WHITE PAPER ON THE SCIENCE AND EFFICACY OF GOLDSHIELD 24 HAND SANITISER, COMPRISED OF BENZALKONIUM CHLORIDE AND AN EFFICACY ANALYSIS OF IT COMBINED WITH THE GOLDSHIELD ORGANOSILANE TECHNOLOGY.

THE SCIENCE OF:
NON-ALCOHOL HAND SANITISER

Benzalkonium chloride ("BAC") is a key active ingredient in the Goldshield 24 **("GS")** hand sanitizer, and this quaternary ammonium chloride has been demonstrated to be highly effective against a broad spectrum of micro-organisms. The Goldshield formula combines BAC with a preservative -3 trihydroxysilylpropyldimethyloctadecyl ammonium chloride, to provide a broader, more effective killing functionality, and a longer term durability of protection with one to four daily applications. This paper was developed to focus on the science and efficacy of BAC and the combined formulation which demonstrated the efficacy against Influenza A virus (H1N1) ATCC VR-1469 which was obtained from the American Type Culture Collection. And a durability analysis against cfu's of 10^8 of MRSA isolates (***see Appendix A & A1)***.

Designated as "GS" HAND SANITISER contains the active quaternary ammonium compound Benzalkonium Chloride (BAC).

HAND SANITISER: a unique product with wide-spectrum efficiency over known pathogenic microbes.

Quaternary ammonium compounds mode of action is as a cationic surfactant with a long carbon ionic charge. This class of chemical reduces the surface tension it interfaces, and is attracted to negatively charged surfaces, including microorganisms.

Quaternary ammonium compounds denature the proteins of the bacterial, viral or fungal cell, affect the metabolic reactions of the cell and allow vital substances to leak out of the cell, finally causing death presenting no known risks of the organism to genetically adapt.

"GS" HAND SANITISER is a ready to use hand sanitizer utilising the active ingredient Benzalkonium Chloride (BAC) at a concentration of 0.1%. BAC is an alcohol-free, antimicrobial compound that has been widely used in the health care industry for more than 60 years in formulas for preservatives, surface cleansers, sterilizing agents, and topical antiseptic sprays.

"GS" FEATURES AND BENEFITS
Alcohol Free - Non-drying to the skin
Non-Flammable
Rinse Free Formula - No soap, water, or towels needed
Non-Irritating and Non-Toxic - Gentle to skin
No Sticky Residue
Antiseptic - Helps prevent infections in minor abrasions
Hypoallergenic

EFFICIENCY OF "GS"
A variety of studies and laboratory tests have been completed in order to demonstrate the efficacy of BAC. The data includes independent laboratory test results, manufacturer's analysis and results, published and non-published study and test results. A compilation of data follows:

In Vitro Test Results- Manufacturer Data

The following data was supplied by Rhone-Poulenc, manufacturer of the raw ingredient Benzalkonium Chloride.

Biological Properties

Phenol Coefficients

Phenol Coefficients of Benzalkonium Chloride (BAC) active were determined by the official A.O.A.C procedure.

Organism	Dilution of BAC Hand Sanitizer in water to get the 10 minute killing	Concentration of BAC Hand Sanitizer (ml/L) to kill in 10 minutes	Phenol	Phenol Coefficient
Brucella abortus	1/152.6	6.55 ml/L	1/110	1.387
Escherichia coli	1/101.25	9.87 ml/L	1/70	1.446
Klebsiella pneumoniae	1/93.75	10.66 ml/L	1/90	1.042
Lactobacillus casei	1/393.75	2.54 ml/L	1/100	3.937
Listeria monocytogenes	1/270	3.70 ml/L	1/100	2.700
Mycobacterium amegmatis	1/78.75	12.70 ml/L	1/65	1.211
Neisseria caiarrbalis	1/64.89	15.41 ml/L	1/70	0.927
Pasteurella multocida	1/202.89	4.92 ml/L	1/110	1.844
Proteus vulgaris	1/45	22.22 ml/L	1/70	0.642
Pseudomonas aeruginosa PRD-10	1/52.25	19.14 ml/L	1/70	0.746
Salmonella gallinarum	1/105	9.52 ml/L	1/80	1.312
Salmonella pullorum	1/93.75	10.66 ml/L	1/90	1.042
Salmonella typhimurium	1/75	13.33 ml/L	1/70	1.071
Salmonella schottumelleri	1/225	4.44 ml/L	1/95	2.368
Salmonella typhosa	1/168.75	5.92 ml/L	1/90	1.875
Shigella sonnei	1/93.75	10.66 ml/L	1/80	1.172
Staphylococcus aureus	1/168.75	5.92 ml/L	1/60	2.812
Streptococcus fecalis	1/562.5	1.77 ml/L	1/70	8.028
Streptococcus pyogenes C-203	1/93.75	10.66 ml/L	1/80	1.172
Streptococcus viridans	1/262.5	3.80 ml/L	1/90	2.916
FUNGI *Saccharomyces cerevisiae*	1/187.5	5.33 ml/L	1/100	1.875
Pityrosporium ovale	1/131.25	7.61 ml/L	1/100	1.312

