# Design and validation for Clean-in-Place (CIP) in a food process

John Johansson

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Swedish University of Agricultural Sciences, SLU
Department of Animal Biosciences
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# Design and validation for Clean-in-Place (CIP) in a food process

*Design och validering för Clean-in-Place (CIP) i en livsmedelsprocess*

John Johansson

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| **Supervisor:** | **Madeleine Moazzami, Swedish University of Agricultural Sciences, Department of Animal Biosciences** |
| **Assistant supervisor:** | Su-lin Hedén, Swedish University of Agricultural Sciences, Department of Molecular Science |
| **Examiner:** | Santanu Basu, Swedish University of Agricultural Sciences, Department of Molecular Science |


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**Swedish University of Agricultural Sciences**

Faculty of Veterinary Medicine and Animal Science

Department of Animal Biosciences

## Abstract

Equipment and facilities for food processes need to be sufficiently clean to prevent the spread of bacteria which could cause disease and spoilage. Clean-in-place (CIP) is a cleaning system that allows for automated cleaning without disassembling. It is important that these systems are functional and that they can be validated. The objective of this study was to design and validate a sampling plan for CIP-operation in a food process. Several methods were chosen based on food safety standards, scientific literature, and the food producer's own information. A total of 13 different methods for validation were used for 13 sampling points each with their own CIP-system. Acceptance criteria were defined for all methods. Total aerobic bacteria and *Enterobacteriaceae* analysis of dip slides, *Enterobacteriaceae* analysis of swab-samples and rinse water gave results under the detection limit. ATP-bioluminescence gave uniform results when compared to the visual inspection. These methods were therefore removed from further validation. In this study, analysis of total aerobic bacteria of swab-samples and rinse water were adequate methods to detect false-negative results when compared to visual inspection. The reduction of the amount of total aerobic bacteria and *Enterobacteriaceae* in batter samples were significant before and after CIP-operations. Conductivity monitoring and UV-light seemed to be useful for detecting chemical residues and mineral coatings. Based on results from this study, useful methods for CIP validation were conductivity, UV-light, analysis of total aerobic bacteria batter samples, rinse water and swab-samples. However, adjusting the acceptance criteria for each method could change the results.



*Keywords:* ATP, Conductivity, Dip slides, Environmental monitoring, Food safety standards, Rinse water, Swabbing, UV-light, Visual inspection.

## Sammanfattning

Utrymmen och utrustning av livsmedelsanläggningar måste rengöras för att undvika spridning av bakterier som kan orsaka sjukdom hos konsumenten och förstöra livsmedlet. Clean-in-place (CIP) är en typ av rengöringssystem som möjliggör automatiserad rengöring utan att utrustningen behöver demonteras. Det är dock viktigt att dessa system är funktionella och att de kan valideras. Syftet med denna studie var att utforma och validera en provtagningsplan för CIP-system i en livsmedelsprocess. Flera metoder valdes ut baserat på standarder för livsmedelssäkerhet, vetenskaplig litteratur och livsmedelsföretagets egen information. Totalt 13 olika metoder för validering användes på 13 provtagningspunkter med egna CIP-system. Gränsvärden valdes ut för alla metoder. Analys av totala aeroba bakterier och *Enterobacteriaceae* på tryckplattor, analys av *Enterobacteriaceae* på svabbprover och sköljvatten gav resultat under gränsvärdena. ATP-bioluminescens gav liknande resultat jämfört med den visuella inspektionen. Dessa metoder togs därför bort från valideringen. I denna studie var analys av totalantal aeroba bakterier i svabbprover och sköljvatten mest användbara metoder för att upptäcka falskt negativa resultat jämfört med visuell inspektion. Reduktionen av mängden totala aeroba bakterier och *Enterobacteriaceae* i smetprover var signifikant före och efter CIP-operationer. Konduktivitet och UV-ljus visade sig vara användbara för att påvisa kemikalier och mineralbeläggningar. Baserat på resultaten från denna studie var de mest lämpliga metoderna för CIP-validering konduktivitet, UV-ljus, analys av totalantal aeroba bakterier i smetprover, sköljprover och svabbprover. Det är dock viktigt att poängtera att en ändring av gränsvärdena skulle kunna förändra resultatet.



*Nyckelord:* ATP, konduktivitet, tryckplattor, miljöövervakning, standarder för livsmedelssäkerhet, sköljvattensprov, svabbprov, UV-ljus, visuell inspektion.

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# Abbreviations

| | |
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| ATP | Adenosine triphosphate |
| CFU | Colony forming units |
| CIP | Clean-in-place |
| RLU | Relative Light Units |
| UV | Ultraviolet |
| µS/cm | Microsiemens per centimeter |

# 1. Introduction

Today's food processes are getting more advanced but still face problems with food spoilage and potential risks for human health (Asioli et al. 2017). The advancements in food production have fortunately led to more sophisticated and advanced ways of cleaning the systems in which the food is produced. One of these types of systems is clean-in-place (CIP), which is an automated cleaning system suitable for food processes with numerous pipelines, tanks and parts that are difficult to dismantle (Ryther 2014). This CIP system will clean the whole facility that is connected to the CIP system without any dismantling and manual labor, as it is integrated into the facility and food process, hence the name clean-in-place.

There are some potential risks when using closed systems in food processes. Examples are foodborne hazards present inside the system that are difficult to detect, such as biofilms (Parkar et al. 2004).

Biofilms are clusters of bacteria attached to a surface, together with proteins and minerals (Bremer et al. 2006). Bacteria can detach from the biofilms and spread through the CIP systems. The system therefore needs to be optimized to prevent the initial formation of biofilms. It is also important that no soil, i.e. food residues or unidentified contaminants, is left in the system after CIP operations. The presence of unclean surfaces in the systems can cause fouling in the pipes, which is also a big challenge for food industries (Escrig et al. 2020). Fouling is unwanted deposition of material from product streams that forms inside of the pipelines, organic and inorganic.

There are food safety standards which describe validation of CIP (FSSC 22000 2023) (BRCGS 2022). These are purposely designed to minimize the risk of contamination of food stuff (Milan et al. 2021). Therefore, food safety standards are used as tools to protect the consumer from diseases caused by pathogenic microorganisms. It should however be noted that it is not mandatory for food companies to use food standards, but nowadays it is expected that they are used.

Validation of the CIP operations is emphasized by food standards. How the validation should be performed is most often not specified. However, the general idea is to set specified specifications for a number of criteria based on the food process and legislation, and then test if these criteria are met (Geigert et al. 1994). This is performed by using suitable types of monitoring and methods. Where monitoring being the practice of observing the quality and methods are established practices. The methods and monitoring should be analyzed, and appropriate action should be taken.

## 1.1 Aim and objective

The objective of this study was to design a sampling plan for CIP operations in a food process, where pancake products were produced and validate the sampling plan.

The aim of this thesis was to: Reduce the risk of food being contaminated with pathogenic and spoilage bacteria through adequate sampling of CIP system in a pancake factory. To further promote public health and the hygienic quality of food products.

# 2. Background

## 2.1 Cleaning

Facilities and equipment in the food industry need to be properly cleaned to avoid contamination of food with microorganisms, such as bacteria (Asioli et al. 2017). Microorganisms can cause food spoilage and human disease.

Automated or manual cleaning also requires a good understanding of where and why contamination occurs (Ryther 2014). The development and validation of cleaning processes is therefore always needed in food production.

## 2.2 Clean-in-place

There are a variety of different systems and operations for cleaning in food processing. There are in general terms three different types of cleaning systems (Ryther 2014). Clean-out-of-place (COP) is a process where the removal and disassembly of equipment is required to enable cleaning and disinfection. Environmental cleaning is the process of cleaning external surfaces in the production facility. External surfaces are mainly surfaces that are not enclosed, such as inside pipelines and tanks. Lastly, CIP is automated cleaning of equipment without disassembly, and without manual labor. This type of cleaning is often used in food processes with a lot of pipeline circuits and is widely used in dairy and brewery industries (Moerman et al. 2014). CIP operations are generally processes within a predefined schedule (Yang et al. 2018). There can be several predefined steps for each CIP operation. The operation is usually automatic and most often follows a set number of cleaning steps. These cleaning steps are affected by several factors. The most prominent are mechanics, chemicals used for cleaning, and programming.

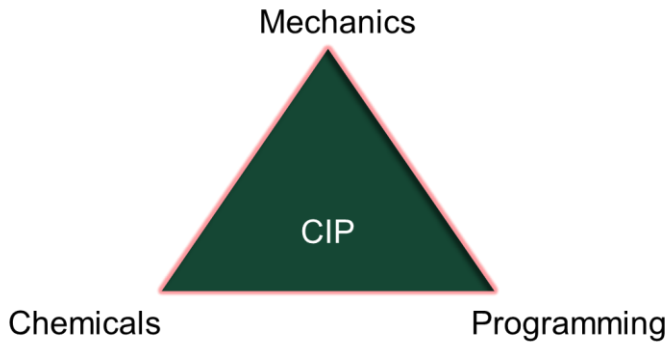


*Figure 1. The most prominent factors in CIP.*

### 2.2.1 Mechanics

How a CIP system is built usually varies considerably, since CIP systems need to interact with the specific food process and the size of the equipment. However, there are still some common factors of CIP systems. Usually, a CIP system is composed of a rinsing tank with a recirculation pump, pumps for applying chemicals, heat exchanger, and automated transmitters for conductivity, temperature and flow measurements (Moerman et al. 2014). This system is then integrated together with the pipework and tanks of the food process. Most food processes that utilize CIP systems for cleaning have line circuits and tanks for its food material, and tanks and pipelines are a common way of dividing the cleaning process.

