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Acronym description

GA	Grant Agreement
RPP	Reusable Plastic Packaging
WP	Work Package

Product glossary

Betelene DB30	Non-foaming alkaline detergent product
Dectocide CDB	Non-foaming detergent-disinfectant product
Green'R Autodish safe	Non-foaming neutral ecological detergent product
Mida AF 622 EM	Anti-foaming product
Mida Chriox 5	Non-foaming disinfectant product
Mida SAN 328 EC	Non-foaming disinfectant product (antibiofilm phase)
Relavit Rinse H	Brightener (helps with the container drying)

Executive Summary

The aim of Task 5.1 is the design and specification of decontamination equipment. It includes the selection of chemical products and the design of cleaning and disinfection procedures optimized and adapted to each use-case. Two industrial washers are members of the project consortium: Eternity Systems and UZAJE. With these purposes, the specifications of washing equipment (including temperatures, times and volumes) have been defined in collaboration with Eternity Systems. In addition, in order to establish optimized cleaning and disinfection procedures for each use- case, it has been necessary to study the initial scenario of used packaging in terms of organic residue and microbiological contamination. For this reason, used existing packaging have been collected from each use-case to characterize organic residues and perform microbiological analyses of total plate counts of bacteria and fungi, as well as specific microorganisms (pathogenic, spoilage or biofilm-forming microorganisms) to describe the initial scenario. The currently used packaging collected and analyzed for each use case is described below:

- AUSOLAN use-case: 9 single-use trays with 3 different residues (3 trays per residue), rinsed and stored during one week at room temperature.
- DAWN-MEATS use-case: 6 units of a similar product (tray with skin film) were bought in Spain and stored in the fridge for one week. After that, the trays were opened and half were rinsed and half were not rinsed. Then they were stored at room temperature for one week.
- VYTAL use-case: 3 trays with 3 different residues were collected (one tray per residue). They were rinsed and stored during one week at room temperature.
- UZAJE use-case: 2 trays with the same residue were collected. They were stored for 5 weeks at room temperature and rinsed.
- ASEVI & SMURFIT KAPPA use-case: 4 bottles were collected and simulate their use for 30 days opening the bottle every day. Then, half of the bottles were rinsed and the other half were not. Finally, they were stored for one week at room temperature.

The results of residue characterization and microbial contamination obtained from used packaging have been used to propose different cleaning and disinfection procedures for each use-case. All these procedures have been tested in the laboratory to assess their capacity to remove organic residues by visual inspection and also to inactivate the microbial contamination by microbiological analysis. Given target was to achieve 90% decrease of contamination. Based on the results obtained, a specific cleaning and disinfection procedure has been defined for each use-case. Thus, a decrease of contamination by, at least, 95% might be achieved. The next step is to scale up and test the selected cleaning and disinfection protocols in Eternity Systems and UZAJE facilities with higher quantity of packaging.

In parallel, Plasmion is doing the training of electronic sensors to detect the volatile organic compounds of different food and chemical residues. Also, wastewater treatment will be addressed when the type of wastewater to be generated by such cleaning and disinfection processes is characterized.

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1. Introduction

The main objective of Task 5.1 is to optimise most relevant cleaning and disinfection technologies following outputs from tasks of analysis of the socio-economic and policy framework for reusable plastic packaging (task 1.1) and its specifications (task 1.3). With this purpose, the aim of this deliverable is to make a first guideline on decontamination equipment, defining not only the necessary specifications required for the equipment but also the properties of chemical products used to clean and disinfect the specific use cases of this project. Also, this deliverable includes the analysis and characterization of the organic residue and microbial contamination of used packaging from each use-case with the aim to propose and evaluate the effect of different cleaning and disinfection procedures not only to eliminate the organic residue but also to inactivate the microbial contamination, achieving a given target of 90% of reduction.

With that purpose, current used packaging from each use-case has been collected and analyzed to characterize the organic residue and the microbial contamination in the initial scenario. After that, different cleaning and disinfection protocols have been proposed for each use-case and they have been tested in the laboratory, analyzing the cleaning result by visual inspection and the microbial reduction by microbiological analysis after each cleaning and disinfection treatment. Finally, the cleaning and disinfection protocol able to achieve the best results has been selected in each case.

In addition, based on the type of residues of each use-case and the chemical products selected for cleaning and disinfection protocols, an electronic sensor is being trained to identify the corresponding volatile organic compounds. Also, the characterization of generated wastewater will be used to establish the best wastewater treatment.

2. Use cases description

In the following, the different use cases defined in WP1 are detailed, as well as the most suitable materials selected in WP3 (from Deliverable 3.1, submitted in September 2024).

- Use Case Vytal: Take-away Food Trays – PBT (Bowl) and CPP (Lid).
- Use Case ASEVI: Refill Personal Care Bottles – 50%rPE (Bottle), PP (Cap).
- Use Case Ausolan: Catering Food Trays – 1 portion PBT (Bowl), CPP (Lid); 8 portion CPET (Tray and Lid).
- Use Case Dawn Meats: Skin Pack Meat – PETG (Tray).
- Use Case Uzaje: Ready-meals Trays – PETG (Tray).
- Use Case Smurfit Kappa: Recyclable Flexible Bag-in-Box® for Personal Care Goods – rPE (Bag-in-Box).

It is important to highlight the most suitable materials chosen in WP3 because it is necessary to know which chemical products may be compatible with the new material design. Thus, a compatibility test will be done when the new designs are released.

3. Decontamination equipment specifications

Considering the specifications defined in previous WP1 (see deliverable 1.3: Technical and economic specifications of reusable plastic packaging) and WP3 (see D3.1. Report on new functional material for reusable packaging) and in collaboration with Eternity Systems the washing and decontamination equipment specifications have been defined.
The decontamination equipment is a tunnel washing machine. At the beginning, the packaging which needs to be washed is arranged directly on the conveyor belt or in gasket on conveyor belt, depending on the size of the

packaging. After that, the packaging moves onto the machine and follows all washing steps described hereafter.