Micro biocidal and Micro biostatic Activity

The antibacterial effectiveness of Benzalkonium Chloride (BAC) hand sanitiser has been measured by an empirical broth dilution procedure in which the highest dilutions capable of inhibiting growth to 48 hours (micro biostatic) and killing all organisms in 24 hours (micro biocidal) are determined.

Organism	Microbicidal	Microbiostatic
Brucella abortus	1/3750	1/7500
Penicillium luteum	1/3	1/6
Penicillium notatum	1/12	1/12
Aerobacter aerogenes	1/120	1/240
Bacillus aerus, var. mycoides	-	1/7500
Bacillus subtilis	-	1/7500
Brevibacterium ammonigenes	-	1/7500
Klebsiella pneumoniae	1/120	1/240
Lactobacillus casei	1/750	1/750
Proteus vulgaris	1/60	1/60
Pseudomonas aeruginosa PRD-10	1/30	1/30
Salmonella gallinarum	1/225	1/225
Salmonella pullorum	1/120	1/120
Salmonella typhimurium	1/120	1/240
Salmonella schottumelleri	1/60	1/240
Salmonella typhosa	1/468.75	1/937.5
Salmonella choleraesuis	1/225	1/225
Shigella sonnei	1/120	1/120
Staphylococcus aureus	1/937.5	1/15000
Trichophyton interdigitale	1/150	1/300
Streptococcus pyogenes C-203	1/375	1/375
Streptococcus viridans	1/1500	1/3000
Saccharomyces cerevisiae	1/750	1/1500
Pityrosporium ovale	1/1500	1/3000

This data shows that the BAC hand sanitiser possesses a broad spectrum of effectiveness against a variety of both gram-positive and gram-negative organisms. Data provided by Rhone-Poulenc.

In Vitro Test Results

The following pathogens were killed within 15 seconds after exposure to the BAC Hand Sanitiser:

Candida albicans
Candida keyfr
Escherichia coli
Enterococcus faecalis
Enterococcus faecium (VRE)
Klebsiella pneumonia
Micrococcus luteus
Pseudomonas aeruginosa
Proteus mirabilis
Salmonella typhimurium
Serratia marcescens
Staphylococcus aureus
Staphylococcus aureus (MRSA)
Salmonella enteritidis
Staphylococcus epidermidis
Staphylococcus haemolyticus
Staphylococcus saprophyticus
Herpes simplex virus Type 1
Human Coronavirus (related to SARS)
Trichophyton mentagrophytes
Apergillis niger
Hepatitis A and B

In vitro tests performed by SCI Laboratories, Inc.; revised protocol of CFR
333.470; revised protocol of CFR 333.470, Viromed Laboratories, Inc.; revised protocol of ASTM E1052, and ATS Laboratories, Inc.; protocol of WLI01041603.COR.

Synopsis

The aim of the project was to determine the germicidal effectiveness of a BAC based hand sanitiser on three bacterial strains:

- Clostridium difficile (ATCC 9689)
- Methicillin-resistant Staphylococcus aureus (ATCC 33591)
- Vancomycin-resistant Streptococcus faecalis (ATCC 51299)

The germicidal effectiveness of the sanitizer was therefore evaluated separately on each of the bacterial strains at contact times of 0, 15, and 30 seconds.
The hand sanitiser showed a germicidal effectiveness:

- Greater or equal to 99.9% against Clostridium difficile (ATCC 9689) in 15 seconds
- Greater or equal to 99.9% against Methicillin-resistant Staphylococcus aureus (ATCC 33591) in 15 seconds
- Greater or equal to 99.9% against Vancomycin-resistant Streptococcus faecalis (ATCC51299) in 15 seconds

In Vitro Test Results

Quaternary Ammonium Chloride based hand sanitizer exhibited strong germicidal activity against a variety of gram-positive and gram-negative bacteria, as well as the yeast Candida albicans.
In most instances viable cell numbers were reduced by greater than 99.99% after a 30-second exposure period.

