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Development of the protocol and realization of experimental tests to evaluate the effectiveness of detergent and sustainable disinfection of the instrument IONICS™ - ION CLEANER SYSTEM in the food sector

Producer: TAND-S srls Via Toscana 13 - Fiume Veneto (PN)

Abstract Expertise technical-scientific evaluation of effectiveness Tool "IONICS™ - Ion Cleaner System"

Protocol 1494/31.7.2021/MAG

The Professional Extenders

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SUMMARY

ABSTRACT

The purpose of this work was the development of the protocol and the realization of experimental tests in ACCREDIA accredited laboratory, to evaluate the sustainable cleaning and disinfecting efficacy of the tool "IONICS™ - Ion Cleaner System" on different contact surfaces and machine tools commonly used for food preparation in industry, retail, food service.

The operation of the instrument is based on the generation of electrolyzed water that, through the ORP (oxidation-reduction potential) is able to remove electrons from the microbial cell membrane making it unstable, so as to devitalize the microorganisms.

For experimental purposes, laboratory tests were performed using two mixes of different microorganisms inoculated into different foods (beef, custard, cooked ham) and contaminating some contact surfaces (marble, plastic material, and steel) and machine tools (slicer, meat grinder).

The experimental approaches adopted an "artificial" contamination of the surfaces, with known charges of the two mixes without the use of the food vehicle, and a "real" contamination of the surfaces with known charges of the two mixes through the food vehicle. In both cases, the surfaces were then treated with IONICS™, adopting the instructions of use suggested by the producer.

As for the results of the tests conducted on "artificial" contamination, the first treatment with IONICS™ lowered the initial charge by 3-5 logarithmic degrees (99.9%-99.999%) while following the second treatment, charges <10 cfu/cm2 were found.

On the other hand, as regards tests with "real" contamination of the steel surface, the treatment eliminated the surface charge of the two mixes used and reduced the microbial charge by 5 logarithmic degrees (99.999%). On the plastic cutting board, the microbial charge was reduced by 3-4 (99.9%-99.99%) logarithmic degrees, as was the case with the marble surface.

It can therefore be seen, in the face of analytical evidence, that the different nature of the materials, the specific roughness and the different foods used for contamination, have influenced, although not in a decisive way, the effectiveness of IONICS™. The data collected, in fact, highlight the cleaning capacity and disinfecting power of the instrument, which from what has emerged, on average has a capacity to reduce the bacterial charge by 3 logarithmic degrees (99.9%) in the presence of organic matter.

The instrument used following the instructions specified by the producer and reported in the pre-operational procedures / program of pre-requirements "Sanitization of environments and work equipment" of the FSMM (Food Safety Management Manual- HACCP System) of OSA, ensures the hygienic-sanitary compliance of contact surfaces and main machine tools in accordance with EC Reg. 852/04 and EC Reg. 853/04

1. DEFINITIONS

1.1. General Definitions

- Bactericide: A substance capable of removing, reducing, or eliminating bacteria.
- CCP (art. 5 EC Reg. 852/04): Critical Control Point. Stage at which it is essential for food safety to carry out a control action to prevent, eliminate or reduce to an acceptable level a danger to food safety.
- Cleaning (Ministerial Decree n° 274/1997): Set of procedures and operations aimed at removing dust, unwanted material or dirt from surfaces, objects, confined spaces and areas. It is also carried out with the aid of alkaline or acidic detergents. Basically, this terminology means the removal of dirt that may be organic (from food) or inorganic (from limescale).
- Detergent (National Center for Chemical Substances, Cosmetic Products and Consumer Protection): Detergent is defined as any substance or mixture containing soaps and/or other surfactants, intended for washing and cleaning activities. These activities do not include those for cleaning the human body.
- Disinfection (Ministerial Decree no. 274/1997): A set of procedures and operations aimed at making certain confined environments and pertinent areas healthy by destroying or inactivating pathogenic microorganisms.
- Ew: Electrolyzed water, an "activated" liquid. A solution that exhibits an increase in chemical activity for a limited period of time.
- Final consumer (art. 3 EC Reg. 178/02): The final consumer of a food product who does not use that product as part of an operation or activity of a food business.
- Food (art. 2 EC Reg. 178/02): foodstuff or foodstuff processed, partially processed or unprocessed substance or product intended to be, or reasonably expected to be, ingested by humans.
- Hazard (art. 3 EC Reg. 178/02): Biological, chemical, or physical agent contained in a food or feed, or conditions in which a food is found, capable of causing a harmful effect on health.
- Logarithmic Degrees: The logarithm of a number in each base is the exponent to which the base must be raised to obtain the number. Logarithmic reduction refers to the percentage of microorganisms physically removed or inactivated by a given process. One logarithmic reduction indicates that 90% of the microorganisms present have been removed or inactivated. Two logarithmic reductions indicate that 99% of the microorganisms have been removed or inactivated. Three reductions indicate 99.9%, 4 reductions 99.99%.
- OPR: Oxidation-reduction potential. Reduction potential is a measure of the tendency of a chemical species to acquire electrons to change to a reduced state. A species with a high reduction potential tends easily to gain electrons and thus to reduce, while a species with a low potential tends easily to lose electrons and thus to oxidize.
- PPM (Parts per million): Expresses the concentration of a substance present in a mixture; in the case of aqueous solutions, 1 ppm corresponds to 1 mg per liter.
- PRP (UNI EN ISO 22000, food safety management systems): Program of Pre-Requirements/Pre-operational Procedures: Practices that, if implemented correctly and in a certain way, create a situation that allows to produce safe food.
- Ready-to-eat food (art. 2 EC Reg. 2073/05): Foodstuffs intended by the producer or manufacturer for direct human consumption, without the need for cooking or other treatment to eliminate or reduce to an acceptable level the microorganisms present.

- Retail (art. 3 EC Reg. 178/02): The handling and/or processing of food and its storage at the point of sale or delivery to the final consumer, including distribution terminals, catering establishments, company and institutional canteens, restaurants and other similar catering facilities, stores, supermarket distribution centers and wholesale outlets.
- Risk (art. 3 EC Reg. 178/02): Function of the probability and severity of a harmful effect on health, resulting from the presence of a hazard.
- Sanitization (Ministerial Decree no. 274/1997): Set of procedures and operations aimed at making certain environments healthy through cleaning and/or disinfection and/or disinfestation activities or through the control and improvement of microclimate conditions about temperature, humidity, and ventilation or with regard to lighting and noise.
- CFU (Colony Forming Unit): This is the unit used to estimate the number of viable bacteria or fungal cells present in a sample and is defined as the ability to multiply through binary fission under controlled conditions.

1.2. Microbiological notions

- Campylobacter: Gram negative bacterium, particularly sensitive to dehydration, heat, and the presence of oxygen (it is not anaerobic, as it can grow at low concentrations). The infection in humans is very frequent, the symptomatology is profuse diarrhea with the possible presence of blood in the stool;
- Clostridium perfringes: is a Gram-positive bacterium, sporigenous and anaerobic, able to produce enterotoxin during sporulation. Symptoms appear after about 8-22 hours from consumption and it is characterized by abdominal cramps and diarrhea;
- E.coli: is a Gram negative bacterium, able to grow in the presence or absence of oxygen, but not in 100% CO2 atmosphere. Its importance is due to the presence of several enterovirulent strains, which can be enterotoxigenic, enteroinvasive, enterohemorrhagic or enteropathogenic and entoroadherent;
- ENT (Enterobacteriacea): The Enterobacteriacea count is a key hygiene parameter in European regulations on microbiological criteria applicable to foodstuffs (Regulation EC 2073/2005). The Enterobacteriaceae family includes important food spoilage agents and some intestinal pathogens;
- Enterotoxin: toxin produced by some bacteria and having action on the intestinal mucosa. Usually it is able to resist to thermal treatments;
- Gram staining: technique of bacterial classification based on the different permeability of a primary dye (Lugol's Reagent). This is due to morphological reasons intrinsic to the structure of the cell wall of the bacterium and can give only two outcomes: full staining ("gram-positivity") or no staining ("gram-negativity");
- Lysteria: this genus includes Gram positive bacteria, motile and able to grow at low temperatures. Among the various species, L. monocytogenes is the only one pathogenic to humans and animals. The disease in humans can be invasive, manifesting itself in a sporadic form with fever, diarrhea, vomiting, meningitis, septicemia, miscarriage and can lead to death; the non-invasive form manifests itself with fever, diarrhea, muscle pain and abdominal cramps;
- Salmonella spp: this genus is represented by Gram-negative, mesophilic bacteria with motility. It is able to survive high salt concentrations and low water activity, but not high temperatures. The disease in humans usually manifests as a nonsevere febrile gastroenteric form. Twenty-two hundred serotypes are known; 20-30 are the most common;

- Staphylococcus aureus: is a Gram-positive bacterium. Its optimal growth is in aerobiosis, but it is capable of growing even at reduced oxygen conditions. The disease in humans is caused by ingestion of enterotoxin preformed in food, and is manifested by nausea, vomiting, abdominal cramps, and profuse diarrhea;
- TBC (Total Bacterial Count): indicates the number of colony forming units (CFU) in a delimited area (example 1 cm^2) of the surface under analysis;

2. INTRODUCTION

2.1. Electrolized Water

Electrolyzed water (Ew) is an "activated" liquid, a solution that shows an increase in chemical activity for a limited period, which is obtained by electrolysis of a very dilute salt solution, represented for example by tap water.

The water passes inside a sealed chamber, in which are placed two electrodes, one positive (anode) and one negative (cathode), separated by a bipolar membrane; after activating the electric current to the electrodes, is obtained this situation:

- from the cathode side, a basic electrolyzed solution with 10.5<pH<11 and oxidation-reduction potential (ORP) of 800mV-900mV is produced, containing sodium hydroxide and a high concentration of dissolved hydrogen, which, due to its strongly reducing potential, is endowed with detergent efficacy;
- on the anode side, an acidic electrolyzed solution is produced (the real electrolyzed water, with 2.4<pH<2.7 and ORP > 1100mV, and an amount of free chlorine equal to approximately 50ppm), containing hydrochloric acid and a high concentration of dissolved oxygen, which has a remarkable antimicrobial power.

Several researches have shown that the main factor affecting the antimicrobial efficacy of electrolyzed water is the oxidation-reduction potential (ORP). Due to the ORP>1100 mV, electrolyzed water sequesters electrons from the microbial cell membrane, making it unstable. This phenomenon favors the oxidizing action of chlorine and therefore the devitalization of the microorganism. The chlorine compounds produced by electrolysis, first the indissociated hypochlorous acid, are in fact responsible for the oxidation that determines the bactericidal effect of Ew.

3. PURPOSE OF THE WORK

L The aim of the work was the development of the protocol and the realization of experimental tests to evaluate the sustainable cleaning and disinfecting efficacy of the tool "IONICS™ - Ion Cleaner System" on different food contact surfaces and machine tools commonly used in food preparation. The operations of cleaning, deterging and disinfection of surfaces and equipment for food processing, are part of the pre-operational procedures / program of pre-requisites "sanitation of environments and work equipment" of FSMM (Food Safety Management Manual – HACCP System), according to EC Reg. 852/04 and EC Reg. 853/04.

4. MATERIAL AND METHODS

4.1. Microorganisms used for contamination

Mix 1	Mix 2
• E. coli ATCC 11775 lot 465-159-2	• S. cerevisiae ATCC 9763 lot L247.18A
• L. monocytogenes ATCC 19115 lot J247.18A	• Arspergillus brasiliensis ATCC 16404 lot G186.19B
• S. enteriditis ATCC 13076 lot 345-106	• CAMPYLOBACTER coli ATCC 43478 lot 121-37
• S. aureus ATCC 25923 lotto 340-459-6	• CL. perfringens ATCC 13124 lotto 318-238

The single bacteria or molds were mixed together: an international reference strain ATCC and a "wild" strain from our collection, isolated from gastronomy products. Moreover, the constitution of the two MIX was necessary to facilitate the inoculation operations on different surfaces avoiding overabundance of liquid that would be difficult to govern.

4.2. Surfaces used for contamination

- n. 3 types of contact surfaces: stainless steel, plastic material (HDPE-PP), marble;
- n. 2 machine tools: slicer, professional meat grinder

4.3. Food used to carry the inoculum

- minced meat;
- custard;
- cooked ham.

4.4. Operational indications for the use of IONICS™

STEP 1: Remove dirt with IONICS.

STEP 2: Press the dispensing button for at least 3 seconds while spraying on the surface to be treated at a distance of 15-20 centimeters and leave for 5 seconds.

Then remove the ionized water with disposable paper or clean cloths. Repeat this process a second time to properly eliminate up to 99.9% of bacteria.

4.5. Experimental approach to protocol development

1 - contamination of surfaces, with known charges of the two MIXEX without the food vehicle

1 ml of each microorganism constituting MIX 1 and MIX 2 was inoculated into the in areas of each surface and then, after a few minutes, a treatment of IONICS™ Step 2 was performed, directly, as in this case there was no obvious organic residue present (4.4).

2 - contamination of surfaces with known charges of the two MIXES through the food vehicle

10g of food (minced meat, custard) and 10 cm2 (slices of cooked ham) are inoculated with the same MIX; this is to replicate what can happen in the reality of everyday work, that is that the presence of organic substance can increase the resistance of microorganisms to the effect of disinfectants / biocides, for the protective action that this substance has on the microorganisms themselves.

After the experimental inoculation, the surfaces contaminated with food were left in the air, in a confined environment for at least 3 hours, so that the inoculated microorganisms could adhere well to the surfaces, always to reproduce as faithfully as possible what can happen in the operative reality.

After this time, a first sample was taken from each surface by means of a sponge bag in order to establish the microbial load inoculated before treatment. Then, on the surfaces, the sanitization test was carried out according to the methods and times foreseen for IONICS™ (4.4).

Immediately afterwards, the specific microbial load remaining on the surfaces treated with IONICS™ was quantified using a sponge bag.

5. OBJECTIVE EVIDENCE FROM EXPERIMENTAL TRIALS

5.1. Objective evidence from experimental trials 1

Experimental tests conducted on different surfaces contaminated with known concentrations of microorganisms, without the organic vehicle, produced a decay of the initial contamination particularly marked following treatment with IONICS™.

