POLAR ICETECH

Dry Ice Blasting Specialists

Demonstrating the efficacy of the Dry Ice Blast Cleaning Process against bacterium Escherichia coli, Salmonella Enteritidis, and Listeria monocytogenes on a range of different substrates

in association with

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Demonstrating the efficacy of the Dry Ice Blast Cleaning Process against bacterium Escherichia coli, Salmonella Enteritidis, and Listeria monocytogenes on a range of different substrates

Study Overview:

Polar IceTech work with a large number of manufacturing and production customers who have requirements for cleaning direct-product contact surfaces. Their cleaning processes must meet the strict cleaning process of the EPA and the FDA.

International claims have been made that Dry Ice Blasting kills 99.9% of all bacteria, however Polar IceTech felt it was important to have these claims independently assessed, and, to have the tests undertaken on substrates in common use, most especially within the food & beverage industry.

Having been successful in an application for research with Enterprise Ireland, Polar IceTech engaged with the Cork Institute of Technology (CIT) and The Centre for Advanced Photonics and Process Analysis (CAPPA) to have this study carried out.

Under the direction of Professor Jim O'Mahony (Project Manager) and Dr Lorraine Endersen (Lead Scientist), four different substrates were heavily contaminated with E Coli, Listeria and Salmonella. These substrates were then cryogenically cleaned with dry ice (also known as Dry Ice Blasting) and swobbed afterwards to see if the process was successful in decontaminating the surface. Adjacent surfaces, which were not originally contaminated with the bateria, were also swobbed after the cleaning to investigare if the process had cross-contaminated these surfaces with the bacteria.

Study Objective

1. To determine if Dry Ice Blasting (cryogenic cleaning) is successful in decontaminating a variety of commonly used substrates which have been contaminated with excessive levels of E coli, Salmonella and Listeria.
2. To determine if the process of Dry Ice Blasting cross transfers and contaminates nearby areas with the aforementioned bacterium.

Substrates tested:

- Nylon
- Stainless Steel (316/304)
- Slate Steel (Mild Steel)
- Ceramic

Results:

Substrate	E. coli		Listeria		Salmonella	
	Test % Reduction	Bank % Reduction	Test % Reduction	Bank % Reduction	Test % Reduction	Bank % Reduction
	Contaminated Surface	Cross Contamination	Contaminated Surface	Cross Contamination	Contaminated Surface	Cross Contamination
Ceramin	100%	100%	99.99%	99.99%	99.96%	100%
Stainless Steel	100%	100%	100%	100%	100%	100%
Slate Steel	99.95%	100%	99.99%	99.99%	99.62%	100%
Nylon	98.73%	100%	99.99%	99.99%	100%	100%
*results show what is the % reduction of the conatminated bacteria following Dry Ice Blasting (aka Cryogenic Cleaning)						

What does this mean for companies who are using Dry Ice Blast Cleaning?

The results of this study offers significant reassurance to manufacturers and producers of consumable products that Dry Ice Cleaning is a safe, non-toxic process which will both clean and decontaminate against harmful bacterial such as E coli, Listeria and Salmonella in one step.

Rights over this Study

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Department of Biological Sciences, Cork Institute of Technology, Rossa Avenue, Bishopstown, Cork.

Project manager: *Professor Jim O'Mahony*

Lead Scientist: *Dr Lorraine Endersen*

Project title: Demonstrating the efficacy of the Dry Ice Blast Cleaning Process against bacterium Escherichia coli, Salmonella Enteritidis, and Listeria monocytogenes on a range of different substrates.

Objectives:

(1) To determine the natural level of contamination/microbial load present on a selection of substrates to be challenged with the dry ice decontamination process during the study.

(2) To determine the viability of *Escherichia coli*, *Salmonella Enteritidis*, and *Listeria monocytogenes* on a selection of four different substrates following dry ice cleaning at set range of predetermined exposure times.

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***Sampling strategy*: Objective 1**

- *This part of the project involved surface sampling of the substrates provided to recover any viable bacterial and fungal isolates that may be present.*
- *For cultivation of bacterial and fungal isolates, sampling was performed on Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA), respectively.*
- *TSA plates were incubated at 37°C for 24 h*
- *SDA plates were incubated at 30°C for 4-7 days*
- *Following incubation relative contamination rates per substrate were calculated by standard enumeration of viable microorganisms.*

Figure 1: *Graphic representation of viable cell recovery procedure from substrates.*

***Sampling strategy*: Objective 2**

- *Cultures of Escherichia coli, Listeria monocytogenes,* and *Salmonella Enteritidis* were prepared in LB broth, and TSA broth, respectively. These cultures were incubated overnight at 37°C. Cultures were subsequently adjusted to the desired starting concentration on each of the four substrates under evaluation.

- Substrates were inoculated via a swabbing method with high (10^5 CFU/mL) numbers of *contaminating* cells. During the trial it was found that the highest number of contaminating cells that could be successfully transferred to each of the substrates were ~1-3 x 10^5 CFU/mL.

- *Swabbing method to achieve contaminating levels of ~10^5 CFU/mL:* Swabs were soaked in overnight cultures that were standardised to ~1 x 10^7 CFU/ml. Soaked

swabs were then used to transfer the culture each substrate respectively. Substrates were left to dry for 5 min and culture was re-applied two more consecutive times. Following the final 5 min drying, substrates were exposed to Dry Ice Cleaning for 10 seconds. Control bottles which were not subjected to Dry Ice Cleaning, were performed in conjunction with the test substrate experiments and each of the above outlined experiments were performed in replicates of two on any given test day.

- Following exposure, fresh swabs were soaked in 1 mL of saline solution (Ringer's) and then used to recover surviving cells from the control and Dry Ice Cleaned substrates. The surface area swabbed is as described above for cell application. The surface area was swabbed three consecutive times with the same swab per substrate to ensure efficient recovery of cells. The 1 mL volume of saline solution containing the recovered cells, was then serially diluted and plated onto LB/TSA agar respectively, for selection and enumeration. Plates were incubated at 37°C overnight and surviving cells were expressed as the mean ± standard deviation.

- The efficacy of the decontamination strategy per strain/substrate was determined by comparative analysis between the test and control cell viability counts.

