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Aqualution Trial Report

Project title	Misting trial at a food processing factory		
Sector	Facilities; Food	Collaborator	Anonymous
Participants	ML, NM, AT		
Start date	20/01/2021	Location	Scotland

Project aim

The aim of this project was to assess the decontamination efficacy of Salvesan applied as a mist in communal spaces. Salvesan is a fast acting biocide based on hypochlorous acid (HOCl). Assessing efficacy was achieved by measuring micro bioburden on hand-touch surfaces / the environment before and after the misting process. Misting was achieved using a high-pressure sprayer provided by Aqualution / Air Pumping. The mist particles are typically 4-5 microns in diameter which allows the mist to float freely in the air (rather than drop quickly) and also prevents wetness on floors / surfaces.

Project Outline

It is imperative that people are kept safe at work, especially in confined spaces / areas with high concentrations of workers / areas with high rates of travel.

SARS-CoV-2 is an enveloped virus approximately 60-140nm in diameter. Transmission occurs through touch or aerosol spreading. During speech, humans emit thousands of oral fluid droplets per second which can remain airborne for 8-14 minutes (Stadnytskyi *et al.*, 2020). SARS-CoV-2 is detectable in surface aerosols for up to three hours, up to four hours on copper, up to 24h on cardboard and up to 2-3 days on plastic and stainless steel (van Doremalen *et al.*, 2020). There are also reports suggesting that coronaviruses can survive for 10 to 100 days in water, depending on the temperature (Gundy and Gerba, 2009), with the virus remaining for longer in lower (<4°C) temperature than higher (>23°C) temperature water.

There is a need to disinfect surfaces potentially exposed to SARS-CoV-2 to prevent / reduce the risk of transmission from healthy but asymptomatic people. An ideal disinfectant and sanitiser must be fast acting, broad spectrum, safe for humans and the environment, easy to use and relatively inexpensive. Hypochlorous acid may be the disinfectant of choice. Salvesan hypochlorous acid is effective against bacteria, viruses, protozoa, fungi, yeasts, moulds and spores (BS EN accreditations include 1276:2009, 13697:2001, 13704:2002, 14476:2013). Figure 1 shows the general order of susceptibility of various classes of microorganisms to disinfectants. Different classes of microorganisms exhibit different degrees of susceptibility due to biochemical and biophysical characteristics of the organism. Enveloped viruses, such as coronavirus, are the most susceptible to disinfectants ie the easiest to kill.

Figure 1: Order of susceptibility of microorganisms to disinfectants

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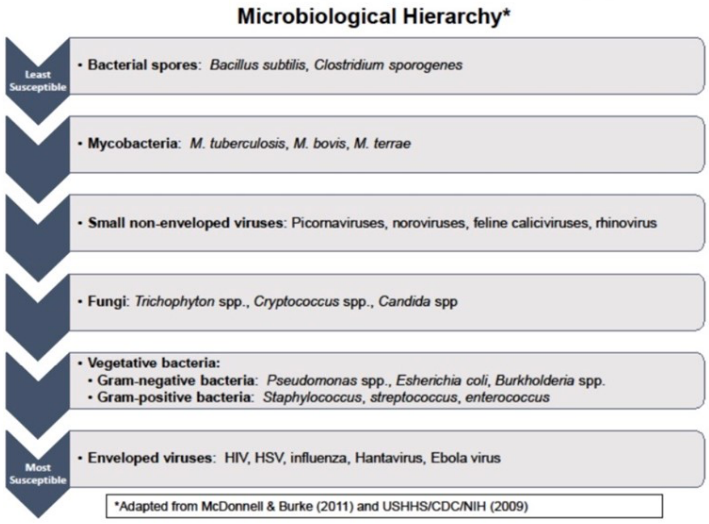
Figure 1. Microbiological disinfection hierarchy. Examples of microorganisms in each category are provided.



Salvesan has very low contact times and is safe for people and the environment. It can be misted into the environment when people are in place with no deleterious effects. It can be sprayed onto a surface and left with no fear of toxic residues. Workers do not need any PPE to handle it. It can be used on soft and hard surfaces. It is extremely versatile and can be used as a dip, spray, mist or fog. It is simple to use therefore compliance is high. It does not lose efficacy at low temperatures. It has a 12 month shelf life if kept under suitable storage conditions. As such Salvesan offers a safe and efficacious disinfection option in domestic, commercial, community and healthcare settings.

