Water control - Baseline	18500000	17750000	7.25	N/A
	Baseline duplicate	17000000			
1 hour	Formula A	21500000	21250000	7.33	0.17
	Formula A duplicate	21000000			
	Formula B	<10	380	2.58	4.92
	Formula B duplicate	380			
	Formula C	32500000	28250000	7.45	0.05
	Formula C duplicate	24000000			
	Formula Ref	28000000	31250000	7.49	0.01
	Formula Ref duplicate	34500000			
	Water control - Baseline	29500000	31750000	7.50	N/A
	Baseline duplicate	34000000			

2 hour	Formula A	31000000	28250000	7.45	-0.04
	Formula A duplicate	25500000			
	Formula B	4200	4750	3.68	3.73
	Formula B duplicate	5300			
	Formula C	29000000	28500000	7.45	-0.05
	Formula C duplicate	28000000			
	Formula Ref	25500000	27500000	7.44	-0.03
	Formula Ref duplicate	29500000			
	Water control - Baseline	20500000	25500000	7.41	N/A
	Baseline duplicate	30500000			
4 hour	Formula A	27000000	29500000	7.47	-0.03
	Formula A duplicate	32000000			
	Formula B	1700	4000	3.60	3.83
	Formula B duplicate	6300			
	Formula C	35500000	34000000	7.53	-0.10
	Formula C duplicate	32500000			
	Formula Ref	26000000	28250000	7.45	-0.02
	Formula Ref duplicate	30500000			
	Water control - Baseline	30000000	27250000	7.44	N/A
	Baseline duplicate	24500000			
6 hour	Formula A	22000000	31250000	7.49	0.03
	Formula A duplicate	40500000			
	Formula B	<10	<10	<1	>6.52
	Formula B duplicate	<10			
	Formula C	27500000	28750000	7.46	0.06
	Formula C duplicate	30000000			
	Formula Ref	24500000	30500000	7.48	0.04
	Formula Ref duplicate	36500000			
	Water control - Baseline	39000000	33250000	7.52	N/A
	Baseline duplicate	27500000			
24 hour	Formula A	29500000	30500000	7.48	0.02
	Formula A duplicate	31500000			
	Formula B	700	375	2.57	4.93
	Formula B duplicate	50			
	Formula C	34500000	35750000	7.55	-0.04
	Formula C duplicate	37000000			

	Formula Ref	35000000	34000000	7.53	-0.02
	Formula Ref duplicate	33000000			
	Water control - Baseline	29000000	32250000	7.51	N/A
	Baseline duplicate	35500000			

Residual Efficacy against Escherichia Coli NZRM 2577 (ATCC 8739)					
Conditioning Time Point	Sample	Result (CFU/Swatch)	Average result	Log 10	Log Reduction
5 minutes	Formula A	36500000	39750000	7.60	-0.03
	Formula A duplicate	43000000			
	Formula B	<10	<10	<1	>6.57
	Formula B duplicate	<10			
	Formula C	35500000	33500000	7.53	0.04
	Formula C duplicate	31500000			
	Formula Ref	42000000	38000000	7.58	-0.01
	Formula Ref duplicate	34000000			
	Water control - Baseline	37000000	36750000	7.57	N/A
	Baseline duplicate	36500000			
30 minutes	Formula A	36500000	31750000	7.50	0.03
	Formula A duplicate	27000000			
	Formula B	<10	10	1.00	6.53
	Formula B duplicate	10			
	Formula C	34500000	33750000	7.53	0.00
	Formula C duplicate	33000000			
	Formula Ref	40000000	34000000	7.53	0.00
	Formula Ref duplicate	28000000			
	Water control - Baseline	32500000	33750000	7.53	N/A
	Baseline duplicate	35000000			
1 hour	Formula A	34500000	27500000	7.44	0.04
	Formula A duplicate	20500000			
	Formula B	<10	<10	<1	>6.57
	Formula B duplicate	<10			
	Formula C	30500000	34750000	7.54	-0.06
	Formula C duplicate	39000000			
	Formula Ref	24000000	18500000	7.27	0.22

	Formula Ref duplicate	13000000			
	Water control - Baseline	34500000	30500000	7.48	N/A
	Baseline duplicate	26500000			
2 hour	Formula A	31000000	29000000	7.46	0.05
	Formula A duplicate	27000000			
	Formula B	75	142.5	2.15	5.35
	Formula B duplicate	210			
	Formula C	31500000	32000000	7.51	0.00
	Formula C duplicate	32500000			
	Formula Ref	39500000	33250000	7.52	-0.01
	Formula Ref duplicate	27000000			
	Water control - Baseline	36500000	32250000	7.51	N/A
	Baseline duplicate	28000000			
4 hour	Formula A	45000000	36750000	7.57	0.07
	Formula A duplicate	28500000			
	Formula B	<10	234500	5.37	2.27
	Formula B duplicate	234500			
	Formula C	30000000	40000000	7.60	0.04
	Formula C duplicate	50000000			
	Formula Ref	32500000	33750000	7.53	0.11
	Formula Ref duplicate	35000000			
	Water control - Baseline	40500000	43500000	7.64	N/A
	Baseline duplicate	46500000			
6 hour	Formula A	34000000	35500000	7.55	0.04
	Formula A duplicate	37000000			
	Formula B	<10	<10	<1	>6.57
	Formula B duplicate	<10			
	Formula C	38500000	38750000	7.59	0.00
	Formula C duplicate	39000000			
	Formula Ref	23500000	24000000	7.38	0.21
	Formula Ref duplicate	24500000			
	Water control - Baseline	41000000	38750000	7.59	N/A
	Baseline duplicate	36500000			
24 hour	Formula A	41000000	42750000	7.63	-0.10