Figure 1. General view of tunnel washing machine.

Also, it is important to emphasize that this equipment does not use nozzles but washing ramps like in figure 2.

Figure 2. Washing ramps.

In more detail, the general process of cleaning and disinfection of packaging must have the general following steps, although the protocol should be adapted to the specific conditions of organic residue and microbial contamination of each use-case: At the beginning of the process, two prewash steps have been considered:

- Prewash 1: the first prewash is carried out at room temperature and needs equipment with 20.5L of capacity. In this step the equipment has two washing ramps.

- Prewash 2: in the second prewash the temperature increases to 50-55 °C and the capacity needed will be 125L. This step is longer than the previous one and possesses nine washing ramps.

Once the prewash steps are finished, the packaging goes through two washing steps:

- Washing 1: in the first washing step the temperature rises to 62-64 °C and the equipment need to have again a capacity of 125L and nine washing ramps.

- Washing 2: a second washing step is necessary, increasing the temperature a little bit to 64-66 ºC and with the same equipment capacity (125L) and washing ramps (9).

After washing, it is very important to do a rinsing and a neutralization step:

- Pre-rinsing and final rinsing: at higher temperature (83-85 °C) the pre-rinsing is carried out with one washing ramp on the top and other on the bottom ensuring a high flow and the final rinsing has one washing ramp on four sides.

- Neutralization: a short step of neutralization is necessary.

Finally, three drying steps have been considered:

- Drying 1: the first drying step is carried out at room temperature.

- Drying 2: in the second drying step, the temperature increases to 73-75 °C.

- Drying 3: in the last drying step, the temperature is maintained at 73-75 °C.

Along all steps of cleaning process, the water pressure used is 0.8 bars and the conveyor belt speed can be adjusted between 1 m/min to 3 m/min. The duration of each step, if we used it in a normalized speed of 1.8 m/min, is summed up in the table 1:

Table 1. Time packaging remains in each step of cleaning process.

Step	Step duration (Seconds)
Infeed	66.7
Prewashing 1	16.7
Prewashing 2	46.7
Washing 1	46.7
Washing 2	46.7
Prerinsing	10.85
Final rinsing	10.85
Neutralization	16.7
Drying 1	30
Drying 2	33.3
Drying 3	46.7
Outfeed	50
Total	421.9 (Approx. 7 min)

4. Initial chemical products selection

Based on the physical-chemical nature and behavior of the different residues and microorganisms that need to be removed from the packaging, different detergents and disinfectants are chosen for this task under specific application conditions in order to obtain packaging surfaces that are safe for reuse.

Food residues expected in the packages include a mixture of proteins, fats and sugars that can be removed using detergents with specific raw materials against each of them (Forsythe & Hayes, 1998; Sansebastiano *et al*., 2007). This process occurs through various physicochemical mechanisms:

- **Surface tension**: Detergents contain substances called surfactants, which reduce the surface tension of water, allowing it to better wet surfaces and penetrate dirt.

- **Emulsification**: Surfactants have a structure that allows them to interact with both water (the hydrophilic part) and fats and oils (the hydrophobic part). This enables them to break down fat and oil particles into small droplets that can be suspended in water, forming an emulsion and facilitating their removal.

- **Solubilization**: Detergents help dissolve water-soluble residues, such as sugars and salts, allowing for their effective removal.

- **Particle suspension**: In addition to emulsifying fats, detergents keep dirt particles suspended in water, preventing them from redepositing on surfaces.

- **Saponification** (in some cases): When detergents contain alkalis, they can transform fats into soluble soaps, a process known as saponification, which also aids in removing greasy residues.

At the same time, incomplete cleaning can lead to the proliferation of undesirable micro-organisms. For this reason, proper disinfection of containers must be applied. With this purpose, two cleaning and disinfection procedures have been proposed for testing in the equipment described:

Procedure 1: this procedure is carried out in a single phase, in which a tertiary amine-based disinfectant detergent and an anti-foaming detergent are mixed. This mixture contains non-ionic surfactants, cationic surfactants, and specific chelating agents for the elimination of this type of residue. At the same time, its alkaline properties favor the saponification process to remove fat.

Procedure 2: this procedure is carried out in two phases, first cleaning and then disinfection. For cleaning, a detergent like the previous one based on biodegradable raw materials is used. In the disinfection phase, a disinfectant based on peracetic acid and hydrogen peroxide with a broad spectrum has been chosen for the elimination of microorganisms. These products are easy to rinse and environmentally friendly. In both cases it will be necessary the application of a rinsing chemical product in order to improve the subsequent drying steps. In addition, we must carry out tests to commit to the compatibility of the selected chemical products with the decontamination process. Both protocols described will be applied to each use case, and they will be explained in greater detail in the following chapters.

5. Cleaning tests and microbial analysis of existing packaging for each use case

Existing commercial plastic packaging of each use case have been collected in order to characterize the initial organic residue and the microbial contamination after using. Presence of organic residues has been evaluated by visual inspection and microbial contamination has been analyzed by sampling surfaces and plate count, after serial dilutions, in general and specific culture mediums to obtain not only the total plate count of bacteria and fungi, but also the plate counts of specific (pathogenic, spoilage or biofilm-forming) microorganisms. This information was used to design

specific cleaning and disinfection procedures for each use case, based on the specifications described in chapter 3 but adapted to particular conditions of each use-case, which are described in the next subchapters.

The products selected for testing in the different use cases are described below:

- **Betelene DB30:** non-foaming alkaline detergent product.
- **Green'R Autodish safe:** non-foaming neutral ecological detergent product.
- **Mida SAN 328 EC:** non-foaming disinfectant product (antibiofilm phase).
- **Dectocide CDB**: non-foaming detergent-disinfectant product.
- **Mida Chriox 5:** non-foaming disinfectant product.
- **Mida AF 622 EM**: anti-foaming product.
- **Relavit Rinse H:** brightener (helps with the drying of the containers).