The line circuits or the pipeline rely on the turbulent flow rate for its proper cleaning (Ryther 2014). This flow needs to be at a velocity that will sufficiently scour the pipeline together with the chosen cleaning chemical (Moerman et al. 2014). It is therefore necessary to set a minimum flow velocity that will be sufficient through the whole pipework (Chisti & Moo-Young 1994). It is also important to consider temperature and chemical concentration when optimizing the flow rate (Moerman et al. 2014). One of the most prominent risks is biofilms and fouling's that cannot be observed inside the pipeline of the CIP-system (Parkar et al. 2004). It is therefore crucial that fluid flow of CIP systems are optimized.

Tanks and other large vessels rely on different kinds of spray devices that spray the whole surface of the tank (Ryther 2014). These spray devices are stationary with a set flow rate out for the chosen chemical (Moerman et al. 2014; Ryther 2014). It is important that the spray device hits all the surface areas in the tank. Areas that are not hit by the spray device are considered as 'dead areas'. These areas are often corners in ceilings of tanks or bends in pipelines. These areas need to be considered and removed in the construction of CIP systems, but it is also important to identify them in CIP systems, because they can be inevitable in some construction designs. It is also important to place the spray balls in the object in a way that eliminates dead areas.

### 2.2.2 Chemicals

The solutions that are used for cleaning in a CIP operation are essential, and it is most common to use both alkaline and acid chemicals in cleaning (Bremer et al. 2006). Sodium hydroxide is the most common alkaline detergent, due to its hydrolytic properties of fat and proteins (Moerman et al. 2014). However, different variations and types of alkaline mixture are today used to match the requirements of cleanliness (Bremer et al. 2006). These mixtures are usually designed for the CIP systems used. Alkaline cleaning chemicals can leave mineral scales and coatings on the internal surface of the pipeline. Therefore, an acidic detergent is needed to remove these scales, but acids also provide bacteriostatic conditions in the pipeline. The most used acidic detergents are nitric acid and sulphuric acid, but other types of blends are also used. Neutral detergents, such as phosphates and citrates, can be used in cases where sodium hydroxide cannot be used (Moerman et al. 2014). There are processes where reactions of sodium hydroxide will damage the process, as in brewing. The most used CIP operations consist of an alkaline detergent step followed by an acid detergent. But the alkaline detergent step is used more frequently (Brasileiro et al. 2023).

### 2.2.3 CIP-Programing

The most common cleaning programs for CIP-systems have standardized steps to achieve efficient cleaning. The steps used vary between operations, but the most common steps in order are: pre-rinse, alkaline cleaning, rinse, acidic cleaning and final rinse (Fan et al. 2015). The pre-rinse step is basically a rinse with water to remove the bulk of remaining food stuff in the pipeline. In the alkaline cleaning step new water is heated with alkaline detergents, the alkaline concentration in the solution is usually 1-3% (Moerman et al. 2014). The alkaline solution is then recirculated in the pipeline and the tank. The heat and the alkaline chemicals usually clean the pipeline and the tanks. This step is the most important step in the cleaning process. The next step is a rinse of water to remove any foodstuff still left in the system, and to remove potential mineral deposits. The next step is acidic cleaning, which should remove any mineral deposits and biofilms formed during the alkaline cleaning step. This step is used occasionally in each CIP-operation (Brasileiro et al. 2023). The last step is a final rinse that will remove chemical residues. This step is usually monitored by measurement of pH and conductivity to ensure that all chemicals are removed (Moerman et al. 2014). Conductivity is a measure of electrical resistance in solutions.

The setting for the steps varies e.g. chemical concentration, temperature and pump strength. It is the type of food process that determines the ideal settings. The programing of the steps is where the factors fluid mechanics and chemicals interact.

## 2.3 Standards regarding CIP

Food safety standards usually mention how CIP-operations should be operated, requirements that should be set and how they should be maintained. Food safety standards are not mandatory by legislations, but are still crucial and often necessary for companies to be trusted as suppliers (Milan et al. 2021). This type of cleaning system is common in the food industry, but other industries also use CIP operations, such as the medical industry (U.S. Food & Drug Administration 2014). The medical industry also uses standards for CIP operations, but these standards differ from food safety standards. Since information on CIP-operations in guidelines and standards is scarce, medical standards could also be useful for the food industry. Two common European food standards are Food Safety System Certification Scheme (FSSC) 22000 and Brand Reputation Compliance Global Standards (BRCGS). In the United States the Food and Drug Administration (FDA) has standards on how CIP operation should be managed in the medical field and is one of the most discussed and used standards.

### 2.3.1 FSSC 22000

FSSC 22000 is developed with open consultation and uses international standards such as ISO 22000 and ISO 9001 in its formation (FSSC 22000 2023). This means that they use third-party certifiers to form the standard. The requirements in FSSC 22000 do not have to be followed strictly but certain parts are emphasized. FSSC 22000 describes an overview of the chemicals used for cleaning operations and how they are suitable for the operation, together with a validation of the methods and how to monitor them. It empathizes that CIP systems and their operations need to have detailed monitoring on its parameters, and a detailed documentation to demonstrate that requirements of the parameters are met.

### 2.3.2 BRCGS

BRCGS has in general more detailed requirements of CIP operations than FSSC 22000. It specifies how an up to date schematic of the CIP pipework should be designed, and recommendations on spray balls position, how to avoid dead spots in the tanks and cross-contamination of food (BRCGS 2022). It also mentions that the efficiency of the chemicals needs to be validated in cleaning the system. BRCGS also includes requirements on how CIP systems should be monitored and maintained. Concentration of chemicals, inspection of spray balls and tank drainage are all important factors that need to be maintained and monitored with a standardized frequency. The monitoring frequency should be determined by risk assessment of the production and the CIP operation.

### 2.3.3 FDA

Even though FDA is targeting medical companies, it provides important knowledge and insights when compared to the food safety standards. The FDA does not have actual guidelines but provides medical companies in the U.S with reference material. (Safety Culture 2024). The main reason for this is because of the large difference between the production and use of CIP-systems between medical companies. This also gives the FDA more liberty in its control of medical companies. However, there are some factors in the validation that FDA always expects from medical producers, and these are communicated through reference material. FDA expects that medical producers should perform tests that validates the cleanliness of each piece of equipment within the CIP system (U.S. Food & Drug Administration 2014). These validations tests need to have acceptance criteria and relevant tests for the equipment. The FDA also expects that the validation and the general procedure for cleaning is documented and approved by suitable personnel who also can ensure that an acceptable level of cleanliness has been reached.

## 2.4 Validation

It is important to have a validated system when working with CIP operations and is also a requirement for the food standard certifications. A validation control cycle describes the workflow for validation (Geigert et al. 1994). Variations of this workflow exist but the simplified implication is usually the same:

1. Set standard.
2. Review the test results and compare them against standard.
3. Act if the standard is not met.
4. Make improvements and repeat from step 1.

The set standard is the chosen criteria for a specific process, most often based on food safety standards and legislation. The food safety standards can vary in their requirements as mentioned before, but legal requirements are most often clear in what they expect. How to meet and prove that the standards are met through testing are in general not strictly set, so there is a large variety of tests that can be used to validate the selected standard. It is common to categorize using indirect and direct microbiological or non-microbiological sampling procedures (Göransson & Petersson 2012). These methods should also be performed after CIP-operations.

### 2.4.1 Indirect microbiological methods

Rinse water samples is an indirect microbiological method. With this technique a large sampling area is achieved and covers areas in the pipeline that are inaccessible to other sampling methods. It is also an easy method to implement as it is only

necessary to sample rinse water at the end of the CIP cycle. The main disadvantage with this method is that any soil stuck on the surface in the system is not sampled, such as biofilms and coatings.
A similar method is sampling of the food stuff produced in the system such as milk, brew, or batter in the sampling procedure is the same as for rinse water sampling, but higher microbiological counts are expected since food samples naturally contain microorganisms.

### 2.4.2 Direct microbiological methods

Direct microbiological methods usually consist of different types of swabbing methods. Swabbing can be performed directly on the surface of CIP-systems pipelines, but the main disadvantage is that inaccessible areas cannot be sampled (Ryther 2014). Swab samples are usually analyzed to detect bacterial populations, such as total aerobic bacteria and bacteria belonging to the family *Enterobacteriaceae*.
Another widely used direct sampling method is Adenosine triphosphate bioluminescence (ATP-B) swabbing. This method detects any type of residues by measuring the ATP content of both living and dead cells. This means that both bacteria and soil are detected when sampling. This method provides rapid results but is non-specific to the source of the ATP (Göransson & Petersson 2012).

### 2.4.3 Non-microbiological methods

Visual inspection and the use of fluorescence methods such as UV-light are non-microbiological indirect methods, meaning that they are not applied directly on a surface. These methods should be conducted before any other type of sampling. Any visible contamination indicates that the CIP operation was insufficient. UV-light can be used to identify non-visible enzyme-linked organisms (Ryther 2014). The main disadvantage of these methods is that inaccessible areas cannot be sampled without disassembly of the CIP system.