In-vitro Antimicrobial Efficacy

Test Microorganisms	Initial Inoculum (cfu/10µL)	Exposure Time (Minutes) 0.5 1.0 2.0 Growth in TSB	Reduction (percent)*
Pseudomonas aeruginosa	3.39×10^5	- - -	99.99
Klebsiella pneumoniae	2.76×10^5	- - -	99.99
Escherichia coli	15.8×10^5	- - -	99.99
Salmonella typhimurium	18.9×10^5	- - -	99.99
Staphylococcus aureus ATTC33591	21.2×10^5	- - - (Methicillin Resistant / MRSA)	99.99
Staph. epidermidis	18.3×10^5	- - -	99.99
Streptococcus faecalis ATTC522A	9.8×10^5	- - - (Vancomycin resistant entercococci/ VRE)	99.99
Streptococcus agalactiae	12.1×10^5	- - -	99.99
Micrococcus luteus	14.4×10^5	- - -	99.99
Candida albicans	12.6×10^5	- - -	99.99
Trichophytin mentogrophytes (Athlete's Foot)	9.6×10^5	- - -	99.99
Salmonella choleraesuis	14.1×10^5	- - -	99.99
Aspergillius niger	11.8×10^5	- - -	99.99
Listeria monocytogenes	17.9×10^6	0 CFU/mL	(15 seconds)
Clostridium difficile	1.1×10^4	0 CFU/mL	(15 seconds)
Human Coronavirus (resembles SARS-like virus family)		0 CFU/mL	(15 seconds)

(*) Indicates percentage reduction in numbers of viable cells evidenced by lack of growth in Trypticase-soy Broth medium.

(-) Indicates no survival of test organisms in the recovery medium.

Synopsis

Results and conclusion:

- **B** hand disinfectant has a cellular lysis effect until 1/10 dilution
- **B** hand disinfectant has a cellular lysis effect until 1/10 dilution after 10 seconds only of treatment.
- **B** can be used to inactivate members of virus family orthomyxo viridae including Avian Influenza A virus in 10 seconds.

The treatment of the influenza virus H1N1 at a concentration of 10 3.77 TCID 50 / (100 µl) with **B** hand disinfectant results in almost instantaneous lysis of cells and inactivation of the influenza virus strain used in this study.

Effectiveness of a Nonrinse, Alcohol-Free Antiseptic Hand Wash (J Am Podiatr Med Assoc 91 (6): 288-293, 2001) Anoosh Moadab, BS Kathryne F. Rupley, BS Peter Wadhams, DPM

Synopsis

This study evaluated the efficacy of a benzalkonium chloride (BAC) hand sanitiser using the US Food and Drug Administration's (USFDA) method for testing antiseptic hand washes that podiatric physicians and other health-care personnel use.

The alcohol-free product was compared with an alcohol based product. Independent researchers from the California College of Podiatric Medicine conducted the study using 40 volunteer students from the class of 2001.

The results show that the BAC hand sanitizer outperformed the alcohol based hand sanitiser and met the regulatory requirements for a hand sanitiser.

The **alcohol based product failed as an antimicrobial hand wash** and was less effective than the control soap used in the study.

Alcohol Free Hand Sanitizer to Combat Infection
AORN Journal, (68 August 1998), p. 239
David L. Dyer, PhD.
Kenneth B. Gerenraich, DPM
Peter S. Wadhams, DPM

Synopsis

Universal precautions require that preoperative health care personnel wash their hands before and after all patient contact. Time constraints, however, can make adhering to universal precautions including proper hand washing, difficult. Some preoperative healthcare workers, therefore, routinely use rinse-free hand sanitizers to supplement normal hand washing.

This study evaluated immediate and persistent antimicrobial effectiveness of two alcohol containing hand sanitisers and a benzalkonium chloride (BAC) hand sanitizer using United State Food and Drug Administration protocol.

Results indicate that all three products were equally effective after a single application.

However, after repeated use, the **alcohol containing sanitisers did not meet the federal Performance standards,** and the alcohol free sanitiser did.

These properties and others, illustrated that a nonflammable, alcohol free hand sanitiser provides better durability in performance standards.