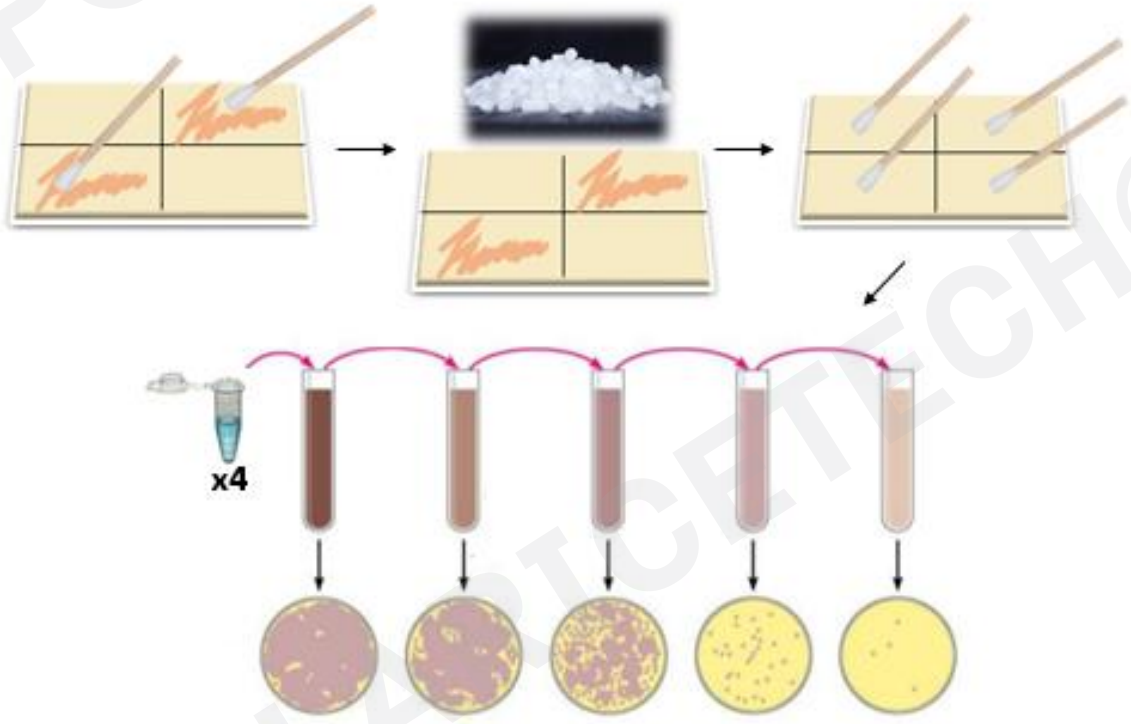

Figure 2: *Graphic representation of sampling application and recovery from substrates during Dry Ice Cleaning challenge experiments.*

Table 1: *Summary of tests performed.*

Microorganism	Substrate type	Replicates	Exposure time	No of tests/1 exposure time
E. coli	*Nylon*	*x2 + control*	*10 sec*	*6*
	Stainless steel	*x2 + control*	*10 sec*	*6*
	Slate steel	*x2 + control*	*10 sec*	*6*
	Ceramic	*x2 + control*	*10 sec*	*6*
S. Enteritidis	*Nylon*	*x2 + control*	*10 sec*	*6*
	Stainless steel	*x2 + control*	*10 sec*	*6*
	Slate steel	*x2 + control*	*10 sec*	*6*
	Ceramic	*x2 + control*	*10 sec*	*6*
L. monocytogenes	*Nylon*	*x2 + control*	*10 sec*	*6*
	Stainless steel	*x2 + control*	*10 sec*	*6*
	Slate steel	*x2 + control*	*10 sec*	*6*
	Ceramic	*x2 + control*	*10 sec*	*6*

Results:

Table 2: *The natural level of contamination/microbial load present on a selection of substrates.*

Total bacteria counts		*Total fungal counts*		
Substrate	***CFU/substrate***	***Substrate***	***CFU/substrate***	***Substrate dimensions***
Ceramic	54	*Ceramic*	1	20 x 20 cm
Nylon A	10	*Nylon A*	0	20.7 x 8 cm
Nylon B	7	*Nylon B*	0	20.7 x 8 cm
Stale Steel A	1	*Stale Steel A*	0	21 x 14.8 cm
Slate Steel B	16	*Slate Steel B*	2	21 x 14.8 cm
Stainless Steel A	17	*Stainless Steel A*	0	29.7 x 21.2 cm
Stainless Steel B	6	*Stainless Steel B*	1	29.7 x 21.2 cm

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E. coli trial

- ***Control*** represents the portion of the ceramic tile that was inoculated with *E. coli* but not dry ice cleaned.
- ***Test*** represents the portion of the ceramic tile that was inoculated with *E. coli* but was cleaned with the dry ice cleaning process.
- ***Blank*** represents the portion of the tile that was tested for cross transfer of *E. coli* across the surface of the substrate during dry ice cleaning of the test portion of the substrate.

