Investigation Into Anti-Bacterial Claims For Polysan Sanitation Solution (TeflexA) - Final Report

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Preface / Disclaimer

The author has over 5 years experience in the biotechnology/pharma industry and has expertise in the discovery, identification, compound profiling, analysis, bioassay and stability assay development, and isolation/purification of anti-infectives, and also specialises in microbial physiology and fermentation.

All work carried out as detailed in this report was performed on the commercially available Polysan work-top sanitiser (referred to 'Polysan' herein)- no individual components were used in microbiological evaluation. This report is intended purely as a scientific evaluation of Polysan's anti-microbial ability and to identify it's limits only and so no other products were used in comparison- it is also not intended as an evaluation of it's safety as this has been covered elsewhere, and no work has been carried out here on compound profiling or chemical analysis of it's components. All experiments detailed in this report were performed in vitro and therefore no guarantees can be made for real-world conditions- the work here should form the basis for other real-world trials.

This is a fully independent study - the author has no affiliation with Polysan's manufacturer, their partners, competitors or distributors, and this work was not carried out for or on behalf of any other company or body.

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Introduction

Aim

The aim of this project was to investigate some of the most notable anti-microbial claims by the manufacturers of a new anti-bacterial surface cleaner called Polysan, and to identify the limits to which it will work *in vitro.*

Summary

The Product

The work carried out as detailed in this report was carried out in order to investigate certain claims, to find at what limit they are true, and to evaluate if this efficacy is suitable for the product's intended purpose. The most notable claims made by Polysan's manufacturer, according to it's advertising literature, are:

- It's ability to kill a host of microbial organisms, including *E. coli, Pseudomonas aeroginosa,* MRSA and *Bacillus* species;
- The speed at which it kills- times vary from organism to organism but it is claimed that it will kill everything in 30 seconds;
- It's efficacy against *Clostridium difficile*, and it's prevention against *C. diff* contamination from spores; and,
- That it forms a bond with surfaces, and therefore prevents cross contamination.

The product tested in all experiments detailed in this report is the **Polysan Anti-Bacterial Clenser**. This specific product is a work-top sanitiser and is packaged in a 500ml spray bottle. It is a clear colourless liquid which has a slightly soap-like consistency, with no odour and no obvious spirit content. Each spray dispenses just over 1ml of product as a fine mist (based on weight). The commercial labeling indicates that it is water soluble, and the directions state that it should be sprayed onto surfaces, and wiped clean after 30 seconds.

Findings

In order to investigate this claims, the minimum bactericidal concentration was identified in growing liquid culture in four of the aerobic organisms which Polysan is reported to be effective against. In these trials, Polysan was found to be effective against each organism tested at a considerably higher level than is probably necessary for such a product.

C. diff spores were also incubated with varying dilutions of Polysan under anaerobic conditions. In this study, Polysan prevented colonisation and growth of *C. diff* cells from spores at the equivalent of roughly 0.5ml per squared meter on Brazier's agar, which is used specifically for the germination of *C. diff* spores.

It was also demonstrated that Polysan does kill all viable cells of *E. coli* in under 30 seconds at a low dilution of product (1/10), and there is also strong evidence that Polysan remains active after a treated surface has dried.

Experimental

1 - Experimental Validation for Testing Against Live Bacterial Culture

Aim

A rudimentary test for anti-microbial properties in Polysan product against *E. coli*. This experiment was performed in order to justify further study, and to find an approximate dilution range which should be studied.

Methods

Dilutions of Polysan

A range of dilutions of Polysan product between 10% and 100% were made up by mixing distilled reverse osmosis (RO) water with Polysan in the ratios shown in Table 1-1.

Culture Preparation

An overnight culture of *E. coli* strain ET12547 was grown by transferring 10µl of a thawed glycerol suspension[1] of the organism to 50ml TSB media[2] in a 250ml conical flask, and shaken at 250rpm at 30°C overnight. 500µl of this was then transfered to another 50ml TSB in a 250ml conical flask, and shaken at 250 rpm at 37°C until an OD_{600} of 0.4 was obtained.

'Kill' Step

500µl of Polysan solution was dispensed into a sterile universal flask for each concentration (table 1.1). 4.5ml of culture was then dispensed in to each, giving a further 1/10 dilution of Polysan in each. The flasks were then vortexed and incubated for 20 minutes at room temperature, then vortexed again. 10µl of this was then transfered and streaked onto MHA, and incubated for 24 hours at 30°C. Plates were then checked for signs of growth.