Alcohol Free Hand Sanitizer Reduces Elementary School Illness Absenteeism
Fam Med 2000;32(9):633-8
David L. Dyer, PhD.
Arnold Shinder, DO
Fay Shinder, RN

Synopsis

A substantial percentage of school absenteeism among children is related to transmissible infection. Rates of transmission can be reduced by hand washing with soap and water, but such washing occurs infrequently.

This study tested whether an alcohol free instant hand sanitiser could reduce illness absenteeism in school age children.

Compared to the hand washing only control group, students using the benzalkonium chloride based hand sanitiser were found to have 41.9% fewer illness related absence days, representing a 28.9% and a 49.7% drop in gastrointestinal and respiratory related illness, respectively. Likewise, absence incidence decreased by 31.7%, consisting of a 44.2% and 50.2% decrease in incidence gastrointestinal and respiratory related illness, respectively. No adverse events were reported during the study.

Reduction of Elementary School Illness Absenteeism in Elementary Schools Using an Alcohol free Instant Hand Sanitizer

The Journal of School Nursing; 17(5) October 2001, p. 258
Catherine G. White, RN, BSN
Fay Shinder, RN, BSN
Arnold Shinder, DO
David L. Dyer, PhD.

Synopsis

Hand washing is the most effective way to prevent the spread of communicable disease.

The purpose of this double-blind, placebo-controlled study was to assess whether an alcohol free, instant hand sanitiser containing benzalkonium chloride could reduce illness absenteeism in a population of 769 elementary school children and serve as an effective alternative when regular soap and water hand washing was not readily available.

Prior to the study, students were educated about proper hand washing technique, the importance of hand washing to prevent transmission of germs, and the relationship between germs and illnesses. Children in kindergarten through 6th grade (ages 5-12) were assigned to the active or placebo hand sanitizer product and instructed to use the product at scheduled times during the day and as needed after coughing or sneezing.

Data on illness absenteeism were tracked. After 5 weeks, students using the active product were 33% less likely to have been absent because of illness when compared with the placebo group.

Research Paper Virucidal Efficacy of GOLDSHIELD® 24 Alcohol-Free Hand Rinse for Inanimate Environmental Surfaces

Dr. Shiyou Li

Research Professor, Director Center for Medicinal Plant Research

Stephan F. Austin University

Feb. 19. 2010

About Dr. Shiyou Li: Dr Li has discovered nine new plant species and invented four cultivars (plant varieties maintained through genetic selection). He has authored four books and eighteen pee- review papers.

Dr Li co-discovered about 30 new natural products including "Katie" one of the first US patents on pharmaceutical crops for anti-cancer drugs..

The following study was conducted to determine the efficacy of a patent –pending alcohol-free QAC and antimicrobial formulation, invented AuPROVISE/ AP Goldshield, which was derived in part from an Emory antimicrobial invention. The invention has been assigned to AP Goldshield LLC and their parent company, AuProvise, S.A. will be marketed under their brand Goldshield.® 24 against Influenza A as a surrogate for H1N1.

The ASTM protocols used were designed to evaluate the efficacy of this formula against the acceptable surrogate for H1N1, classified by WHO as a "Pandemic".

Virucidal efficacy of GOLDSHIELD ™ 24 (Lot # Lab-C0810-18)

Hand Rinse Foam used for inanimate environmental surfaces

1. General information

1.1 Test samples:

GOLDSHIELD ™ 24 (Lot # Lab-C0810-18 Hand Rinse Foam) containing, benzalkonium chloride 0.1%, 3 trihydroxysilylpropyldimethyloctadecyl ammonium chloride, Propylene Glycol, Methyl Anthranilate and purified water.

1.2 Protocol used:

ASTM E1053 - 97(2002) Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces,

ASTM E1482 - 04 Standard Test Method for Neutralization of Virucidal Agents in Virucidal Efficacy Evaluations

U.S. E.P.A. Pesticide Assessment Guidelines, Subdivision G: Product Performance, Section 91-2 (f), and Section 91-30, (d), (e), November 1982.

1.3 Virus and cell culture

1.3.1 MDCK cell culture:

Madin Darby Canine Kidney (MDCK) ATCC CCL-34 was obtained from the American Type Culture Collection. MDCK cells were cultured in DMEM Dulbecco's Minimum Essential Medium (DMEM) with 2.50 mM L-Glutamine and 15 mM HEPES Buffer, and 10% fetal bovine serum (FBS). The MDCK cultures were incubated under 5% CO_2 at 37°C for 24~48 hours to form monolayers before testing.