## 2.5 Regulations and acceptance criteria

Acceptance criteria should be used for microbiological or non-microbiological methods. Specified acceptance criteria are not included in legislation and regulations, but it is mandatory to set acceptance criteria based on microbiological guidelines and studies (Lee et al. 2021). The acceptance criteria to evaluate cleanliness should be based on food safety policies and regulations, both national and international (Schmitt & Moerman 2016). It is essential that the food producers select a maximum upper limit to confirm sufficient cleanliness. The acceptance

criteria are crucial in the validation of any type of food process because it sets the standard and target of the validation. FSSC 22000 (2023) emphasizes that hazard identification is the basis of determining acceptance levels. It mentions that each hazard identified needs to have acceptance criteria including appropriate control measures. BRCGS (2022) mentions how acceptance criteria should be based on sampling of the food, both visually determined and through microbiological testing. It also includes a list of sampling methods and requirements which should be used, and how the EU legislation concerning raw material needs to be followed. Commission Regulation No 178/2002 is the fundamental regulation on food safety in the EU. It mentions how risk analysis and identification of emerging risks is crucial in food safety.

It is important to note that all the mentioned standards and regulations do not give any maximum or minimum limit on acceptance criteria, but instead propose measures on how to set limits. However, the Commission Regulation No 2073/2005 include microbiological limits for some microorganisms and food stuff. This regulation is therefore often the basis of acceptance criteria used in many food processes. The regulation is mandatory for all member states of the EU. Limits in the regulation are either strict limit or has a maximum limit (EU Regulation 2073/2005). The batter in this production is mostly made of wheat flour and milk, and these ingredients are used to set the acceptance criteria of the batter. The strict limit in the EU regulation is usually expressed as absence in x grams.

## 2.6 Indicators for cleanliness

Total aerobic bacteria, Enterobacteriaceae and relative light units (RLU) are common parameters used in validation (Ryther 2014).

Total aerobic bacteria are bacteria that grows and survive in environments with oxygen (Borisov & Verkhovsky 2015). It should be noted that the number of total aerobic bacteria does not indicate that pathogens and toxins are present in the sample. This means that a high number of total aerobic bacteria should not necessarily be a risk for human health, but it can be used as an indicator for cleanliness.

Bacteria belonging to the family *Enterobacteriaceae* are a large group of gram-negative and facultative anaerobic bacteria (Baylis 2006). Members of this family are some of the most studied species of bacteria, some having a significant impact on human health. This includes *Shigella dysenteriae, Salmonella enterica* and *Escherichia coli.* Both pathogenic bacteria and bacteria cause food spoilage, and are included in this family. *Enterobacteriaceae* is therefore an important indicator for food quality (Nasopoulou et al. 2012).

ATP-B swabbing is, a common method for determining cleanliness (Göransson & Petersson 2012). It measures the relative amount of ATP in relative light units

(RLU) (Whiteley et al. 2016). The swab uses a luciferase enzyme that reacts with ATP, the light is then analyzed with a luminometer. It is important to mention that different organic materials will give different results in RLU. Animal based products will give higher values of RLU than plant-based products (Møretrø et al. 2012). The scale and the number of RLU is also different when comparing different brands of luminometers (Whiteley et al. 2016).

# 3. Materials and methods

## 3.1 CIP system used

A CIP system of a pancake factory was used in this study. Pancakes were produced Monday-Saturday and was only interrupted during CIP operations. The food facility had a capacity of producing 600 000 pancakes a day. The CIP system consisted of 13 objects, each with its own unique CIP operation system. Ten of the objects were tanks with pipelines and three were standalone pipelines. Three dosage endings were also included. All locations used in the validation are found in table 1 and 2. All pipelines were of similar size and had a diameter of approximately 10 cm. The tanks and pipelines were made exclusively out of stainless steel. These 13 objects together with the three dosages for the food products were chosen as sampling points. The samplings were performed on nine occasions. The CIP operations were mainly programmed to use pre-rinse, alkaline cleaning (sodium hydroxide and potassium hydroxide 3% w/v), and a final rinse. All operations could also use acid cleaning (phosphoric acid and nitric acid 3% w/v), but this step was only used approximately six times on six individual sampling points.

*Table 1. Tanks used in validation.*

| Tank 1 (mixer) | Tank 2 | Tank 3 | Tank 4 | Tank 5 | Tank 6 | Tank 7 | Tank 8 | Tank 9 | Tank 10 |
|---|---|---|---|---|---|---|---|---|---|

*Table 2. Pipelines and dosage endings used in validation.*

| Pipeline 1 with dosage | Dosage 2 | Dosage 3 | Pipeline 2 | Pipeline 3 |
|---|---|---|---|---|

## 3.2 Sampling methods

Sampling methods used for validation of the CIP-process were swabbing with swabs, ATP-B, dip slides, conductivity, visual inspection, UV-light, rinse water, and batter sampling. All methods were conducted after CIP-operations, except the batter which was sampled both before and after CIP-operation.

*Swab:* Pre-moistened swabs (Steriswab™, Great Britain) were used to swab an area of 100cm$^2$. The swab was analyzed quantitatively by SGS Analytics AB (Sweden). Analysis by SGS were usually performed within 24 h after sampling. Aerobic microorganisms at 30°C and *Enterobacteriaceae* were enumerated using NMKL 86(5$^{th}$ Ed. 2013) and NMKL 144 (3$^{rd}$ Ed. 2005), after an incubation time of 72 and 24 h respectively. The detection limit was 1.0 log CFU/area.

*ATP-Bs*: ATP-B swabs (UXL100 Clean-Trace™ Surface ATP-swabbing, USA) were used to swab a 100 cm$^2$ surface. These swabs were then analyzed qualitatively in a luminometer (3M Clean-Trace™ Luminometer LM1, USA). Which gave results in RLU after 10 seconds.

*Dip-slides*: Dipslides (Envirocheck® Contact E Total viable count, Germany) with different nutrient media on each side were used. One side had plate count agar for obtaining a total aerobic bacteria count, and the other side had violet, red bile dextrose agar for isolation and visibility of bacteria belonging to the *Enterobacteriaceae* family. The total area of both sides of the dip-slide was 19 cm$^2$. The dip slides were pressed against the surfaces of the objects.

*Conductivity:* The conductivity was measured with a conductivity meter (Greisinger GMH 3431, Germany) from the final rinse of the CIP operations. The conductivity was measured in microsiemens per centimeter (µS/cm) with a temperature compensation to 25°C.

*Visual inspection:* The visual inspection was based on visibly clean descriptions previously described (Kohli 2012) and this method was used as a reference method to set the initial cleanliness of the sampling point. All forms of batter and coatings were noted, and any remaining batter and coatings meant the object were assessed as not sufficiently clean. This method was conducted before other samplings.

*UV-light:* The UV LED light (Labino BB 2.0 Artemis, USA) used UVA irradiation intensity of 22.000 µW/cm$^2$ at 38 cm which gives a high level of detection in low light environments.

*Rinse water:* Rinse water sampling was based on previously described methods but with some modifications (Kohli 2012). Approximately 200 ml of the final rinses liquid was collected in a sterile plastic jar and was sent to analyzes SGS Analytics AB (Sweden), were total aerobic bacteria at 30° C and Enterobacteriaceae were enumerated using NMKL 86(5$^{th}$ Ed. 2013) and NMKL 144 (3$^{rd}$ Ed. 2005) The detection limit was 2.3-6.6 log CFU/g for total aerobic bacteria and 2.0-4.0 log CFU/g for *Enterobacteriaceae*.

*Batter sampling:* The batter sampling was conducted in the same way as the rinse water sampling, but the batter was collected instead of the final rinse water. Approximately 150 ml of batter was collected in sterile plastic jars before and after CIP operations, and were sent to analysis by SGS Analytics AB. The detection limit was 3.3-6.6 log CFU/g for total aerobic bacteria and 2.0-4.0 log CFU/g for *Enterobacteriaceae*.

## 3.3 Sampling procedures

All sampling methods could not be performed at every location (table 3), mostly due to location/object design, size, and placement. This were determined before starting the sampling process.

*Swabbing*: Swabbing was performed on all locations except the CIP-system tank. The sampling in the sampling point varied but were usually an arm's length down if it was a tank, or 30 cm in if a pipeline. The swab was kept at a temperature of 4°C until the analysis. The time between sampling and analysis was approximately 24-48 h.

*ATP-B:* ATP-B was conducted in the same locations and was done in the same manner as for swabbing. ATP-B was not done on the exact same surface as swabbing but on a surface adjacent to the swabbing surface. The ATP-B was gently shaken according to the manufacturer's instructions, immediately after performed swabbing. The ATP-B was then inserted into the luminometer and the result was documented.

*Dip-slides*: Dip-slides were only used in tanks because they could not fit inside of pipelines. The sampling surface in the tanks varied but they were usually performed an arm's length down the tank and adjacent to swabbing and ATP-B sampling surfaces. Each side of the dip-slide was pressed gently against the surface for 5 seconds. After incubation at 37°C for 48 hours. The dip-slides were enumerated and expressed in CFU/19cm$^2$.

*Conductivity*: The conductivity of the final rinse water was measured at the same time and location as the rinse water samples were collected, but by using other jars. The value of the conductivity meters was documented.