	Formula A duplicate	44500000	119250	5.08	2.46
	Formula B	102500			
	Formula B duplicate	136000			
	Formula C	45500000	44750000	7.65	-0.12
	Formula C duplicate	44000000			
	Formula Ref	44000000	40500000	7.61	-0.07
	Formula Ref duplicate	37000000			
	Water control - Baseline	36000000	34250000	7.53	N/A
	Baseline duplicate	35500000			

Summary of Residual Efficacy Results (Formulation A)

24 hours			Result (cfu/swatch)	Average result (cfu/swatch)	Log 10	Log reduction
Staphylococcus aureus NZRM 147 (ATCC 6538)	Formula	Repeat 1	29500000	30500000	7.48	0.02
		Repeat 2	31500000			
	Water Control	Repeat 1	29000000	32250000	7.51	
		Repeat 2	35500000			

24 hours			Result (cfu/swatch)	Average result (cfu/swatch)	Log 10	Log reduction
Escherichia coli NZRM 2577 (ATCC 8739)	Formula	Repeat 1	41000000	42750000	7.63	-0.10
		Repeat 2	44500000			
	Water Control	Repeat 1	36000000	34250000	7.53	
		Repeat 2	32500000			

5 Summary of Residual Efficacy Results (Formulation B)

24 hours			Result (cfu/swatch)	Average result (cfu/swatch)	Log 10	Log reduction
Staphylococcus aureus NZRM 147 (ATCC 6538)	Formula	Repeat 1	700	375	2.57	4.93
		Repeat 2	50			
	Water Control	Repeat 1	29000000	32250000	7.51	
		Repeat 2	35500000			

24 hours			Result (cfu/swatch)	Average result (cfu/swatch)	Log 10	Log reduction
Escherichia coli NZRM 2577 (ATCC 8739)	Formula	Repeat 1	102500	119250	5.08	2.46
		Repeat 2	136000			
	Water Control	Repeat 1	36000000	34250000	7.53	
		Repeat 2	32500000			

Summary of Residual Efficacy Results (Formulation C)

24 hours			Result (cfu/swatch)	Average result (cfu/swatch)	Log 10	Log reduction
Staphylococcus aureus NZRM 147 (ATCC 6538)	Formula	Repeat 1	34500000	35750000	7.55	-0.04
		Repeat 2	37000000			
	Water Control	Repeat 1	29000000	32250000	7.51	
		Repeat 2	35500000			

24 hours			Result (cfu/swatch)	Average result (cfu/swatch)	Log 10	Log reduction
Escherichia coli NZRM 2577 (ATCC 8739)	Formula	Repeat 1	45500000	44750000	7.65	-0.12
		Repeat 2	44000000			
	Water Control	Repeat 1	36000000	34250000	7.53	
		Repeat 2	32500000			

- 5 By way of summary, Formulation B (at RTU volume) demonstrated residual efficacy with log reduction of between 2.27 and >6.57 throughout the 24 hour's period (at 5 mins, 30 mins, 1hr, 2hr, 4hr, 6hr and 24hr) against Staphylococcus aureus NZRM 147 and Escherichia coli NZRM 2577. In comparison, Formulations A and C did not exhibit effective residual efficacy against either bacterial strain (maximum log reduction for either organism did not exceed 0.2 at any
- 10 time point).

Formulation B demonstrated residual biocidal activity against both Staphylococcus aureus and Escherichia coli after 24 hours (4.93 and 2.46 log reduction, respectively). This translates to a >99.99% and >99% reduction in the count of Staphylococcus aureus and Escherichia coli, even

15 if exposure to either organism occurs 24 hours after application of Formula B. Conversely, Formulation A exhibited a low log reduction against Staphylococcus aureus (0.02) after 24 hours, in addition to exhibiting a negative log reduction against Escherichia coli (-0.10),

indicating growth of organism. Similarly, Formula C resulted in negative log reductions against both *Staphylococcus aureus* (-0.04) and *Escherichia coli* (-0.12) after 24 hours, indicating organism growth.

- 5 This is significant as it indicates that Formula B exhibits residual efficacy against microorganisms on synthetic skin, even after significant time has passed following application of the formula. This is believed to be related to a synergistic interaction provided by the Benzylkonium and Organosilane ingredients in Formulation B.