Selecting the most suitable disinfectant is one of the two components essential for effective environmental disinfection. The other component is ensuring that the disinfectant contacts all surfaces, is given the correct dwell time and that manufacturers label instructions are followed. There has been considerable interest from all sectors in the potential of mist as a decontamination strategy: environmental misting potentially allows a disinfectant to reach areas that would not normally be sanitised, requires minimal human input and provides visual reassurance that something positive is being done.

At Aqualution Systems we have carried out extensive misting work over the last decade and have several commercial applications where misting is highly effective, both against bacteria and viruses (eg the decontamination of raspberries amid fears of Hepatitis A and Norovirus). To date our experiments in healthcare have consistently demonstrated that there is no substitution for "elbow grease" and that the highest decontamination rates are achieved using a traditional spray and wipe technique. This is because dust, dirt and organic matter can create protective reservoirs for pathogens if not effectively removed.

However, we have found that misting after physical cleaning can achieve additional decontamination benefits above physical cleaning alone.

The aim of the current study was to assess how effective misting Salvesan is in the communal areas of a working chilled food factory. The trial was carried out in four communal areas of the factory: canteen, handwashing area (pre entrance to the packing hall), female WCs and male WCs. We investigated decontamination rates on a range of surfaces immediately after environmental misting with Salvesan. Surfaces of different materials were chosen. The majority of the surfaces (22 of the 28) were hand touch sites; three of the remaining surfaces were floors (to give a worst case scenario) and the reservoirs of hand dryers (as they potentially harbour significant biofilm).

Methodology

The site was asked not to clean the area prior to our arrival to ensure that it was well trafficked and had sufficient levels of microbial contamination. Sampling sites were chosen across a range of surfaces in the chosen areas. The areas were light, warm and visually clean.

All samples were taken using sterile trans-swabs. Sampling sites were fitted with temporary templates (area c 100cm^2) to ensure that pre and post treatment swabbing areas were the same. Some sampling sites were not sufficiently large to accommodate a template so they were swabbed free hand. When swabbing free hand care was taken to choose an area that would be easy to replicate i.e. the whole fridge handle and the whole label on the bottle of hand sanitiser. Initial samples were taken under "as found" conditions. The area was then misted. Misting took 4-10 minutes depending on the size of the area. This was followed by a 5 minute dwell time. The same areas were then re-swabbed by the same person using the same technique – these were the post-mist samples.

A total of 28 sampling sites were swabbed so 56 swabs were obtained in total (28 pre treatment and 28 post treatment per sampling site). Swabs were couriered to the lab (One Scientific, Avonmouth) to arrive before 9am the following morning. Swabs were analysed for aerobic colony counts (ACC) using the pour-plate technique. All swabs were cultured successfully, and all results were reported.

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Results: Table 1: Aerobic Colony Counts (ACC) before and after treatment (colony forming units, cfu)

Swab area	Sampling site	Material	Hand touch?	Swabbing area		ACC (cfu per swab)		Log		
				Template	Free hand	Pre	Post	Pre	Post	Reduction
Canteen	Locker #138	Painted metal	Y		*	30	250,000	1	5	+ 4
Canteen	Microwave door	Plastic	Y	*		10	10	1	1	-
Canteen	Table 1	Veneer	Y	*		3,600,000	<10	6	0	6
Canteen	Table 2	Veneer	Y	*		37,000	560	4	2	2
Canteen	Sink drainer	Stainless steel	Y	*		10,000,000	10,000,000	7	7	-
Canteen	Washing up liquid label	Paper	Y		*	20	<10	1	0	1
Canteen	Chair seat	Plastic	Y	*		330,000	290,000	5	5	-
Canteen	Chair back	Plastic	Y	*		10,000,000	<10	7	0	7
Canteen	Fridge handle	Plastic	Y		*	1,600	<10	3	0	3
Canteen	Floor	Lino	N	*		10,000,000	10,000,000	7	7	-
Canteen	Hand gel label	Paper	Y		*	810	<10	2	0	2
Canteen	Face mask bin lid	Plastic	Y		*	30,000	26,000	4	4	-
Female WC	Door plate (entrance)	Stainless steel	Y	*		5,000	<10	3	0	3
Female WC	Counter by sinks	Veneer	Y	*		10,000,000	<10	7	0	7
Female WC	Floor	Lino	N	*		10,000,000	10,000,000	7	7	-