*Visible inspection*: Visual inspection was performed all locations. A LED-light was used to assist the inspection. Any foodstuff in the sampling point meant that it was considered not clean. Coatings of minerals was determined as coatings.

*UV-light:* The UV-light was used at a 38 cm distance to the surface of the objective. The same sampling points were used in the visible inspection, and the same methodology was used for determining cleanliness and coatings.

*Rinse water:* Rinse water were collected on sampling points where collection of final rinse water were possible.

*Batter sampling:* Batter was collected on sampling points where it was possible to track the batter, from mixing to its dosage (figure 2). The sampling operations of each sampling point with each method were performed.
Acceptance criteria for each method were based on literature or discussions with suppliers of sampling material (table 4). Where each method were performed is described in table 3.

*Table 3. Sampling locations and on which sampling point they were performed. The X indicates that the sampling method was performed at the specified location*

| Location | Dip slides | Swab | ATP-B | Rinse water | Batter | Conductivity | Visible inspection | UV-light |
|---|---|---|---|---|---|---|---|---|
| CIP-system tank | | | | X | | X | | |
| Tank 1 (mixer) | X | X | X | | X | X | X | X |
| Tank 2 | X | X | X | X | | X | X | X |
| Tank 3 | X | X | X | X | | X | X | X |
| Tank 4 | X | X | X | X | X | X | X | X |
| Tank 5 | X | X | X | X | X | X | X | X |
| Tank 6 | X | X | X | X | X | X | X | X |
| Tank 7 | X | X | X | X | X | X | X | X |
| Tank 8 | X | X | X | X | | X | X | X |
| Tank 9 | X | X | X | X | | X | X | X |
| Tank 10 | X | X | X | X | X | X | X | |
| Pipeline 1 with Dosage 1 | | X | X | X | X | X | X | |
| Dosage 2 | | X | X | | X | | X | |
| Dosage 3 | | X | X | | X | | X | |
| Pipeline 2 | | X | X | X | X | X | X | |
| Pipeline 3 | | X | X | | X | | X | |

*Table 4. Acceptance criteria used for each method.*

| Method | Acceptance criteria | | Source |
|---|---|---|---|
| | Total aerobic bacteria | Enterobacteriaceae | |
| Dip-slides | <0.65 log CFU/19cm$^2$ | <0.69 log CFU/19cm$^2$ | Discussion with supplier |
| Swab | <1.0 log CFU/100cm$^2$ | <1.0 log CFU/100cm$^2$ | Detection limit |
| Batter | >5.7 log CFU/g | >2.0 log CFU/g | Discussion with food producer (EU Regulation 2073/2005) |
| Rinse water | >2.3 log CFU/g | >2.0 log CFU/g | Detection limit |
| ATP-B | 150 RLU/100 cm$^2$ | | Discussion with supplier |
| Conductivity | 500 µS/cm | | Discussion with supplier |
| Visual inspection | Visually clean (0) | | (Kohli 2012) |
| UV-light | Visually clean (0), no coatings | | (Kohli 2012) |

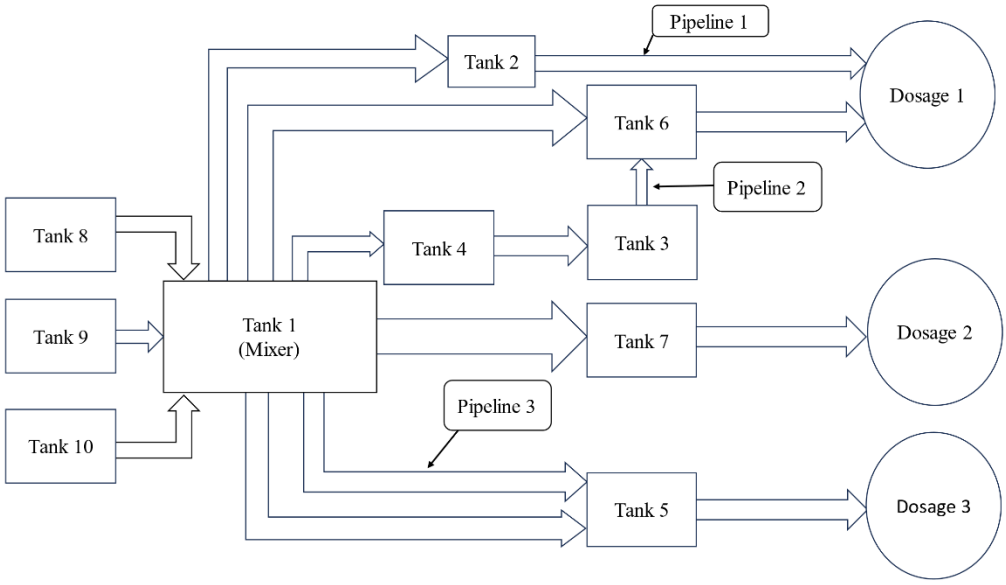


*Figure 2. Pipeline and facility map. Showcases how all tanks and pipelines integrate*

## 3.4 Statistical analysis

Statistical analysis and structural division of all obtained data was mainly performed using Microsoft Excel and R Studio 4.4.0. Microsoft Excel was used for table formulations and R studio 4.4.0 was used for Wilcox signed-rank test. MathWorks® Matlab was used for the formulation of 3D bar charts.

The Null hypothesis for the Wilcox signed-rank test is that there is no difference between paired samples ($H_0$: $\mu = \mu_0$), and the alternative hypothesis is that there is a difference ($H\alpha$: $\mu \neq \mu_0$). The data needs to be fully numerical for the statistical test, therefore some values are adjusted. Values of <2.0 were adjusted to 0. Values of >6.6 were adjusted to 6.7 and values of >3.2 were adjusted to 3.3. A total of 32 values were <2.0, 27 values were >6.6 and 34 values were >3.2.

# 4. Results

## 4.1 Bacteriological analysis

The detection limit was used as the acceptance criteria for swab samples, rinse water and batter samples (table 5). Dip-slides had an acceptance criteria for both total aerobic bacteria and *Enterobacteriaceae*, which had been set in accordance with supplier (table 6). The amount of samples that were above the detection limit were; 95% for the total aerobic bacteria of the batter, 45% for the *Enterobacteriaceae* of the batter, 43% for the total aerobic bacteria of the rinse water and 17% for the total aerobic bacteria of the swab samples (table 5). All swab and rinse water samples had an *Enterobacteriaceae* count below the limit of detection. The amount of total aerobic bacteria and the *Enterobacteriaceae* count of all dip-slides were below the acceptance criteria (table 5) (table 6). Other methods result also indicate that all methods cannot be 0% minimum values (table 7).

*Table 5. Methods and the number of measurements with detection limit and the total number of measurements. S.=swab samples, R.=rinse water and B.= batter samples. Aerob= analysis of total aerobic bacteria, and Entero= bacteria belonging to the family Enterobacteriaceae.*

| | S.Aerob | S.Entero | R.Aerob | R.Entero | B.Aerob | B.Entero |
|---|---|---|---|---|---|---|
| Detection limit | <1.0 log *CFU/100cm²* | <1.0 log *CFU/100cm²* | <0.36 log *CFU/g* | <0.30 log *CFU/g* | <0.51 log *CFU/g* | <0.30 log *CFU/g* |
| Detection % of total | 17% | 0% | 43% | 0% | 95% | 45% |

*Table 6. Total aerobic bacteria and Enterobacteriaceae of the dip-slides, the number of measurements below acceptance criteria and the total number of measurements. S.=swab samples, R.=rinse water and B.= batter samples. Aerob= analysis of total aerobic bacteria, and Entero= bacteria belonging to the family Enterobacteriaceae.*

| | Dip-slide total aerobic bacteria | Dip-slide *Enterobacteriaceae* |
|---|---|---|
| Acceptance criteria | <45 *CFU/19cm²* | <5 *CFU/19cm²* |
| Acceptance % of total | 0% | 0% |

*Table 7. Percentage of acceptably clean (0) and not clean (1) samples for each method. S.=swab samples, R.=rinse water and B.= batter samples. Aerob= analysis of total aerobic bacteria, and Entero= bacteria belonging to the family Enterobacteriaceae.*

| | S.Aerob *n=117* | ATP *n=132* | Visual inspection *n=132* | R.Aerob *n=79* | B.Aerob *n=40* | B.Entero *n=40* |
|---|---|---|---|---|---|---|
| 0 | 82,9% | 93,9% | 70,5% | 59,5% | 57,5% | 67,5% |
| 1 | 17,1% | 6,1% | 29,5% | 40,5% | 42,5% | 32,5% |

## 4.2 Comparison of clean and not clean

The ATP-B was the method that most times determined the sampling point to be clean while the visual inspection determined it not to be clean (33 times, 25%) (table 8). This indicates that ATP-B gives false negative results when compared to visual inspection. While the total aerobic bacteria of the batter did these a few times (7 times, 17.5%) and indicates that this method gives the least amount of false negative results.

All methods had a high proportion of samples that agreed with the visual inspection, indicating that the sampling point was clean. ATP-B showed highest amount of agreement with the visual inspection (91 times, 69%). Agreed results of visual inspection of not clean were generally lower. But total aerobic bacteria batter samples had the highest number of agreeing with not clean (7 times, 17.5%), while ATP-B had the lowest number (6 times, 4.5%).