10 **Other Forms of Benzlkonium**

While preferred formulations according to the invention utilise Benzlkonium chloride, in other embodiments this may be substituted by other forms of Benzlkonium, for example Benzalkonium free base or one or more alternative Benzalkonium salts (e.g. Benzalkonium Bromide, Benzalkonium Saccharinate and Acesulfame Benzalkonium). In each case the weight amounts would be adjusted to provide more or less equivalent function.

Other Organosilanes

Similarly, while preferred forms of the invention utilise 3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium chloride, in other embodiments this may be substituted by other organosilanes, for example one or more of 3-(trihydroxysilyl) propyl dimethyl octadecyl ammonium chloride (CAS 199111-50-7) and n,n-dimethyl-n-[3-(trimethoxysilyl)propyl]-1-tetradecanaminium chloride (CAS 41591-87-1). In each case the weight amounts would be adjusted to provide more or less equivalent function.

25 **Preservatives, Humectants and Surfactants**

Further, formulations according to the invention may use alternative preservative, humectant and/or surfactant materials to those of Formulations A, B and C, adjusting the weight amounts as appropriate to provide more less equivalent function. Examples of these are as follows –

Preservative	Humectant	Surfactants
- Chlorhexidine gluconate 1, 2-Hexanediol - Benzoic acid	- Propylene glycol - Glycerol - Aloe vera	Alternative organosilanes, e.g. 3-(trihydroxysilyl) propyl dimethyl octadecyl ammonium chloride (CAS 199111-50-7) and/or n,n-dimethyl-n-[3-

• Benzethonium chloride • Ethylparaben		(trimethoxysilyl)propyl]-1-tetradecanaminium chloride (CAS 41591-87-1)
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In terms of disclosure, this document hereby discloses each item, feature or step mentioned herein in combination with one or more of any of the other item, feature or step disclosed herein, in each case regardless of whether such combination is claimed.

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While some preferred embodiments of the invention have been described by way of example it should be appreciated that modifications and improvements can occur without departing from the scope of the following claims.

CLAIMS

1. The use of Organosilane and Benzalkonium in the manufacture of a disinfectant comprising:
 - a) about 0.3% wt 3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium chloride; and
 - b) about 0.1% wt Benzalkonium chloride;wherein the disinfectant is for providing, and provides, effective sanitising protection on human skin for about 24 hours or more.
2. The use according to claim 1, wherein the disinfectant comprises:
 - a) 0.3 % wt 3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium chloride; and
 - b) 0.1 % wt Benzalkonium chloride.
3. The use according to claim 1 or 2, wherein the sanitising protection is against Coronavirus.
4. The use according to claim 1, 2 or 3, wherein the sanitising protection is against SARS-CoV-1 and/or SARS-CoV-2 (eg CoVid-19 causing Coronavirus).
5. The use according to any one of the preceding claims, comprising Phenoxyethanol which functions in the disinfectant as a preservative.
6. The use according to claim 5, wherein the Phenoxyethanol is present in the disinfectant at 0.7% - 1.3% wt.
7. The use according to claim 5, wherein the Phenoxyethanol is present in the disinfectant at 0.8% - 1.2% wt.
8. The use according to claim 5, wherein the Phenoxyethanol is present in the disinfectant at 0.9% - 1.1% wt.
9. The use according to claim 5, wherein the Phenoxyethanol is present in the disinfectant at about 1% wt.

10. The use according to any one of the preceding claims, comprising Ethylhexylglycerin which functions in the disinfectant as a preservative.
- 5 11. The use according to claim 10, wherein the Ethylhexylglycerin is present in the disinfectant at 0.07% - 0.13% wt.
12. The use according to claim 10, wherein the Ethylhexylglycerin is present in the disinfectant at 0.08% - 0.12% wt.
- 10 13. The use according to claim 10, wherein the Ethylhexylglycerin is present in the disinfectant at 0.09% - 0.11% wt.
14. The use according to claim 10, wherein the Ethylhexylglycerin is present in the disinfectant at about 0.1% wt.
- 15 15. The use according to any one of the preceding claims, wherein the sanitising protection is against one or more of E.hirae, P.aeruginosa, S.aureus, E.coli, Norovirus, Candida, MRSA, Salmonella, Listeria, Flu Virus and MHV1 virus.
- 20 16. The use according to any one of the preceding claims, wherein the sanitising protection is against one or more of Enterococcus hirae, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.
17. The use according to any one of the preceding claims wherein the disinfectant is for providing, and provides, effective sanitising protection on human hands for about 24 or more hours.
- 25 18. A disinfectant prepared according to the use of any one of the preceding claims.
- 30 19. A disinfectant substantially as below-

Component	Approximate % by Weight
Glycerin	2.0%
Phenoxyethanol	0.9%

Benzalkonium Chloride	0.1%
Ethylhexylglycerin	0.1%
3-(trimethoxysilyl)propyl dimethyl octadecyl ammonium chloride	0.3%
Water	to 100%