The initial charges, in fact, were reduced by 3-5 logarithmic degrees; following the second treatment, most surfaces reported charges <10 cfu/cm2. However, it should be noted that L. monocytogenes colonies of 30 cfu/cm2 remained on the steel surface after the second treatment with IONICS™.

This aspect, however, assumes a minimal dimension if one considers the tenacity of the microorganism and the dose present on the surface before treatment 16500 (cfu/cm2).

This scenario, and the probability that this charge is present on the work surfaces is really unlikely, in fact it is generally present in charges around 10-100 ufc/g/cm2. E. coli, however, after the second treatment, compared to a starting contamination of 19800 cfu/cm2, is detected with charges of 10 cfu/cm2 on marble and on the cutting board in plastic material.

5.2. Objective evidence from experimental trials 2

To comment the results of this experiment, it is necessary to consider the real microbial contamination found on each surface thanks to the "footprint" of the food used for the test, inoculated with the respective mix.

This contamination was the starting point for judging the sanitizing effect of IONICS™. It should be noted that this situation was taken to the extreme in order to evaluate and appreciate the effectiveness of the product tested and, never in reality, especially in food, can occur overlapping scenarios. In the case of the experimental test, in fact, foods have been inoculated, then, contaminated, with particularly high loads of microorganisms and among these some very pathogenic.

IONICS™ on the surface of steel, contaminated with minced meat and custard, with both microbial preparations Mix 1 and Mix 2, has produced satisfactory results, zeroing the surface charge of the two mixes used and lowered the microbial load of 5 logarithms (99.999%).

IONICS™ on the plastic cutting board has determined a reduction, compared to the initial charge, of 3-4 logarithmic degrees both in case it was contaminated with minced meat and with custard. The same marble, soiled and contaminated with the same foodstuffs, showed reduced charges compared to the initial ones. In the comparisons of the experimental contamination with custard, following the second treatment with IONICS™, it showed residual surface charges <10 cfu/cm2. After the two treatments with IONICS™, the test conducted on the slicer, contaminated with cooked ham, produced a decrease in bacterial load equal to 4 logarithms. However, it was less effective against S. aureus, for which the reduction was 2 logarithms. As far as the meat grinder is concerned, considering the complexity of the mechanical part and the opportunity given by the same to the microorganisms to get into the holes with which it is provided, the results obtained were very interesting: from an initial average charge of 10^5 cfu/cm2 of Mix 1, a reduction of about 3 logarithms was achieved, while from a starting charge of 10^102-3 cfu/cm2 of Mix 2, final charges of <10 cfu/cm2 were found.

6. CONCLUSION

The data emerging from the two different experimental tests conducted on the effectiveness of IONICS™, agree with the accredited scientific bibliography.

The use of electrolyzed water, in all its formulations and combinations, is more effective against many microorganisms, when they are not combined with organic matter, whether animal or vegetable.

The effectiveness of IONICS™ against microorganisms as they are, has been particularly significant, demonstrating excellent qualities of sanitization of treated surfaces.

The different surfaces, their roughness, the composition of the materials and the different foodstuffs used for contamination have conditioned in part, as expected, the effectiveness of IONICS™, highlighting the need for proper maintenance by users.

The data collected, however, highlight the undoubted disinfecting capacity, which even in the presence of organic matter, corresponds to a reduction of 3 logarithms, or 99.9%.

The instrument is also innovative in several respects:

safety and health in the workplace: it avoids the use of potentially harmful chemicals for operators, during the handling, dilution and use phases that may be the cause of occupational risk, and consequently, it is not necessary for them to be equipped with PPE;

environmental sustainability: it avoids the disposal of packaging and containers of chemical products, also safeguards the water supply system that can be polluted in case the products are not properly diluted.

The tool tested, following the instructions specified by the manufacturer and reported in the pre-operational procedures / pre-requirements program "sanitization of environments and work equipment" of MGSA (Food Safety Management Manual), represents a valuable contribution to ensure in a sustainable way the hygiene of the process and the safety of products sold or administered to the consumer. The data emerging from the two different experimental trials conducted on the efficacy of IONICS™, agree with the accredited scientific bibliography.

7. MAIN LEGISLATIVE REFERENCES

- EC Regulation 178/2002 "Laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety".
- EC Regulation 852/2004 "On the hygiene of foodstuffs".
- EC Regulation 853/2004 "Laying down specific hygiene rules for food of animal origin".
- EU Regulation 2017/625 "On official controls and other official activities carried out to ensure the enforcement of food and feed law, animal health and animal welfare rules, plant health rules and plant protection products".
- EU Regulation 2073/2005 et seq. "On microbiological criteria applicable to foodstuffs".
- EU Regulation 528/2012 "On the making available on the market and use of biocidal products".
- Ministerial Decree no. 274/1997 "Regulation implementing articles 1 and 4 of Law no. 82 of 25 January 1994, governing cleaning, disinfection, pest control, deratization and sanitization activities.
- GFSI Benchmarking Requirements - GFSI Guidance document Version 2020.
- UNI EN ISO 22000: Food Safety Management System.
- FSSC 22000 "Food Safety Systems Certification".
- IFS Food Standard v7.
- BRC Global Standard for Food Safety v8.
- FDA CFR Code of Federal Regulations Title 21 PART 120 - (HACCP Systems, Subpart A General Provisions, Sec. 120.6 Sanitation standard operating procedures).

8. MAIN BIBLIOGRAPHICAL REFERENCES

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9. ANNEX 1

Table of Results – Experimental Approach 1 – Artificial Contamination

				STEEL		MARBLE		SLICER		CUTTER	
Microorganism	Concentration starting point cfu/ml	Sown 1ml on 100 cm2 of concentration - cfu/ml	Concentration in the spongebag with 100 ml thinner cfu/ml	POST 1st treatment - cfu at dil -1	POST 2nd treatment - cfu at dil -1	POST 1st treatment - cfu at dil -1	POST 2nd treatment - cfu at dil -1	POST 1st treatment - cfu at dil -1	POST 2nd treatment - cfu at dil -1	POST 1st treatment - cfu at dil -1	POST 2nd treatment - cfu at dil -1
E.COLI	$1{,}98 \times 10^{9}$	$1{,}98 \times 10^{6}$	$1{,}98 \times 10^{4}$	<10	<10	30	10	160	<10	50	10
YEASTS	$1{,}45 \times 10^{8}$	$1{,}45 \times 10^{8}$	$1{,}45 \times 10^{6}$	<10	<10	10	<10	100	<10	30	<10
MOLDS	4×10^{5}	4×10^{5}	40×10^{3}	<10	<10	10	<10	<10	<10	<10	<10
SALMONELLA	$1{,}73 \times 10^{9}$	$1{,}73 \times 10^{6}$	$1{,}73 \times 10^{4}$	<10	<10	20	<10	30	<10	<10	<10
S.AUREUS	$7{,}4 \times 10^{8}$	$7{,}4 \times 10^{5}$	$7{,}4 \times 10^{3}$	20	<10	<10	<10	40	<10	<10	<10
L. MONOCYTOGENES	$1{,}65 \times 10^{9}$	$1{,}65 \times 10^{6}$	$1{,}65 \times 10^{4}$	50	30	20	<10	10	<10	<10	<10
CAMPY	$4{,}8 \times 10^{5}$	$4{,}8 \times 10^{5}$	$4{,}8 \times 10^{3}$	<10	<10	<10	<10	10	<10	20	<10
CL. PER.	$1{,}05 \times 10^{8}$	$1{,}05 \times 10^{5}$	$1{,}05 \times 10^{3}$	<10	<10	<10	<10	<10	<10	<10	<10

Table of Results – Experimental Approach 2 – Real contamination

				STEEL + MEAT						
Microorganism	Concentration starting point cfu/ml	Contaminated 100 cm2 of surface with a number of microorganism equal	Theorical concentration of microorganism in the spongebag with 100 ml thinner - cfu/cm2	Area in contact with food pre-treatment cfu/cm2	Area in contact with food + Mix 1 Pre-treatment cfu/cm2	Area in contact with food + Mix 1 Post 1° contact cfu/cm2	Area in contact with food + Mix 1 Post 2° contact cfu/cm2	Area in contact with food + Mix 2 Pre-treatment cfu/cm2	Area in contact with food + Mix 2 Post 1° contact cfu/cm2	Area in contact with food + Mix 2 Post 2° contact cfu/cm2
E.COLI	1,33 x 10^9	1,33 x 10^9	1,33 x 10^7	<10	150.000	30	<10	-	-	-
SALMONELLA	1,35 x 10^9	1,35 x 10^9	1,35 x 10^7	<10	16.000	<10	<10	-	-	-
S.AUREUS	7,1 x 10^8	7,1 x 10^8	7,1 x 10^6	<10	37.000	<10	<10	-	-	-
L. MONOCYTOGENE	1,56 x 10^9	1,56 x 10^9	1,56 x 10^7	<10	100.000	10	<10	-	-	-
CAMPY	3,2 x 10^5	3,2 x 10^5	3,2 x 10^3	<10	-	-	-	80	<10	<10
CL. PER.	7,3 x 10^7	7,3 x 10^7	7,3 x 10^5	<10	-	-	-	1.600	<10	<10
YEASTS	4,9 x 10^5	4,9 x 10^5	4,9 x 10^3	50	-	-	-	390	<10	<10
MOLDS	5 x 10^4	5 x 10^4	5 x 10^2	<10	-	-	-	<10	<10	<10
TBC				1.000.000	450.000	180	40	560.000	90	50
ENT				3.800	184.000	10	20	610	<10	<10

				STEEL + CUSTARD						
Microorganism	Concentration starting point cfu/ml	Contaminated 100 cm2 of surface with a number of microorganism equal	Theorical concentration of microorganism in the spongebag with 100 ml thinner - cfu/cm2	Area in contact with food pre-treatment cfu/cm2	Area in contact with food + Mix 1 Pre-treatment cfu/cm2	Area in contact with food + Mix 1 Post 1° contact cfu/cm2	Area in contact with food + Mix 1 Post 2° contact cfu/cm2	Area in contact with food + Mix 2 Pre-treatment cfu/cm2	Area in contact with food + Mix 2 Post 1° contact cfu/cm2	Area in contact with food + Mix 2 Post 2° contact cfu/cm2
E.COLI	1,33 x 10^9	1,33 x 10^9	1,33 x 10^7	<10	750	80	10	-	-	-
SALMONELLA	1,35 x 10^9	1,35 x 10^9	1,35 x 10^7	<10	30	<10	<10	-	-	-
S.AUREUS	7,1 x 10^8	7,1 x 10^8	7,1 x 10^6	<10	11.000	70	<10	-	-	-
L. MONOCYTOGENE	1,56 x 10^9	1,56 x 10^9	1,56 x 10^7	<10	110.000	50	<10	-	-	-
CAMPY	3,2 x 10^5	3,2 x 10^5	3,2 x 10^3	<10	-	-	-	40	<10	<10
CL. PER.	7,3 x 10^7	7,3 x 10^7	7,3 x 10^5	<10	-	-	-	<10	<10	<10
YEASTS	4,9 x 10^5	4,9 x 10^5	4,9 x 10^3	<10	-	-	-	110	<10	<10
MOLDS	5 x 10^4	5 x 10^4	5 x 10^2	<10	-	-	-	<10	<10	<10
TBC				<10	23.000	200	10	350	30	<10
ENT				<10	1.000	60	<10	<10	<10	<10

				CUTTER + MEAT						
Microorganism	Concentration starting point cfu/ml	Contaminated 100 cm2 of surface with a number of microorganism	Theorical concentration of microorganism in the spongebag with 100 ml thinner - cfu/cm2	Area in contact with food pre-treatment cfu/cm2	Area in contact with food + Mix 1 Pre-treatment cfu/cm2	Area in contact with food + Mix 1 Post 1° contact cfu/cm2	Area in contact with food + Mix 1 Post 2° contact cfu/cm2	Area in contact with food + Mix 2 Pre-treatment cfu/cm2	Area in contact with food + Mix 2 Post 1° contact cfu/cm2	Area in contact with food + Mix 2 Post 2° contact cfu/cm2
E.COLI	$1{,}33 \times 10^9$	$1{,}33 \times 10^9$	$1{,}33 \times 10^7$	<10	340.000	460	180	-	-	-
SALMONELLA	$1{,}35 \times 10^9$	$1{,}35 \times 10^9$	$1{,}35 \times 10^7$	<10	23.000	30	20	-	-	-
S.AUREUS	$7{,}1 \times 10^8$	$7{,}1 \times 10^8$	$7{,}1 \times 10^6$	<10	98.000	1.000	370	-	-	-
L. MONOCYTOGENE	$1{,}56 \times 10^9$	$1{,}56 \times 10^9$	$1{,}56 \times 10^7$	<10	370.000	1.600	450	-	-	-
CAMPY	$3{,}2 \times 10^5$	$3{,}2 \times 10^5$	$3{,}2 \times 10^3$	<10	-	-	-	90	<10	<10
CL. PER.	$7{,}3 \times 10^7$	$7{,}3 \times 10^7$	$7{,}3 \times 10^5$	<10	-	-	-	320	<10	<10
YEASTS	$4{,}9 \times 10^5$	$4{,}9 \times 10^5$	$4{,}9 \times 10^3$	<10	-	-	-	140	<10	<10
MOLDS	5×10^4	5×10^4	5×10^2	<10	-	-	-	<10	<10	<10
TBC				2.500.000	2.500.000	7.900	2.200	620.000	13.000	6.200
ENT				9.000	420.000	600	290	500	50	20

				CUTTER + CUSTARD						
Microorganism	Concentration starting point cfu/ml	Contaminated 100 cm2 of surface with a number of microorganism	Theorical concentration of microorganism in the spongebag with 100 ml thinner - cfu/cm2	Area in contact with food pre-treatment cfu/cm2	Area in contact with food + Mix 1 Pre-treatment cfu/cm2	Area in contact with food + Mix 1 Post 1° contact cfu/cm2	Area in contact with food + Mix 1 Post 2° contact cfu/cm2	Area in contact with food + Mix 2 Pre-treatment cfu/cm2	Area in contact with food + Mix 2 Post 1° contact cfu/cm2	Area in contact with food + Mix 2 Post 2° contact cfu/cm2
E.COLI	$1{,}33 \times 10^9$	$1{,}33 \times 10^9$	$1{,}33 \times 10^7$	<10	31.000	1.500	110	-	-	-
SALMONELLA	$1{,}35 \times 10^9$	$1{,}35 \times 10^9$	$1{,}35 \times 10^7$	<10	2.100	220	10	-	-	-
S.AUREUS	$7{,}1 \times 10^8$	$7{,}1 \times 10^8$	$7{,}1 \times 10^6$	<10	360.000	1.600	250	-	-	-
L. MONOCYTOGENE	$1{,}56 \times 10^9$	$1{,}56 \times 10^9$	$1{,}56 \times 10^7$	<10	200.000	530	20	-	-	-
CAMPY	$3{,}2 \times 10^5$	$3{,}2 \times 10^5$	$3{,}2 \times 10^3$	<10	-	-	-	160	<10	<10
CL. PER.	$7{,}3 \times 10^7$	$7{,}3 \times 10^7$	$7{,}3 \times 10^5$	<10	-	-	-	<10	<10	<10
YEASTS	$4{,}9 \times 10^5$	$4{,}9 \times 10^5$	$4{,}9 \times 10^3$	<10	-	-	-	320	<10	<10
MOLDS	5×10^4	5×10^4	5×10^2	<10	-	-	-	<10	<10	<10
TBC				<10	310.000	2.800	440	13.000	<10	<10
ENT				<10	26.000	1.700	80	10	<10	<10

				MARBLE + MEAT						
Microorganism	Concentration starting point cfu/ml	Contaminated 100 cm2 of surface with a number of microorganism equal	Theorical concentration of microorganism in the spongebag with 100 ml thinner - cfu/cm2	Area in contact with food pre-treatment cfu/cm2	Area in contact with food + Mix 1 Pre-treatment cfu/cm2	Area in contact with food + Mix 1 Post 1° contact cfu/cm2	Area in contact with food + Mix 1 Post 2° contact cfu/cm2	Area in contact with food + Mix 2 Pre-treatment cfu/cm2	Area in contact with food + Mix 2 Post 1° contact cfu/cm2	Area in contact with food + Mix 2 Post 2° contact cfu/cm2
E.COLI	$1{,}33 \times 10^{9}$	$1{,}33 \times 10^{9}$	$1{,}33 \times 10^{7}$	<10	76.000	15.000	100	-	-	-
SALMONELLA	$1{,}35 \times 10^{9}$	$1{,}35 \times 10^{9}$	$1{,}35 \times 10^{7}$	<10	9.000	140	60	-	-	-
S.AUREUS	$7{,}1 \times 10^{8}$	$7{,}1 \times 10^{8}$	$7{,}1 \times 10^{6}$	<10	27.000	50	<10	-	-	-
L. MONOCYTOGENE	$1{,}56 \times 10^{9}$	$1{,}56 \times 10^{9}$	$1{,}56 \times 10^{7}$	<10	130.000	270	40	-	-	-
CAMPY	$3{,}2 \times 10^{5}$	$3{,}2 \times 10^{5}$	$3{,}2 \times 10^{3}$	<10	-	-	-	110	<10	<10
CL. PER.	$7{,}3 \times 10^{7}$	$7{,}3 \times 10^{7}$	$7{,}3 \times 10^{5}$		-	-	-	660	<10	<10
YEASTS	$4{,}9 \times 10^{5}$	$4{,}9 \times 10^{5}$	$4{,}9 \times 10^{3}$	<10	-	-	-	180	<10	<10
MOLDS	5×10^{4}	5×10^{4}	5×10^{2}	<10	-	-	-	<10	<10	<10
TBC				280.000	630.000	3.000	590	530.000	4.000	790
ENT				230	72.000	1.400	150	1.600	680	40

				MARBLE + CUSTARD						
Microorganism	Concentration starting point cfu/ml	Contaminated 100 cm2 of surface with a number of microorganism equal	Theorical concentration of microorganism in the spongebag with 100 ml thinner - cfu/cm2	Area in contact with food pre-treatment cfu/cm2	Area in contact with food + Mix 1 Pre-treatment cfu/cm2	Area in contact with food + Mix 1 Post 1° contact cfu/cm2	Area in contact with food + Mix 1 Post 2° contact cfu/cm2	Area in contact with food + Mix 2 Pre-treatment cfu/cm2	Area in contact with food + Mix 2 Post 1° contact cfu/cm2	Area in contact with food + Mix 2 Post 2° contact cfu/cm2
E.COLI	$1{,}33 \times 10^{9}$	$1{,}33 \times 10^{9}$	$1{,}33 \times 10^{7}$	<10	940	20	<10	-	-	-
SALMONELLA	$1{,}35 \times 10^{9}$	$1{,}35 \times 10^{9}$	$1{,}35 \times 10^{7}$	<10	180	<10	<10	-	-	-
S.AUREUS	$7{,}1 \times 10^{8}$	$7{,}1 \times 10^{8}$	$7{,}1 \times 10^{6}$	<10	21.000	150	<10	-	-	-
L. MONOCYTOGENE	$1{,}56 \times 10^{9}$	$1{,}56 \times 10^{9}$	$1{,}56 \times 10^{7}$	<10	150.000	190	20	-	-	-
CAMPY	$3{,}2 \times 10^{5}$	$3{,}2 \times 10^{5}$	$3{,}2 \times 10^{3}$	<10	-	-	-	70	<10	<10
CL. PER.	$7{,}3 \times 10^{7}$	$7{,}3 \times 10^{7}$	$7{,}3 \times 10^{5}$	<10	-	-	-	<10	<10	<10
YEASTS	$4{,}9 \times 10^{5}$	$4{,}9 \times 10^{5}$	$4{,}9 \times 10^{3}$	<10	-	-	-	90	<10	<10
MOLDS	5×10^{4}	5×10^{4}	5×10^{2}	<10	-	-	-	<10	<10	<10
TBC				<10	31.000	190	10	260	<10	<10
ENT				<10	1.300	30	<10	<10	<10	<10

				MEAT GRINDER + MEAT						
Microorganism	Concentration starting point cfu/ml	Contaminated 100 cm2 of surface with a number of microorganism equal	Theorical concentration of microorganism in the spongebag with 100 ml thinner - cfu/cm2	Area in contact with food pre-treatment cfu/cm2	Area in contact with food + Mix 1 Pre-treatment cfu/cm2	Area in contact with food + Mix 1 Post 1° contact cfu/cm2	Area in contact with food + Mix 1 Post 2° contact cfu/cm2	Area in contact with food + Mix 2 Pre-treatment cfu/cm2	Area in contact with food + Mix 2 Post 1° contact cfu/cm2	Area in contact with food + Mix 2 Post 2° contact cfu/cm2
E.COLI	1,33 x 10^9	1,33 x 10^9	1,33 x 10^7	<10	1.200.000	42.000	7.400	-	-	-
SALMONELLA	1,35 x 10^9	1,35 x 10^9	1,35 x 10^7	<10	72.000	9.000	160	-	-	-
S.AUREUS	7,1 x 10^8	7,1 x 10^8	7,1 x 10^6	<10	200.000	18.000	6.100	-	-	-
L. MONOCYTOGENE	1,56 x 10^9	1,56 x 10^9	1,56 x 10^7	<10	75.000	10.000	4.000	-	-	-
CAMPY	3,2 x 10^5	3,2 x 10^5	3,2 x 10^3	<10	-	-	-	8	<0,25	<0,25
CL. PER.	7,3 x 10^7	7,3 x 10^7	7,3 x 10^5	<10	-	-	-	1.100	58	23
YEASTS	4,9 x 10^5	4,9 x 10^5	4,9 x 10^3	<10	-	-	-	250	18	<0,25
MOLDS	5 x 10^4	5 x 10^4	5 x 10^2	<10	-	-	-	<0,25	<0,25	<0,25
TBC				120.000	3.000.000	160.000	28.000	140.000	5.500	3.000
ENT				15.000	1.800.000	60.000	12.000	3.300	190	88

				SLICER + COOKED HAM						
Microorganism	Concentration starting point cfu/ml	Contaminated 100 cm2 of surface with a number of microorganism equal to:	Theorical concentration of microorganism in the spongebag with 100 ml thinner - cfu/cm2	Area in contact with food pre-treatment cfu/cm2	Area in contact with food + Mix 1 Pre-treatment cfu/cm2	Area in contact with food + Mix 1 Post 1° contact cfu/cm2	Area in contact with food + Mix 1 Post 2° contact cfu/cm2	Area in contact with food + Mix 2 Pre-treatment cfu/cm2	Area in contact with food + Mix 2 Post 1° contact cfu/cm2	Area in contact with food + Mix 2 Post 2° contact cfu/cm2
E.COLI	1,33 x 10^9	1,33 x 10^9	1,33 x 10^7	260	140.000	4.000	250	-	-	-
SALMONELLA	1,35 x 10^9	1,35 x 10^9	1,35 x 10^7	220	160.000	400	40	-	-	-
S.AUREUS	7,1 x 10^8	7,1 x 10^8	7,1 x 10^6	350	250.000	1.100	1.000	-	-	-
L. MONOCYTOGENE	1,56 x 10^9	1,56 x 10^9	1,56 x 10^7	440	280.000	750	80	-	-	-
CAMPY	3,2 x 10^5	3,2 x 10^5	3,2 x 10^3	<10	-	-	-	120	10	<10
CL. PER.	7,3 x 10^7	7,3 x 10^7	7,3 x 10^5	<10	-	-	-	10.000	140	10
YEASTS	4,9 x 10^5	4,9 x 10^5	4,9 x 10^3	<10	-	-	-	8.500	340	10
MOLDS	5 x 10^4	5 x 10^4	5 x 10^2	<10	-	-	-	20	<10	<10
TBC				3.400	1.000.000	4000	1100	15.000	1.600	10
ENT				1.900	412.000	530	8.000	1.500	20	<10