The total aerobic bacteria of the rinse water did not agree with the results of clean with visual inspection the most times (22 times, 27.8%), and the total aerobic bacteria of batter samples did this also a high number of times (10 times, 25.0%). ATP-B did this the lowest number of times (2 times, 1.5%).

*Table 8. Comparison of visual inspection used as a reference with other methods. 0=clean, 1=not clean. S.=swab samples, R.=rinse water, B.= batter samples. Aerob= analysis of total aerobic bacteria, and Entero= bacteria belonging to the family Enterobacteriaceae. The cells in the middle of each comparison shows the number of measurements and the percentage where both methods agree or disagree if a place is clean or not clean.*

| | | **Visual** | |
|---|---|---|---|
| | | **0** | **1** |
| **ATP-B** | **0** | 69.0% (91) | 25.0% (33) |
| | **1** | 1.5% (2) | 4.5% (6) |
| | | **Visual** | |
| | | **0** | **1** |
| **S.Aerob** | **0** | 59.0% (69) | 23.9% (28) |
| | **1** | 10.3% (12) | 6.8% (8) |
| | | **Visual** | |
| | | **0** | **1** |
| **R.Aerob** | **0** | 36.7% (29) | 22.8% (18) |
| | **1** | 27.8% (22) | 12.7% (10) |
| | | **Visual** | |
| | | **0** | **1** |
| **B.Aerob** | **0** | 40.0% (16) | 17.5% (7) |
| | **1** | 25.0% (10) | 17.5% (7) |
| | | **Visual** | |
| | | **0** | **1** |
| **B.Entero** | **0** | 47.5% (19) | 20.0% (8) |
| | **1** | 17.5% (7) | 15.0% (6) |

## 4.3 Batter

A total of 164 batter samples were collected from six sampling points before and after CIP operations. The batter was also sampled enabling batches of batter to be tracked, from the mixer to the tank and then to the dosage. The maximum, average and standard deviation before and after CIP in each place can be found in table 9. This data shows if there is a statistical difference between the paired batters before and after CIP, from mixer to tank and tank to dosage.

The confidence interval of the test was set to 95%. The results showed that there was a significant difference between batter samples before and after CIP-operations, while differences between mixer to tank and tank to dosage were not deemed significant (table 10). A comparison between the average values for each method can be found in figure 2, 3 and 4.

*Table 9. Maximum, average, and standard deviation of batter samples analyzed with Wilcox signed-rank test, in CFU/g before and after clean-in-place (CIP). Aerob= analysis of total aerobic bacteria, and Entero= bacteria belonging to the family Enterobacteriaceae.*

| | Before | | After | | Aerob | | | Entero | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Aerob | Entero | Aerob | Entero | Mixer | Tank | Dosage | Mixer | Tank | Dosage |
| Maximum | 6.9 | 3.7 | 6.7 | 3.2 | 6.7 | 6.7 | 6.9 | 3.2 | 3.7 | 3.2 |
| Average | 6.1 | 2.8 | 5.0 | 0.9 | 5.4 | 5.7 | 5.9 | 1.6 | 1.9 | 2.0 |
| Standard deviation | 0.7 | 0.9 | 1.6 | 1.0 | 1.2 | 1.6 | 1.1 | 1.5 | 1.6 | 1.5 |

*Table 10. Result of Wilcox signed-rank test of batter samples. Aerob= analysis of total aerobic bacteria, and Entero= bacteria belonging to the family Enterobacteriaceae.*

| | Before and after CIP | | Mixer to tank | | Tank to dosage | |
|---|---|---|---|---|---|---|
| | Aerob | Entero | Aerob | Entero | Aerob | Entero |
| P-value | <0.0001 | <0.0001 | 0.131 | 0.098 | 0.488 | 1.000 |
| 95% confidence | 0.750 | 1.950 | -0.950 | -2.000 | -0.450 | -1.450 |
| interval | -1.800 | -3.100 | -0.200 | 0.900 | 0.150 | 0.800 |
| $H_0$ | Rejected | Rejected | Not Rejected | Not Rejected | Not Rejected | Not Rejected |

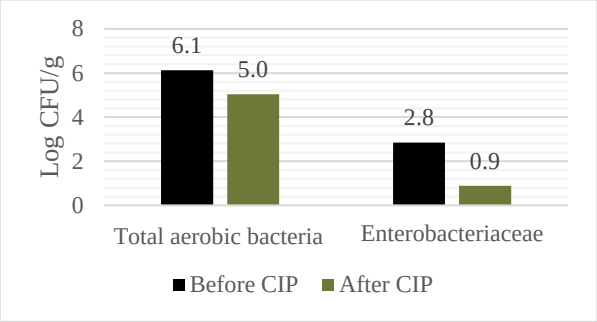


*Figure 3. Comparison of average values of total aerobic bacteria and Enterobacteriaceae in batter samples expressed in log CFU/g before and after CIP-operation.*

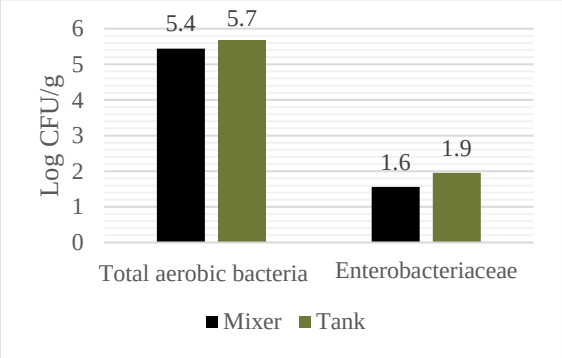


*Figure 4. Comparison of average values of total aerobic bacteria and Enterobacteriaceae in batter samples expressed in log CFU/g between mixer and tank.*

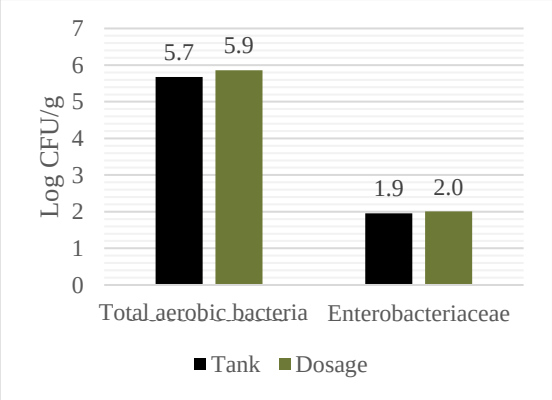


*Figure 5. Comparison of average values of total aerobic bacteria and Enterobacteriaceae in batter samples expressed in log CFU/g between tank and dosage.*

## 4.4 Conductivity

A total of 107 conductivity measures were performed on 12 sampling points after CIP-operations. The water collected was the final rinse water after a CIP-operation. The result from all locations, occasions and measurement can be found in figure 5. The collection and measurement of rinse water was not always successful, which is why some bars are missing in the bar graph. The CIP-tank was the water tank that was used for each CIP-operation and its conductivity value was interpreted as the normal value for conductivity in the water in the food process. The average value of conductivity for the CIP-tanks water was 345,4 µS/cm (table 11).

The majority of values were below 500 µS/cm and the values were similar to the CIP-tanks average values (table 12) (figure 5). However, conductivity values over 500 µS/cm were observed on seven occasions in tank 6 and on three occasions in tank 3 (figure 5) (figure 6).

*Table 11. Average and median values of conductivity in µS/cm of each location after CIP operations. T=tank, PD=pipeline with dosage.*

| | CIP-tank | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | PD1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average *µS/cm* | 345.4 | 362.1 | 347.2 | 482.3 | 349.8 | 359.3 | 601.1 | 349.5 | 351.4 | 359.4 | 366.0 | 351.6 |
| Median *µS/cm* | 345.0 | 351.5 | 350.0 | 353.5 | 349.5 | 356.0 | 499.5 | 351.0 | 352.5 | 354.0 | 364.0 | 351.5 |

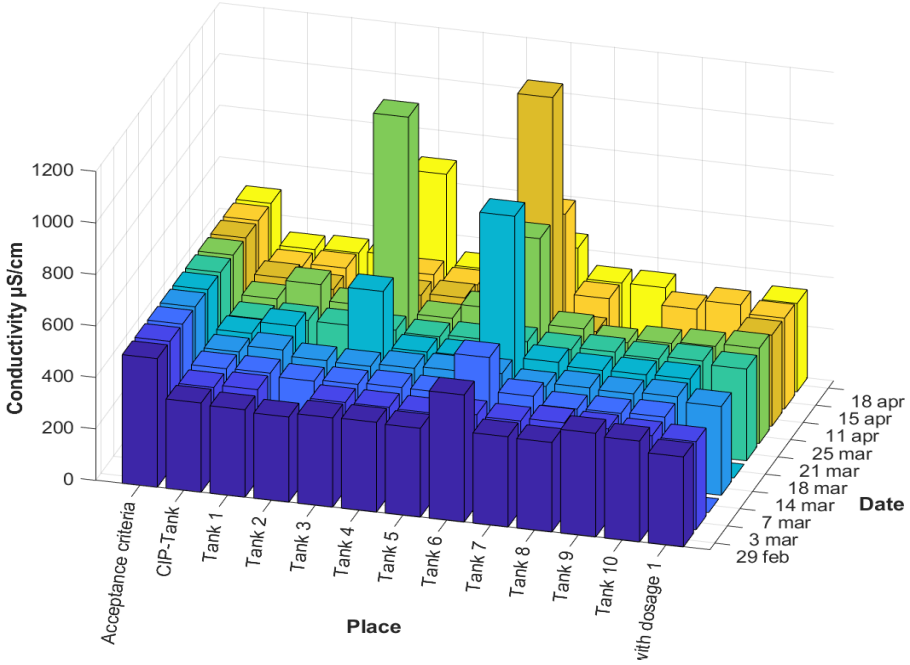


*Figure 6. Bar chart of conductivity. Each bar represents the conductivity in µS/cm of each location and occasion.*

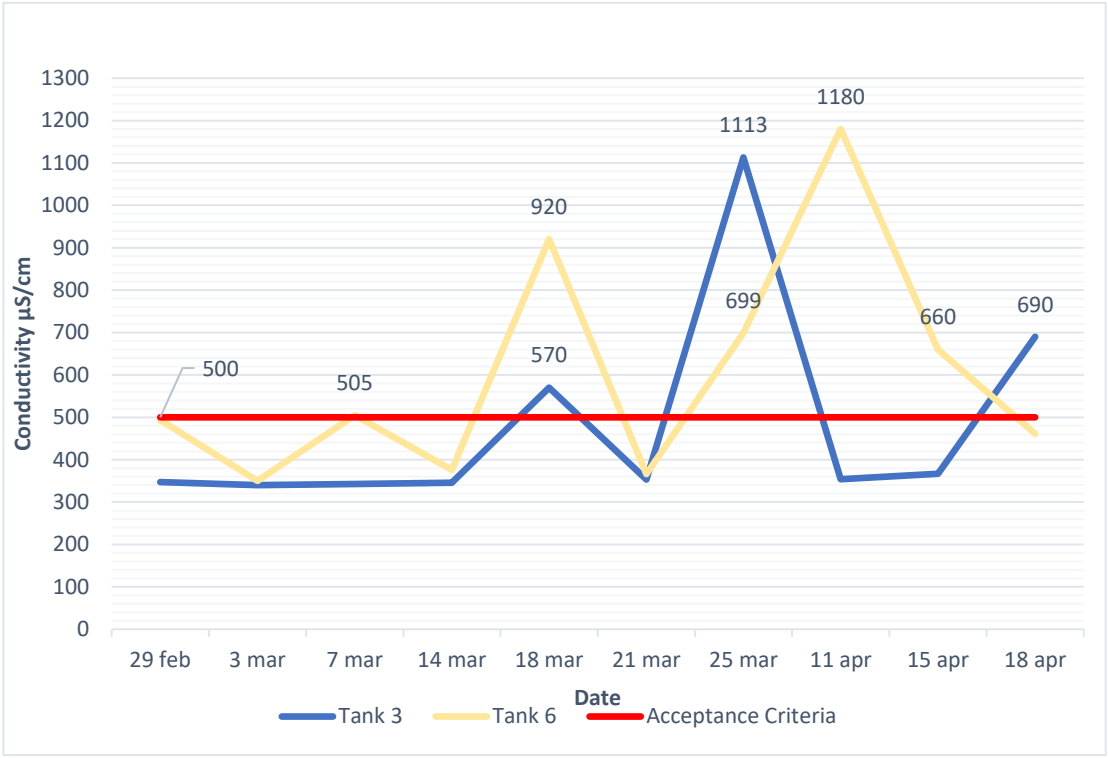


*Figure 7. Trendline overview of tanks with values over 500 µS/cm, depending on the conductivity and occasion. Acceptance criteria is 500 µS/cm and each date with conductivity over 500 µS/cm is specified*

## 4.5 UV-light

A total of 25 UV-light measurements were recorded (table 12). Visual inspection was always performed at the same occasion as the UV-light measurement. The result of the UV-light measurement was in accordance with the results of the visual inspection on every occasion. However, the UV-light could detect different types of coatings on the surfaces.

*Table 12. UV-light measurements.*

| | |
|---|---|
| Clean | 9 |
| Not clean | 9 |
| Not clean + coatings | 7 |
| Total occasions | 25 |

# 5. Discussion

The objective for this study was to design and validate a sampling plan for CIP-operation in a food process. The mentioned food standards FSSC 22000 (2023), BRCGS (2022) and FDA guidelines (2019) emphasize the need for validation and monitoring of CIP-operations. To validate the CIP-operation common methods to assess cleanliness in the food industry were used, and acceptance criteria were selected for each method, either through use of the detection limit, EU legislations (2073/2005) or with discussion with supplier. Conductivity and UV-light are non-microbiological methods and do not compare good with other used methods. But they are good compliments to the microbiological methods.

The validation of the CIP-operation was performed with methods that had previously not been used in the food process before. This meant that all methods were on trial, and some of these methods gave results with similar outcomes. It became clear that some methods were not suitable for validating the CIP-operation in this food premise. Analysis of *Enterobacteriaceae* of swab-samples, rinse water and dip-slides, and the analysis of total aerobic bacteria of dip-slides, all gave results under the acceptance criteria, which indicates that these methods are not suitable for validation in this food process. It should however be noted that *Enterobacteriaceae* analysis of swabs and rinse water had a set detection limit, (<1.0 log CFU/100 $cm^2$ and <2.3 log CFU/g) meaning that the presence of *Enterobacteriaceae* could still be present, but in small amounts. This can change the results but should not impact the food safety, because of the amount being low. The dip slides were never above the acceptance criteria, but different values of CFU could still be noted. The reason why the methods gave results under the acceptance criteria could be because of the methods detection limit but also because of the acceptance criteria being set too high.

Visual inspection was used as a reference method, because visually not clean is always without doubt not clean. When a surface is considered not clean with a method that provides immediate results such as visual inspection or ATP, actions need to be taken and the surface should be cleaned again. However, bacteriological methods are necessary even though the object is clean through visual inspection, because contamination can still be present which is nonvisible. The methods that

most times gave not clean as a result when the sampling point was deemed as visually clean was rinse sampling of total aerobic bacteria, followed by batter sampling of total aerobic bacteria, and then batter sampling of *Enterobacteriaceae*. On the majority of the occasions, ATP-B mostly agreed with the results of visual inspection that the sampling point was clean (69%). ATP-B only determined the sampling point not to be clean at 1.5% of the occasions when the visual inspection determined the sampling points to be clean. This indicates that ATP-B agreed with the visual inspection and may not be useful as a validation method in this CIP-process.

Batter sampling was performed before and after CIP-operations on objects where the same batter could be tracked. The null hypothesis could be rejected for comparison between batters before and after CIP-operations, which means that there is a significant difference of the bacterial populations after CIP-operations and that this method can be used in further validation. The null hypothesis was not rejected for batter between objects, so there is no statistical significance between objects. This may mean that sampling before and after CIP-operations are important and a valid method to analyze the microbiological population in the batter, but tracking the flow of batter seems not to be necessary because of no significant difference between objects.

The measurements for the conductivity showed that two tanks (tank 3 and 6) had higher conductivity in the rinse water, which indicates that rests of alkaline chemicals were still present in the tanks after the CIP-operation. Because they had conductivity measurements over 500 µS/cm several times. These two tanks had visual errors, meaning that they were not fully functional. This had been noted by the food process management, and it could be concluded that the alkaline is not always successfully rinsed away. This indicates that measuring conductivity can be a useful method for detecting errors in construction and in settings of the objects. Meaning that this method could be the basis of finding dead areas with alkaline water and detecting if the settings such as the time for final rinse needs to be extended.

UV-light showed potential to detect coatings, which were not visible with visual inspection. The problem with UV-light was that there was a risk for the workers as the lights had to be turned off in in the food process for the UV-light. For safety reasons, the light should not be turned off in all parts of the food premise, therefore all sampling points could not be inspected with UV-light. But it could be concluded that UV-light is a good extension of visual inspection.

Some of the data collected was analyses by an accredited laboratory, which used a minimum and maximum detection limit. Batter samples gave results within the range of <2.0 and >6.6 log CFU/g. This means that the values could have been much higher. This would change the results of the validation and is something that needs to be considered. But other methods than batter samples, where the acceptance criteria were just considered do not change this result.

Some of the methods used in the validation were deemed as not suitable for the validation of this food process. The result showed that the results from ATP-B usually were the same as for visual inspection. Whiteley et al (2016) also discussed ATP-B in validation, where the method was found to have high variability but was useful to pair together with visual inspection because of its ability to find contamination not visible by visual inspection. This was not in agreement with the findings of this study. One important thing to note is that it was impossible to reach the bottom of the tanks when sampling with both ATP-B and swab. This is where the batter and rinse water were collected and usually the place in the tank deemed as not clean during visual inspection. This is something that most probably affected the result of this study. This indicates that ATP-B is not suitable for trial of large tanks were the bottom surface cannot be reached. However, ATP-B could still be a useful tool for measurements of pipelines, were rinse and batter samples are difficult to collect. Kohli (2012) has previously observed ATP-B to be a suitable method for smaller areas, because swabbing a small area of a large object may not be representative. This is something that might advocate the use of swab and ATP-B for pipelines in food processes.

Rinse water and batter sampling were two methods that seemed adequate for validation in this pancake factory. However, Kohli (2012) discusses that there is a risk of unacceptably high amounts of contaminants, such as bacteria and soil in the pipelines that may be missed when sampling with rinse water or food stuff. This is something that needs to be considered in this validation process. Visual inspection and swabbing for total aerobic bacteria are methods that showed to be useful in combination with rinse water and batter sampling in this food process.

# 6. Conclusion

Validation of a sampling plan of a CIP system was performed and was based on food safety and medical standards. The standards had requirements on the need for acceptance criteria's, which were set for each used method. These acceptance criteria were selected using EU legislations, detection limits and in discussion with supplier.

Based on the results in this thesis a suggested sampling plan for the CIP-operation of the food process should include the following steps: Visual inspection, which determines if the object is clean or not clean and sampling of rinse water for conductivity measurement. No other method is needed to follow-up the visual inspection and the conductivity test, if the sampling point is determined not clean and/or have alkaline left in the rinse water. Rinse water analysis and swab-sampling seemed to be adequate methods to verify the daily inspections. Batter sampled before and after the CIP-operation of the object should also be performed. Followed by UV-light inspection, if it does not make a risk to turn off the lights during the sampling. This proposed sampling plan could be performed on each sampling point on a regular basis, and when changes have been made on the object or in the CIP-settings. The sampling frequency for sampling validation should be set by producer.

Some of the acceptance criteria were based on detection limits and discussion with supplier of the sampling material, which is according to legislations and food standards. However, the results of this study could have been different if other acceptance criteria would have been selected.

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# Popular science summary

There are many ways of producing food in factories, but that also means that there are a lot of ways for the food to spoil or spread diseases to humans. It is therefore very important to have good and up to date systems for cleaning in food production. Clean-in-place (CIP) is a type of system that cleans all the pipelines and tanks without disassembly. CIP is an integrated system within the pipelines and tanks that can clean everything with just the press of a button. But it is important to control this system so that everything becomes clean. The danger is that food and other contaminants get stuck inside the pipelines and tanks and is not visible. So there needs to be a way of proving that the system works or not, and this is what this study explores.

The objective of this study was to design and validate a sampling plan for CIP-operation in a food process. This was made by first studying food safety standards and medical standards to see what demands are set on CIP-systems. Food safety standards and medical standards vary in their demand on CIP-operations, but they all expect a validated plan for how to ensure cleanliness. Methods were chosen and acceptance criteria for each method were set, either by EU-legislations, detection limit or by discussion with supplier. Acceptance criteria were used to determine if the method says that the object is clean or not clean. A total of 13 different methods were used on 13 different objects with individual CIP-systems, and three additional locations were also included. These objects were either tanks or pipelines. Trials were then repeated on each object and location on nine occasions and were always done after CIP-operations. Microbiological methods detected the number of total aerobic bacteria and *Enterobacteriaceae*. This is because it was deemed to be good to get a general picture of the cleanliness and bacterial population. These methods were analyzed by SGS analytics and had a fixed detection limit.

It became clear that some methods were not suitable for validation after all trials were done. Some methods had given the same result under the detection limit for all trials and were excluded from further analysis. Visual inspections were deemed as a good initial method to assess if the object was clean or not. It was therefore used as a reference method, and its results were compared together with other results. ATP-B was a method that gave quick results that were compared with visual inspection. The comparison showed that this method almost always agreed with visual inspection, and ATP-B was deemed unnecessary in this validation. There

were however some methods that could detect false negatives when compared to visual inspection. Meaning methods that did not agree with visual inspection when it determined the object clean. These methods were analysis of total aerobic bacteria of swab-samples and rinse water. Together with analysis of total aerobic bacteria and *Enterobacteriaceae* batter samples. These methods are deemed useful for the validation to confirm cleanliness. Batter samples were taken before and after each CIP-operation in the same places. This was to see if there was a change in results before and after cleaning, but also to see if the batter's bacterial population changed when travelling between objects. The statistical analysis of the batters showed a significant difference before and after CIP-operations, but no significant difference between objects. Conductivity measurements were made in the rinse water after each CIP-operation. Conductivity can detect if there are any chemical cleaners left after the CIP-operation. This method could detect chemicals and was deemed as good for validation. UV-light was also used to find coatings inside objects that were not visible to the naked eye. It was proven successful in doing this but came with a risk, because all other light needed to be turned off in the factory.

The trials showed which method was fit for the food process. So, the suggested sampling plan used for validation would begin with visual inspection and conductivity measurements. No further methods would be needed if the visual inspection deemed the object as not clean and/or conductivity measurements could detect chemicals. But further methods would be needed to be certain if the object is clean if the visual inspection and conductivity measurements deemed it so. So, rinse water analysis and swab-sampling would be used, and batter samples would be taken at the next CIP-operation. UV-light should also be used if possible.

It is important to note that the results of the microbiological methods were sometimes "over this value". This means that these results are impossible to know and could have affected the result. It should also be mentioned that ATP-B samples that were taken did not reach the bottom of the tanks where most of the food stuff were. This means that ATP-B could be a good method for the pipelines but not the tanks. The acceptance criteria for the methods were sometimes set by discussion with supplier or the detection limit of the analysis because no food safety standards or legislations had any suggestions. This creates uncertainty about the validation and other acceptance criteria that would affect the results. Further research is therefore needed in determining acceptance criteria.

# Acknowledgements

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# Appendix I

Results of the methods; Dip-slides, swabbing, ATP-B, UV-light, visual and rinse water samples. Place ID is the place where the measurement was taken. Replication ID is the date of the measurement. DS. Aerob and Entero are the Dip-slide result of total aerobic bacteria and *Enterobacteriaceae*. S. Aerob and Entero are the swab results of Aerobic bacteria and *Enterobacteriaceae*. R. Aerob and Entero are the rinse water results of total aerobic bacteria and *Enterobacteriaceae*.

| PlaceID | Replication ID | DS.Aerob *(CFU/sample)* | DS.Entero *(CFU/sample)* | S.Aerob *(CFU/sample)* | S.Entero *(CFU/sample)* | ATP *(RLU/100cm$^{2)}$* | UV-light *(clean/not clean)* | Visual inspection *(clean/not clean)* | R.Aerob *(log CFU/g)* | R.Entero *(log CFU/g)* |
|---|---|---|---|---|---|---|---|---|---|---|
| T1 | 1 | 0 | 0 | | | 9 | | 0 | | |
| T1 | 2 | 0 | 0 | <10 | <10 | 97 | 1 | 1 | | |
| T1 | 3 | 1 | 1 | <10 | <10 | 3 | | 1 | | |
| T1 | 4 | | | 50 | <10 | 2 | 1 | 1 | | |
| T1 | 5 | | | 70 | <10 | 8 | | 0 | | |
| T1 | 6 | 0 | 0 | <10 | <10 | 3 | 0 | 0 | | |
| T1 | 7 | 0 | 0 | <10 | <10 | 7 | | 1 | | |
| T1 | 8 | 0 | 0 | 10 | <10 | 2 | | 0 | | |
| T1 | 9 | 0 | 0 | <10 | <10 | 2 | | 0 | | |
| T2 | 1 | 0 | 0 | | | 5 | | 0 | <3.3 | <1.0 |
| T2 | 2 | 0 | 0 | <10 | <10 | 5 | 1 | 1 | | |
| T2 | 3 | 0 | 0 | <10 | <10 | 7 | | 0 | <5.0 | <2.0 |
| T2 | 4 | | | 700 | <10 | 11 | 1 | 1 | 3.7 | <2.0 |
| T2 | 5 | | | 70 | <10 | 9 | | 0 | 3.2 | <2.0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| T2 | 6 | 0 | 0 | <10 | <10 | 6 | | 0 | 2.6 | <2.0 |
| T2 | 7 | | | | | | | | | |
| T2 | 8 | | | | | | | | | |
| T2 | 9 | 0 | 0 | <10 | <10 | 2 | | 0 | <2.3 | <2.0 |
| T3 | 1 | 0 | 0 | | | 6 | | 0 | <3.3 | <1.0 |
| T3 | 2 | 3 | 1 | <10 | <10 | 5 | 0 | 0 | <3.3 | <2.0 |
| T3 | 3 | 3 | 0 | <10 | <10 | 6 | | 0 | <2.3 | <2.0 |
| T3 | 4 | 4 | 1 | 30 | <10 | 416 | 1 | 1 | <2.3 | <2.0 |
| T3 | 5 | | | 10 | <10 | 8 | | 0 | 3.2 | <2.0 |
| T3 | 6 | 0 | 0 | <10 | <10 | 5 | | 1 | 2.6 | <2.0 |
| T3 | 7 | 0 | 0 | <10 | <10 | 8 | | 0 | <2.3 | <2.0 |
| T3 | 8 | 0 | 0 | >2500 | <10 | 2385 | | 1 | 2.6 | <2.0 |
| T3 | 9 | 4 | 0 | <10 | <10 | 10 | | 0 | 3.3 | <2.0 |
| T4 | 1 | 0 | 1 | | | 11 | | 1 | <3.3 | <1 |
| T4 | 2 | 0 | 0 | <10 | <10 | 2 | 0 | 0 | <3.3 | <2.0 |
| T4 | 3 | 1 | 1 | <10 | <10 | 5 | 1 | 1 | <2.3 | <2.0 |
| T4 | 4 | | | 20 | <10 | 3 | 0 | 0 | 4.2 | <2.0 |
| T4 | 5 | | | <10 | <10 | 5 | | 0 | <2.3 | <2.0 |
| T4 | 6 | 0 | 0 | <10 | <10 | 5 | 1 | 1 | 2.3 | <2.0 |
| T4 | 7 | 0 | 0 | <10 | <10 | 4 | | 1 | <2.3 | <2.0 |
| T4 | 8 | 0 | 0 | <10 | <10 | 5 | 1 | 1 | 2.3 | <2.0 |
| T4 | 9 | 2 | 0 | <10 | <10 | 46 | | 1 | <2.3 | <2.0 |
| T5 | 1 | 0 | 0 | | | 3 | | 1 | <3.3 | <1.0 |
| T5 | 2 | 0 | 0 | <10 | <10 | 2 | 1 | 1 | <3.3 | <2.0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| T5 | 3 | 1 | 0 | <10 | <10 | 12 | 1 | 1 | <5.0 | <2.0 |
| T5 | 4 | | | 30 | <10 | 6 | 1 | 1 | 4.4 | <2.0 |
| T5 | 5 | | | <10 | <10 | 6 | | 0 | 3.3 | <2.0 |
| T5 | 6 | 0 | 0 | <10 | <10 | 4 | 1 | 1 | 2.6 | <2.0 |
| T5 | 7 | 0 | 0 | <10 | <10 | 6 | | 1 | <2.3 | <2.0 |
| T5 | 8 | 0 | 0 | <10 | <10 | 2 | 1 | 0 | <2.3 | <2.0 |
| T5 | 9 | 0 | 0 | <10 | <10 | 5 | | 1 | | |
| T6 | 1 | 0 | 0 | | | 4 | | 1 | <3.3 | <2.0 |
| T6 | 2 | 0 | 2 | <10 | <10 | 9 | 1 | 1 | <3.3 | <2.0 |
| T6 | 3 | 1 | 0 | <10 | <10 | 4 | | 1 | <5.0 | <2.0 |
| T6 | 4 | 0 | 0 | 30 | <10 | 10 | 1 | 1 | 2.3 | <2.0 |
| T6 | 5 | | | <10 | <10 | 5 | | 1 | 2.3 | <2.0 |
| T6 | 6 | 0 | 0 | <10 | <10 | 4 | | 1 | <2.3 | <2.0 |
| T6 | 7 | 1 | 0 | <10 | <10 | 228 | | 1 | <2.3 | <2.0 |
| T6 | 8 | 0 | 0 | 10 | <10 | 4 | | 1 | <2.3 | <2.0 |
| T6 | 9 | 1 | 0 | <10 | <10 | 12 | | 1 | 2.6 | <2.0 |
| T7 | 1 | 0 | 1 | | | 15 | | 0 | <3.3 | <1.0 |
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| T7 | 3 | 0 | 0 | <10 | <10 | 16 | | 0 | <5.0 | <2.0 |
| T7 | 4 | 1 | 0 | 30 | <10 | 6 | 1 | 1 | 2.3 | <2.0 |
| T7 | 5 | | | <10 | <10 | 10 | | 0 | 3.2 | <2.0 |
| T7 | 6 | 0 | 0 | <10 | <10 | 6 | | 0 | <2.3 | <2.0 |
| T7 | 7 | 6 | 1 | <10 | <10 | 71 | | 1 | <2.3 | <2.0 |
| T7 | 8 | 0 | 0 | <10 | <10 | 6 | | 0 | <2.3 | <2.0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| T7 | 9 | 0 | 0 | <10 | <10 | 11 | | 0 | <2.3 | <2.0 |
| T8 | 1 | 1 | 1 | | | 6 | | 0 | 3.6 | <1.0 |
| T8 | 2 | 1 | 0 | <10 | <10 | 5 | 0 | 0 | 5.3 | <2.0 |
| T8 | 3 | 0 | 0 | <10 | <10 | 3 | 0 | 0 | 3 | <2.0 |
| T8 | 4 | 0 | 0 | 30 | <10 | 5 | | 0 | 4.9 | <2.0 |
| T8 | 5 | | | <10 | <10 | 4 | | 1 | 3.6 | <2.0 |
| T8 | 6 | 0 | 0 | <10 | <10 | 3 | | 0 | <2.3 | <2.0 |
| T8 | 7 | 0 | 0 | <10 | <10 | 5 | | 0 | | |
| T8 | 8 | 0 | 0 | <10 | <10 | 4 | | 1 | | |
| T8 | 9 | 0 | 0 | <10 | <10 | 11 | | 0 | <2.3 | <2.0 |
| T9 | 1 | 0 | 1 | | | 4 | | 0 | 3.3 | <1.0 |
| T9 | 2 | 0 | 0 | <10 | <10 | 4 | | 0 | <3.3 | <2.0 |
| T9 | 3 | 0 | 0 | <10 | <10 | 64 | 0 | 0 | <2.3 | <2.0 |
| T9 | 4 | | | 30 | <10 | 3 | | 0 | 4.3 | <2.0 |
| T9 | 5 | | | <10 | <10 | 4 | | 0 | 2.9 | <2.0 |
| T9 | 6 | 0 | 0 | <10 | <10 | 7 | | 0 | <2.3 | <2.0 |
| T9 | 7 | 0 | 0 | <10 | <10 | 3 | | 0 | | |
| T9 | 8 | 0 | 0 | <10 | <10 | 4 | | 0 | 2.3 | <2.0 |
| T9 | 9 | 0 | 0 | <10 | <10 | 3 | | 0 | | |
| T10 | 1 | 0 | 0 | | | 3 | | 0 | 3.3 | <1.0 |
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| T10 | 4 | | | 30 | <10 | 3 | | 0 | <2.3 | <2.0 |
| T10 | 5 | | | <10 | <10 | 3 | | 0 | 3.2 | <2.0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| T10 | 6 | 0 | 0 | <10 | <10 | 4 | | 0 | <2.3 | <2.0 |
| T10 | 7 | 0 | 0 | <10 | <10 | 3 | | 0 | | |
| T10 | 8 | 0 | 0 | <10 | <10 | 8 | | 0 | <2.3 | <2.0 |
| T10 | 9 | 1 | 0 | <10 | <10 | 8 | | 0 | | |
| PD1 | 1 | | | | | 4 | | 0 | <3.3 | <1.0 |
| PD1 | 2 | | | <10 | <10 | 6 | | 0 | <3.3 | <2.0 |
| PD1 | 3 | | | <10 | <10 | 24 | | 0 | <2.3 | <2.0 |
| PD1 | 4 | | | 10 | <10 | 7 | | 0 | | |
| PD1 | 5 | | | <10 | <10 | 9 | | 0 | 3.2 | <2.0 |
| PD1 | 6 | | | <10 | <10 | 203 | | 0 | <2.3 | <2.0 |
| PD1 | 7 | | | <10 | <10 | 8 | | 0 | <2.3 | <2.0 |
| PD1 | 8 | | | >2500 | <10 | 29 | | 0 | 3.3 | <2.0 |
| PD1 | 9 | | | <10 | <10 | 6 | | 0 | <2.3 | <2.0 |
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| D2 | 2 | | | <10 | <10 | 5 | | 0 | | |
| D2 | 3 | | | <10 | <10 | 8 | | 0 | | |
| D2 | 4 | | | <10 | <10 | 4 | | 0 | | |
| D2 | 5 | | | 10 | <10 | 3 | | 0 | | |
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| D3 | 1 | | | | | 5 | | 0 | | |
| D3 | 2 | | | <10 | <10 | 5 | | 0 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| D3 | 3 | | | <10 | <10 | 17 | | 0 | | |
| D3 | 4 | | | 50 | <10 | 6 | | 0 | | |
| D3 | 5 | | | <10 | <10 | 7 | | 0 | | |
| D3 | 6 | | | 220 | <10 | 123 | | 1 | | |
| D3 | 7 | | | <10 | <10 | 7 | | 1 | | |
| D3 | 8 | | | 1100 | <10 | 9 | | 0 | | |
| D3 | 9 | | | <10 | <10 | 6 | | 0 | | |
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| P2 | 2 | | | <10 | <10 | 6 | | 0 | | |
| P2 | 3 | | | <10 | <10 | 7 | | 0 | | |
| P2 | 4 | | | 20 | <10 | 6 | | 0 | | |
| P2 | 5 | | | <10 | <10 | 7 | | 0 | | |
| P2 | 6 | | | <10 | <10 | 4 | | 0 | | |
| P2 | 7 | | | | | | | | | |
| P2 | 8 | | | <10 | <10 | 5 | | 0 | | |
| P2 | 9 | | | <10 | <10 | 2 | | 0 | | |
| P3 | 1 | | | | | 4 | | 0 | | |
| P3 | 2 | | | <10 | <10 | 4 | | 0 | | |
| P3 | 3 | | | <10 | <10 | 3 | | 0 | | |
| P3 | 4 | | | >2500 | <10 | 55 | | 0 | | |
| P3 | 5 | | | <10 | <10 | 3 | | 0 | | |
| P3 | 6 | | | <10 | <10 | 4 | | 0 | | |
| P3 | 7 | | | <10 | <10 | 7 | | 0 | | |
| P3 | 8 | | | <10 | <10 | 94 | | 0 | | |

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