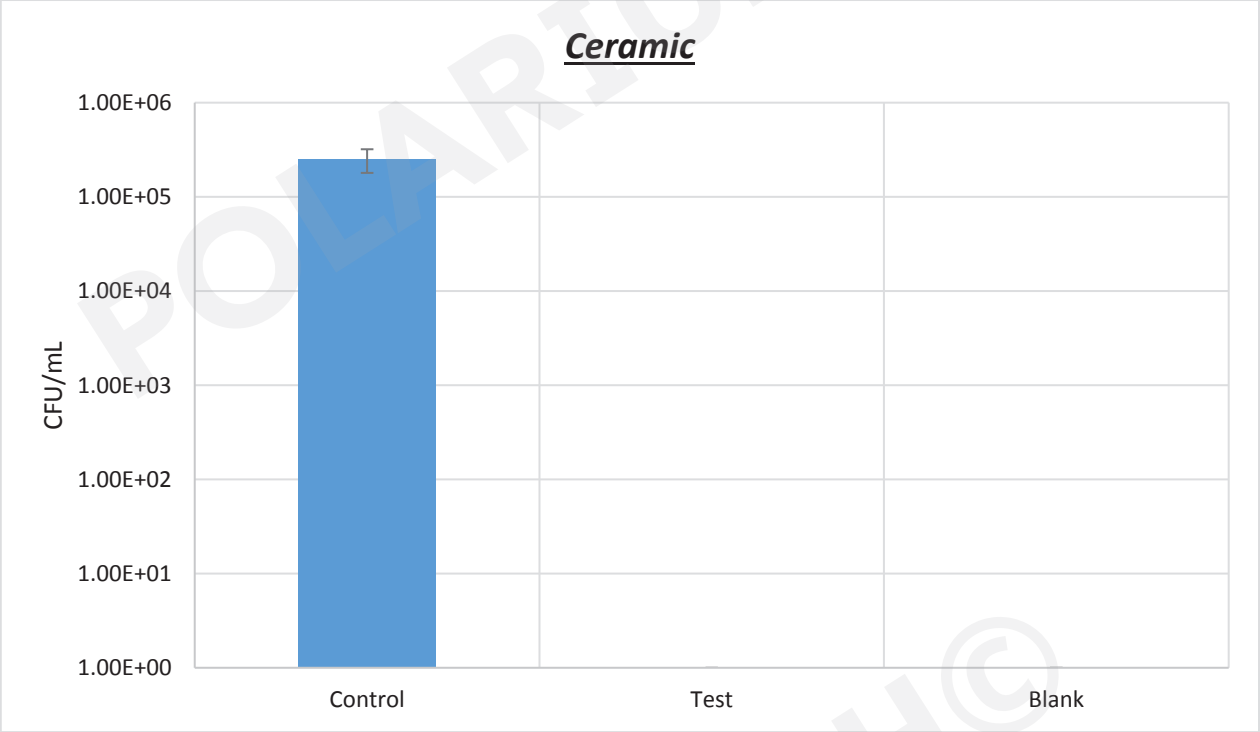

Fig 1: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *E. coli* on ceramic.

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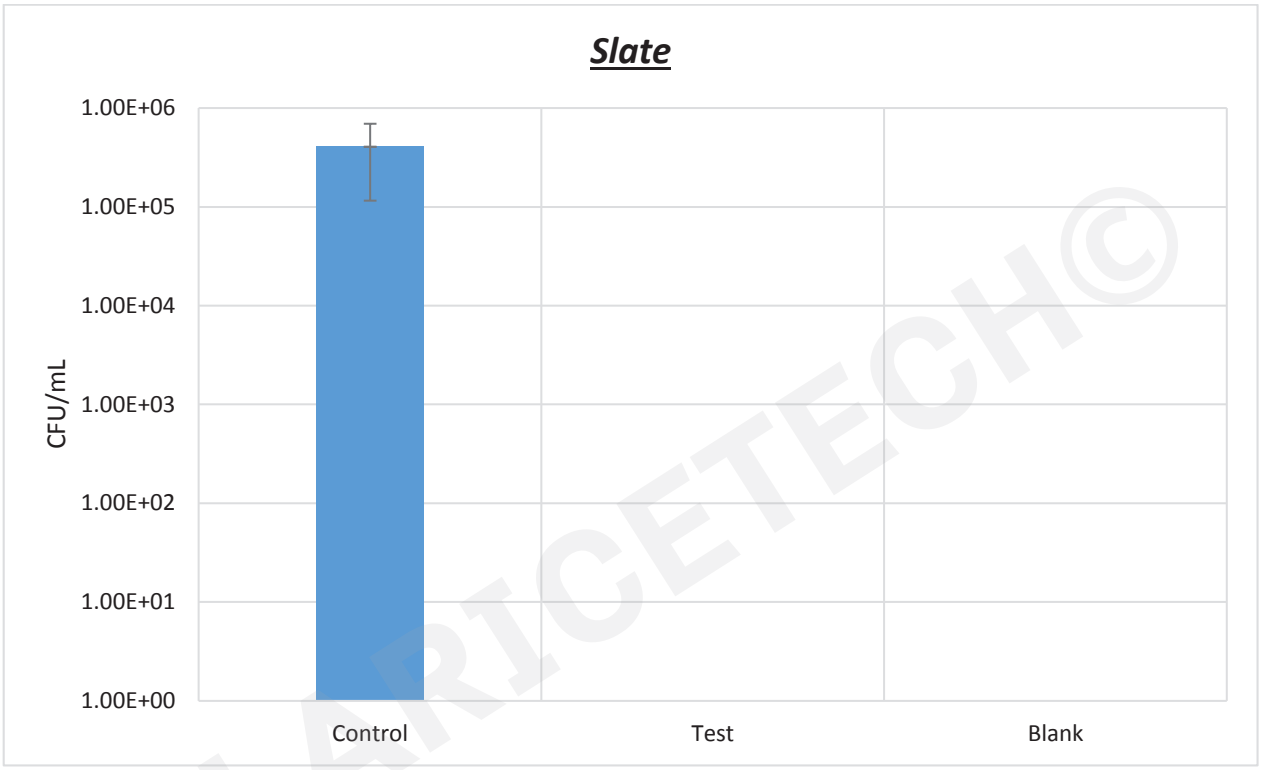

Fig 2: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *E. coli* on slate steel.