1.3.2 Virus:

Influenza A virus (H1N1) ATCC VR-1469 was obtained from the American Type Culture Collection. Typically, monolayer MDCK cultures were washed twice with PBS buffer. Then, 10~15% VR-1469 were inoculated and incubate at 37°C for 1 hour. Interval 15 min shaking the flask to allow the virus attach to MDCK cell. After 1 hour, remove medium and wash with PBS. Add maintenance medium (DMEM with 2 ug/ml TPCK-trypsin without FBS). Put the culture back to 37°C incubator, culture 48~72 h (or see the CPE, if the CPE come out more than 90%), and then harvest the virus. The virus stock culture was stored at -80ºC freezer until testing.

1.4 Calculation of TCID50

Viral titers are expressed as 50% titration end point infectivity ($TCID_{50}$) by the Reed-Muench method.

Proportional distance formula =[(% positive value > 50%)-50%]/[(% positive value > 50%)-(% positive value <50%)]

Log infectious dose 50= (log dilution >50%) + (proportional distance × dilution factor)

2. Test procedures

2.1 Neutralization control test:

Place a small wad of glass wool in a 5-cc syringe. Add Sephacryl S-1000 SF into the syringe to form a 3-cc column volume. Calibrate the column with 10 mL PBS. Centrifuge the column at 600 × g for 3 min. 0.6 mL GS solution was pipetted onto the column. Place the column on a 15 mL conical centrifuge tube. Centrifuge for 3 min at 600 × g. Prepare a set of 10-fold dilutions of the filtrate and add 50 µl amounts into monolayer MDCK cultures on 96-well plates. Incubate for 1 hr and add 100 µl DMEM medium. Incubate at 37°C and observe cell growth (neutralization cytotoxicity control). Additionally, each 10-fold dilution of the filtrate was mixed with 2 log_{10} virus. Add the mixture into monolayer MDCK cultures on 96-well plates to observe viral infectivity (neutralization infectivity control).

2.2 Virus film control:

Preparation of virus film: Spread 0.2 mL virus suspension on 60 mm petri plates. Allow the virus film to dry at room temperature for 1 hour.

Add 2 mL PBS over the virus film. Stand for 10 min and then scrape surface with sterile rubber policeman to suspend virus. Pipet 0.6 mL amounts onto the Sephacryl S-1000 column. After centrifuge the column at 600 × g for 3 min, prepare serial 10-fold dilutions of the filtrate. Add 50 µl of each dilution onto the monolayer MDCK cultures. Allow one hour at 37°C for virus absorption and then add 100 µl virus maintenance medium. Incubate at 37°C and observe cell growth for evidence of cytopathic effect.

2.3 Virucidal test:

On each virus film, add 2 mL use dilution of GS. Stand for 10 min at room temperature. Scrape petri plate surface to suspend virus. Pipet 0.6 mL virus/GS mixture onto the column, and centrifuge the column at 600 × g for 3 min. Prepare serial 10-fold dilutions of the filtrate, and add 50 µl of each dilution onto the monolayer MDCK cultures. Stand one hour at 37°C and then add 100 µl virus maintenance medium. Incubate at 37°C and observe cell growth for evidence of cytopathic effect.

3. Test results

The following Table 1 showed results of GOLDSHIELD ^{TM} 24 (Lot # Lab-C0810-18 Hand Rinse Foam) exposed to Influenza A virus (H1N1) ATCC VR-1469 on inanimate surface. The titer of virus control and virus dry film control were 4.66 and 3.33 $\log_{10}$ respectively. In neutralization cytotoxicity control test, the GOLDSHIELD ^{TM} 24 filtrate didn't show cytotoxicity against MDCK cells. After the virus film exposure to GOLDSHIELD ^{TM} 24, the viral titer was tested as $\leq 10^{0.5}$, and the reduction of influenza A virus VR-1469 was $\geq$ 2.83 $\log_{10}$.