Before and after the application of each cleaning and disinfection procedure, cleaning has been evaluated by visual inspection. Disinfection has been evaluated by microbiological analysis. Specifically, the microbiological analysis has been based on:

- Total Bacteria Count
- Total Fungi Count
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Listeria* spp. and *Listeria monocytogenes*
- *Salmonella* spp.
- *Escherichia coli*
- *Clostridium* spp.

Microbial analysis has been carried out following the next steps:

- Sampling the surface of packaging using a wipe.
- Suspend the wipe in 100 mL of buffered peptone water.
- Homogenize using a Stomacher.
- Serial dilution in buffered peptone water tubes.
- Plate count in general media for total plate count of bacteria (Tryptic Soy Agar) and total plate count of fungi (Sabouraud Agar) and in selective media for each specific microorganism:
    - *Pseudomonas aeruginosa* – Cetrimide Agar.
    - *Staphylococcus aureus* – Baird Parker Agar.
    - *Listeria* spp. and *Listeria monocytogenes* – ALOA.
    - *Salmonella* spp. – Rambach Agar.
    - *Escherichia coli* – ColiID Agar.
    - *Clostridium* spp. – Sulfite Polymyxin Sulfadiazine Agar.

- Incubate at appropriate temperature for each microorganism and plate count.

5.1 AUSOLAN USE-CASE:

Nine single-use trays (Figure 3) with three different residues, three trays per residue, have been received from AUSOLAN. Each tray has been rinsed and stored during one week at room temperature. The three types of residues were the following:

- Residue 1: pinto beans.
- Residue 2: battered pork loin with "piperada" (green and red pepper and tomato sauce).
- Residue 3: pasta with tomato.

Figure 3. AUSOLAN single-use trays.

For each residue described three commercial beige single use plastic trays were received. The dimensions of each packaging were 20 cm long, 15 cm wide and 5 cm high. The trays have been divided and cut in four portions to use each one to test different cleaning (C) and disinfection (D) procedures. Initially, the procedures T1 and T2 described in Table 2, were proposed:

Table 2. Cleaning and disinfection procedures proposed to AUSOLAN use-case.

C+D procedure	Phase	Products	Dose (%w/w)	T (°C)	Time(s)
T1	1	Dectocide CDB + Mida AF 622 EM	3.0 + 0.2	60	240
	2	Relavit Rinse H	0.0002	60	20
T2	1	Green'R Autodish Safe	2.0	60	60
	2	Mida Chriox 5	1.0	60	60
	3	Relavit Rinse H	0.0002	60	20

The results of the cleaning test and microbial analysis of each residue with each cleaning (C) and disinfection (D) procedure are shown in Annex 1.
It is shown that, in all cases, a red residue (lycopenes, beta-carotenes, curcumin and anthocyanins) remains in the packaging due to the fact that the packaging contains tomato residues. This issue is already being considered in WP3 (D3.1. Report on new functional material for reusable packaging) and will be taken into account in the choice of material and color of the RPP. The microbiological results after the application of these cleaning and disinfection procedures are also not completely satisfactory because they do not achieve acceptable levels.

Based on the results obtained in the first two treatments, another three treatments were proposed with the objective of improving cleaning and disinfection. The treatments are described in table 3.

Table 3. Additional cleaning and disinfection procedures proposed to AUSOLAN use-case.

C+D procedure	Phase	Products	Dose (%w/w)	T (°C)	Time (s)
T3	1	Green Autodish Safe	2.0	60	60
	2	Mida SAN 328 EC	3.0	60	60
	3	Dectocide CDB + Mida AF 622 EM	3.0 + 0.2	60	120
	4	Relavit Rinse H	0.0002	60	20
T4	1	Green'R Autodish Safe	2,0	60	60
	2	Dectocide CDB + Mida AF 622 EM	3.0 + 0.2	60	120
	3	Relavit Rinse H	0.0002	60	20
T5	1	Betelene DB30	2.0	60	60
	2	Mida SAN 328 EC	3.0	60	60
	3	Dectocide CDB + Mida AF 622 EM	5.0 + 0.2	60	120
	4	Relavit Rinse H	0.0002	60	20

The results of cleaning tests and disinfection evaluation by microbiological analysis of three new proposed procedures are described in Annex 1.
Based on the results obtained, T4 have been discarded because biofilm-forming microorganisms have been detected in pre-treatment so an antibiofilm treatment is necessary (two phases of disinfection: antibiofilm products + disinfectant). What we expect is to detect microorganisms after the first phase (antibiofilm product) because the aim of this phase is to break the matrix of the biofilm and then to achieve complete inactivation or a reduction of microbial contamination to acceptable levels after the application of disinfectant.

The results obtained have allowed validation of T5 treatment described in Table 4.

Table 4. Selected cleaning and disinfection procedure to AUSOLAN use-case.

C+D protocol	Phase	Products	Dose (%w/w)	Tª (ºC)	Time (s)	Cleaning results	Microbial analysis
T5	1	Betelene DB30	2.0	60	60	☑	☑
	2	Mida SAN 328 EC	3.0	60	60		
	3	Dectocide CDB + Mida AF 622 EM	5.0 + 0.2	60	120		
	4	Relavit Rinse H	0.0002	60	20		

5.2 DAWN-MEATS USE-CASE:

Six units of a similar packaging to DAWN-MEATS', containing aged meat, have been bought and an absorbent pad has been attached to the packaging. The absorbent material usually consists of layers of cellulose or absorbent paper, coated with a perforated plastic film. The cellulose acts as the main absorbent agent. Its structure usually consists of several layers. The top layer is in contact with the meat and allows liquids to pass through it to the inner layer, where they are retained. The bottom layer, usually composed of an impermeable film, prevents liquids from escaping from the tray. The main function they play in packaging is to achieve:
1) Moisture control: Absorb liquids released by the meat to prevent them from accumulating in the tray.
2) Improve hygiene: By retaining juices, it reduces the risk of bacterial growth and minimizes contact between meat and liquid.
3) Presentation: Keeping the meat visually more attractive, without excess liquid in the tray.