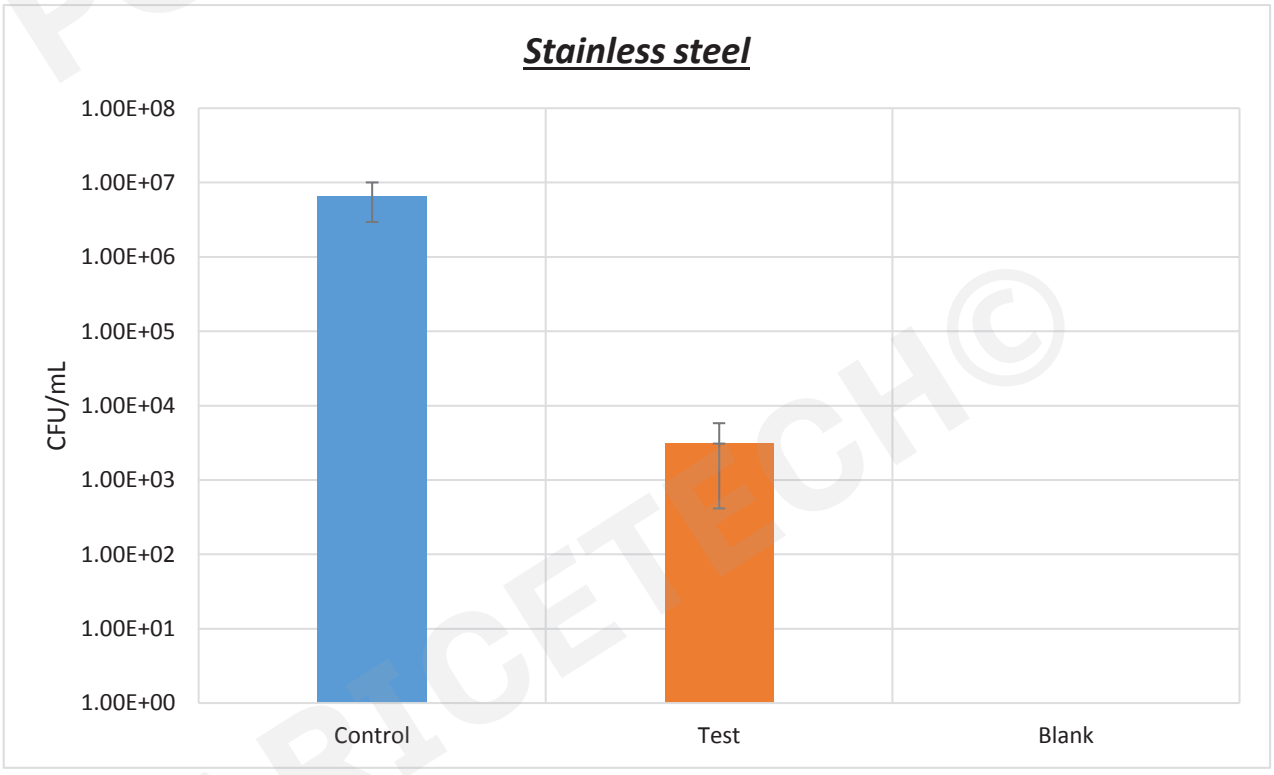

Fig 3: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *E. coli* on stainless steel.

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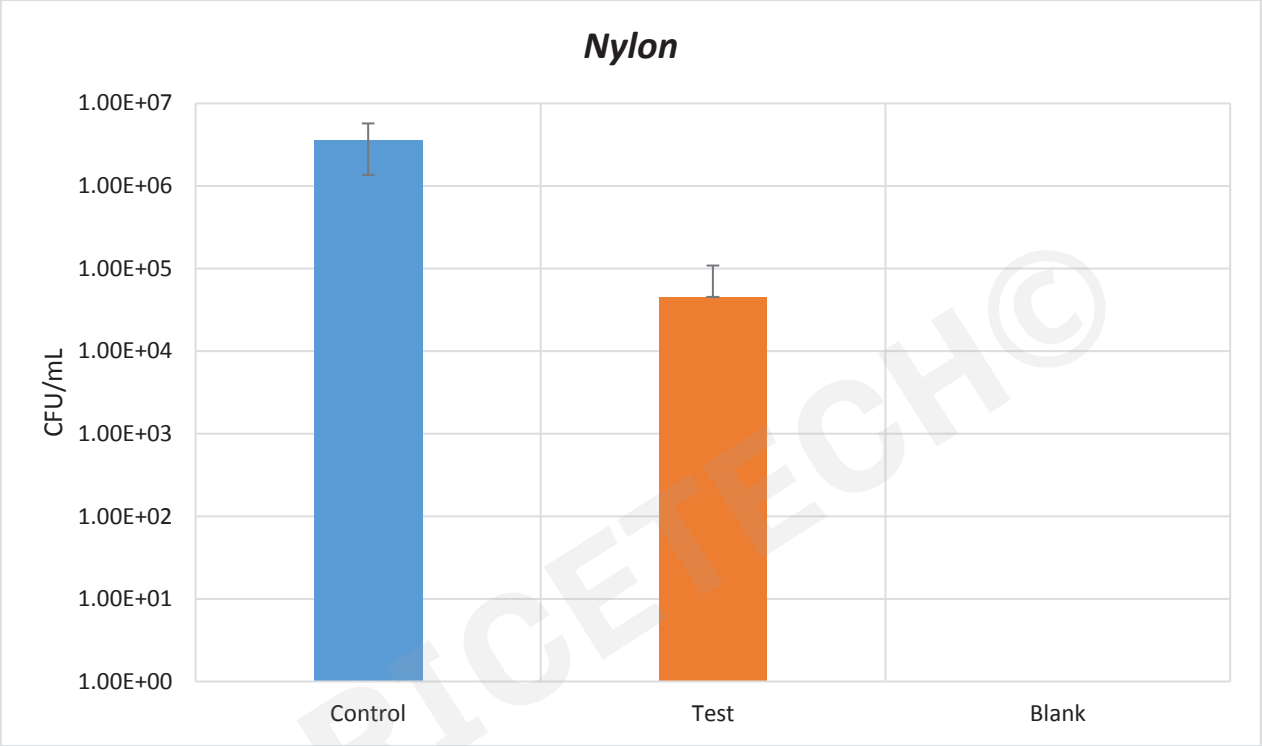

Fig 4: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *E. coli* on nylon.

S. enteritidis trial

- ***Control*** represents the portion of the ceramic tile that was inoculated with *S. enteritidis* but not dry ice cleaned.
- ***Test*** represents the portion of the ceramic tile that was inoculated with *S. enteritidis* but was cleaned with the dry ice.
- ***Blank*** represents the portion of the tile that was tested for cross transfer of *S. enteritidis* across the surface of the substrate during dry ice cleaning of the test portion of the substrate.