Table 1. Virucidal efficacy test of Goldshield™ AP GOLDSHIELD ™2 (GS)

Dilution	Virus titer	Virus dry film + column control	Virus film + GS5	Neutralization infectivity control	Neutralization cytotoxicity control
10^{-1}	+ + + +	+ + + +	————	+ + + +	————
10^{-2}	+ + + +	+ + + +	————	+ + + +	————
10^{-3}	+ + + +	+ + + —	————	+ + + +	————
10^{-4}	+ + + +	————	————	+ + + +	————
10^{-5}	+ ————	————	————	+ + + +	————
10^{-6}	————	————	————	+ + + +	————
10^{-7}	————	————	————	+ + + +	————
$TCID_{50}$/0.1 mL	$10^{4.66}$	$10^{3.33}$	$\leq 10^{0.5}$		

+ virus-specific cytopathic effect observed; — no virus-specific cytopathic effect or cytotoxicity observed.

APPENDIX "A1" GOLDSHIELD HAND SANITISER PROJECT: MRSA DURABILITY STUDY

Test done: 03/10/10 Test completed: 03/12/10

PROJECT ID: 2010512

TEST PARAMETERS

Size of the slide: 1 x 1 inch

Original inoculum Conc.: 10^8 (Mac #1)

Inoculum used: 0.05ml

Amount of Sanitizer used: 1 spray

Amount of Tween 80 used: 0.1ml

Organism used: MRSA –BAA 44

Amount of Diluent used (DE Neutralizer Broth): 5 ml

Contact time: 1hr, 3hr, 6hr, 8hr & 24hr

Shaker time: 5 min

Drying time in Incubator: 40min

Replicates: 5

Serial dilution: For control: 10^1

PROCEDURE:

1. Prepared 10 ml of the inoculum of Mac #1 from a 24 hr stock culture.
2. Aseptically added 0.05 ml inoculum to the sterile 1 x 1 inch slide and spread evenly.
3. Let air dry in the incubator at 35° C for 40 min.
4. After air dry, sprayed the slides with 1 spray (approximately 0.75ml) of the sanitizer.
5. Let the slides for the contact time for 1 hr, 3hr, 6hr, 8hr and 24hr.
6. After each contact time, transferred the slides aseptically into sterile specimen cup and added 5 ml of the neutralizer broth (DE broth).
7. Agitated for 5 min on the shaker.
8. Sub cultured on to the plates at various dilutions. For control, serial dilution done.
9. The counts and dilution are recorded at 24hrs.

RESULTS:

Contact time	Sample	Sanitizer			Control		
		Raw count	Dilution read	Final count	Raw count	Dilution read	Final count
1 HR	1	NG		NG	128	0.001	6.4×10^6
	2	19	0.01	9.5×10^3	116	0.001	5.8×10^6
	3	2	0.001	1.0×10^4	160	0.001	8.0×10^6
	4	11	0.001	5.5×10^4	108	0.001	5.4×10^6
	5	25	0.001	1.2×10^5	76	0.001	3.8×10^6
3 HR	1	53	0.001	2.6×10^5	168	0.001	8.4×10^6
	2	22	0.001	1.1×10^5	144	0.001	7.2×10^6
	3	7	0.001	3.5×10^4	132	0.001	6.6×10^6
	4	25	0.001	1.2×10^5	112	0.001	5.6×10^6
	5	40	0.001	2.0×10^5	236	0.001	1.1×10^7
6HR	1	56	0.001	2.8×10^5	192	0.001	9.6×10^6
	2	52	0.001	2.6×10^5	168	0.001	$8.4x\ 10^6$
	3	50	0.001	2.5×10^5	168	0.001	8.4×10^6
	4	6	0.001	3.0×10^4	184	0.001	9.2×10^6
	5	46	0.001	2.3×10^5	176	0.001	8.8×10^6
8 HR	1	42	0.001	2.1×10^5	152	0.001	7.6×10^6
	2	2	0.001	1.0×10^4	172	0.001	8.6×10^6
	3	3	0.001	1.5×10^4	132	0.001	6.6×10^6
	4	8	0.01	4.0×10^3	172	0.001	8.6×10^6
	5	34	0.001	1.7×10^5	132	0.001	6.6×10^6
24 HR	1	30	0.001	1.5×10^5	154	0.001	7.7×10^6
	2	3	0.001	1.5×10^4	224	0.001	$1.1x\ 10^7$
	3	11	0.001	5.5×10^4	157	0.001	7.8×10^6
	4	33	0.001	1.6×10^5	180	0.001	9.0×10^6
	5	8	0.01	4.0×10^3	128	0.001	$6.4\ x\ 10^6$

CALCULATION:

For Challenge: Raw count x dilution read x Amount of Diluent (DE broth) 5 ml **For Control**: Raw count x Dilution read x Amount of Diluent (DE broth) 5 ml x Serial dilution (10^1)