The meat trays were sealed with cling film glued to the top. It is important to evaluate the behavior of the adhesive glue it contains so that the selected detergents are able to remove it completely.

The following procedure has been applied to simulate the real procedure of using and collecting the packaging to be cleaned and disinfected:

- Seven days in the fridge (4ºC).
- Open the packaging:
  - No rinse
  - Rinse
- Seven days at room temperature.
- Microbiological analysis
- Application C+D procedure.
- Microbiological analysis and cleaning validation.

The trays have been divided and cut in three parts to apply different cleaning and disinfection treatments:

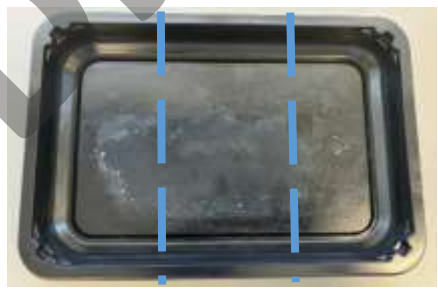

Figure 4. Similar packaging to Dawn-meats use-case.

The cleaning and disinfection procedures proposed to DAWN-MEATS use-case are described in Table 5.

Table 5. Cleaning and disinfection procedures proposed to DAWN-MEATS use-case.

C+D protocol	Phase	Products	Dose (%w/w)	Temperature (ºC)	Time (s)
T1	1	Green Autodish Safe	2.0	60	60
	2	Mida SAN 328 EC	3.0	60	60
	3	Dectocide CDB + Mida AF 622 EM	3.0 + 0.2	60	120
	4	Relavit Rinse H	0.0002	60	20
T2	1	Green'R Autodish Safe	2,0	60	60
	2	Mida SAN 328 EC	3.0	60	60
	3	Dectocide CDB + Mida AF 622 EM	5.0 + 0.2	60	120
	4	Relavit Rinse H	0.0002	60	20
T3	1	Betelene DB30	2.0	60	60
	2	Mida SAN 328 EC	3.0	60	60
	3	Dectocide CDB + Mida AF 622 EM	5.0 + 0.2	60	120
	4	Relavit Rinse H	0.0002	60	20

The results of cleaning tests and microbiological analysis before and after each cleaning and disinfection procedure are shown in Annex 2. The results show a high number of total bacteria and total fungi in the pre-treated trays, and specifically a high number of *Salmonella* spp. was detected. T2 was discarded as biofilm-forming microorganisms were detected after this treatment. T3 was the best cleaning and disinfection protocol taking into account a reduction higher than 90% of total microorganisms, a complete reduction of *Salmonella* species and the absence of biofilm-forming microorganisms after its use.
Based on the results obtained, the selected cleaning and disinfection procedure is described in Table 6.

Table 6. Selected cleaning and disinfection procedure to DAWN-MEATS use-case.

C+D protocol	Phase	Products	Dose (%w/w)	Tª (ºC)	Time (s)	Cleaning results	Microbial analysis
T3	1	Betelene DB30	2.0	60	60	☑	☑
	2	Mida SAN 328 EC	3.0	60	60		
	3	Dectocide CDB + Mida AF 622 EM	5.0 + 0.2	60	120		
	4	Relavit Rinse H	0.0002	60	20		

In DAWN-MEATS use-case, it is necessary to remark that it would be recommended to discard the absorbent pads and the skin packaging after use in order to avoid dried glue residues which can interfere in the cleaning and disinfection procedure.

5.3 ASEVI USE-CASE:

Four 1L bottles of Marsella's detergent have been received from ASEVI and the following procedure has been applied to simulate the real procedure of using and collecting the packaging to be cleaned and disinfected:

1) 30 days simulating the normal use.
2) When the product was finished:
    1) No Rinse (bottle and cap).
    2) Rinse (bottle and cap).
3) One week storage at room temperature.
4) Microbiological analysis.
5) Application C+D procedure.
6) Microbiological analysis and cleaning validation.

Figure 5. ASEVI bottles.

Bottles were divided and cut in three portions to analyze the effect of cleaning and disinfection procedures in different parts of the bottle.

The next table shows the proposed cleaning and disinfection procedures:

Table 7. Cleaning and disinfection procedures proposed to ASEVI use-case.

C+D protocol	Phase	Product	Dose (%w/w)	Temperature (ºC)	Time (s)
T1	1	Dectocide CDB + Mida AF 622 EM	2.0 + 0.2	60	60
T2	1	Mida SAN 328 EC	2.0	60	60
	2	Dectocide CDB + Mida AF 622 EM	1.0 + 0.2	60	60
T3	1	Quacide PQ60 EC	3.0	60	60

The results of cleaning tests and microbiological evaluation before and after the application of each cleaning and disinfection procedure are described in Annex 3.

The results obtained show that microbial contamination is observed in the unrinsed bottles, especially *Pseudomonas*, a biofilm-forming microorganism. This is the reason why the selected cleaning and disinfection treatment must include an antibiofilm phase. Also, if the bottles were rinsed (by filling the bottle with water and shaking vigorously three times), the initial microbial load was reduced.
After the cleaning and disinfection treatments tested, the treatment that is able to reduce both the specific microorganisms and the total bacterial and fungal count to acceptable levels is the treatment that includes an antibiofilm step; it is described in Table 8.

Table 8. Selected cleaning and disinfection procedure to ASEVI use-case.

C+D protocol	Phase	Product	Dose (%w/w)	Tª (ºC)	Time (s)	Cleaning results	Microbial analysis
T2	1	Mida SAN 328 EC	2.0	60	60	☑	☑
	2	Dectocide CDB + Mida AF 622 EM	1.0 + 0.2	60	60		

5.4 VYTAL USE-CASE:

Three trays with three different residues have been received from VYTAL (Figures 6, 7 and 8). They were all rinsed and stored for one week at room temperature.