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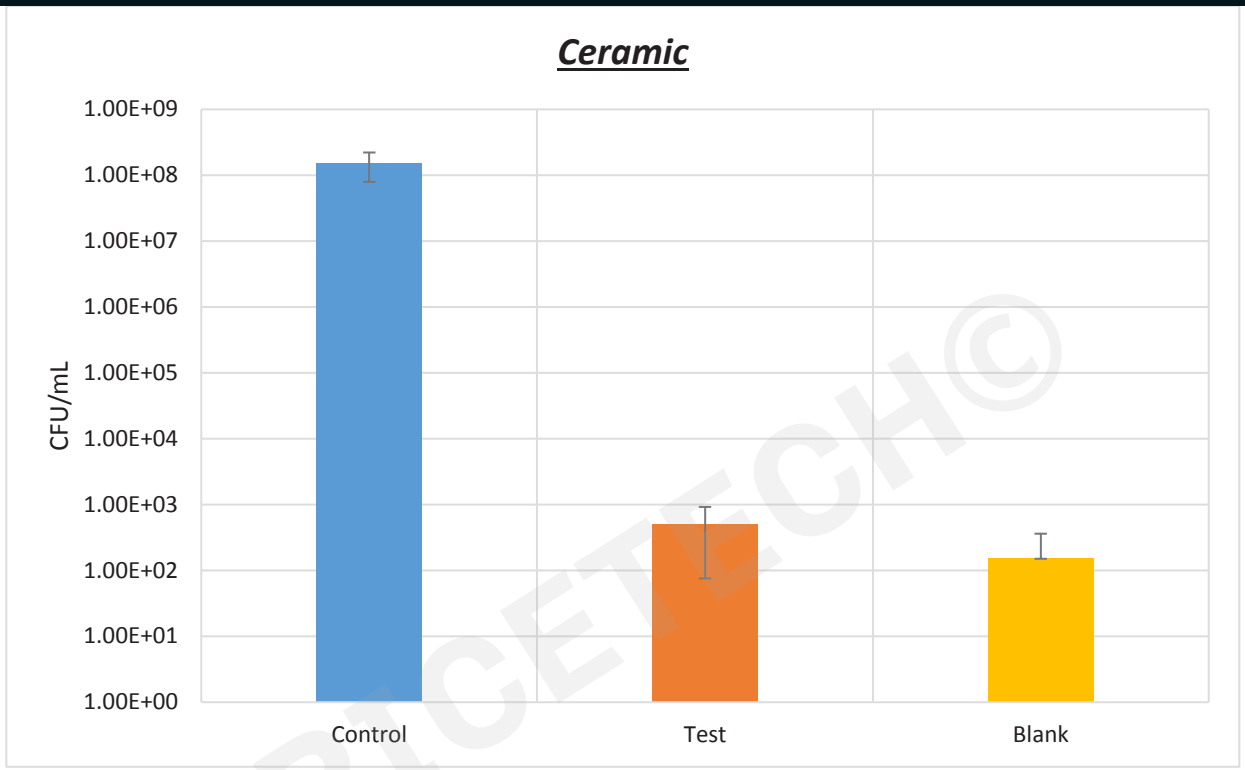

Fig 5: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *S. enteritidis* on ceramic.

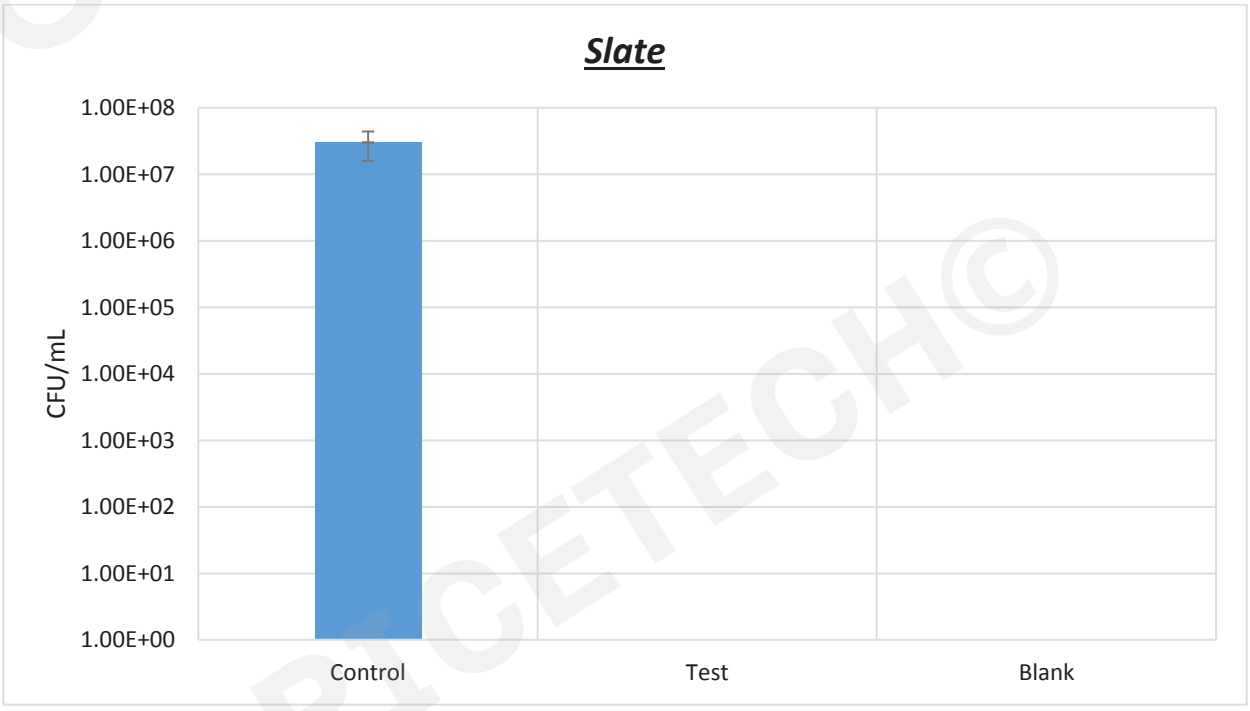

Fig 6: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *S. enteritidis* on slate.

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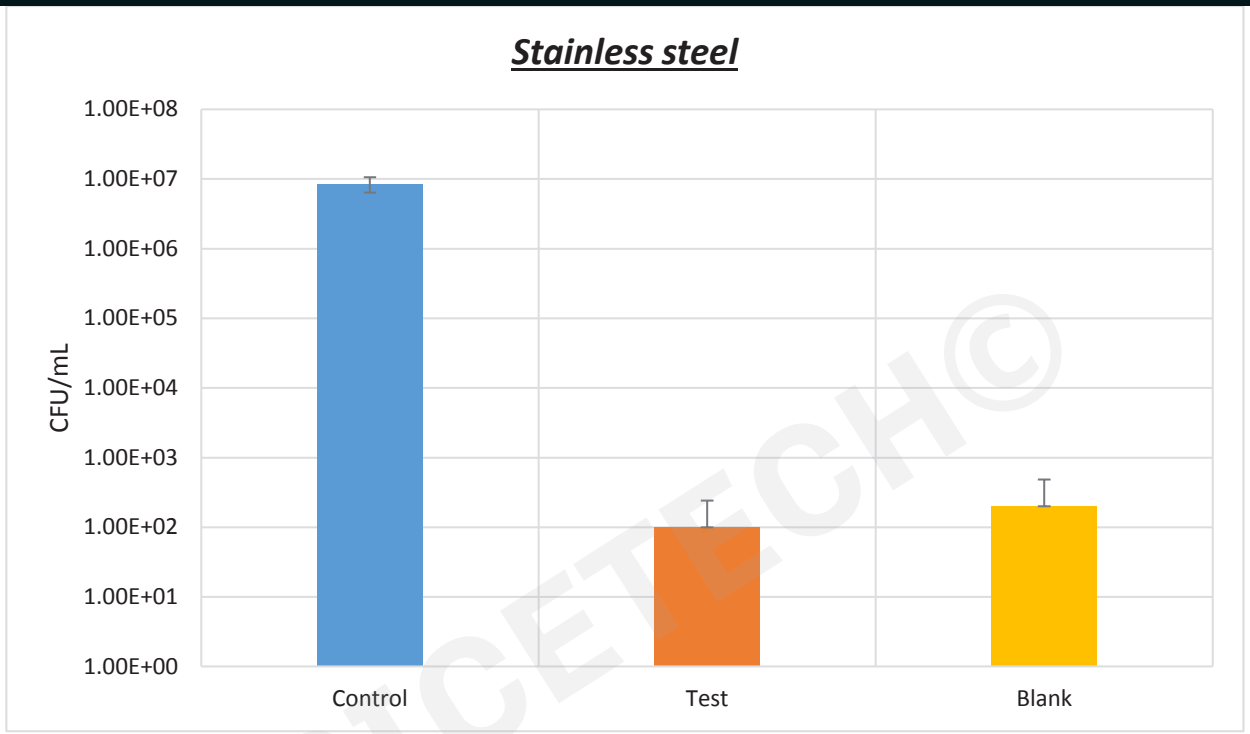