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[1] For strain preparation and storage see Appendix 2, P16

[2] For media composition see Appendix 1, P15

Conc. Polysan stock sol'n (%)	Vol. Polysan sol'n added (ml)	Vol. culture broth added (ml)	Final conc.Polysan
0	0.5	4.5	0%
10	0.5	4.5	1%
20	0.5	4.5	2%
30	0.5	4.5	3%
40	0.5	4.5	4%
50	0.5	4.5	5%
75	0.5	4.5	7.5%
100	0.5	4.5	10%

Table 1-1 - Polysan Dilutions

Results

Confluent growth was observed for the control. No growth was observed at 4% overall concentration of Polysan and above. Also, when viewed under a microscope, the broth/Polysan mixture showed an abundance of rod shaped cells for the control, but no cells were observed when treated with a 5% overall concentration of Polysan, both after 1 minute of incubation. Therefore there is obviously antimicrobial activity in Polysan, and the concentrations used during this experiment give a good indication of the approximate dilutions needed for generating MBC data- 10% and below Polysan is effective, therefore serial dilutions of this will be ideal for identifying the minimum.

2 - Experimental Validation for Testing Against *Clostridium Difficile* Spores

Aim

To validate experimental methods into testing Polysan against *Clostridium difficile*, this experiment is designed to be a rudimentary way of determining if Polysan inhibits growth from spores, to justify further study.

Methods

4 plates of 20ml Braizer's agar[1] were treated by adding 1ml of Polysan solution at concentrations of 0%, 20%, 50% and 100% respectively. Plates were allowed to dry and then 20µl *C.diff* strain 19126 spores[2] transfered and streaked onto each. Plates incubated under anaerobic conditions at 37°C for 48 hours and then inspected for growth.

Conclusion

These results could mean that presence of Polysan inhibits spore germination, kills vegetative cells after germination, or renders spores inactive or damages them in some way. They also show that though this is a valid method to use, lower dilutions of Polysan should be used to find the MIC.

3 - Find The Minimum Bactericidal Concentration (MBC) for Polysan Against a Small Panel Of Organisms

Aim

To test Polysan against a small panel of organisms selected based on claims in Polysan's promotional literature, in order to determine the minimum bactericidal concentration (MBC) for these organisms in culture during growth phase.

Methods

Culture preparation

Strains selected for this trial were based on some of the organisms which Polysan is claimed to be effective against (Table 3-1), with the exception of *Clostridium difficile*, which will be studied separately. For each replicate, 50ml of Tryptic Soy Broth (TSB)[1] media in 250ml conical flasks was inoculated with 10µl glycerol stock suspension[2] of each strain and incubated overnight, shaking at 30°C 250rpm. 500µl of this was then transfered to 50ml fresh TSB media in 250ml conicals and shaken at 250rpm, 37°C until an OD_{600} of 0.3-0.4 was achieved.

Claim	Strain	Gram	Morphology
B. subtillis	DSM4584 (Aventis)	+	Rod
S. aureus (MRSA)	12232 (Uni. of Leeds)	+	Cocci
E. coli	ET12567 (Merck)	-	Rod
P. aeruginosa	ATCC27853	-	Rod

Table 3-1 - Organisms tested for MBC of Polysan

Minimum Bactericidal Concentration

Stock solutions of a 1/2 serial dilution of Polysan were prepared[3], and 500µl of each were dispensed into sterile universal flasks. 4.5ml culture broth was then added to each flask to give a further 1/10 dilution of Polysan, vortexed, then incubated at room temperature for 30 minutes, then vortexed again. 10µl from each flask was then transfered and streaked onto Muller Hinton Agar (MHA)[2] with a sterile loop. This was incubated for 24 hours at 30°C, and checked for growth to find the MBC for each organism.