Figure 6. Sung Sook F residue.

Figure 7. Reenhold H residue.

Figure 8. Skylar residue.

The microbiological analysis in the initial situation showed high microbial contamination in all cases (see the results in Annex 4).

Due to the high concentration of microorganisms in the initial situation, the cleaning and disinfection treatment that was proposed were with T1; it is described in Table 9.

Table 9. Proposed cleaning and disinfection procedure to VYTAL use-case.

C+D protocol	Phase	Products	Dose (%w/w)	Temperature (ºC)	Time (s)
T1	1	Betelene DB30	3.0	60	60
	2	Mida SAN 328 EC	3.0	60	60
	3	Dectocide CDB + Mida AF 622 EM	5.0 + 0.2	60	120
	4	Relavit Rinse H	0.0002	60	20

Due to the good results obtained, the same cleaning and disinfection procedure is proposed for this use-case; it is described in Table 10.

Table 10. Selected cleaning and disinfection procedure to VYTAL use-case.

C+D protocol	Phase	Products	Dose (%w/w)	Tª (ºC)	Time (s)	Cleaning results	Microbial analysis
T1	1	Betelene DB30	3.0	60	60	☑	☑
	2	Mida SAN 328 EC	3.0	60	60		
	3	Dectocide CDB + Mida AF 622 EM	5.0 + 0.2	60	120		
	4	Relavit Rinse H	0.0002	60	20		

5.5 UZAJE USE-CASE:

Two samples of the same residue have been received from UZAJE (Figure 9), stored for five weeks at room temperature and then rinsed:

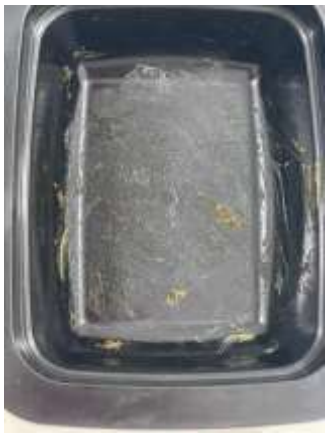

Figure 9. Used packaging from UZAJE.

After use, the samples were stored for five weeks at room temperature to simulate the time required to collect the samples and to send them to the cleaning and disinfection facilities.
The initial microbial tests showed that the microbiological contamination pre-treatment was low, probably due to the five weeks between use and cleaning and disinfection during which both the residue and the microbial contamination have been drying (see the results in Annex 5).
Considering the results of microbiological contamination in the initial situation, the cleaning and disinfection procedures described in Table 11 were proposed.

Table 11. Proposed cleaning and disinfection procedures to UZAJE use-case.

C+D protocol	Phase	Products	Dose (%w/w)	Temperature (ºC)	Time (s)
T1	1	Betelene DB30	3.0	60	60
	2	Mida SAN 328 EC	3.0	60	60
	3	Dectocide CDB + Mida AF 622 EM	3.0 + 0.2	60	120
	4	Relavit Rinse H	0.0002	60	20
T2	1	Betelene DB30	3,0	60	60
	2	Mida SAN 328 EC	3.0	60	60
	3	Dectocide CDB + Mida AF 622 EM	5.0 + 0.2	60	120
	4	Relavit Rinse H	0.0002	60	20

The results of cleaning tests and microbial analysis after each cleaning and disinfection procedure are described in Annex 5.
Based on the results obtained, the best cleaning and disinfection procedure was selected considering the achievement of a complete organic residue and microbial elimination; it is described in Table 12.

Table 12. Selected cleaning and disinfection procedure to UZAJE use-case.

C+D protocol	Phase	Products	Dose (%w/w)	Tª (ºC)	Time (s)	Cleaning results	Microbial analysis
T1	1	Betelene DB30	3.0	60	60	☑	☑
	2	Mida SAN 328 EC	3.0	60	60		
	3	Dectocide CDB + Mida AF 622 EM	3.0 + 0.2	60	120		
	4	Relavit Rinse H	0.0002	60	20		

6. Conclusions

The conclusions that can be drawn from task 5.1 are as follows:

- Used packaging has been collected from each use case to characterize the organic residues and the microbiological contamination. Different types of organic residues have been characterized (sugars, proteins and fats) and also pathogenic, spoilage and biofilm-forming microorganisms have been identified. Based on these results, specific cleaning and disinfection procedures have been developed for each use-case.
- Cleaning and disinfection procedures have been proposed for each use-case and tested in the laboratory, analyzing their effect against organic residue by visual inspection and against microbial contamination by microbiological analysis. The best protocol has been selected to completely remove organic material and achieve a reduction of microbial contamination of more than 90%.

  The specifications of the decontamination equipment (temperatures, times, volumes) and products have been defined to apply cleaning and disinfection procedures for each use-case based on the results obtained:

  1) AUSOLAN and DAWN MEATS use-cases:

Table 13. Selected cleaning and disinfection procedure of AUSOLAN and DAWN-MEATS use-cases.

Phase	Products	Dose (%w/w)	Tª (ºC)	Time (s)
1	Betelene DB30	2.0	60	60
2	Mida SAN 328 EC	3.0	60	60
3	Dectocide CDB + Mida AF 622 EM	5.0 + 0.2	60	120
4	Relavit Rinse H	0.0002	60	20

  2) VYTAL use-case:

Table 14. Selected cleaning and disinfection procedure of VYTAL use-case.

Phase	Products	Dose (%w/w)	Tª (ºC)	Time (s)
1	Betelene DB30	3.0	60	60
2	Mida SAN 328 EC	3.0	60	60
3	Dectocide CDB + Mida AF 622 EM	5.0 + 0.2	60	120
4	Relavit Rinse H	0.0002	60	20

3) UZAJE use-case:

Table 15. Selected cleaning and disinfection procedure of UZAJE use-case.