Fig 7: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *S. enteritidis* on stainless steel.

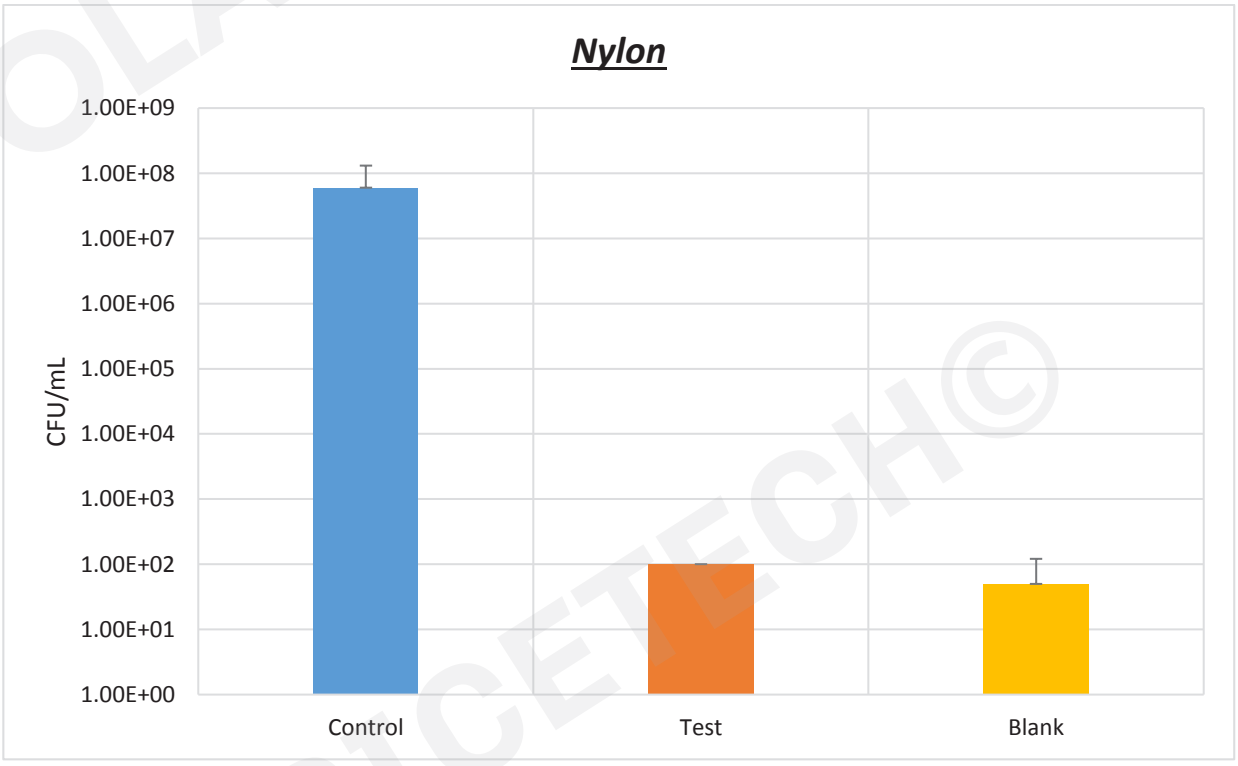

Fig 8: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *S. enteritidis* on nylon.

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***L. monocytogenes* trial**

- ***Control*** represents the portion of the ceramic tile that was inoculated with *L. monocytogenes* but not dry ice cleaned.
- ***Test*** represents the portion of the ceramic tile that was inoculated with *L. monocytogenes* but was cleaned with the dry ice process.
- ***Blank*** represents the portion of the tile that was tested for cross transfer of *L. monocytogenes* across the surface of the substrate during dry ice cleaning of the test portion of the substrate.