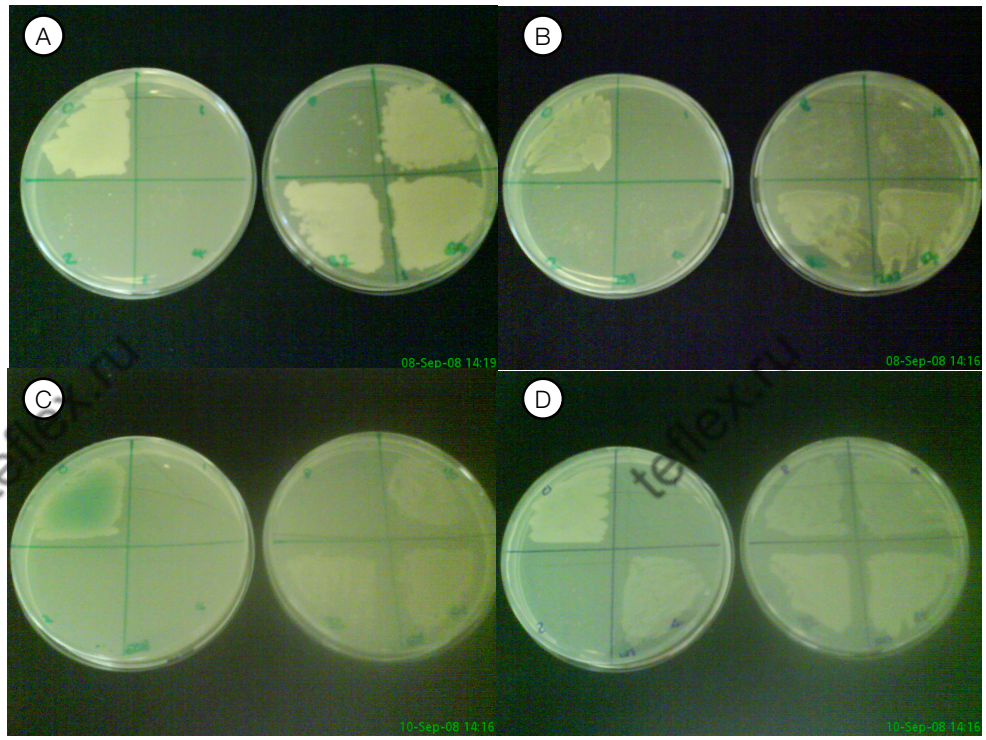
Results

Table 3-2 summarises the MBC data, and diagram 3-1 shows replicate plates from this experiment.

[3] For serial dilution details see Appendix 3, P16

	Overall Polysan Dilution							
Test Organism	0	1/10	1/20	1/40	1/80	1/160	1/320	1/640
B. subtillis	+	-	-	-	-	+/-	+	+
S. aureus (MRSA)	+	-	-	-	-	+/-	+	+
E. coli	+	-	-	+	+	+	+	+
P. aeruginosa	+	-	-	-	+	+	+	+

Table 3-2 - Growth detected for each dilution on MHA, where + is positive, - negetive, and +/- sparse growth



Diagrams 3-1A-E

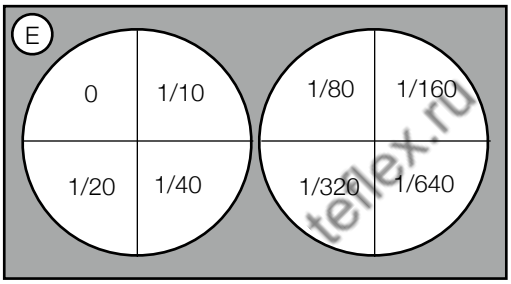
A - Bacillus subtillis

B - Staphylococcus aureus MRSA

C - Pseudomonas aeroginosa

D - Escherichia coli

E - Locations of dilution subculture on plates



Conclusion

This study has indicated that this Polysan product is effective at killing MRSA, *E. coli, Pseudomonas aeroginosa and Bacillus subtillis* in growing culture at between OD_{600} 0.3 and 0.4, at dilutions of between 1/20 and 1/80.

The conditions used during this experiment were designed to test the limit to which Polysan may work. The product is intended as a surface sanitiser, and not as a product to decontaminate culture. In a real-life situation, Polysan would be used to eliminate cross-contamination- infections of organisms such as MRSA are commonly spread from contact between surfaces with the organism present. Therefore, in this situation, there would be concentrations of Polysan several orders of magnitude higher than those demonstrated to be effective here, and it can therefore be concluded that Polysan is active enough for this purpose.

4 - Find The Minimum Inhibition Concentration (MIC) for Polysan Against *C. diff* Spores

Aim

To deduce the amount of Polysan per meter squared necessary to prevent colonisation of *C. diff* from spores.

Methods

This experiment was designed based around the traditional MIC methodology employed to deduce efficacy of antibiotics against selected organisms, whereby a dilution series of a known concentration of the antimicrobial agent is introduced to growing culture, and the minimum concentration for growth inhibition is found. In this case, however, the commercial product is designed to be applied to a surface, so it is therefore more relevant to measure efficacy in terms of volume per area treated.

Stock solutions of a 1/2 serial dilution of Polysan were prepared[3], and 500µl of each were dispensed and spread onto 20ml Braizer's agar[1] plates, and allowed to dry- table 4-1 details the equivalent volume to area coverage for each. Once dried, 20µl of *C. diff* strain 19126 spore suspension[2] was streaked out onto each plate, then incubated at 37°C under anaerobic atmosphere for 48 hours. Plates were then checked for growth.

Stock Dilution	Volume Dispensed (ml)	∴ Volume Polysan Dispensed (ml)	Plate Area (cm2)	∴ Polysan Coverage (ml/cm^2)
1	0.5	0.5	56.75	8.811
1/2	0.5	0.25	56.75	4.405
1/4	0.5	0.125	56.75	2.203
1/8	0.5	0.0625	56.75	1.101
1/16	0.5	0.03125	56.75	0.551
1/32	0.5	0.015625	56.75	0.275
1/64	0.5	0.0078125	56.75	0.138
0	0.5	0	56.75	0.000

Table 4-1 - Dilutions of Polysan and the equivalent coverage per squared metre

Results

Table 4-2 summarises the results from this experiment, and example plates are shown in diagrams 4.1A-C. There was no growth observed on plates treated with dilutions of between 1 and 1/8, sparse growth observed on plates treated with 1/16 dilution of Polysan and decreasingly reduced growth on all other plates treated with higher dilutions.

Dilution	Equivalent Coverage of Polysan (ml/m^2)	Growth Observed
1	8.81	No growth
1/2	4.41	No growth
1/4	2.20	No growth
1/8	1.10	No growth
1/16	0.55	Sparse growth
1/32	0.26	Reduced growth
1/64	0.14	Reduced growth
0	0.00	Confluent growth

Table 4-2 - Dilutions & equivalent area coverage of polysan, and groth observed

Diagram 4-1 - Plates after 48h anaerobic incubation: A- No polysan; B- 1/16 dilution Polysan; C- 1/8 dilution

Conclusion

These are compelling results and show evidence of Polysan's efficacy against the colonisation by *C. diff* spores. As seen here, it is effective on CCEY agar, under anaerobic conditions, which are ideal conditions for spores to colonise, yet one spray is effective over 1m^2 (at 1ml per spray)- even after the Polysan was allowed to dry out.

This could indicate either that Polysan kills *C. diff* spores outright or at least renders them inviable, it kills vegetative cells after spores germinate, or it sticks to spores when and kills vegetative cells when germinated. An assay to investigate this had started to be developed whereby spores were treated with Polysan & washed with water in water. No conclusive data was yielded in the timeframe but preliminary bioassay trials indicate significantly less growth after treating spores with Polysan & washing in water.

The conclusion from this experiment is that, after treating a surface with 1 spray per m2 of this product, *C. diff* spores are unable to form colonies on it.

5 - Subculture Polysan-Treated Culture At Various Times

Aim

Microscopic checks of Polysan treated culture suggest that all or most cells are destroyed within a minute of adding the product. Therefore in order to quantify this observation, a subculture at several time points after adding the product should be carried out.

Methods

In this experiment, the *E. coli* strain used previously in this project will be used to evaluate if all cells are killed within 30 seconds- it showed the lowest susceptibility in the MBC experiment, and has less safety implications than some of the other organisms used in these experiments.

50ml of TSB[1] in a 250ml conical flask was inoculated with 10µl thawed glycerol suspension[2] of *E. coli* ET12567, and shaken at 30°C, 250rpm overnight. 500µl of this was then sub-cultured into 50ml fresh TSB in a 250ml conical flask, then shaken at 250rpm, 37°C until an OD_{600} of 0.3-0.4 was obtained. This was streaked onto MHA[1] as a control for the experiment (t=0)

9ml of this was then added to 1ml 100% Polysan in a sterile universal flask and vortexed. At regular intervals shown in table 5-1, 10µl was transferred with a sterile loop and streaked onto MHA. Plates were then incubated at 30°C for 24 hours and inspected for growth.

Results

Timepoint	Growth Observed
0s	Confluent growth
15s	Hazy growth; thin layer of cells
30s	No growth
60s	No growth
2m	No growth
5m	No growth
10m	No growth
20m	No growth

Table 5-1 - Growth observed at each timepoint

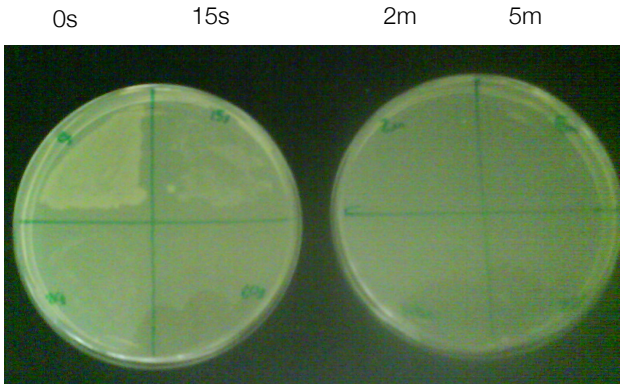


Diagram 5-1 - Plates showing subcultures at each timepoint after addition of Polysan

Conclusion

The results from this experiment suggest that Polysan is as fast acting as is claimed- though it would probably be prudent to run this experiment on other organisms as well- though in this case the assumption was that, with a higher MBC, *E. coli* would give a clear baseline for how fast this acts. It is also worth noting that in Polysan's advertising literature a time of 12.5 seconds is quoted for killing *Escherchia* cells which is the longest kill time noted- and bearing in mind the considerably lower concentration of Polysan used here, it is a claim clearly backed up by this evidence.

6 - Residual Inhibition of Bacteria

Aim

To find evidence that the claim that Polysan remains as a residual layer on treated surfaces which helps prevent cross-contamination.

Experimental

It is difficult to scientifically evaluate this claim, and ideally real-world trials should be undertaken under different conditions, whereby swabs are taken in areas with and without Polysan treatment. However, two approaches were used in order to test this claim.

Dry Culture Swabs

Clean plastic petri plates were treated with 200µl Polysan 1/2 serial dilutions[3], which was spread across the plastic surface with a sterile spreader. This was allowed to dry out completely, and 100µl of E. coli cell culture at OD_{600} 0.3-0.4 (as experiment 5 in this report) was streaked across the same surface on each plate. This too was allowed to dry out completely, after which a sterile swab was used to transfer a sample of the residue onto MHA[1] plates, which were then incubated for 24 hours at 30°C. Table 6-1 shows the result of this, and diagram 6-1 shows the cultures obtained.

Dilution	Growth Observed
0	Confluent growth
1	No growth
1/2	No growth
1/4	No growth
1/8	No growth
1/16	No growth
1/32	No growth
1/64	Reduced growth

Table 6-1 - Dilutions of Polysan used to treat plates, and the growth observed after swabbing the residue

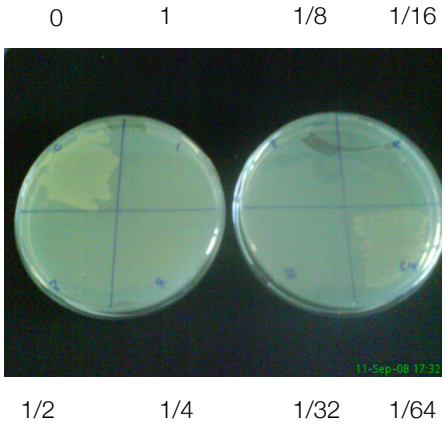
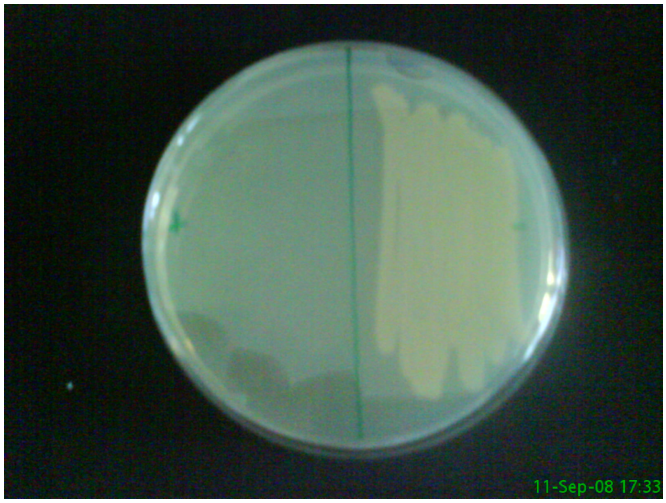


Diagram 6-1 - Plates showing growth after swabs were taken of residue from dried culture on Polysan treated surfaces.

Culture Contact With Surface

As with the previous method, empty plastic petri plates were treated with 200µl Polysan solution, though this time only two levels were tested- 100% Polysan, and sterile RO water. After these had dried, 20µl of culture as used previously were applied to the plates, mixed with a sterile loop, then transfered to MHA, which was incubated at 30°C for 24 hours. Total contact time between culture and the surface was 30 seconds. Diagram 6-2 shows the growth on the agar plate.

With Polysan



Without Polysan

Diagram 6-2 - Growth on MHB after inocula was in contact with treated plate surfaces

Conclusion

There is truth in the fact that Polysan leaves a layer which is still active against bacteria.

It is very fast acting in the latter experiment, as live culture only has 30 seconds contact and was not allowed to dry out- which is more favourable to the organism than in the first experiment. In this first experiment, which is likely to me more like real-life conditions, it is shown that dried polysan at the equivalent of 0.26ml per m^2 is sufficient to kill *E. coli* cells which come in to contact with it at the concentrations used here.

One reason this happens is that, when culture is applied it is in liquid, which could re-dissolve active compound, kills cells and leaves no viable cells for re-growth. This is still encouraging as when bacteria are normally transferred there is a certain amount of moisture transfered with them anyway.

Final Conclusions

As set out at the beginning of this report, the aim was to confirm several claims about Polysan's efficacy against bacteria by it's manufacturer.

Firstly, it was claimed that it is effective against a host of organisms which are linked to the spread of disease. The more notable of these were *Clostridium difficile,* methicillin-resistant *Staphylococcus aureus, Escherichia coli, Bacillus* species and *Pseudomonas aeroginosa.* There are many more organisms which Polysan is reported to kill, but for the purposes of this project it was decided to limit the organisms screened to the most noteworthy. Also, with the different morphology displayed between the organisms tested, it is a fair indicator of Polysan's potential broad anti-microbial spectrum.

In these studies it was found that it was effective against all of the aerobic organisms in growing culture. As discussed before, this gave the organisms some advantage, as they were treated during the log phase of growth when the cells were dividing rapidly and were in abundance, the temperature was just below the optimum for growth and they were suspended in a solution with all the nutrients necessary for growth. In the real world, Polysan would be used against a much smaller number of cells, which would not be in an environment optimised for growth and survival, which is why it was concluded that Polysan is effective at a much higher rate than is probably necessary.

It could be argued that during these real world conditions, stress factors would cause certain organisms to employ survival measures- the best example of course is sporulation- and therefore more difficult to eradicate than cells in log growth phase. However, as shown here, Polysan is able to stop colonisation of *C. diff* spores on Braizer's agar, which is employed specifically for that purpose. As already discussed this could be down to anything between the active compound(s) destroying the cells on germination to complete destruction of the spores- more insight into this could be yielded from further tests but either way the results are very impressive- it was also proven that the kill time of Polysan is less than 30 seconds, as described, even in conditions which favour the organism.

Another impressive boast about Polysan is that is forms a layer which helps prevent cross contamination for up to 7 days. In order to prove this extensive real-world evaluation should be performed, and also as no chemical analysis of any of the components has been made here, there is no direct evidence that this is true. However the *in vitro* studies detailed in this report suggest that it is the case- other agents used for decontamination, such as alcohol, are volatile, and once evaporated leave no bactericidal barrier to prevent cross contamination. Polysan at least has this property.

Overall, Polysan has lived up to each of the claims which have been tested in this report which should encourage confidence in this product, and on the basis of the work carried out here Polysan should be considered a serious contender in it's market.

Appendix I - Media

Tryptone Soy Broth (TSB)

Manufacturer: **Oxoid**

Product: CM0129B

Preparation: Xg is dissolved in 1000ml RO.H_2O, and 100ml aliquots are autoclaved at at 121°C 15 mins in medical flats. This is aseptically transfered to shakeflasks as needed.

Muller Hinton Agar (MHA)

Manufacturer: **Oxoid**

Product: **CM0337B**

Preparation: Xg is dissolved in 300ml RO.H_2O and dispensed into a 500ml Duran bottle. This is autoclaved at 121°C 15 mins. 30ml molten agar is then dispensed into sterile petri plates and allowed to dry before inoculation.

CCEY / Braizer's Agar

Manufacturer: **Bioconnections**

Product: **BC2160**

Preparation: Suspend and soak 24g of agar powder in 500ml RO.H_2O. Sterilise at 121°C 15 mins. Molten agar is then cooled to 47°C and 5ml of lysed defibrinated horse blood, supplements S2093 cycloserine/cefoxitin (5g), and 20ml S2073 egg yolk emulsion are added. 20ml of agar is dispensed into sterile petri plates and allowed to dry before inoculation. These plates are stored at 4°C.

Appendix II - Stock Culture

Clostridium Difficile Spores

Subculture *C. difficile* on to Braziers (C.C.E.Y.) agar. Incubate at 37°C in anaerobic atmosphere for 48 h. Pre-reduce in anaerobic atmosphere 10 fresh blood agar (FBA) plates overnight. From single plate inoculate (with sterile swabs) all 10 FBA plates. Incubate in anaerobic atmosphere at 37°C for ~ 10 days for sporulation. Scrape spore growth from all 10 plates (with sterile swabs) into 1 ml sterile NaCl (0.9%) and add equal volume 100% EtOH (EtOH will kill any vegetative cells leaving only spores). Alcohol shock for 1 hour at room temperature then centrifuge at 3000G for 10 minutes, and re-suspend in 1ml sterile NaCl (0.9%). Keep at 4°C indefinitely for working stock.

Glycerol Suspension Stocks

Each organism was cultured overnight in 50ml TSB in 250ml flasks, shaken at 250rpm 30°C. Culture was then mixed 1:1 with 20% glycerol, dispensed into cryogenic vials and stored at -80°C indefinitely. Vials thawed before use.

Appendix III - Test Dilutions

Dilutions of Polysan Used In Minimum Bactericidal Concentration (MBC) Experiments

A 1/2 dilution series of Polysan in sterile RO.H_2O was made for the stock solutions used in all MBC experiments detailed in this report. These were made and stored in sterile universal flasks. A 20ml aliquot of Polysan was dispensed, and 10ml was transfered to 10ml sterile RO.H_2O to make a 1/2 dilution. This series was continued until a 1/64 concentration was made (diagram A3-1).

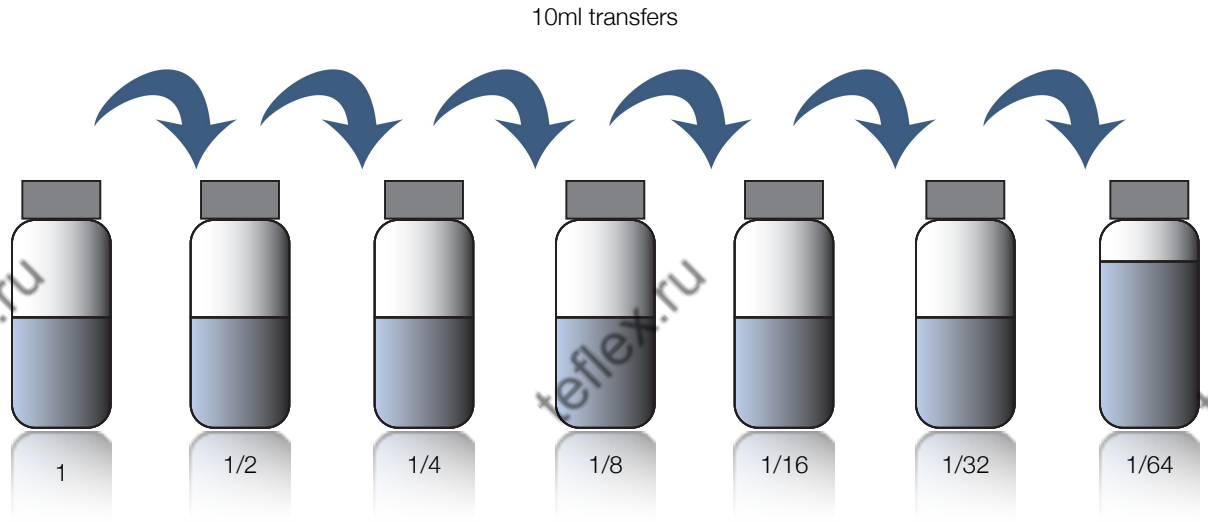


Figure A3-1 - Serial dilutions of Polysan