Phase	Products	Dose (%w/w)	Tª (ºC)	Time (s)
1	Betelene DB30	3.0	60	60
2	Mida SAN 328 EC	3.0	60	60
3	Dectocide CDB + Mida AF 622 EM	3.0 + 0.2	60	120
4	Relavit Rinse H	0.0002	60	20

4) ASEVI use-case:

Table 16. Selected cleaning and disinfection procedure of ASEVI use-case.

Phase	Product	Dose (%w/w)	Tª (ºC)	Time (s)
1	Mida SAN 328 EC	2.0	60	60
2	Dectocide CDB + Mida AF 622 EM	1.0 + 0.2	60	60

- The next step is to scale up these results and test the selected cleaning and disinfection procedure for each use case at Eternity Systems facility during Task 5.5 of the project. As the tests have been carried out with existing packaging, compatibility tests with the materials selected to new packaging designs will be necessary.

In parallel, Plasmion is doing the training of electronic sensors to detect the volatile organic compounds of different food and chemical residues. Also, wastewater treatment will be addressed when the type of wastewater to be generated by such cleaning and disinfection processes is characterized.

7. References

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2. Sansebastiano, G., Zoni, R., Bigliardi, L. (2007). Cleaning and Disinfection Procedures in the Food Industry General Aspects and Practical Applications. In: McElhatton, A., Marshall, R.J. (eds) Food Safety., vol 1. Springer, Boston, MA. https://doi.org/10.1007/978-0-387-33957-3_13

8. Annexes

Annex 1: AUSOLAN use-case

Cleaning results - Treatment 1 and 2 – Residue 1:

Table 17. Results of cleaning tests applying treatment 1 and 2 with residue 1 in AUSOLAN packaging.

C+D procedure	Result	Before treatment	After treatment
T1	Partial cleaning		
T2	Complete cleaning		

Cleaning results - Treatment 1 and 2 – Residue 2:

Table 18. Results of cleaning tests applying treatment 1 and 2 with residue 2 in AUSOLAN packaging.

C+D procedure	Result	Before treatment	After treatment
T1	Partial cleaning		
T2	Complete cleaning		

Cleaning results - Treatment 1 and 2 – Residue 3:

Table 19. Results of cleaning tests applying treatment 1 and 2 with residue 3 in AUSOLAN packaging.

C+D procedure	Result	Before treatment	After treatment
T1	Incomplete cleaning		
T2	Partial cleaning		

Microbiological results - Treatment 1 and 2 – Residue 1:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 20. Results of microbiological analysis expressed in cfu/cm^2 before and after the application of treatment 1 and 2 with residue 1 by triplicate in AUSOLAN packaging.

C+D procedure	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Pre-treatment	26,15 Contaminated 815,38 Contaminated 680,77 Contaminated	Not detected Not detected 688,46 Contaminated	Not detected Not detected Not detected	3,85 Contaminated Not detected 2,50 Contaminated	Not detected Not detected Not detected	Not detected 106,67 Contaminated Not detected	0,77 Acceptable Not detected 0,38 Acceptable	Not detected Not detected Not detected
Post-treatment (T1)	3,08 Contaminated 5,38 Contaminated 0,77 Acceptable	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected
Post-treatment (T2)	12,31 Contaminated Not detected 31,15 Contaminated	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected

Microbiological results - Treatment 1 and 2 – Residue 2:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 21. Results of microbiological analysis expressed in cfu/cm^2 before and after the application of treatment 1 and 2 with residue 2 by triplicate in AUSOLAN packaging.

C+D procedure	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Pre-treatment	43,46 Contaminated 2000,00 Contaminated 65,00 Contaminated	2,31 Contaminated 30,38 Contaminated Not detected	Not detected Not detected Not detected	Not detected 35,77 Contaminated Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	0,38 Acceptable Not detected Not detected	Not detected Not detected Not detected
Post-treatment (T1)	0,77 Acceptable Not detected 0,38 Acceptable	Not detected Not detected Not detected	Not detected Not detected 0,15 Acceptable	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected
Post-treatment (T2)	Not detected Not detected 0,38 Acceptable	Not detected 0,38 Acceptable Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected

Microbiological results - Treatment 1 and 2 – Residue 3:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 22. Results of microbiological analysis expressed in cfu/cm^2 before and after the application of treatment 1 and 2 with residue 3 by triplicate in AUSOLAN packaging.

C+D procedure	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Pre-treatment	43,08 Contaminated 13,08 Contaminated 27,31 Contaminated	0,77 Acceptable 1,15 Acceptable Not detected	Not detected Not detected Not detected	0,77 Acceptable 1,15 Acceptable Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected 1,54 Acceptable	Not detected Not detected Not detected
Post-treatment (T1)	0,38 Acceptable Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected
Post-treatment (T2)	Not detected 3,85 Contaminated 4,23 Contaminated	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected

Results – Cleaning test R1 – Treatment 3, 4, 5:

Table 23. Results of cleaning tests applying treatment 3, 4 and 5 with residue 1 in AUSOLAN packaging.

C+D procedure	Result	Before treatment	After treatment
T3	Partial cleaning of the residue		
T4	Partial cleaning of the residue		
T5	Almost complete cleaning of the residue		

Results – Cleaning test R2 - Treatment 3, 4, 5:

Table 24. Results of cleaning tests applying treatment 3, 4 and 5 with residue 2 in AUSOLAN packaging.

C+D protocol	Result	Before treatment	After treatment
T3	Partial cleaning of the residue		
T4	Partial cleaning of the residue		
T5	Almost complete cleaning of the residue		

Results – Cleaning test R3 - Treatment 3, 4, 5:

Table 25. Results of cleaning tests applying treatment 3, 4 and 5 with residue 3 in AUSOLAN packaging.

C+D procedure	Result	Before treatment	After treatment
T3	Partial cleaning of the residue		
T4	Partial cleaning of the residue		
T5	Almost complete cleaning of the residue		

Microbiological analysis – Residue 1 - Treatment 3, 4, 5:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 26. Results of microbiological analysis expressed in cfu/cm^2 before and after the application of treatment 3, 4 and 5 with residue 1 by triplicate in AUSOLAN packaging.