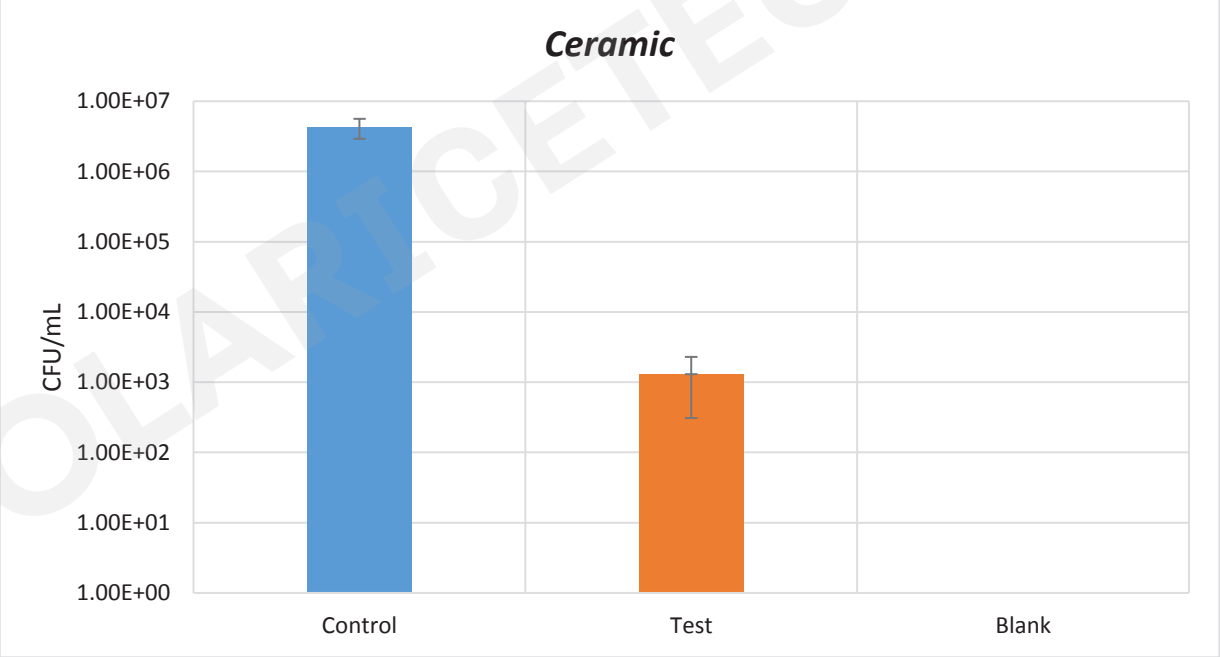

Fig 9: Demonstrating the efficacy of the Dry Ice cleaning process in decontaminating against *L. monocytogenes* on ceramic.

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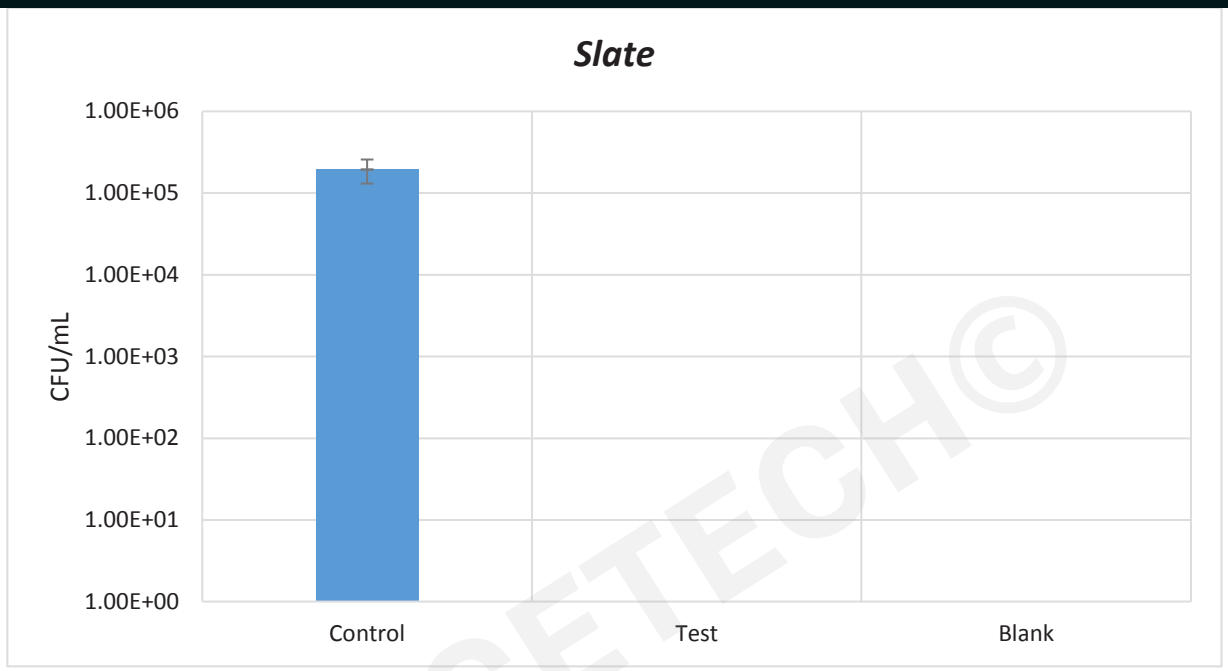

Fig 10: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *S. enteritidis* on slate steel.

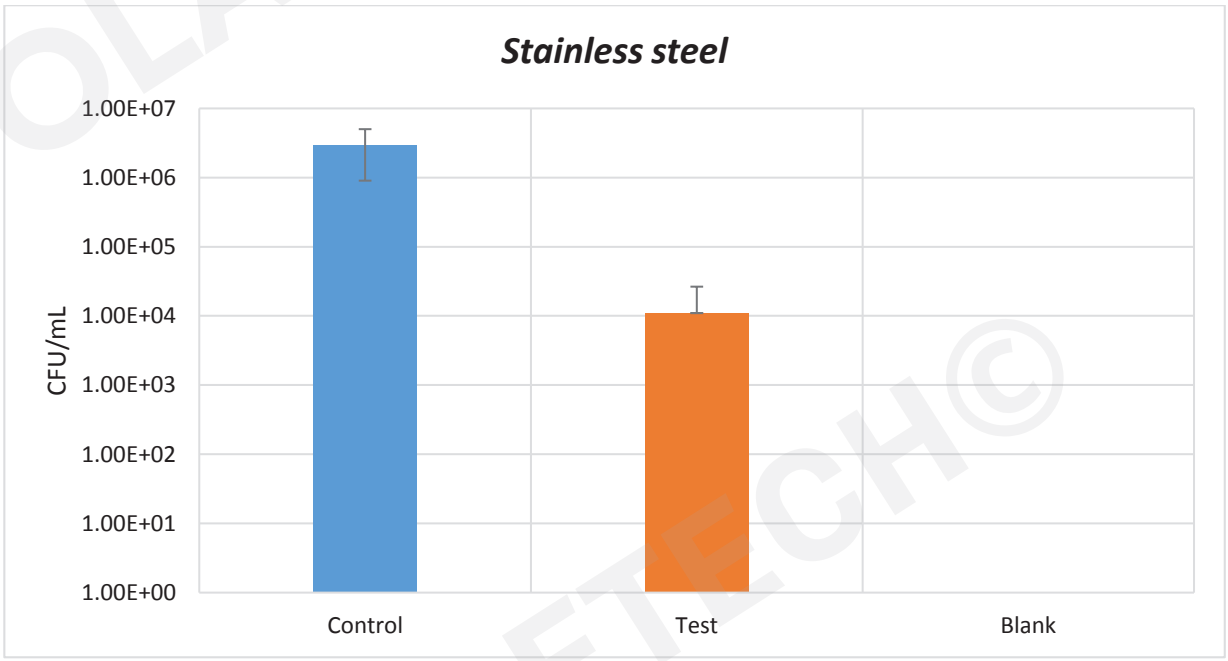

Fig 11: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *S. enteritidis* on stainless steel.

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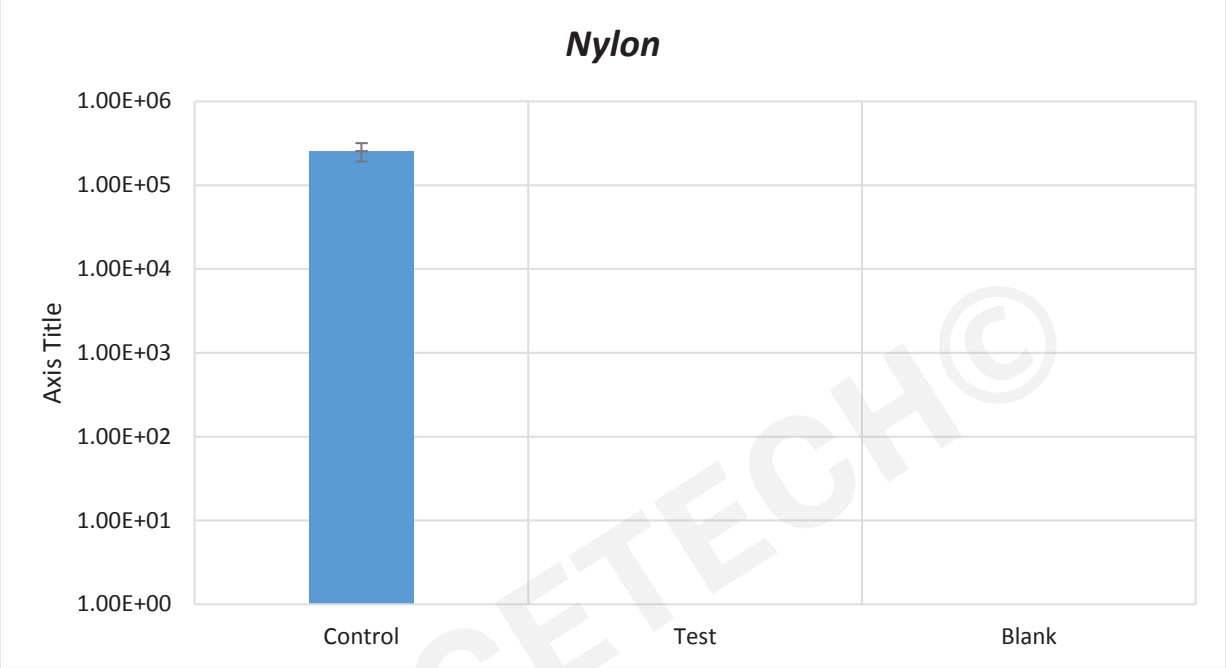

Fig 12: Demonstrating the efficacy of the Dry Ice Cleaning process in decontaminating against *S. enteritidis* on nylon.

Summary of data to part 1 of the study:

Investigating the microbial load present on a range of different substrates.

- A total of 7 different substrates were screened for the presence of bacteria and fungi.
- All substrates were found to harbour very low levels of contaminating microbes.
- 100% of the substrates harboured cultural bacterial contamination, and 42% of substrates were found to be contaminated with fungi.
- Total microbial counts ranged from 0-54 colony forming units (cfu)/substrate.
- Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) were the media of choice for isolating general microbial load.

Summary data to part 2 of the study:

Investigating the efficacy of the Dry Ice Process on artificially contaminated substrates using a selection of Gram-positive and Gram-negative food related pathogenic microorganisms.

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Table 1: D value* inactivation rates of *E. coli* on a range of different substrates following 10 s exposure to the Dry Ice Cleaning.

Substrate	Test % reduction	Blank % reduction
Ceramic	100 %	100 %
Slate steel	100 %	100 %
Stainless steel	99.95 %	100 %
Nylon	98.73 %	100 %

**D value of an organism refers to the time required at a given set of conditions to result in a 10-fold reduction in cell numbers - expressed as a percentage.*

Table 2: D value inactivation rates of *S. Enteritidis* on a range of different substrates following 10 s exposure to the Dry Ice Cleaning.

Substrate	Test % reduction	Blank % reduction
Ceramic	99.99%	99.99%
Slate steel	100%	100%
Stainless steel	99.99%	99.99%
Nylon	99.99%	99.99%

**D value of an organism refers to the time required at a given set of conditions to result in a 10-fold reduction in cell numbers - expressed as a percentage.*

Table 3: D value inactivation rates of *L. monocytogenes* on a range of different substrates following 10 s exposure to the Dry Ice Cleaning.

Substrate	Test % reduction	Blank % reduction
Ceramic	99.96%	100%
Slate steel	100%	100%
Stainless steel	99.62%	100%
Nylon	100%	100%

**D value of an organism refers to the time required at a given set of conditions to result in a 10-fold reduction in cell numbers - expressed as a percentage.*

Executive summary:

It is evident from the results obtained that the Dry Ice Cleaning process is an effective method for inactivating both Gram-positive and Gram-negative bacteria from a series of different substrates when exposed for a defined 10 seconds exposure. Inactivation rates ranged from 98.73 – 100%, 99.99 – 100%, and 99.62 – 100% for *E.* coli, *S. Enteritidis,* and *L. monocytogenes*, respectively. In addition, preliminary results suggest that little to no microbial transfer to clean portions of the substrates occur during Dry Ice Cleaning, with 0.01% transfer evident during the *S. Enteritidis* trial, and 0.00% evident for the *E. coli* and *L. monocytogenes* challenge experiments. In addition, it was established that the natural level of contamination found on these substrates is generally low. The different concentrations of

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bacteria used to artificially contaminate the substrates in part 2 of the study are well in excess of the levels generally found in the environment and accordingly, highlights the significant potential of this novel Dry Ice Cleaning process as a novel decontamination device against a selection of Gram-positive and Gram-negative bacteria - most notably those heavily associated with the food industry.

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- 2016 Winner Cork Business R&D and Innovation Award
- 2017 Overall Winner Local Enterprise Awards
- 2017 Finalist Business All Star Innovation Awards
- 2017 Finalist National Enterprise Awards