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Female WC	Cubicle door	Veneer	Y	*		10,000,000	<10	7	0	7
Female WC	Cubicle door lock	Stainless steel	Y		*	10,000,000	<10	7	0	7
Female WC	Dyson dryer	Plastic	N		*	190	10,000,000	2	7	+ 5
Handwash	Soap dispenser RHS	Plastic	Y		*	10	<10	1	0	1
Handwash	Soap dispenser LHS	Plastic	Y		*	10,000,000	4,500	7	3	4
Handwash	Sanitiser LHS	Plastic	Y		*	130,000	<10	5	0	5
Handwash	Hand cream RHS	Plastic	Y		*	65,000	<10	4	0	4
Handwash	Towel dispenser	Plastic	Y		*	6,200	400,000	3	5	+ 2
Male WC	Dyson dryer	Plastic	N		*	1,200,000	10,000,000	6	7	+ 1
Male WC	Door plate (entrance)	Stainless steel	Y	*		240	<10	2	0	2
Male WC	Floor by urinal	Lino	N	*		10,000,000	10,000,000	7	7	-
Male WC	Cubicle door	Veneer	Y	*		10,000,000	<10	7	0	7
Male WC	Cubicle door lock	Stainless steel	Y		*	10,000,000	<10	7	0	7

Results

The average count for all 56 swabs was 3,149,593 cfu per swab. The average pre treatment count for all 28 swabs was 4,121,647 cfu (range 10 to 10,000,000 cfu) and the average for the 28 post treatment swabs was 2,177,539 cfu (range <10 to 10,000,000 cfu). However, these figures are somewhat misleading. Normally when looking at data the different measurements are distributed on a bell curve with most lying "somewhere in the middle" and a few high and a few low. In this dataset 11 of the 28 pre treatment samples (40%) and 6 of the post treatment samples (21%) achieved swab counts of 10,000,000 per swab. These very high numbers mask what is really happening if we just rely on averages. Microbiology tends to yield very high and very low results which means that the data are skewed and averages are not representative. For this reason microbiology datasets are often described and compared using median (the middle number in a set) and mode (the number which occurs most frequently) as these better reflect trends.

The areas we chose to sample were a mixture of hand touch sites (where we expected good success rates), floors (where we did not expect good success due to the organic loading present) and other surfaces which were neither hand touch or floor (eg the groove in the base of the Dyson hand dryers). The following tables summarise the mean, median and mode log counts for pre and post samples in all areas (all 28 sites, table 2) and hand touch sites only (22 sampling sites, table 3).

Table 2: Mean, median and mode results for all sampling sites

	Pre (cfu)	Post (cfu)	Pre Log	Post Log	Log reduction	% cfu reduction
Mean	4,121,647	2,177,539	5	2	3	99.9
Median	230,000	<10	5	0	5	99.999
Mode	10,000,000	<10	7	0	7	99.99999

The numerical average reductions appear to be unremarkable. However, once log transformed there is an average 3 log reduction (ie 99.9% of bacterial burden is removed through the misting process). The median figure pre misting was 230,000 cfu per swab. After misting it was <10 which represents a 5 log reduction. The most frequently observed result (mode) pre misting was 10,000,000 per swab (11 observations) and after misting was <10 cfu per swab (15 observations).

The highest results, as expected were seen in the floor swabs. The mist was not effective here due to high organic loading. It may be that longer exposure time would increase effectiveness. The trial looked purely at non-contact disinfection. The Aqualution team requested an uncleaned environment to give the misting process a tough challenge. If the misting process was used as a disinfection step after a detergent clean then reductions in

floor swabs may be seen. The other surprising area was the Dyson hand dryers which were sampled in the female WC and the handwashing area: pre-treatment swab results were significantly lower than post treatment results in both sites. The method of sampling was to rub the swab along the whole length of the groove in the base of the dryer 10 times. The groove could potentially harbour water and therefore biofilms may have an opportunity to adhere and thrive. We have observed increased post treatment cfu counts in a small number of swabbing areas in our work in the healthcare environment (NHS Intensive Care Unit). The clinicians involved in that study hypothesised that the hypochlorous acid was breaking down the biofilms present and releasing the trapped bacteria held within the biofilm matrix which is why the counts were higher after treatment. This is a possibility in the hand dryers.

Table 3: Mean, median and mode results for all hand touch sites

	Pre (cfu)	Post (cfu)	Pre Log	Post Log	Log reduction	% cfu reduction
Mean	3,826,178	497,504	4	1	3	99.9
Median	97,500	<10	5	0	5	99.999
Mode	10,000,000	<10	7	0	7	99.99999

The trends are very similar when looking solely at hand touch sites, though the median pre counts were, as expected, slightly lower when the floors were excluded. Again the most commonly observed pre-treatment count was 10,000,000 cfu per swab (8 observations, 36%) and the most commonly observed post treatment count was <10 cfu per swab (15 observations, 68%). The veneer cubicle door and stainless steel lock mechanisms in both the female and male WC were all 10,000,000 cfu prior to treatment and all <10 cfu after treatment. Misting Salvesan conferred a median 5 log reduction on surface bacteria on hand touch sites ie 99.999% of bacteria were removed by the treatment.

There are no standard acceptable levels of surface bioburden for hand touch sites in hospitals. However, proposals of <2.5cfu/cm^2 have been proposed by NHS researchers / clinicians. This equates to 250 cfu/swab. Of the 22 hand touch areas chosen, 77% failed this criteria prior to misting. After misting hypochlorous acid 73% of sites passed (with 94% of those passes achieving results of <1 cfu/swab). This pass rate would be even greater if the Salvesan was used as a disinfection step after a traditional detergent clean.

These studies looked at bacterial counts. We look at bacteria because they are relatively easy and cost effective to analyse. This picture shows how resistant (ie hard to kill) the different micro-organism groups are to disinfectant agents. The hardest organisms to kill are bacterial spores. Efficacy testing against different classes of micro-organisms is carried out by independent laboratories in accordance with strict British Standards testing criteria. The tests are based on a range of target organisms and in order to pass a disinfectant must achieve a

minimum kill percentage within a specified time frame. The organisms are chosen to reflect the range of organisms in that class eg both gram positive and gram negative bacteria species are included in the bactericidal tests. The idea is that if a disinfectant passes the relevant BS EN test then it will work against other micro-organisms in that category eg the bacterial tests usually feature *Pseudomonas*, *Staph aureus*, *E. coli* and *Enterococcus* species as the target organisms but any disinfectant that passes can claim efficacy against other bacteria. This standardises testing and minimising the amount of testing required to claim efficacy. We have demonstrated that Aqualution products are effective against bacterial spores (eg BS EN 13704:2002), mycobacteria (eg BS EN 14204:2012), non-enveloped viruses (eg BS EN 14476:2013), fungi (eg BS EN 13697:2001) and enveloped viruses (eg BS EN 14476:2013). SARS-Cov-2 is an enveloped virus and is therefore the most susceptible of all the categories listed ie it is very easy to kill (outside the body). Whilst we cannot make official claims for efficacy against SARS-Cov-2 we have proven efficacy studies against a range of other enveloped viruses eg HIV, influenza, hepatitis. British standards viral tests require that 99% of virons are killed after 30 minutes contact time AND 99.99% after one hour. Aqualution products achieved a 4 log (99.99%) kill in 5 minutes which is the first possible testing time in these studies.

Conclusions

Misting with Salvesan hypochlorous acid resulted in a significant and repeatable effect on surface log counts achieving 5 log (99.999%) median reductions. Modal figures suggested a 7 log reduction ie 99.99999% of surface bacteria were removed.

Surface bioburden was <1 cfu/cm^2 on 73% of hand touch surfaces after misting. The high pressure air system is suitable for applying Salvesan in a range of environments (ambient and chilled) and its high output rate means it can treat large areas efficiently and quickly.