C+D protocol	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Pre-treatment	26,15 815,38 680,77 Contaminated	Not detected Not detected 688,46 Contaminated	Not detected Not detected Not detected	Not detected 3,85 2,50 Contaminated	Not detected Not detected Not detected	Not detected Not detected 106,67 Contaminated	Not detected 0,77 0,38 Acceptable	Not detected Not detected Not detected
Post-treatment (T3)	1,15* Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	5,00** Contaminated	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T4)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T5)	3,85* Contaminated	0,38 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	1,92** Acceptable	1,15 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

*After antibiofilm phase
**After disinfection

Microbiological analysis – Residue 2 - Treatment 3, 4, 5:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 27. Results of microbiological analysis expressed in cfu/cm^2 before and after the application of treatment 3, 4 and 5 with residue 2 by triplicate in AUSOLAN packaging.

C+D protocol	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Pre-treatment	43,46 2000,00 65,00 Contaminated	Not detected 2,31 30,38 Contaminated	Not detected Not detected Not detected	Not detected 35,77 Contaminated Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	0,38 Acceptable Not detected Not detected	Not detected Not detected Not detected
Post-treatment (T3)	0,38* Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	Not detected**	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T4)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T5)	4,00* Contaminated	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	1,92** Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

*After antibiofilm phase
**After disinfection

Microbiological analysis – Residue 3 - Treatment 3, 4, 5:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 28. Results of microbiological analysis expressed in cfu/cm^2 before and after the application of treatment 3, 4 and 5 with residue 3 by triplicate in AUSOLAN packaging.

C+D protocol	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Pre-treatment	43,08 13,08 27,31 Contaminated	0,77 1,15 Acceptable Not detected	Not detected Not detected Not detected	0,77 1,15 Acceptable Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected 1,54 Acceptable	Not detected Not detected Not detected
Post-treatment (T3)	1,54* Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	Not detected**	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T4)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T5)	2,31* Contaminated	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	Not detected**	0,38	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

*After antibiofilm phase
**After disinfection

- Annex 2: DAWN-MEATS use-case

Results – Cleaning tests no rinsed trays:

Table 29. Results of cleaning tests applying treatment 1, 2 and 3 in no rinsed trays similar to DAWN-MEATS packaging.

C+D protocol	Result	Before treatment	After treatment
T1	Complete cleaning of the residue		
T2	Complete cleaning of the residue		
T3	Complete cleaning of the residue		

Results – Cleaning tests rinsed trays:

Table 30. Results of cleaning tests applying treatment 1, 2 and 3 in rinsed trays similar to DAWN-MEATS packaging.

C+D protocol	Result	Before treatment	After treatment
T1	Complete cleaning of the residue		
T2	Complete cleaning of the residue		
T3	Complete cleaning of the residue		

Microbiological analysis - No rinsed trays:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 31. Results of microbiological analysis expressed in cfu/cm^2 before and after the application of treatment 1, 2 and 3 by triplicate with no rinsed trays similar to DAWN-MEATS packaging.

C+D protocol	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Pre-treatment	4760,96 147788,14 22961,71 Contaminated	Not detected 50089,27 Contaminated Not detected	Not detected 87,28 Contaminated Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	8,93 17357,67 Contaminated Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected
Post-treatment (T1)	0,50* Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	7,89** Contaminated	0,99 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T2)	Not detected*	0,25 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	Not detected**	Not detected	46,87 Contaminated	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T3)	Not detected*	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	1,24** Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

*After antibiofilm phase
**After disinfection

Microbiological analysis - Rinsed trays:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 32. Results of microbiological analysis expressed in cfu/cm^2 before and after the application of treatment 1, 2 and 3 by triplicate with rinsed trays similar to DAWN- MEATS packaging.

C+D protocol	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Pre-treatment	93731,40 Not detected 9720,29	15522,71 Not detected 99,19	63,98 Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected	Not detected Not detected Not detected
Post-treatment (T1)	Not detected*	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	Not detected**	0,25 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T2)	0,74* Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	Not detected**	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T3)	Not detected*	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	Not detected**	0,50 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

*After antibiofilm phase
**After disinfection

- Annex 3: ASEVI use-case

Results – Cleaning tests no rinsed packaging:

Table 33. Results of cleaning tests applying treatment 1, 2 and 3 in no rinsed ASEVI bottles.

C+D protocol	Result	Before treatment	After treatment
T1	Complete cleaning of the residue		
T2	Complete cleaning of the residue		
T3	Complete cleaning of the residue		

Table 34. Results of cleaning tests applying treatment 1 and 2 in no rinsed ASEVI caps.

C+D protocol	Result	Before treatment	After treatment
T1	Complete cleaning of the residue		
T2	Complete cleaning of the residue		

Results – Cleaning tests rinsed packaging:

Table 35. Results of cleaning tests applying treatment 1, 2 and 3 in rinsed ASEVI bottles.

C+D protocol	Result	Before treatment	After treatment
T1	Complete cleaning of the residue		
T2	Complete cleaning of the residue		
T3	Complete cleaning of the residue		

Table 36. Results of cleaning tests applying treatment 1 and 2 in rinsed ASEVI caps.

C+D protocol	Result	Before treatment	After treatment
T1	Complete cleaning of the residue		
T2	Complete cleaning of the residue		

Microbiological analysis – No rinsed bottles:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 37. Results of microbiological analysis expressed in cfu/cm^2 before and after the application of treatment 1, 2 and 3 by triplicate with no rinsed ASEVI packaging.

C+D protocol	Part of the bottle	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*C. albicans*
Pre-treatment	Cap	9,95	Not detected	25,86	Not detected	Not detected	Not detected
	Upper	6,14	0,20	>3000 Highly Contaminated	Not detected	Not detected	1,43
	Lower	2,53	Not detected	>3000 Highly Contaminated	Not detected	Not detected	15,33
Post-treatment (T1)	Cap	Not detected	Not detected	20,05 Contaminated	Not detected	Not detected	Not detected
	Upper	6,55 Contaminated	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T2)	Cap	9,82* Contaminated	Not detected	Not detected	Not detected	Not detected	Not detected
		2,89** Contaminated	Not detected	Not detected	Not detected	Not detected	Not detected
	Upper	6,13* Contaminated	Not detected	Not detected	Not detected	Not detected	Not detected
		Not detected**	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T3)	Lower	437,74 Contaminated	Not detected	0,41 Acceptable	Not detected	Not detected	Not detected

*After antibiofilm phase
**After disinfection

Microbiological analysis – Rinsed bottles:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 38. Results of microbiological analysis expressed in cfu/cm^2 before and after the application of treatment 1, 2 and 3 by triplicate with rinsed ASEVI packaging.

C+D protocol	Part of the bottle	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*C. albicans*
Pre-treatment	Cap	5,78 Contaminated	9,95 Contaminated	Not detected	Not detected	Not detected	Not detected
	Upper	4,30 Contaminated	2,05 Contaminated	Not detected	Not detected	Not detected	Not detected
	Lower	1,62 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T1)	Cap	3,27 Contaminated	Not detected	Not detected	Not detected	Not detected	Not detected
	Upper	1,44 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T2)	Cap	Not detected*	Not detected	Not detected	Not detected	Not detected	Not detected
		Not detected**	Not detected	Not detected	Not detected	Not detected	Not detected
	Lower	1,80* Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected
		2,00** Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected
Post-treatment (T3)	Upper	111,68 Contaminated	Not detected	Not detected	Not detected	Not detected	Not detected

*After antibiofilm phase
**After disinfection

- Annex 4: VYTAL use-case

Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Pre-treatment microbiology results:

Table 39. Results of microbiological analysis expressed in cfu/cm^2 before applying cleaning and disinfection treatment in VYTAL packaging.

C+D protocol	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Pre-treatment R1	T 377778,99 Highly contaminated	T 180760,08 Highly contaminated	T 554,71 Highly contaminated	T Not detected	T Not detected	T Not detected	T Not detected	T Not detected
	L 62166,16 Highly contaminated	L 43994,52 Highly contaminated	L 22,76 Highly contaminated	L Not detected	L Not detected	L Not detected	L Not detected	L Not detected
Pre-treatment R2	T TNTC Highly contaminated	T 10,46 Highly contaminated	T 778,51 Highly contaminated	T Not detected	T Not detected	T Not detected	T Not detected	T Not detected
	L 516494,94 Highly contaminated	L 0,42 Acceptable	L 30,97 Highly contaminated	L Not detected	L Not detected	L Not detected	L Not detected	L Not detected
Pre-treatment R3	T 1099425,59 Highly contaminated	T 664064,08 Highly contaminated	T 2094,14 Highly contaminated	T Not detected	T 3,79 Highly contaminated	T TNTC Highly contaminated	T Not detected	T Not detected
	L TNTC Highly contaminated	L 72330,63 Highly contaminated	L 166,70 Highly contaminated	L Not detected	L 4,06 Highly contaminated	L TNTC Highly contaminated	L Not detected	L Not detected

*After antibiofilm phase
**After disinfection
TNTC: Too number to count. T: tray. L: lid.

Results – Cleaning tests:

Table 40. Cleaning results in VYTAL trays.

Residue	Result	Before treatment	After treatment
R1	Complete cleaning of the residue		
R2	Complete cleaning of the residue		
R3	Complete cleaning of the residue		

Table 41. Cleaning results in VYTAL lids.

Residue	Result	Before treatment	After treatment
R1	Complete cleaning of the residue		
R2	Complete cleaning of the residue		
R3	Complete cleaning of the residue		

Microbiological results:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 42. Microbiological results expressed in cfu/cm^2 after the application of cleaning and disinfection treatment in different VYTAL packaging.

C+D protocol	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Post-treatment R1	T 2,39* Contaminated T Not detected** L Not detected* L Not detected**	T 0,10* T 0,19** Acceptable L Not detected* L 0,10* Acceptable	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**
Post-treatment R2	T 5,15* Contaminated T 0,17** Acceptable L 6,45* Highly contaminated L Not detected**	T Not detected* T 0,13** Acceptable L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**
Post-treatment R3	T 1,17* Contaminated T Not detected** L 1,17* Contaminated L 0,07** Acceptable	T Not detected* T 0,07** Acceptable L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**	T Not detected* T Not detected** L Not detected* L Not detected**

*After antibiofilm phase
**After disinfection
T: tray. L: lid.

- Annex 5: UZAJE use-case

Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Pre-treatment microbiology results:

Table 43. Results of microbiological analysis expressed in cfu/cm^2 before applying cleaning and disinfection treatment in UZAJE packaging.

C+D protocol	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
Pre-treatment	10,50 Highly contaminated	1,00 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	57,00 Highly contaminated	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

Results – Cleaning tests:

Table 44. Cleaning results in UZAJE trays.

Treatment	Result	Before treatment	After treatment
T1	Complete cleaning of the residue		
T2	Complete cleaning of the residue		

Results of microbiological analysis after the cleaning and disinfection treatments:
Microbiological contamination higher than 3 cfu/cm^2 is considered highly contaminated, between 2 and 3 cfu/cm^2 is considered contaminated and lower than 2 cfu/cm^2 is considered acceptable.

Table 45. Microbiological results after the application of cleaning and disinfection treatments in UZAJE packaging.

C+D protocol	Total Bacteria Count	Total Fungi Count	*P. aeruginosa*	*S. aureus*	*E. coli*	*Salmonella*	*Listeria*	*Clostridium*
After antibiofilm	7,50 Highly contaminated	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
After disinfectant 3%	1,50 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
After disinfectant 5%	2,00 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
	1,00 Acceptable	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected