



Final report

Evaluation of an innovative processing technology for production efficiencies and cost savings in food processing including red meat

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Abstract

The proposed project will bring commercially proven air and surface purification technology in global applications to Australia for validation in a specific Stage 1 application in red meat processing. The current project proposes to evaluate the application of an innovative processing technology for production efficiencies and cost savings in red meat processing, specifically in ready retail meat products. Specifically, the project will evaluate air and surface purification technology to deliver more efficient and cost effective hygienic processes than the current manual wet cleaning methods required prior and periodically throughout red meat production.

The project aims to evaluate **Active Photocatalytic Oxidation (PCO)** technology to reduce &/or **eliminate airborne and surface pathogens**, including SARS-CoV-2 (novel coronavirus) and its variants, for its potential to **extend shelf life of food products** with inclusion of red meat. In addition to reviewing the potential effectiveness of reducing the health risks in the workplace and improved food safety, this project will also evaluate specific applications for red meat processing and the process steps for successful implementation. The expected profitability gains through widespread introduction are likely to be shared up and down the value chain with producers, suppliers and customers.

Executive summary

Background

Australian red meat processing has exemplar food hygiene standards, which comes at significant cost to the business. It is generally considered that the cost of maintaining the required high hygienic standards is in the top 10 highest costs of meat production. Specifically, retail ready operations estimate the overall cost of maintaining a high hygienic standard could be 10-15% of the cost of production. There are significant production efficiencies and opportunity cost savings that could be delivered by **more efficient and cost effective hygiene maintaining procedures**. Coles, RROA and MLA are partnering on an innovative processing technology that is new to Australia and food processing including red meat.

The current project proposes to evaluate the application of an innovative processing technology for production efficiencies and cost savings in red meat processing, specifically in retail ready meat products. While used in various proven applications in medical and food production applications globally, this will be the first application of this technology in Australia and for red meat processing. There are expected to be significant benefits and impacts to the red meat industry with the application of this new technology in production efficiencies and cost savings to maintain and likely exceed the current high food hygienic standards.

To mitigate the very real and present risks of harm caused by exposure to **SARS-CoV-2 (novel coronavirus)** and the resulting disease, **COVID-19**, and other pathogens, this project seeks to evaluate new technology, specifically Active Photocatalytic Oxidation (PCO), that aims to reduce &/or eliminate airborne and surface pathogens, including SARS-CoV-2 (novel coronavirus), with the additional benefit of potential to **extend shelf life of food products** including red meat, reduce packaging material contaminants and **reductions in HVAC pathogens such as mould**.

The PCO phenomena was originally discovered by **Akira Fujishima**¹ in 1967, the so-called Honda Fujishima effect. This phenomenon was developed into an air purification system by NASA scientists in the 1990s for use on the Space Shuttle and International Space Station. Some of the key patents for this NASA technology were later acquired by **Puradigm**², who in 2011, made a breakthrough that took this passive technology and developed it into an active, safe and scalable technology capable of reaching every corner of an enclosed space, **inactivating pathogens and volatile organic compounds in both the air and on surfaces**.

Real-world evidence as reported by certified labs from several years of use at various hospitals, indoor grow facilities, food processing plants, university, school, office and retail spaces around the world, show high levels of reduction in both airborne and surface microbial loads, are achievable at scale.

The technology has been successfully tested at the **CSIRO's³ Australian Centre for Disease Preparedness against SARS-CoV-2, Delta variant** which showed that the device has an active agent which was able to inactivate the virus on surfaces remote from the device, without elevating ozone above safe or pathogenic levels. The study did not investigate what the active agent is, as this has been investigated through many previous studies which showed it is comprised of water vapour surrounded by a non-thermal plasma (NTP) ie H+ and -O₂ ions, basically a **charged water aerosol**. The ions are naturally produced outdoors and our bodies are adapted to live with them, so it is **totally safe, yet very effective** at destroying both bacteria and volatile organic compounds and inactivating

viruses of all types. When used continuously, this NTP, doesn't allow pathogens to build up and so doesn't need to be as harsh as general sanitation chemicals such as alcohol or other disinfectants. The most effective implementations are preventative rather than as a remediation method ie **prevention is better than cure**. However remediation is certainly possible, but just requires longer exposure times, as was shown in the results within this project for heavily contaminated surfaces.

The proposed project will bring this air and surface purification technology to Australia for validation in a specific Stage 1 applications within red meat processing. The project will provide insights and practical knowledge on how this publicly **available technology can be applied to benefit red meat production operations across the whole value chain**. It is envisaged that this may lead into further research aimed at incorporating the technology into MAP, Utilities, odour control in waste management and increased sanitation in equipment cleaning applications to provide additional microbial reduction with associated cost savings and efficiency gains.

This Stage 1 project will bring the air and surface purification technology to Australia for validation on specific applications in red meat processing in multiple phases. It will initially evaluate the air and surface purification technology in the Chef Fresh facility's packaging material decontamination rooms (previously ozone rooms). Subsequent phases will cover trials in real-world settings in RROA focusing on pathogen reduction in the HVAC system, implementation challenges for production environments, shelf life extension of food products and enhancing food safety trust.

The outcomes of this stage 1 validation in red meat processing will be applied to develop recommendations for adoption in red meat processing. The outcome will also be used to identify and inform any potential future R&D.

1. Akira Fujishima - [Akira Fujishima - Wikipedia](#)
2. Puradigm is a US-based company (www.puradigm.com) with local Australian distributor Pandara (<https://pandara.life>)
3. "Performance testing of Active PCO technology for SARS-CoV-2 inactivation on surfaces", CSIRO. Only available in full with permission by Coles Group Limited. The study aimed to confirm the existing study reports and enable internal Coles evaluation of the technology, and should not be deemed as an endorsement of the technology by the CSIRO.

Objectives

The overall objective is to evaluate air and surface purification technology for packaging material surface pathogen reduction, extended shelf-life and HVAC pathogen reduction in food processing, including in red meat. The project aims to evaluate new pathogen reduction technology that aims to reduce &/or eliminate airborne and surface pathogens, including SARS-CoV-2 (novel coronavirus), for its potential to extend shelf life of food products and reduce pathogen bio-loads in various settings in Coles' manufacturing facilities with inclusion of red meat.

Specifically, the project aims are to:

- Gain an understanding of the efficacy of Active PCO technology in real-world environments against various microbial loads that are possible in red meat processing facilities with the view of continuing to provide leading edge hygiene standards with reduced need for interventionist cleaning regimes which interrupt production.
- Gain an insight into the efficacy of Active PCO technology in reducing intra-day and intra-species cleaning while maintaining shelf-life of food products, including red meat.
- Develop implementation designs for the various real-world environments to manage challenges such as air movements, large scale spaces, fast moving product, wash down procedures, etc.
- Identify use cases for the technology across the facility at RROA and Chef Fresh.

Methodology

Milestone 1 will develop the detailed test schedules for the overall project, including Chef Fresh packaging decontamination, RROA HVAC pathogen reductions and RROA shelf-life extension trials.

Milestone 2 will focus on evaluating the technology in real-world settings against standard microbiological pathogens expected in the Chef Fresh packaging material decontamination rooms (old ozone rooms).

Milestone 3 will focus on evaluating the technology in real-world settings within the RROA facility, testing against standard microbiological pathogens found in the air and on surfaces of the HVAC environments.

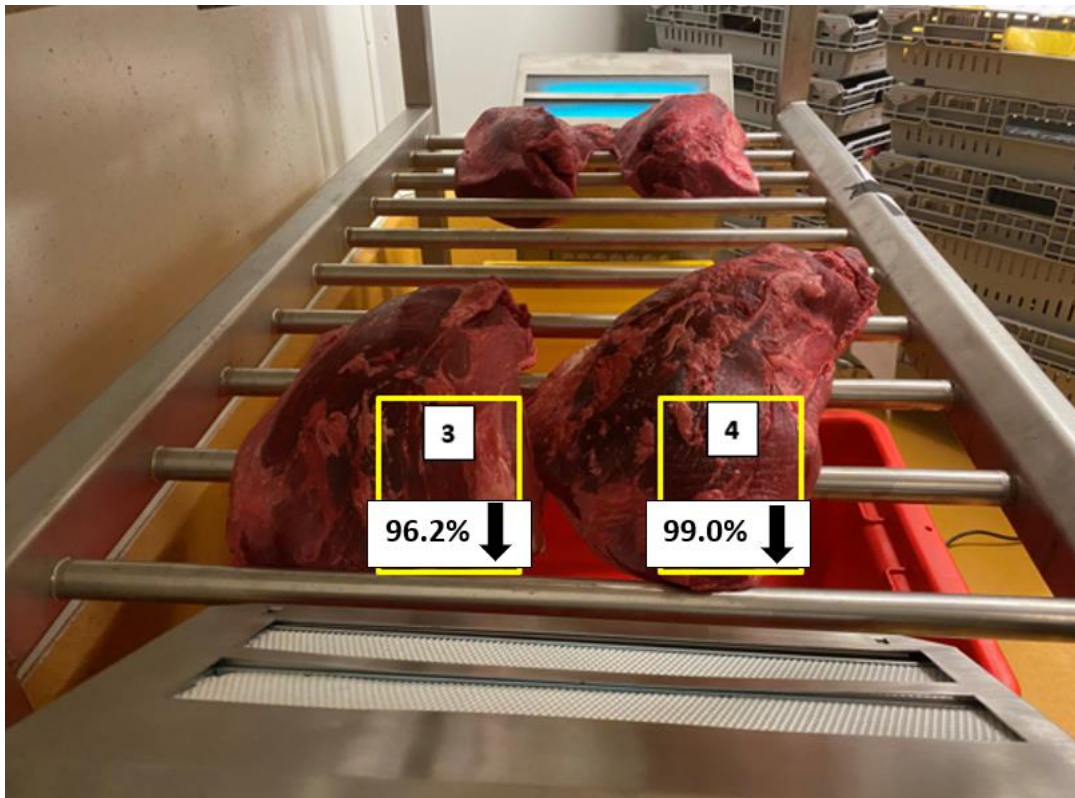
Milestone 4 will evaluate the technology to investigate shelf life extension of food products and enhancing food safety trust.

Milestone 5 is the final report detailing the outcomes of the packaging decontamination, shelf life extension, HVAC microbiological reduction and shelf life extension in combination with Denba.

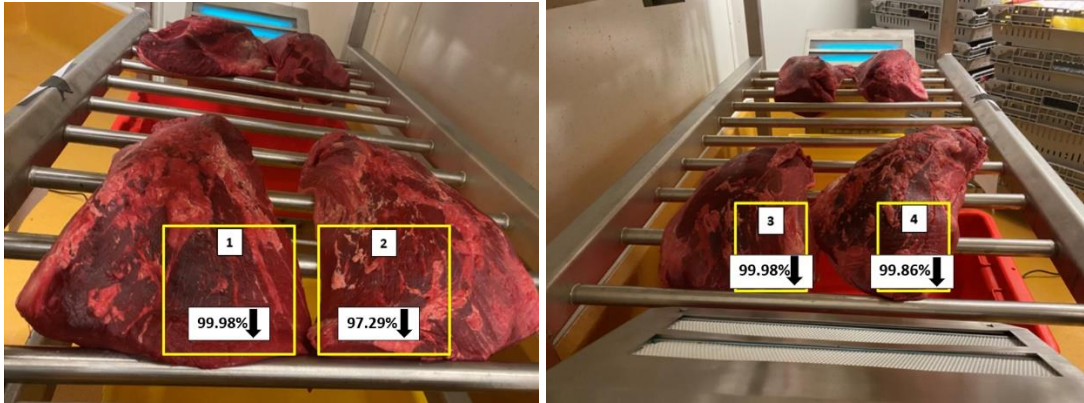
Results/key findings

Microbiological Reductions on Primal Surfaces for various exposure times

24hr Exposures – 99% / 2 Log Reductions (35 day aged primals / Extremely high count)



>24hr Exposures – 99.99% / 4 Log Reductions (35 day aged primals / Extremely high count)



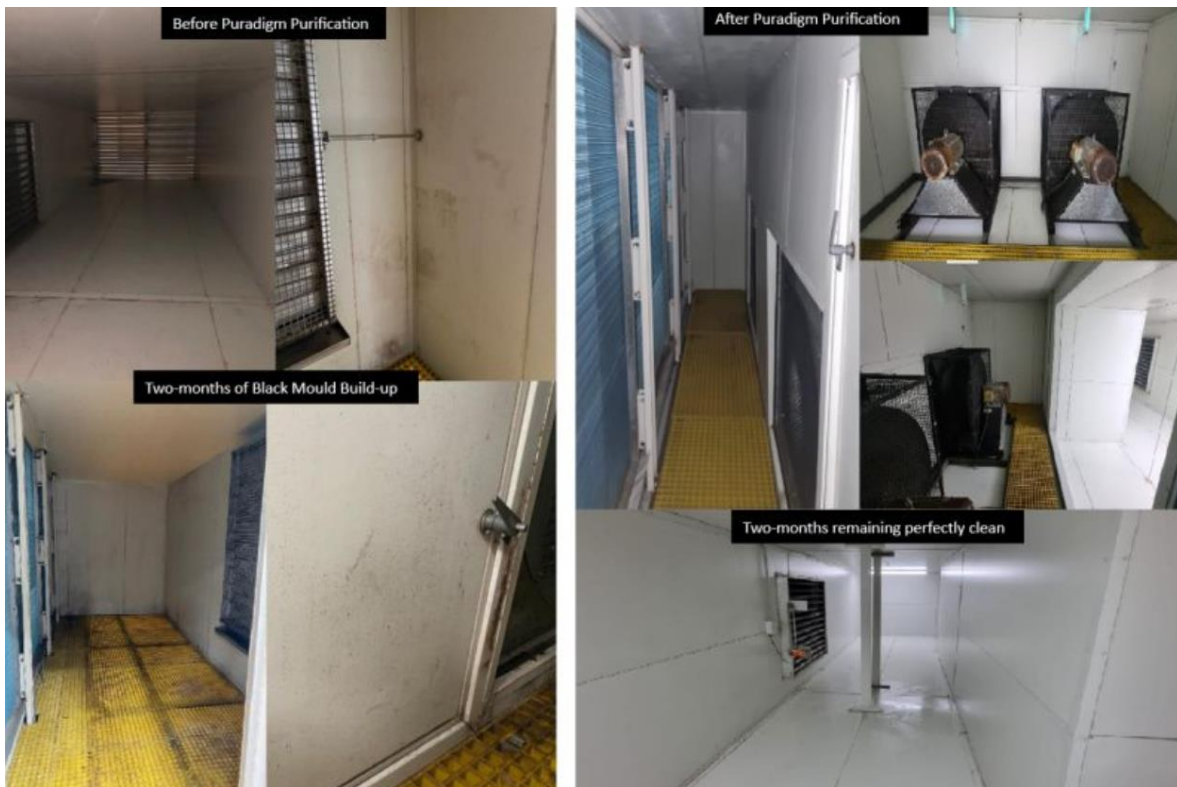
5 min Exposures – 90% / 1 Log Reductions (Fresh primals 10 day aged / Low count)

10 min Exposures – 100% / >8 Log Reductions (Fresh primals 10 day aged / Low count)



Microbiological Reductions on HVAC Air Handling Unit (AHU) Surfaces

Black mould is one of the most difficult microbiological pathogens to remove from air and surfaces. **Puradigm was able to keep the HVAC's AHU surfaces clean well beyond the usual 2 month cleaning period.** It should be noted these surfaces experience high humidity every night but this posed no issue for the Puradigm charged water aerosols. The air is being circulated during the day at very **high flow rates of 20 m³/s** per AHU. Every evening the plant is cleaned which produces large quantities of steam (**100% humidity**) and the AHU air flow rates are lowered to around 5 m³/s and ventilated to atmosphere. In this way the AHUs are subjected to some extreme conditions which are **more severe than seasonal humidity changes.**



Microbiological Reductions on Packaging Surfaces covered in Water

High concentration of staphylococcus submerged in water – no reductions

Large quantities of water will block the effect of Puradigm’s charged water aerosols. However, as can be seen in the HVAC AHU trial, damp or dry surfaces experience significant and rapid reductions.

The Chef Fresh facility’s packaging decontamination has two rooms, one with raw product packaging which is very wet and the other with dry packaging material. Puradigm is only suitable for the dry packaging material room, while alternate decontamination technology, is required for the heavily wet packaging material surfaces.



Benefits to industry

These trials confirm the effectiveness of Puradigm’s air and surface purification technology within various red meat industry environments and detail the implementation considerations which are specific to this industry. ***The following benefits are based on global research findings as well as those carried out in this project.***

The benefits for the red meat industry are:

- Primary processors - ***Carcass surface purification*** over short or long exposure times – reduced reliance on chemical washes or DNA damaging interventions such as UV. ***Enhanced shelf-life of products.***

- Primary & Secondary processors - **Primal surface purification** over short exposures prior and during portioning. **Enhanced shelf-life of products.**
- Primary & Secondary processors – **Cleaning of Air Handling Units, refrigeration coils** and surfaces, 24/7 cleaning, **reducing chemical clean frequency, usage and cost**
- Primary, Secondary and Supermarkets - reducing air borne & surface pathogens including **SARS-CoV-2, cold, influenza, norovirus and other bacteria and viruses** and thereby keeping staff & customers safe, **reducing absenteeism** and enhancing the well-being of people, plants and animals.
- Primary & Secondary processors – **Purification of external packaging surfaces** prior to opening within high risk production areas.
- Primary & Secondary processors – Purification of production surfaces between chemical cleaning periods, **to minimise the risk of outbreaks such as listeria**, and **enhance shelf-life** of products.
- Supermarket – **Reduce surface pathogens in deli and fresh produce aisles** to **enhance shelf-life** of products
- Puradigm is over **2400x faster than HEPA alone purification**, as the air is not required to pass through the Puradigm device, but instead every part of the volume of an indoor space is filled with the **natural charged water aerosols**, deactivating pathogens in seconds rather than 40 mins or more.
- **Safer and more effective than UV** which requires slow air flow and close proximity, and requires the air to be brought to the device. UV can also be blocked by shadowing and damages the DNA of products thereby changing its taste and texture.
- **Puradigm is safe for continuous operation in occupied spaces** keeping people and products protected at all times, never allowing pathogens to build up to unsafe levels which would then require harsh chemical interventions.

Future research and recommendations

Further research aimed at incorporating the technology into **MAP packaging, odour control** in waste management, **minimise offensive odours such as those from fish, equipment cleaning**, transportation of **fresh produce, transportation sanitation for mould control**, and **combined applications with Denba** to provide additional microbial reductions/control **for enhanced shelf-life of products**, reduced energy consumption for frozen products and enhance frozen products to fresh or sashimi-grade on defrosting.

Trials in supermarkets and primary processing to investigate optimal implementation strategies for the benefits listed above should be conducted.

Export market research analysis aimed at the benefits of certifying processing plants as COVID-19 safe production environments.

This project has confirmed that Puradigm represents a paradigm shift in purification, promising a wide range of benefits to all industries, including the red meat industry.

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1 Milestone Description

1.1 Milestone 1

Design and detailed work testing schedule.

Design trials & testing methodologies, evaluate safety aspects and develop operating protocols for the purification technology. Procure test units. Develop a detailed project testing schedule. Identify key value propositions/ potential areas of application in red meat. Project steering group formed.

Milestone 1 Report: Progress report on design and detailed project schedule submitted to the project steering group & approved by MLA.

1.2 Milestone 2

Practical trials and initial testing in production environment, including:

- Microbiological reductions on packaging material within old ozone rooms
- Analysis of environmental conditions and design to maximise Ion efficiencies in new and old ozone rooms

Milestone 2: Progress report on practical trials and testing to be submitted to the project steering group and approved by MLA.

1.3 Milestone 3

Commission and production testing at Coles RROA.

Install trial units in RROA production HVAC locations. Design production trial methodologies. Collect and analyse test samples, including:

- Microbiological reductions in air and on surfaces within the HVAC environment
- Microbiological reductions on conveyor or machine surface

Shelf life trials

- Microbiological reduction trials over 24, 96 and 144hrs

Milestone 3: Progress report on commission of production trials and testing to be submitted to the project steering group and approved by MLA.

1.4 Milestone 4

Commission and production testing at Coles RROA.

Start testing RROA production HVAC locations. Collect and analyse test samples, including:

- Microbiological reductions in air and on surfaces within the HVAC environment

Shelf life trials

- Microbiological trials on fresh primals to determine speed of reduction for short duration exposure
- Design for combination of Denba and Puradigm for maximum reductions

Milestone 4: Progress report on commission of production trials and testing to be submitted to the project steering group and approved by MLA.

1.5 Milestone 5

Deliver key functions such as:

Practical trials in production environment, including:

- Microbiological reduction final report on effectiveness in various conditions and timeframes.
- Results for HVAC trials in Air Handling Units, for extremely high speed air currents
- Puradigm and Denba combined shelf life extension trials
- Packaging decontamination final report on effectiveness

Milestone 5: Final report on practical trials and testing to be submitted to the project steering group and approved by MLA.

2 Background

2.1 Project purpose & scope

The overall objective is to evaluate air and surface purification technology for packaging material surface pathogen reduction, extended shelf-life and HVAC pathogen reduction in food processing, including in red meat. The project aims to evaluate new pathogen reduction technology that aims to reduce &/or eliminate airborne and surface pathogens, including SARS-CoV-2 (novel coronavirus), for its potential to extend shelf life of food products and reduce pathogen bio-loads in various settings in Coles' manufacturing facilities with inclusion of red meat.

Specifically, the project aims are to:

- Gain an understanding of the efficacy of Active PCO technology in real-world environments against various microbial loads that are possible in red meat processing facilities with the view of continuing to provide leading edge hygiene standards with reduced need for interventionist cleaning regimes which interrupt production.
 - Gain an insight into the efficacy of Active PCO technology in reducing intra-day and intra-species cleaning while maintaining shelf-life of food products, including red meat.
 - Develop implementation designs for the various real-world environments to manage challenges such as air movements, large scale spaces, fast moving product, wash down procedures, etc.
- Identify use cases for the technology across the facility at RROA and Chef Fresh

2.2 Project Target Outcomes

Value proposition and benefits to the Australian red meat industry

The primary proposition of the project is to deliver significantly more efficient and cost effective hygienic processes than the current manual wet cleaning methods required prior, and periodically throughout, red meat production or the dangerous use of ozone or UV. It is proposed that continuous sanitation throughout production will deliver benefits to labour and utilities (energy and water savings), and asset utilisation improvement with minimal manual washdowns between change of production runs and within the HVAC system. Additionally, the technology aims to reduce and/or eliminate airborne and surface pathogens (including viruses such as SARS-CoV-2), and thereby potentially extending shelf life of retail ready red meat products. Reduction and/or elimination of pathogen and viruses, could provide safer workplace environments in meat processing and minimal disruption to production at times of operator illness or outbreaks.

The value proposition for the Australian Red Meat Industry of utilising Active Photocatalytic Oxidation technology may be:

- i. Log Reduction (for example, in applications the production surfaces are continuously sanitised throughout production achieving results of up to 95% reduction in microbial loads)
- ii. Water Saving - The sanitisation process achieved within the air handling systems and production rooms may reduce the need to perform the same frequency of intra-day or intra-species cleaning which requires sanitisation of components with traditional hot water methods, thus resulting in significant water saving benefits.
- iii. Labour Saving – significant labour resources are spent in maintaining GMP standards. These resources would be significantly reduced with the application of this continuous sanitisation technology.
- iv. Power Saving – significant power is consumed in generating the required air movements through HEPA filtration systems in order to remove airborne pathogens. The number of air movements and thus power consumption could be reduced with the employment of this technology.

Asset Utilisation Improvement – with the reduction of intra-day and intra-species cleaning through intermittent traditional hot water interventions, production line utilisation will be increased thereby providing higher levels of operational efficiencies and increased capacity.

- vi. Continuous sanitisation - The benefit of this system is the fact that the production surfaces are sanitised throughout the entire process as opposed to the unreliability of intermittent water sanitisation.
- vii. First industrial applications in Australia – New breakthrough in Active PCO technology enabling effective microbial load reduction in both indoor air and surfaces. Limited application expertise in food and red meat processing.
- viii. Maintenance – Low cost of ownership and maintenance.
- ix. Health and Safety – Continuous risk reduction from infectious pathogens in the workplace environment will reduce absenteeism caused by associated sickness, maintaining production efficiencies.
- x. Shelf-life extension red meat processing – use of Active PCO technology to maintain and possibly enhance Australia's exemplar shelf-life through continuous reduction of surface microbial loads in red meat processing environments despite reduction of traditional cleaning methods.

The expected outcomes of the project will be to provide insights and practical knowledge on how this publicly available technology can be applied to benefit red meat production operations across the whole value chain. It is envisaged that this may lead into further research aimed at incorporating the technology into MAP, Utilities, odour control in waste management, increased sanitation in HVAC and equipment cleaning applications to provide additional microbial reduction. The outcomes of this stage 1 validation in red meat processing will be applied to develop recommendations for adoption in red meat processing.

The project will provide insights and practical knowledge on how this publicly available technology can be applied to benefit red meat production operations across the whole value chain.

The proposed project will bring the technology to Australia for validation in a specific Stage 1 application in air and surface purification. It is envisaged that this may lead into further research aimed at incorporating the technology into MAP, Utilities, odour control in waste management, increased sanitation in HVAC and equipment cleaning applications to provide additional microbial reduction.

Further R&D may include:

- The project will provide insights and practical knowledge on how this publicly available technology can be applied to benefit red meat production operations across the whole value chain.
- Shelf-life extension red meat retail - a reduced surface microbial load on red meat deli products in the retail environment may produce shelf-life extension
- Customer market analysis - associated with elimination of odours in the retail environment associated with fresh products, particularly seafood in the deli sections, enhancing customer perceptions of freshness.
- Customer market analysis – customer perceptions associated with COVID-19 safe production facilities and products with possible development of premium product lines.
- Export market – Research the effects of Active PCO technology on primals with possible impact on the good lactic acid bacteria
- Transportation – Although primals are vacuumed sealed and packed in crates, leaving minimal exposed surfaces, it would be prudent to investigate the impacts of limiting microbial loads on the packaging surfaces during transportation which could be introduced into processing facilities. Likewise sanitation of transport from processing to retail would be beneficial to avoid introducing contamination into retail environments.
- Further research aimed at incorporating the technology into MAP, Utilities, odour control in waste management, increased sanitation in HVAC and equipment cleaning applications to provide additional microbial reduction.
- Investigating the benefits to worker health through introduction of Puradigm units in work environments and collecting data on absenteeism reductions due to reductions in person to person transmission of viruses or bacteria such as flu, cold and gastro pathogens.

The overall roadmap of the project is as follows:

Milestone 1 will develop the detailed test schedules for the overall project, including Chef Fresh packaging decontamination, RROA HVAC pathogen reductions and RROA shelf-life extension trials.

Milestone 2 will focus on evaluating the technology in real-world settings against standard microbiological pathogens expected in the Chef Fresh packaging material decontamination rooms (old ozone rooms).

Milestone 3 will focus on evaluating the technology in real-world settings within the RROA facility, testing against standard microbiological pathogens found in the air and on surfaces of the HVAC environments.

Milestone 4 will evaluate the technology to investigate shelf life extension of food products and enhancing food safety trust.

Milestone 5 is the final report detailing the outcomes of the packaging decontamination, shelf life extension, HVAC microbiological reduction and shelf life extension in combination with Denba.

3 Milestone Outcomes

3.1 Milestone 1

Milestone 1 focuses on the development of the test schedule for the project. The project is divided into 3 main sections:

1. Packaging Decontamination
2. HVAC Pathogen Reduction
3. Shelf-Life Extension

A project steering committee has been formed to manage and execute the project. The members are formed from staff from Technical, Engineering and the Senior Leadership Team.

Project Steering Committee:

Patrick Youil:	Team Leader
Suvir Salins:	Project Manager
Sheetal Maharaj:	Technical/Quality Manager
George Salloum:	Site Manager
Justin Meehan:	Chef Fresh Engineering

3.2 Test Schedule – Packaging Decontamination Room

Currently at the Chef Fresh site ozone rooms are being utilised to disinfect the outer packaging material of tray packages. While the trays are kept sterilised within the packages, the outer material can't be guaranteed to be sterile before being opened, and thus ozone was being used to perform the disinfection.

3.2.1 Test Schedule – Packaging Decontamination Room - Safety

Ozone is not safe at levels above 0.1 ppm for more than 6.6hrs according to safe work Australia and EPA safety standards. These high levels of ozone can cause lung or eye damage. However, to be sufficiently anti-pathogenic, levels of ozone above 1ppm are required which are even more dangerous.

Levels measured with an ultra-low ozone sensor show that the current ozone room is generating dangerous levels of ozone.



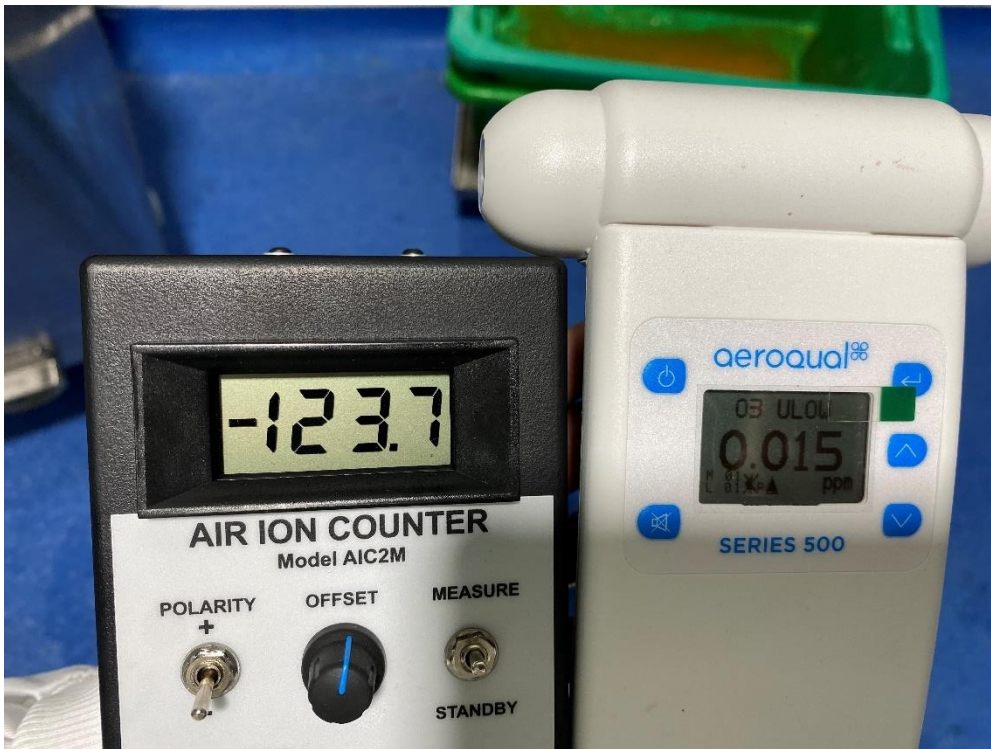
To control the safety and protect staff, safety doors were used to control the entry of personnel into the ozone room in such a way as to only allow access when the room had been purged of ozone. Lower levels of ozone (0.1ppm) were utilised over longer periods of time in order to retain efficacy while further enhancing safety.

Puradigm presents an alternative to the use of harmful ozone. Puradigm can inactivate viruses and destroy bacteria on surfaces remote to the device, without requiring the air to be brought back to the device for treatment. Devices which simply treat the air can't sufficiently treat the surfaces of objects. Puradigm's unique capability of producing long lasting, safe, charged water vapour ie water droplets covered with protons and electrons, which is a non-thermal plasma (NTP), and can treat both the air and surfaces within a large volume of space, is the perfect alternative.

Puradigm Zone 100s installed in the Ozone Room



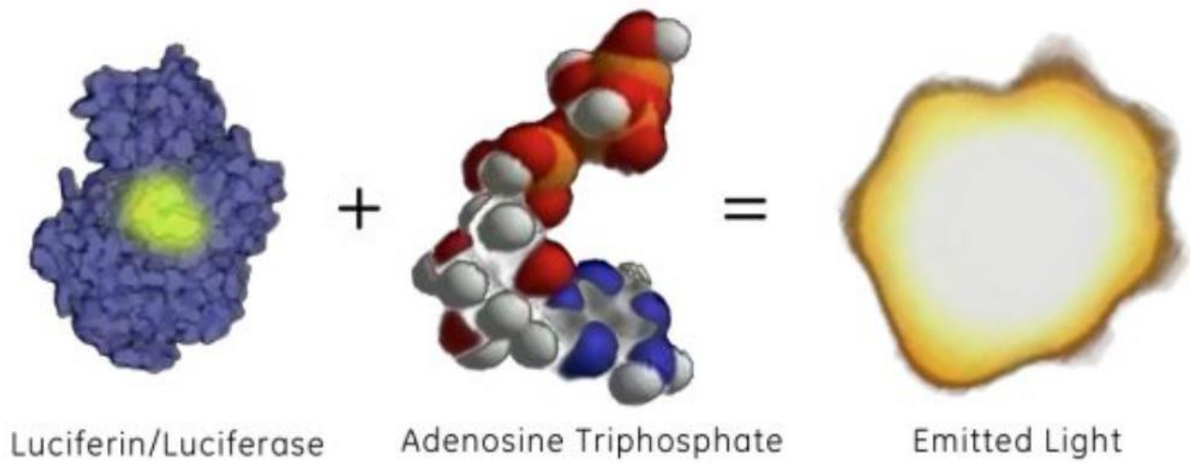
Using Puradigm - Ozone well within safety limits, NTP at levels over 123k ions/cm³



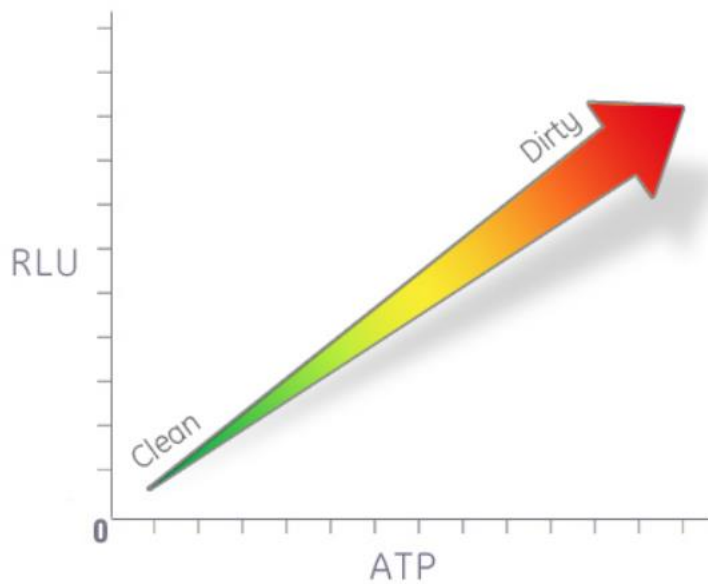
3.2.2 Test Schedule – Packaging Decontamination Room – Test Method

The test protocol to be used to validate the efficacy of the Puradigm units in pathogen reduction of the packaging material, will utilise ATP testers to check the cleanliness of the packaging material before and after exposure to the Puradigm technology within the packaging decontamination room.

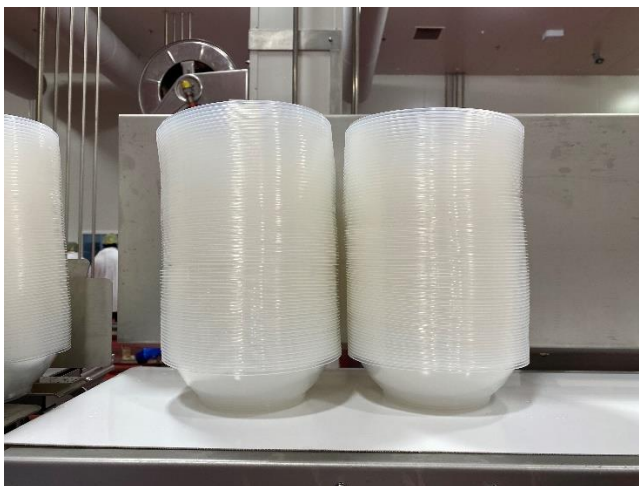
ATP testers measure Adenosine triphosphate, which is the energy molecule of all living organisms. Using an enzyme derived from fire flies a chemical reaction is utilised to generate photons of light if ATP is present. The number of photons generated is directly proportional to the amount of ATP present.



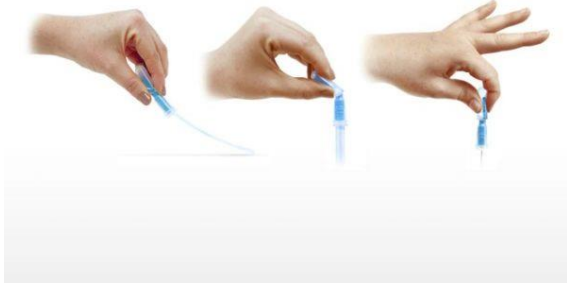
The ATP Tester generates a result measured in Relative Light Unit (RLU). The higher the RLU the dirtier the surface being tested.



Packaging outer material will be swabbed with the ATP UltraSnap swab.



The UltraSnap swab is wiped across the material in a grid pattern vertically and horizontally. The swab is placed back in the holder, snapped to activate and then squeezed to deliver the enzyme to the swab.

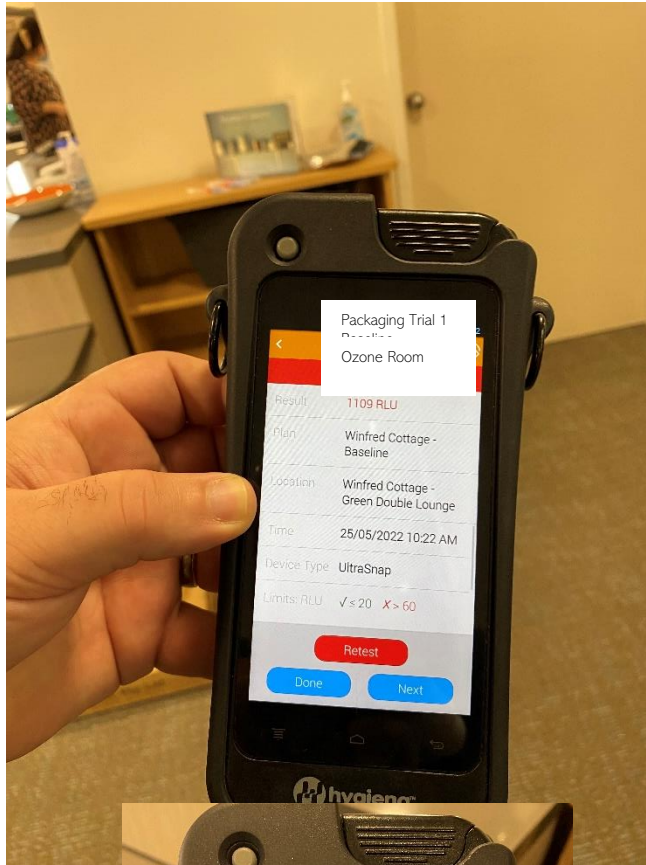


The swab is then placed into the testing unit.

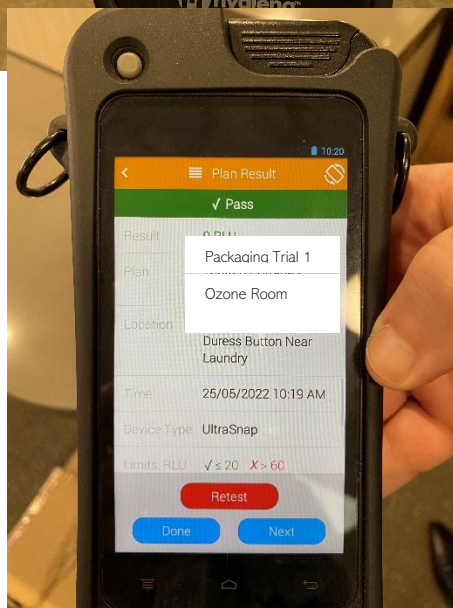


If the RLU result is less than 20 the material is clean, a green result. If the result is between 20-60 RLU the material is moderately unclean, an orange result. If the RLU is greater than 60 the material is dirty, a red result.

Baseline measurement of the packaging material before exposure



Measurement of the packaging material after exposure



Packaging Trial 1
Baseline
Ozone Room

3.2.3 Test Schedule – Packaging Decontamination Room – Test Schedule

Place four packages in four locations around the room.



Measure the ATP on packages before and after 8hours of Puradigm exposure for all four packages.

Record results in the table below for 3 separate trials.

Trial	Packaging 1 Result (RLU)	Packaging 2 Result (RLU)	Packaging 3 Result (RLU)	Packaging 4 Result (RLU)
1 – Baseline Before Exposure				
1 – After 8hr Puradigm Exposure				
2 – Baseline Before Exposure				
2 – After 8hr Puradigm Exposure				
3 – Baseline Before Exposure				
3 – After 8hr Puradigm Exposure				

Based on results above the position of packaging may need to be altered to get better results, or the units themselves may need to be lowered, or possibly stronger units, such as the PRO, may need to be trialled.

Repeat trials above for each change.

3.3 Test Schedule – HVAC

The Coles Retail Ready Operations Australia manufacturing facility utilises Air Handling Units (AHU) to maintain cold chain compliance within the facility. The units circulate air through heat exchangers, down to the plant via air socks and then back via return air vents. During washdown periods at the end of each day significant hot water is used which generates large quantities of steam. During this period the AHUs are switched to a normally ventilated mode which brings in fresh air from outside. This process allows mould spores into the facility. Coupled with the organic material flowing in from the plant, the moisture and mould spores create a mould problem within each AHU.



3.3.1 Test Schedule – HVAC – Safety

Chronic coughing and sneezing, irritation to the eyes, mucus membranes of the nose and throat, rashes, chronic fatigue and persistent headaches can all be symptomatic of black mould exposure or black mould poisoning. Currently hygiene staff use harsh chemicals to clean AHU surfaces and to treat the coils to maintain their cleanliness. Despite these treatments, mould outbreaks continue to occur over a 1-3 month period.

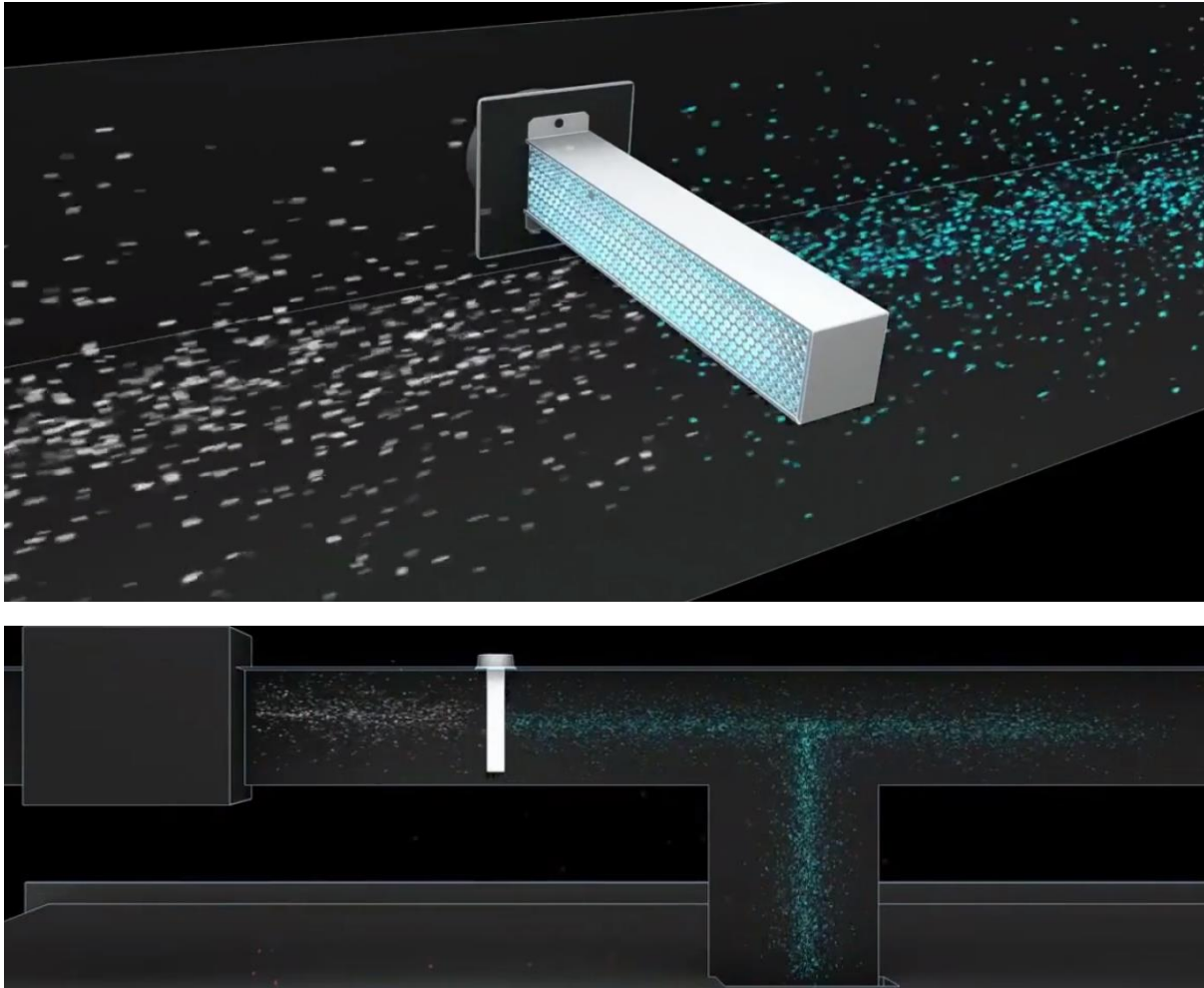
Puradigm represents a safe alternative to the use of harsh chemical cleaners. No harmful ozone, hydrogen peroxide or volatile organic compounds are generated by Puradigm technology as compared to other Photo-Catalytic Oxidation (PCO) technology. Only a safe non-thermal plasma (NTP) is generated, which is a safe product that is naturally produced in outdoor environments as the sun's UV rays interact with the ambient moisture in the air.

3.3.2 Test Schedule – HVAC – Test Method

Prior to the testing phase, the Hygiene team will wash down the walls within the RR11 AHU. RROA Technical team will then take air plates and swabs of supply locations within RR11 AHU5 as a baseline. At least 10 locations within the AHU will be tested, walls, floor, ceiling and coils.



The Puradigm HVAC units will be mounted through the ceiling of the AHU and will treat the air flowing through them as shown below.



3.3.3 Test Schedule – HVAC – Test Schedule

The Puradigm units will be activated once the AHU has been cleaned and baseline readings have been taken. Samples of air plates and swabs will then be taken at the 1 week, 1 month, 2 month and 3 month points after the initial activation of the units.

If results do not show at least a 1-3 log reduction in pathogen counts, the units may be moved around in the AHU or additional units may be installed and then test process repeated for each change.

3.4 Test Schedule – Shelf-Life

Coles RROA periodically check the shelf-life of primals received from our primary processing partners. Primals are debagged and swabbed to measure the total plate count (TPC) ie total microbiological counts on the surface of the primals. Primals are also left bagged and tested at certain periods up to 90 days.

Most primals are fairly clean with TPC under 1000 for freshly received product. This value can exceed 100s of millions of counts at the end of the shelf life period ie at 90 days.

Any reduction in TPC in the early stages of a primal’s life can make significant impact on the overall shelf life of the product. Furthermore if micro contamination can be minimised, as a product is handled through the secondary processing phases within the RROA facility, additional shelf life extension can be achieved beyond current levels.

3.4.1 Test Schedule – Shelf-Life – Safety

Puradigm technology is registered for use in organic agriculture in the US (Appendix 2 – Certifications), but in essence it doesn’t need certification to be regarded as organic since it doesn’t rely on chemicals to purify, but rather just a charged water aerosol, with trace amounts of ozone and hydrogen peroxide which then gets consumed by the catalytic process (Appendix 2 – Certifications). This technology does not produce any harmful or artificial substances. The NTP is a naturally occurring substance in outdoor environments that all organisms encounter and benefit from. Thus this technology is totally safe for humans, pets and plants and of course also safe for food manufacturing facilities.

Food product, including red meat, will be exposed to the Puradigm device’s output, the NTP. All exposed surfaces of the food product will experience reductions in microbial counts only on the surface, not internally. The NTP, being comprised of charged molecules, can’t penetrate the surface of multi-cellular organisms due to the chemical and structural nature of the cell membrane.

The Puradigm PRO is the largest Puradigm purifier delivering 2,200 billion NTP clusters/min. With a stainless steel form factor, this device is suitable for use in food grade environments.



3.4.2 Test Schedule – Shelf-Life – Test Method

Static Tests

The validation testing will be conducted in the RROA Retention Room used by the Technical/Quality team.

There are no daily washdowns performed in this area, making it suitable for the Puradigm device without a splash guard. During the twice monthly general cleans, the portable devices will be removed from the room and brought back in after the cleaning is complete.

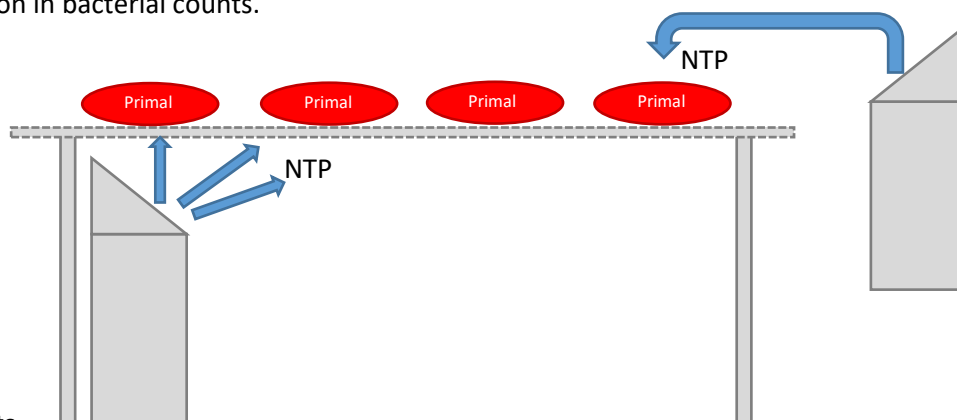
Primals will be placed on a tray, possibly with a mesh floor to allow treatment from the Puradigm device on the top and bottom of the primal surfaces.

Avoid primals stacked on top of one another, or too close to each other as shown below, as this will block NTP clusters from contacting surfaces which are occluded by adjoining primals.



Swabs will be taken from each primal at intervals and total plate counts measured by lab partners.

The expectation is to achieve 2-4 log reductions in surface microbiology counts after several minutes ie 99% to 99.99% reduction in bacterial counts.

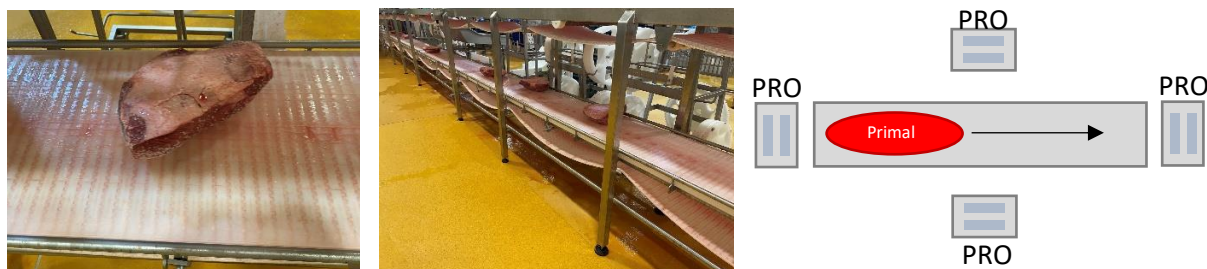


Dynamic Production Tests

If the static tests are successful and achieve the desired log reduction of surface micro counts, as detailed above, production trials with primals being transferred along conveyors, will be conducted.

Puradigm units will be placed strategically along conveyor sections in order to maximise the time primals are exposed to the NTP clusters.

Swabs will be taken of treated primals to determine what log reductions can be achieved.



3.4.3 Test Schedule – Shelf-Life – Test Schedule

Long Exposure trial – Primal and Conveyor surfaces

Static Trials – long exposures	Primal 1 Result (TPC)	Conveyor exposed to primal – 1 (TPC)	Conveyor exposed to primal – 2 (TPC)	Conveyor control (TPC)
1 – Baseline Before Exposure				
1 – After 15 mins Puradigm Exposure				
1 – After 2 hrs Puradigm Exposure				
1 – After 8hrs Puradigm Exposure				

If the long exposure trials are successful, then proceed to short exposure trials.

Short Exposure trial – primal surfaces

Static Trials – short exposures	Primal 1 Result (TPC)	Primal 2 Result (TPC)	Primal 3 Result (TPC)	Primal 4 Result (TPC)
2 – Baseline Before Exposure				
2 – After 1 min Puradigm Exposure				
2 – After 2 min Puradigm Exposure				
2 – After 5 min Puradigm Exposure				
2 – After 10 min Puradigm Exposure				
2 – After 15 min Puradigm Exposure				

Short Exposure trial – conveyor surfaces

Static Trials – short exposures	Primal 1 Result (TPC)	Conveyor exposed to primal – 1 (TPC)	Conveyor exposed to primal – 2 (TPC)	Conveyor control (TPC)
2 – Baseline Before Exposure				
2 – After 1 min Puradigm Exposure				
2 – After 2 mins Puradigm Exposure				
2 – After 5 mins Puradigm Exposure				
2 – After 10 mins Puradigm Exposure				
2 – After 15 mins Puradigm Exposure				

Repeat trial with various orientations and distances of PROs relative to the primals, to determine minimum exposure time required and maximum effective distance between PRO and primal.

Use results from static tests to determine ideal positions of Puradigm units around the production line, to maximise exposure time and reduction effect on primals travelling on the conveyor, for the dynamic tests.

Swab primals before they are placed on the conveyor and then after they exit the conveyor section which is being treated by the Puradigm devices.

Dynamic Trials	Primal 1 Result (TPC)	Primal 2 Result (TPC)	Primal 3 Result (TPC)	Primal 4 Result (TPC)
1 – Baseline Before Exposure				
1 – After Puradigm Exposure				

Repeat the above with various orientations and distances of units around the conveyor until optimum results are achieved.

4 Milestone 2

Milestone 2 focuses on the implementation through practical trials and initial testing in a production environment, including:

- Analysis of environmental conditions and design to maximise Ion efficiencies in new and old ozone rooms
- Microbiological reductions on packaging material within old ozone rooms

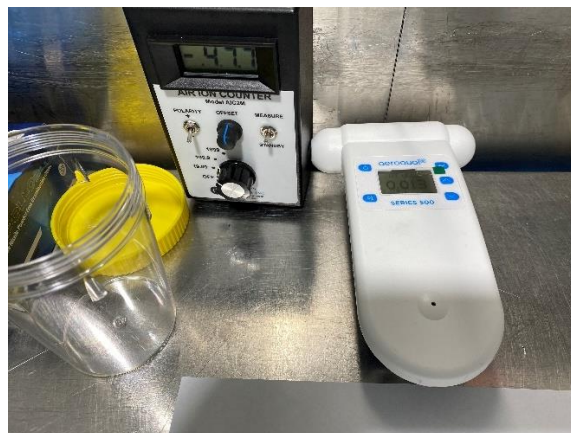
4.1 Packaging Decontamination Room – Analysis of Environmental Conditions

The Non-thermal plasma, charged water vapour, that the Puradigm units produce are generated at concentrations of 2 million/cm³. To be effective concentrations of 5K/cm³ are required.

Ion concentrations were measured around the ozone room at various heights. The concentration was an average 20K/cm³ at 1m high, increasing to 35-40K/cm³ at 2m high. It was decided to reduce the height of the higher unit to the same level as the unit on the opposite wall to increase the concentration at lower levels which is where the majority of the packaging will be located.



After the unit was moved, the ion concentration readings increased to 30-50K/cm³ at the bench level



4.2 Packaging Decontamination Room – Microbiological Reductions

The QA department at Chef Fresh worked with their laboratory partners to design a test plan to measure the microbiological reductions on the packaging material. It was felt, that although ATP monitors could be used during ongoing spot checks, when the system is transitioned to full production, the trial would be best suited to standard micro swabs which are sent to the lab for analysis.

The packaging material is generally quite clean and so no dramatic changes would be seen using ATP. As such the testing protocol will include the inoculation of the test surfaces with a known concentration of microbiological contaminant and then test at various intervals to measure any reductions.

4.2.1 Packaging Decontamination Test Protocol

1. Inoculate 1ml of 10^6 concentration of coagulase positive staphylococcus onto a sterilised, blank petri dish
2. Baseline measurement: Swab petri dish and obtain recovered count. Count is to be used as a baseline for the log reductions
3. Inoculate 6 petri dishes with 1ml of inoculum
4. Place the 6 petri dishes x 2 on the test bench in the ozone room
5. At various time intervals swab the petri dishes associated with that time period in both the test room and control area, and store swabs in the WIP freezer for lab pick up and analysis
6. Lab will incubate swabs and determine the count of each petri dish to measure the reduction relative to the control samples
7. Deduct the baseline count from each petri dish count to determine the log reduction for that time point

Petri Dish 0:	Blank dish 0 hour
Petri Dish 1:	Expose 1 hour
Petri Dish 2:	Expose 3 hours
Petri Dish 3:	Expose 5 hours
Petri Dish 4:	Expose 8 hours
Petri Dish 5:	Expose 12 hours
Petri Dish 6:	Expose 24 hours

Inoculating the test samples in duplicate for each time period T0, T1, T3, T5, T8, T12, T24



Ion concentration and ozone measurements during the trial



Unfortunately despite controlling the access to the room quite strictly, at various points through the day, material had to be moved through the room several times. The result was a noticeable drop in ion concentration from 30-50k/cm³ down to 15-20K/cm³ which would have slowed reductions down.

Ozone levels stayed well within safety limits and also played very little role in reductions staying in the 0.008ppm – 0.014ppm which are the same as outdoor ambient conditions (note safe levels are <0.05ppm, with dangerous levels above 0.1ppm according to Australian Safety Standards).

Control Area samples for time periods T0 to T24 set up outside of the test room



4.2.2 Packaging Decontamination Test Results

Unfortunately the lab made a mistake in their preparation of the test samples. The critical step of drying the staph inoculum onto the surface of the petri dishes was not done. 1ml solution represented a lot of liquid which both fed the pathogen as well as protected it from the active output of the Puradigm devices. Other technologies such as ozone will not penetrate a liquid barrier either.

The results of the lab analysis confirmed expectations with counts of staph increasing in both the test and control samples between the baseline at T=0hrs and T=24hrs. However it should be noted that at T=24hrs the test samples were 59-70% lower than the control samples for the same time point.

The reductions between test and control at T=24hrs can be explained by some of the staph migrating to the surface of the liquid which allowed the non-thermal plasma to deactivate the staph.

The experiment will have to be repeated but this time with all test and control samples being dried prior to the test starting. Using a 0.1mL solution will aid this drying process.

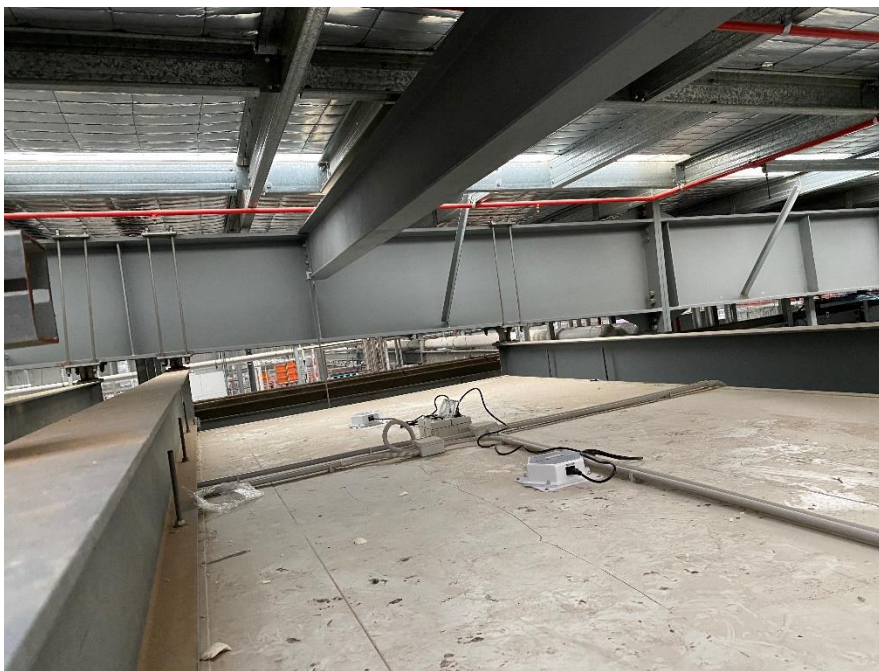
Table showing actual microbiological counts for time periods T0, T1, T3, T5, T8, T12, T24 in duplicate

Sample No.	Overall Description	Sample	Sample Details	Status	Received	Certificate Issued D	FM0023 Coag Pos	Reductions against T0	Reductions Test vs Control
FS2243308-033 (1)	FS2243308-033 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm Bla	Completed	2022-08-04	2022-08-15	<10		
FS2243308-032 (1)	FS2243308-032 Preliminary Puradigm Unit Verification Study	-	Paradigm Blank	Completed	2022-08-04	2022-08-15	<10		
FS2243308-031 (1)	FS2243308-031 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T2	Completed	2022-08-04	2022-08-15	3,100,000	-107%	
FS2243308-030 (1)	FS2243308-030 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T2	Completed	2022-08-04	2022-08-15	3,800,000	-124%	
FS2243308-029 (1)	FS2243308-029 Preliminary Puradigm Unit Verification Study	-	Paradigm T24	Completed	2022-08-04	2022-08-15	2,800,000	-65%	59%
FS2243308-028 (1)	FS2243308-028 Preliminary Puradigm Unit Verification Study	-	Paradigm T24	Completed	2022-08-04	2022-08-15	1,700,000	15%	70%
FS2243308-027 (1)	FS2243308-027 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T1	Completed	2022-08-04	2022-08-15	2,000,000	-18%	
FS2243308-026 (1)	FS2243308-026 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T1	Completed	2022-08-04	2022-08-15	1,500,000	0%	
FS2243308-025 (1)	FS2243308-025 Preliminary Puradigm Unit Verification Study	-	Paradigm T12	Completed	2022-08-04	2022-08-15	1,500,000	25%	0%
FS2243308-024 (1)	FS2243308-024 Preliminary Puradigm Unit Verification Study	-	Paradigm T12	Completed	2022-08-04	2022-08-15	2,900,000	-71%	-53%
FS2243308-023 (1)	FS2243308-023 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T8	Completed	2022-08-04	2022-08-15	1,400,000	7%	
FS2243308-022 (1)	FS2243308-022 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T8	Completed	2022-08-04	2022-08-15	1,900,000	-12%	
FS2243308-021 (1)	FS2243308-021 Preliminary Puradigm Unit Verification Study	-	Paradigm T8	Completed	2022-08-04	2022-08-15	1,300,000	35%	5%
FS2243308-020 (1)	FS2243308-020 Preliminary Puradigm Unit Verification Study	-	Paradigm T8	Completed	2022-08-04	2022-08-15	1,900,000	-12%	0%
FS2243308-019 (1)	FS2243308-019 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T5	Completed	2022-08-04	2022-08-15	1,400,000	7%	
FS2243308-018 (1)	FS2243308-018 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T5	Completed	2022-08-04	2022-08-15	1,900,000	-12%	
FS2243308-017 (1)	FS2243308-017 Preliminary Puradigm Unit Verification Study	-	Paradigm T5	Completed	2022-08-04	2022-08-15	1,800,000	-6%	6%
FS2243308-016 (1)	FS2243308-016 Preliminary Puradigm Unit Verification Study	-	Paradigm T5	Completed	2022-08-04	2022-08-15	1,400,000	30%	0%
FS2243308-015 (1)	FS2243308-015 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T3	Completed	2022-08-04	2022-08-15	1,900,000	-12%	
FS2243308-014 (1)	FS2243308-014 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T3	Completed	2022-08-04	2022-08-15	1,500,000	0%	
FS2243308-013 (1)	FS2243308-013 Preliminary Puradigm Unit Verification Study	-	Paradigm T3	Completed	2022-08-04	2022-08-15	1,800,000	10%	-15%
FS2243308-012 (1)	FS2243308-012 Preliminary Puradigm Unit Verification Study	-	Paradigm T3	Completed	2022-08-04	2022-08-15	2,300,000	-35%	-24%
FS2243308-011 (1)	FS2243308-011 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T1	Completed	2022-08-04	2022-08-15	2,700,000	-59%	
FS2243308-010 (1)	FS2243308-010 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T1	Completed	2022-08-04	2022-08-15	2,100,000	-40%	
FS2243308-009 (1)	FS2243308-009 Preliminary Puradigm Unit Verification Study	-	Paradigm T1	Completed	2022-08-04	2022-08-15	2,800,000	-40%	-5%
FS2243308-008 (1)	FS2243308-008 Preliminary Puradigm Unit Verification Study	-	Paradigm T1	Completed	2022-08-04	2022-08-15	2,200,000	-29%	-6%
FS2243308-007 (1)	FS2243308-007 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T0	Completed	2022-08-04	2022-08-15	1,700,000		Baseline
FS2243308-006 (1)	FS2243308-006 Preliminary Puradigm Unit Verification Study	-	Non-Paradigm T0	Completed	2022-08-04	2022-08-15	1,500,000		Baseline
FS2243308-005 (1)	FS2243308-005 Preliminary Puradigm Unit Verification Study	-	Paradigm T0	Completed	2022-08-04	2022-08-15	1,700,000		Baseline
FS2243308-004 (1)	FS2243308-004 Preliminary Puradigm Unit Verification Study	-	Paradigm T0	Completed	2022-08-04	2022-08-15	2,000,000		Baseline
FS2243308-003 (1)	FS2243308-003 Preliminary Puradigm Unit Verification Study	-	Innoculum level	Completed	2022-08-04	2022-08-15	2,000,000		
FS2243308-002 (1)	FS2243308-002 Preliminary Puradigm Unit Verification Study	-	Innoculum level	Completed	2022-08-04	2022-08-15	800,000		
FS2243308-001 (1)	FS2243308-001 Preliminary Puradigm Unit Verification Study	-	Innoculum level	Completed	2022-08-04	2022-08-15	1,400,000*		

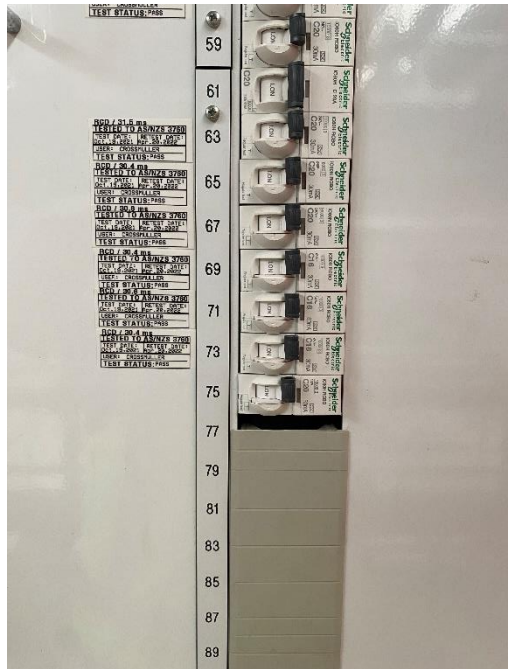
4.3 HVAC Installation

In preparation for the upcoming trials within the Air Handling Units (AHUs), electrical services have been installed on the roof of the units as shown below.

Penetrations have then been cut into the roof of the AHUs and the HVAC 14” Puradigm Units installed.

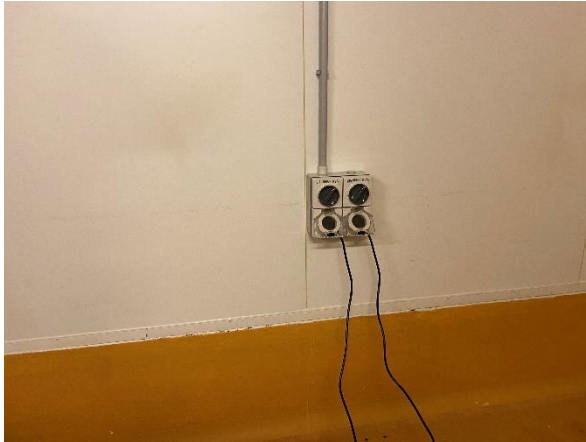


41			42	
43	20	Data Rack Power (IDF-CH1)	44	
45	20	Data Rack Power (IDF-CH2)	46	
47	20	Data Rack Power (IDF-IN)	48	
49	20	Data Rack Power (IDF-RR1)	50	
51	20	Camera Power Outside Pole	52	
53	20	GPO Fire Compressor	54	
55	20	GPO (1) hicken Process	56	
57	20	GPO (1) DeCartoning Bin	58	
59	20	Security Panel	60	
81	20	Roller Door Chicken Chiller	62	
63	20	Heat Trace Hydrant/Shower Grid Floor	64	
65	20	GPO Adj. SWB 2D	66	
67	20	GPO Side Of Distribution Board	68	
69	16	GPO Line 24 VA Room East Wall	70	
71	16	GPOs VA Mixing Room North Wall	72	
73	16	GPO Line 30 VA Room West Wall	74	
75	20	GPD AHU's - Roof Units	76	
77			78	
79			80	
81			82	
83			84	
85			86	
87			88	
89			90	
91			92	
93			94	



4.4 Shelf-Life Room – Trial Preparation

In preparation for the upcoming trials within the QA Retention cool room, electrical GPOs have been installed as shown below.



4.5 Shelf-Life Room – Analysis of Environmental Conditions and 24hr Microbiological Reduction Trial

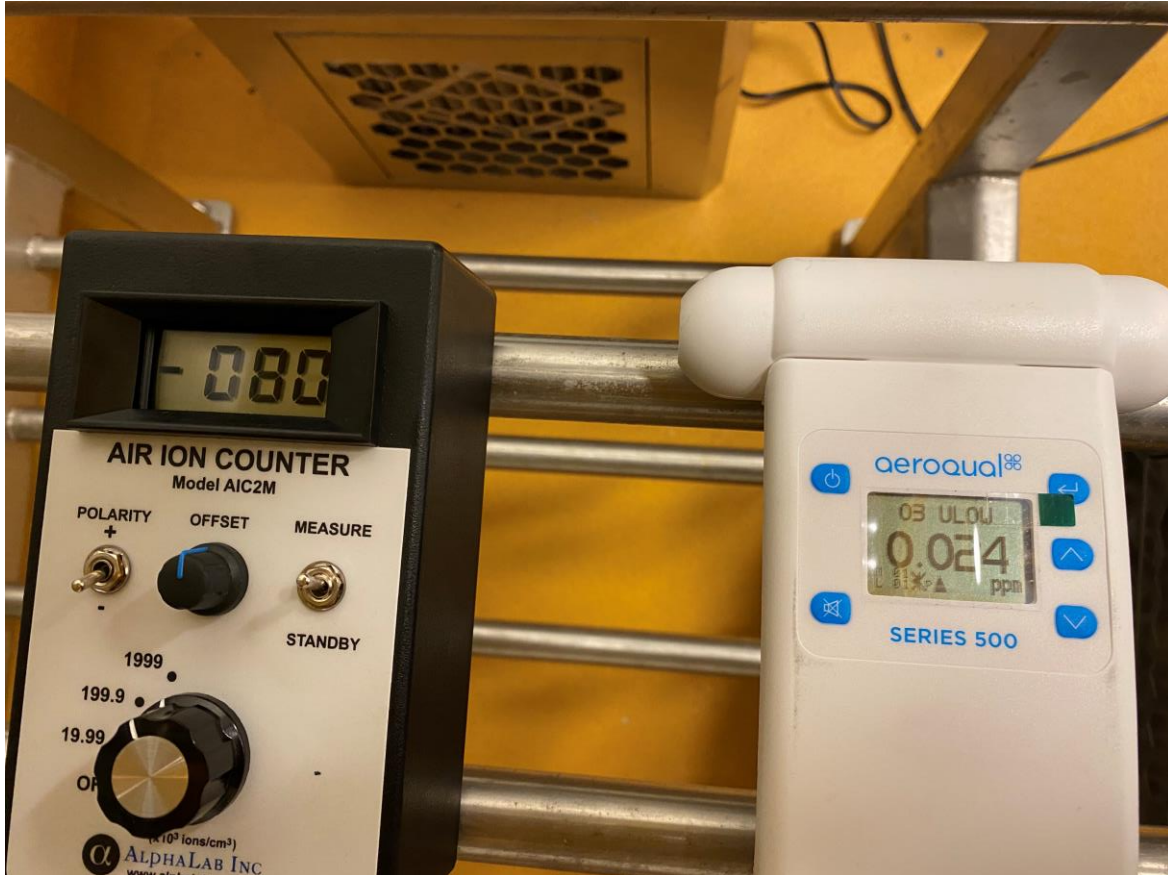
An initial trial on shelf-life extension was conducted in Milestone 2 in preparation for the production trials scheduled for Milestone 2. This trial was conducted in the QA Retention Chiller Room where samples are stored for analysis. The temperature of the room is 4°C. Note that the room's temperature increased to 5°C with 2 x Puradigm PROs running constantly in the room consuming 168W each. The room's temperature was dropped by 1°C to compensate and return ambient conditions to 4°C. There was also a ***strong opposing air flow*** which will ***show if the Puradigm NTP can still have an effect in this real-world condition.***

4.5.1 Shelf-Life 24hr Microbiological Reduction Test Protocol

1. Use Outside flat primals aged in vac bags for 35 days. Expect 1 million total plate counts (TPC) at this age
2. Baseline measurement: Cut open vac bags outside the retention room and take swabs of a 10cm x 10cm area of each primal after pat drying the surfaces with blue paper towelling to remove excess moisture, using FlexiSwabs (sponge swabs). Label swabs accordingly.
3. Move primals into the retention room which contains 2 x Puradigm PROs which have been running continuously for 1 week.
4. Place primals in the ordered they were swabbed on the rack that the Puradigm PROs are treating
5. Measure ion counts and ozone levels at T=0hrs
6. Measure ion counts and ozone levels at T=24hrs
7. Test measurement: Take swabs of a 10cm x 10cm area of each primal in the same order the baseline swabs were taken, using FlexiSwabs. Label T=24hrs and 1 to 4 for each primal.
8. Send to the ALS laboratory for analysis of TPC reductions measured over the 24hr period.

4.5.2 Shelf-Life 24hr Microbiological Reduction Test Results – Test 1

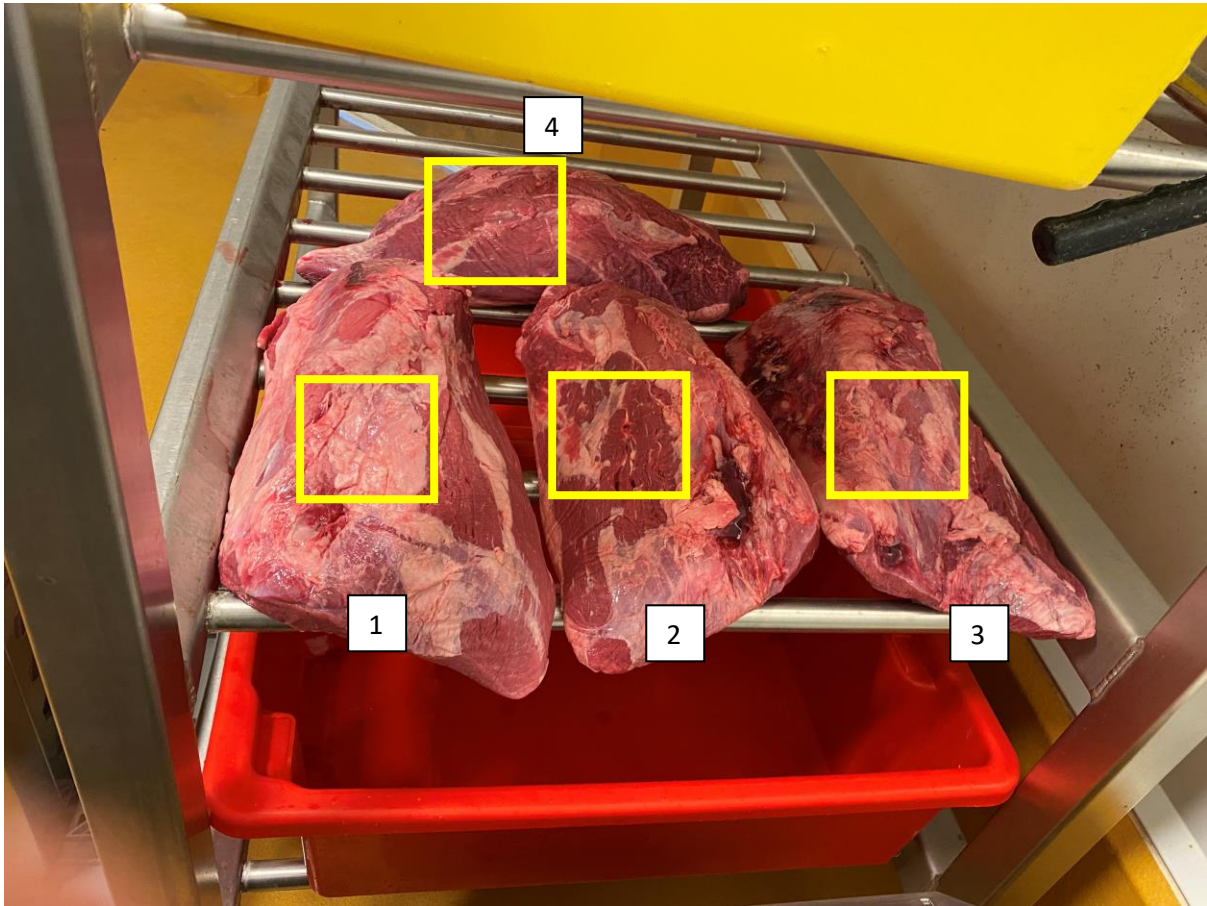
Check Ion Count (1000 ions/cm³) and Ozone levels (ppm) at T=0hrs Baseline (16th August, 2022)



It should be noted that due to very **dry conditions and low temperatures** these **ion counts are lower than usual**, with 500K-2million ions/cm³ usual, not 100-200K ions/cm³ at the outlet of each device.

The **ozone levels are well within safety levels** at 0.024ppm, and well below the 1ppm that ozone needs to reach before it starts reducing pathogens. Any **reductions** will thus only be **attributable to the non-thermal plasma (NTP) ie charged water aerosols**.

Swabs at T=0hrs, Baseline, in 10cm x 10cm surface regions (16th August, 2022)



Swabs kept in the fridge for lab pick-up



Check Ion Count (1000 ions/cm³) and Ozone levels (ppm) at T=24hrs Baseline (17th August, 2022)



It was noted that one of the units seemed to be off with the power cable slightly loose. Possibly knocked by QA personnel passing through the room. However the other device was operating and ion and ozone levels were similar to the baseline levels.

Swabs at T=24hrs, in 10cm x 10cm surface regions (17th August, 2022)



Swabs kept in the fridge for lab pick-up



ALS Laboratory Microbiology Results



Retail Ready Operations Australia Pty Ltd
PO Box 97
St Clair NSW, 2759

Attention: Sheetal Maharaj

Overall Description: Puradigm 24-hour Log Trial on Primal Surface Environmental FlexiSwab - 17/08/2022

Our Ref No: FS2246510

Your Ref:

Project:

Report Date: 24 Aug 2022

Samples Received: 17 Aug 2022

Temperature when received: 2.8

Testing Commenced: 17 Aug 2022

This report cannot be reproduced except in full, without written approval from the laboratory. Samples tested as received into the laboratory, unless the sampling was conducted by ALS.

Sample Details: Test Description	Results	Units	Site
001 Sample 1. T=0 (16/08/2022) (NATA Accredited)			
FM0010	Aerobic Plate Count	>200,000	cfu/swab. NSW
002 Sample 2. T=0(16/08/2022) (NATA Accredited)			
FM0010	Aerobic Plate Count	>200,000	cfu/swab. NSW
003 Sample 3. T=0(16/08/2022) (NATA Accredited)			
FM0010	Aerobic Plate Count	>200,000	cfu/swab. NSW
004 Sample 4. T=0(16/08/2022) (NATA Accredited)			
FM0010	Aerobic Plate Count	>200,000	cfu/swab. NSW
005 Sample 1. T=24h(17/08/2022) (NATA Accredited)			
FM0010	Aerobic Plate Count	~28,000	cfu/swab. NSW
006 Sample 2. T=24h(17/08/2022) (NATA Accredited)			
FM0010	Aerobic Plate Count	>200,000	cfu/swab. NSW
007 Sample 3. T=24h(17/08/2022) (NATA Accredited)			
FM0010	Aerobic Plate Count	>200,000	cfu/swab. NSW
008 Sample 4. T=24h(17/08/2022) (NATA Accredited)			
FM0010	Aerobic Plate Count	~32,000	cfu/swab. NSW

¹NATA accreditation does not cover the performance of this service. Measurement of Uncertainty values for your compliance are available at <https://www.alsglobal.com/au/services-and-products/food-safety/laboratory-downloads/client-downloads>.

Signatories

This document has been electronically signed by the authorized signatories indicated below. Electronic signing has been carried out in compliance with procedures specified in 21 CFR Part 11.

Signatories	Position	Accreditation Category
Tran Tu	Laboratory Supervisor - Microbiology	Food Microbiology



General Comments

N/D = Not Detected, < = Less than, > = Greater than, cfu = colony forming unit, MPN = Most Probable Number, PN = Probable Number, Y = Yeast, M = Mould, ~ = Estimate

These tests are based on **35 day aged primals with very high pathogen loads** which are generally not the target product, but are being used to show how **this technology handles an extreme case**.

Primals 1 and 4 showed **at least an 86% and 84% reduction** in aerobic plate count for surface samples based on the T0 200,000 counts. Expected surface counts for primals at day 35 is expected to be 1,000,000+ counts, so the **actual reduction may be closer to 97.2% and 96.8% ie 2 log reduction**.

A 2 log reduction is what is expected. However the primals which did not show a reduction, may have reduced from 1million+ to just above 200K, but the lab was not able to show those levels. **The next tests will be based on better count resolution to capture the true reduction amount.**

Additionally adding tubs of water to enhance humidity and moving the trial out of the direct air flow of the condenser fan may enhance the effectiveness of the Puradigm units. These will be trialled in the next milestone.

5 Milestone 3

Milestone 3 focuses on the implementation through practical trials and initial testing in a production environment, including:

- Analysis of environmental conditions and design to maximise Ion efficiencies in new and old ozone rooms
- Microbiological reductions on packaging material within old ozone rooms

Install trial units in RROA production HVAC locations. Design production trial methodologies. Collect and analyse test samples, including:

- Microbiological reductions in air and on surfaces within the HVAC environment
- Microbiological reductions on conveyor or machine surfaces

Continue shelf-life trials

- Microbiological reductions on the surface of primals

5.1 Shelf-Life Room – Analysis of Environmental Conditions and 24hr Microbiological Reduction Trial

A repeat trial on shelf-life extension was conducted in Milestone 3 in preparation for the production trials scheduled for Milestone 4. This trial was conducted in the QA Retention Chiller Room where samples are stored for analysis. The temperature of **the room is 4°C**. This temperature is a **challenge for NTP production as the UV bulb generally like temperatures above 10°C**.

The previous trial showed that ion levels were lower than expected. As such a tub of water was introduced near the PROs to **enhance the humidity of the room**. The NTP production is related to the ambient humidity and as such levels were expected to be higher.

Negative ion levels in Test 1 of Milestone 2, were 80K ions/cm³. Measurements for Test 2 of Milestone 3 showed levels around 100-130K ions/cm³ at the primals between T=0hrs and T=24hrs.

The targets were 35 day aged primals with very high pathogenic loads to again show how the technology handles an extreme case.

Test 2 24hr Microbiological Reduction – 1st September, 2022



5.1.1 Shelf-Life 24-96hr Microbiological Reductions Test Results – Test 2 & 3

Check Ion Count (1000 ions/cm³) and Ozone levels (ppm) at T=0hrs Baseline (1st September, 2022)



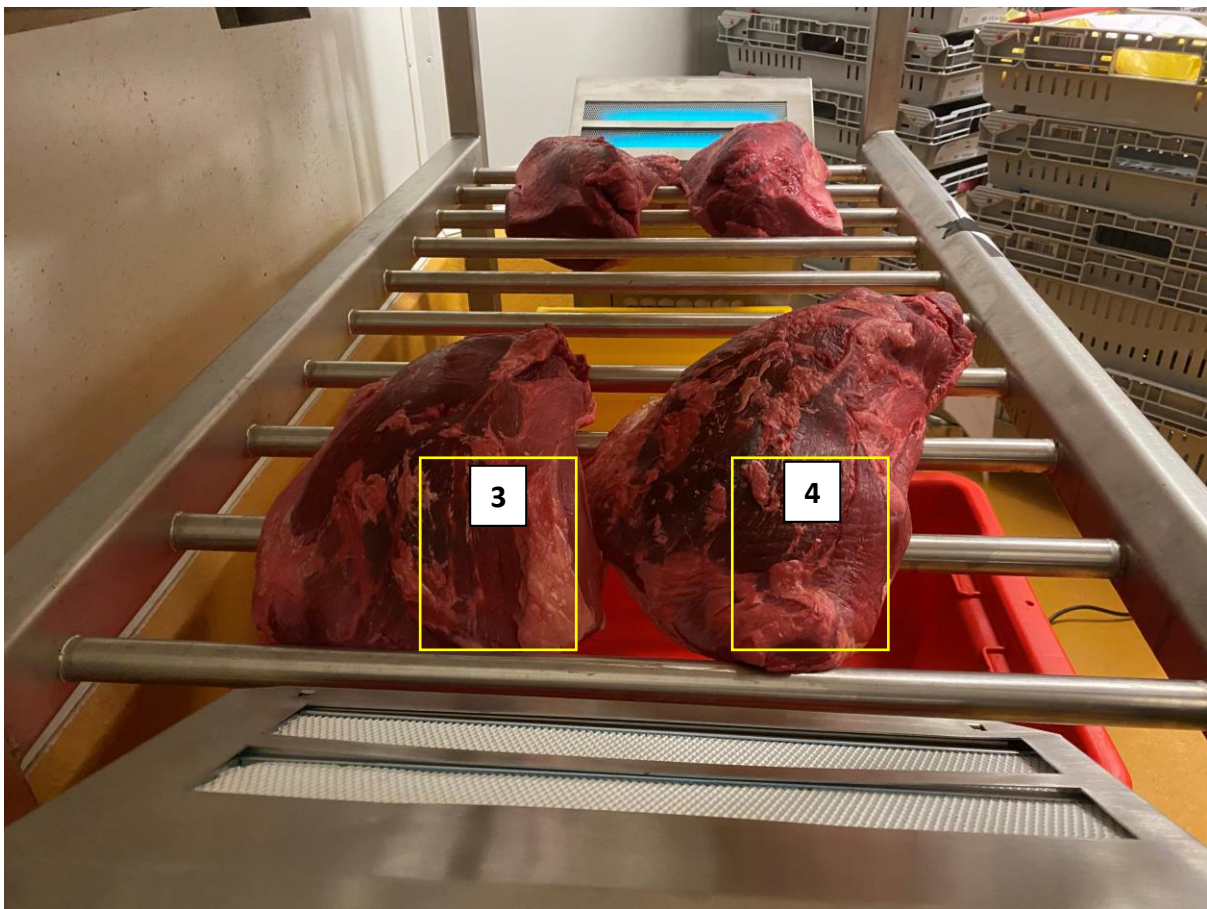
It should be noted that due to **very dry conditions and low temperatures these ion counts are lower** than usual, with 500K-2million ions/cm³ usual, not 100-200K ions/cm³ at the outlet of each device. However the levels have increased relative to Test 1 Milestone 2, most likely due to the addition of the tub of water to increase relative humidity levels.

The **ozone levels are well within safety levels** and well below the 1ppm that ozone needs to reach before it starts reducing pathogens. Any **reductions will thus only be attributable to the non-thermal plasma (NTP) ie charged water aerosols.**

Swabs at T=0hrs, Baseline, in 10cm x 10cm surface regions (1st September, 2022)







Check Ion Count (1000 ions/cm³) and Ozone levels (ppm) at T=24hrs Baseline (2nd September, 2022)



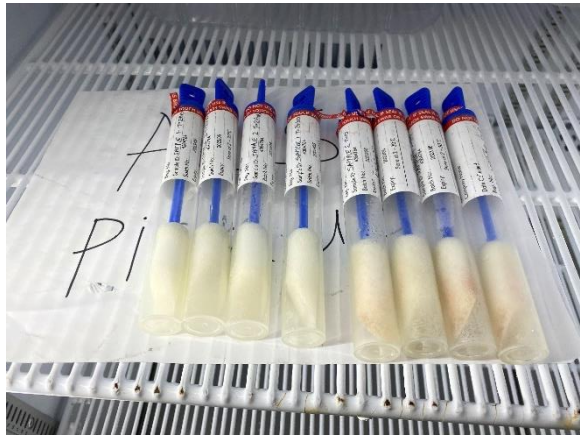
Ion levels showed improvement with the use of the tub of water enhancing the humidity of the air.



The setup was still in the direct path of the **opposing, high speed air flow** from the condenser fan which would be blowing ions away from primals 3 and 4 from the second PRO unit, while primals 1 and 2 are downstream from the first PRO unit which is blowing ions in the same direction as the room's condenser fan.

Ozone levels were still low relative to safety standards and the ability of ozone to reduce pathogens, ensuring that any ***reductions seen would be solely due to the NTPs***.

Swabs at T=24hrs, in 10cm x 10cm surface regions (2nd September, 2022)



ALS Laboratory Microbiology Results – Test 2 – 24hrs



Certificate of Analysis

Retail Ready Operations Australia Pty Ltd
PO Box 97
St Clair NSW, 2759

Attention: Sheetal Maharaj

Overall Description: Puradigm 24-hour Log Trial on Primal Surface Environmental FlexiSwab - 02/09/2022

Our Ref No: FS2250055 Your Ref:
Project: Report Date: 07 Sep 2022
Samples Received: 02 Sep 2022 Temperature when received: 3.1
Testing Commenced: 02 Sep 2022

This report cannot be reproduced except in full, without written approval from the laboratory.
Samples tested as received into the laboratory, unless the sampling was conducted by ALS.

Sample Details: Test Description	Results	Units	Site
001 Sample 1. T=0 (NATA Accredited)			
FM0010 Aerobic Plate Count	~50,000	cfu/swab.	NSW
002 Sample 2. T=0 (NATA Accredited)			
FM0010 Aerobic Plate Count	4,800	cfu/swab.	NSW
003 Sample 3. T=0 (NATA Accredited)			
FM0010 Aerobic Plate Count	~50,000	cfu/swab.	NSW
004 Sample 4. T=0 (NATA Accredited)			
FM0010 Aerobic Plate Count	~56,000	cfu/swab.	NSW
005 Sample 1. T=24h (NATA Accredited)			
FM0010 Aerobic Plate Count	2,700	cfu/swab.	NSW
006 Sample 2. T=24h (NATA Accredited)			
FM0010 Aerobic Plate Count	4,500	cfu/swab.	NSW
007 Sample 3. T=24h (NATA Accredited)			
FM0010 Aerobic Plate Count	1,900	cfu/swab.	NSW
008 Sample 4. T=24h (NATA Accredited)			
FM0010 Aerobic Plate Count	540	cfu/swab.	NSW

¹NATA accreditation does not cover the performance of this service.
Measurement of Uncertainty values for your compliance are available at <https://www.alsglobal.com/au/services-and-products/food-safety/laboratory-downloads/client-downloads>.

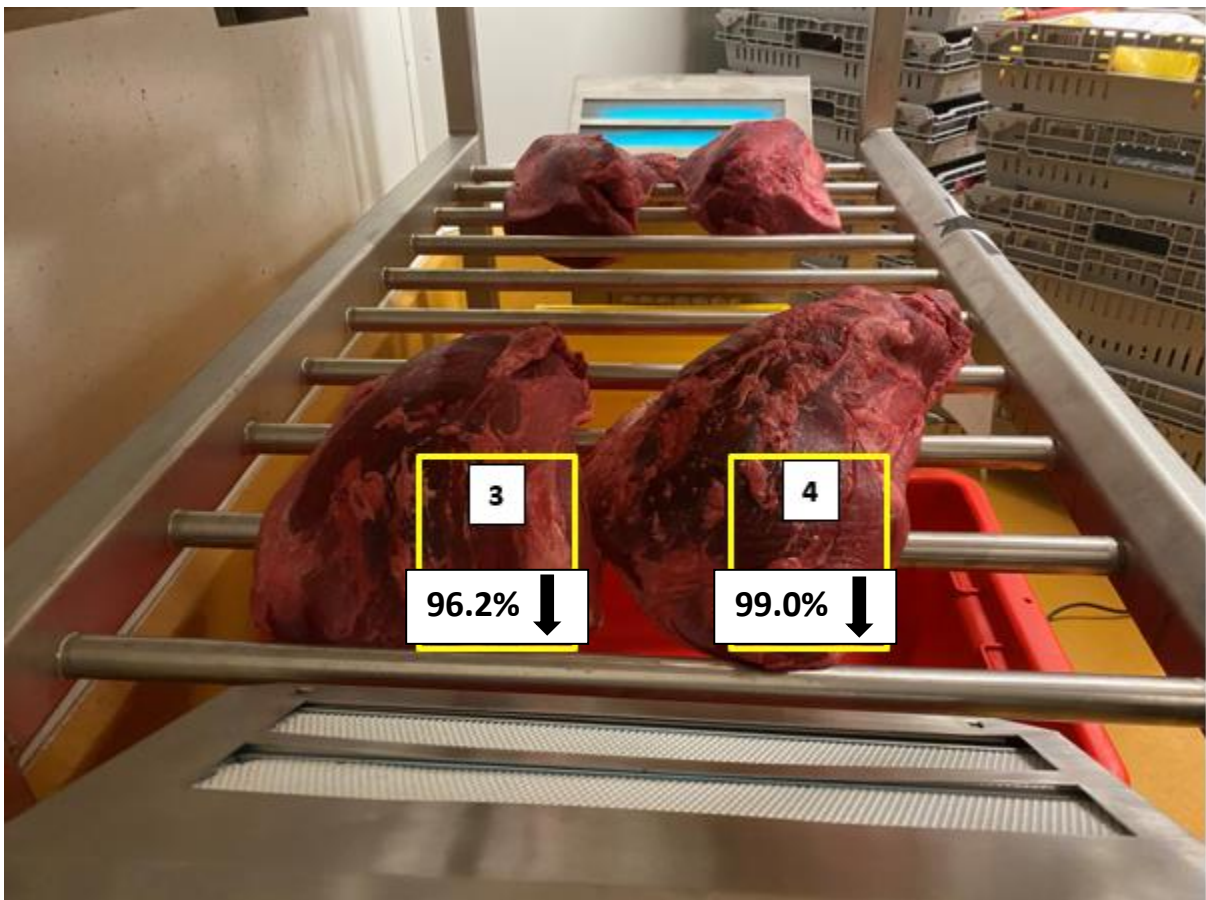
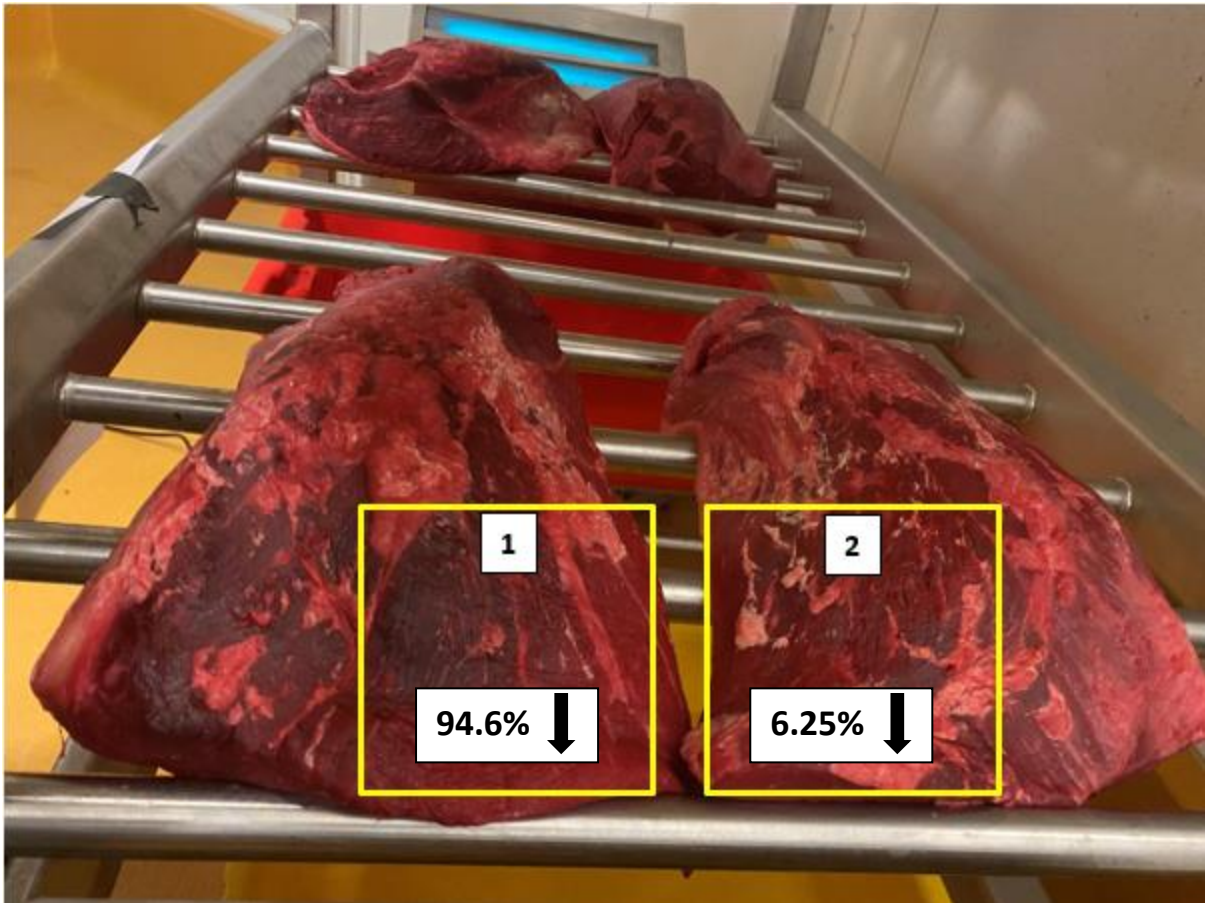
Signatories

This document has been electronically signed by the authorized signatories indicated below. Electronic signing has been carried out in compliance with procedures specified in 21 CFR Part 11.

Signatories	Position	Accreditation Category
Jason Alvarez	Laboratory Manager	Food Microbiology





Using the 10:1 dilution factor instead of the 1000:1 dilution factor used in Test 1 allowed a more accurate T=24hr relative to T=0hr count to be measured. The **results clearly showed 2 log reductions in surface counts**. Primals 1, 3 & 4 showed **95%, 96% and 99% reduction** in aerobic plate count for surface samples based on the T0 50,000, 50,000 and 56,000 counts respectively. Primal 2 showed an anomalous T0 count of only 4,800 with a final count of 4,500 which is only a 6.25% reduction. If Primal 2's T0 count was 50,000, its reduction would have been 91%. Possibly the **T0 swab was not thorough**.



ALS Laboratory Submission Form – Test 3 – 96hrs

The laboratory will measure TPC at T=96hrs for the 4 primal samples. Used a dilution factor of 1:10 (limit of test <10 cfu/swab) to ensure we get closer to the true counts should any counts be very high, however expecting the counts to be quite low at this stage.

FOOD MICROBIOLOGY - SAMPLE SUBMISSION FORM			
 <p>ALS Food & Pharmaceutical 10/2-8 South St Rydalmere NSW 2116 T: +61 2 8832 7500 F: +61 2 9898 3472</p> <p>Office use only: Affix ALS Workorder label</p>	Company Name: Retail Ready Operations Australia Contact Person: Sheetal Maharaj	Office use only: Date: Time: Condition: Opened by:	
	Street Address: 54 Templar Road Erskine Park NSW 2759		Print & Email to ALS 
	Email Address: Sheetal.Maharaj@coles.com.au		
	Phone: 0437 967 591		
Purchase Order No. 05/09/2022	Special Instructions: Puradigm 24-hour Log Trial on Primal Surface Environmental FlexiSwab. Separate invoice for this test. Limit of the tests as <10 cfu/swab T=96h (05/09/2022)		

Sample Name	Testing Required (please tick tests required)																Other (please specify)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Sample 1. T=96 h		X															
Sample 2. T=96h		X															
Sample 3. T=96h		X															
Sample 4. T=96h		x															

Check Ion Count (1000 ions/cm³) and Ozone levels (ppm) at T=96hrs (5th September, 2022)



ALS Laboratory Microbiology Results – Test 3 – 96hrs



Retail Ready Operations Australia Pty Ltd
 PO Box 97
 St Clair NSW, 2759

Attention: Sheetal Maharaj

Overall Description: Puradigm 24-hour Log Trial on Primal Surface Environmental FlexiSwab. T=96h (05/09/2022)- 06/09/2022

Our Ref No: FS2250508

Your Ref: 05/09/2022

Project:

Report Date: 13 Sep 2022

Samples Received: 06 Sep 2022

Temperature when received: 3.2

Testing Commenced: 06 Sep 2022

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Sample Details: Test Description	Results	Units	Site
001 Sample 1. T=96 h (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	cfu/swab.	NSW
002 Sample 2. T=96h (NATA Accredited)			
FM0010 Aerobic Plate Count	130	cfu/swab.	NSW
003 Sample 3. T=96h (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	cfu/swab.	NSW
004 Sample 4. T=96h (NATA Accredited)			
FM0010 Aerobic Plate Count	80	cfu/swab.	NSW

*NATA accreditation does not cover the performance of this service. Measurement of Uncertainty values for your compliance are available at <https://www.alsglobal.com/au/services-and-products/food-safety/laboratory-downloads/client-downloads>.

Signatories

This document has been electronically signed by the authorized signatories indicated below. Electronic signing has been carried out in compliance with procedures specified in 21 CFR Part 11.

Signatories	Position	Accreditation Category
Jason Alvarez	Laboratory Manager	Food Microbiology



General Comments

N/D = Not Detected, < = Less than, > = Greater than, cfu = colony forming unit, MPN = Most Probable Number, PN = Probable Number, Y = Yeast, M = Mould, ~ = Estimate

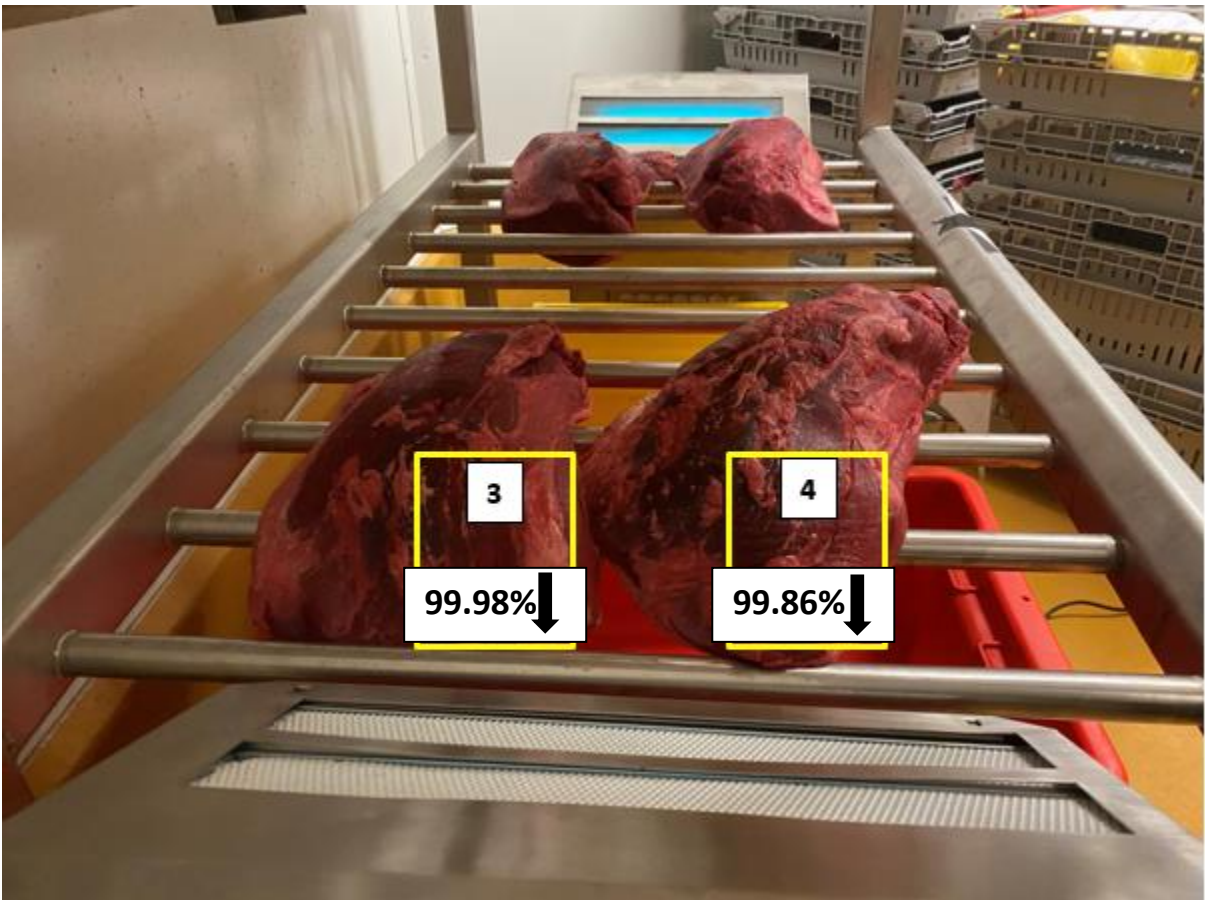
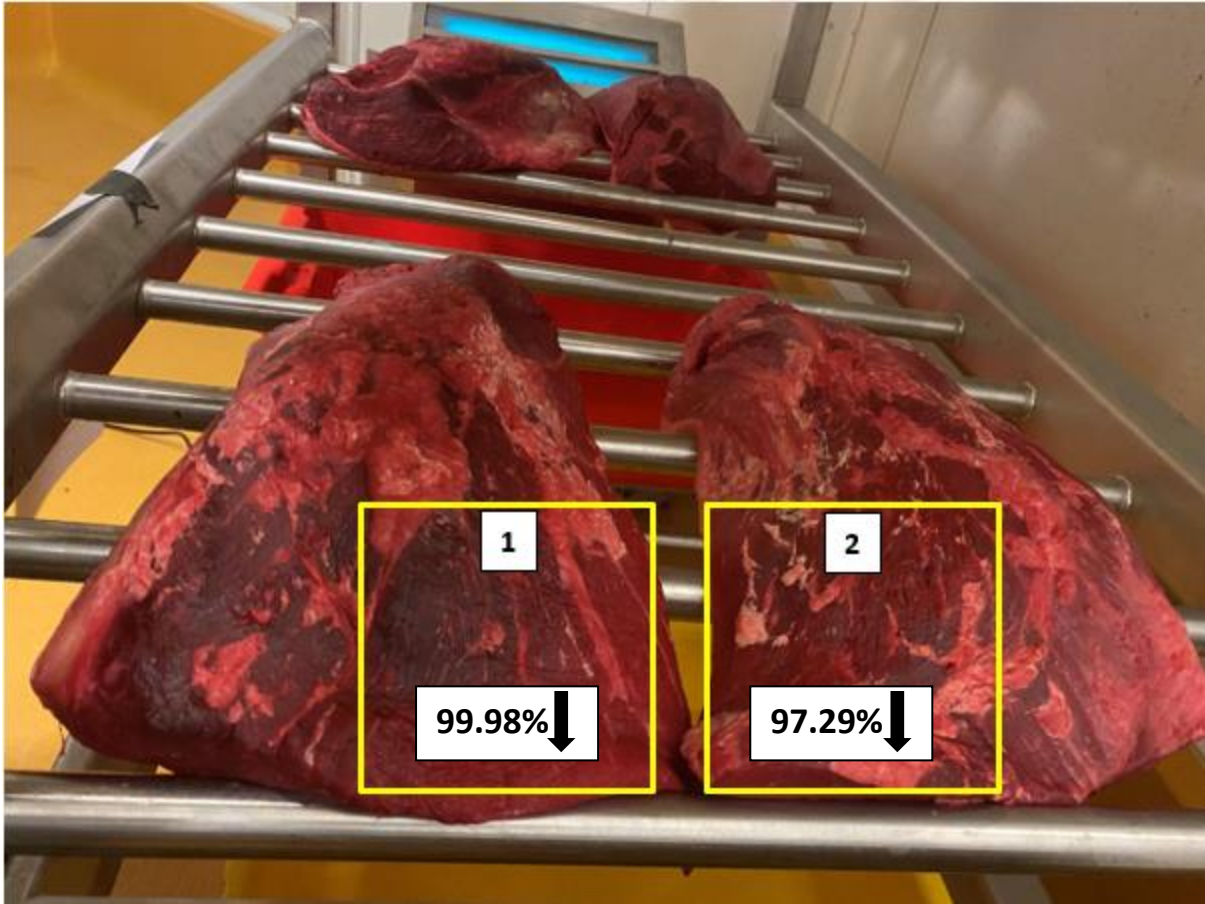
Primals 1 & 3 had counts below the level of detection ie <10 cfu/swab, while primals 2&4 were very low. Log reductions from the baseline counts were as follows:

Primal 1: 50000 down to <10 is >99.98% reduction ie >4 log

Primal 2: 4800 down to 130 is >97.29% reduction ie >2 log (If T0 was 50000 then reduction is >99.74%)

Primal 3: 50000 down to <10 is >99.98% reduction ie >4 log

Primal 4: 56000 down to 80 is >99.86% reduction ie >3 log



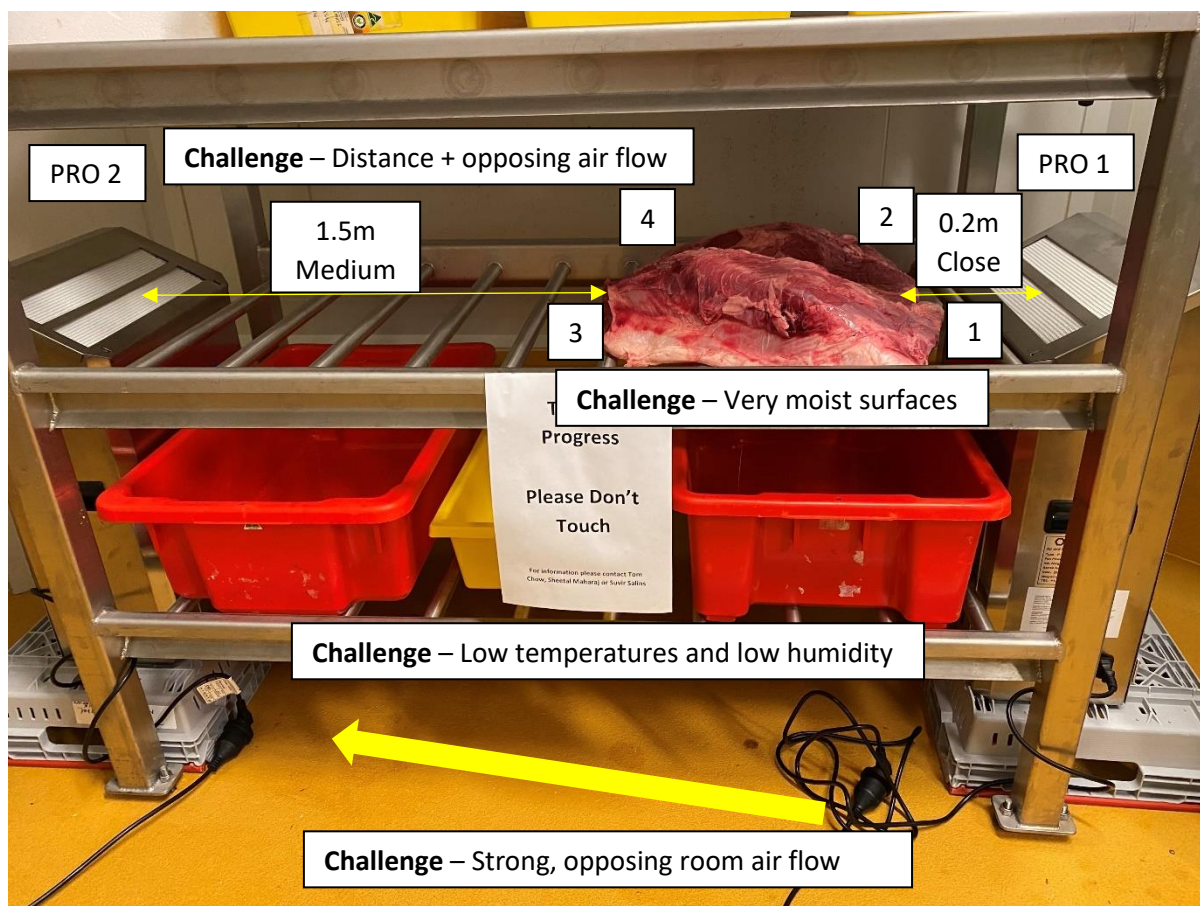
The trial results show that despite the **challenge of a cold environment and an opposing, fast ambient air flow and extremely high pathogen loads from 35 day aged primals** this trial of **close proximity, long duration**, with some moisture in the air but a dry primal surface, produced results are as expected, ranging from **2 to 4 log reductions**.

The trial has passed the first stage gate of one extreme.

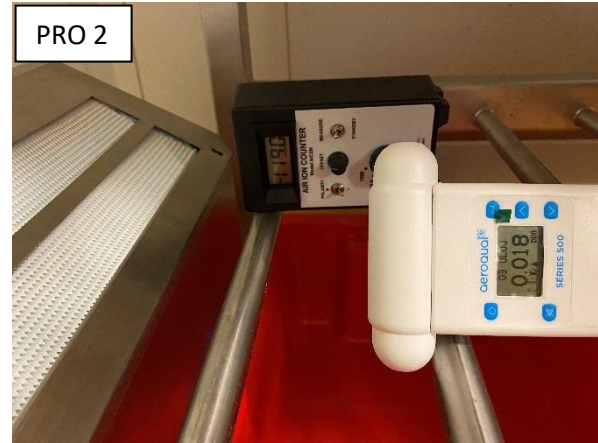
The next trials will confirm that **shorter durations, greater distances and moist surfaces** also experience **significant reductions**.

5.1.2 Shelf-Life 24-144hr 2hourly Microbiological Reductions Test Results – Test 4

The next trial will focus on how quickly the reduction results are actually achieved, on **extreme pathogen loads using 35 day aged primals**, by conducting another 24hr trial and taking samples every 2hrs to see whether the **shorter exposure times** result in significant log reductions. This trial only used 2 primals with one end close (0.2m) to PRO 1 while the other end is 1.5m away from PRO 2 to see if there is a significant difference to reductions and time with distance. Furthermore it should be noted that the ends of the primal facing PRO 2 are at a **disadvantage being downstream from the strong air flow within the room**. Additionally sample point 3 does not see the peak of the non-thermal plasma (NTP) flow from PRO 2, while sample point 4 sees the peak NTP

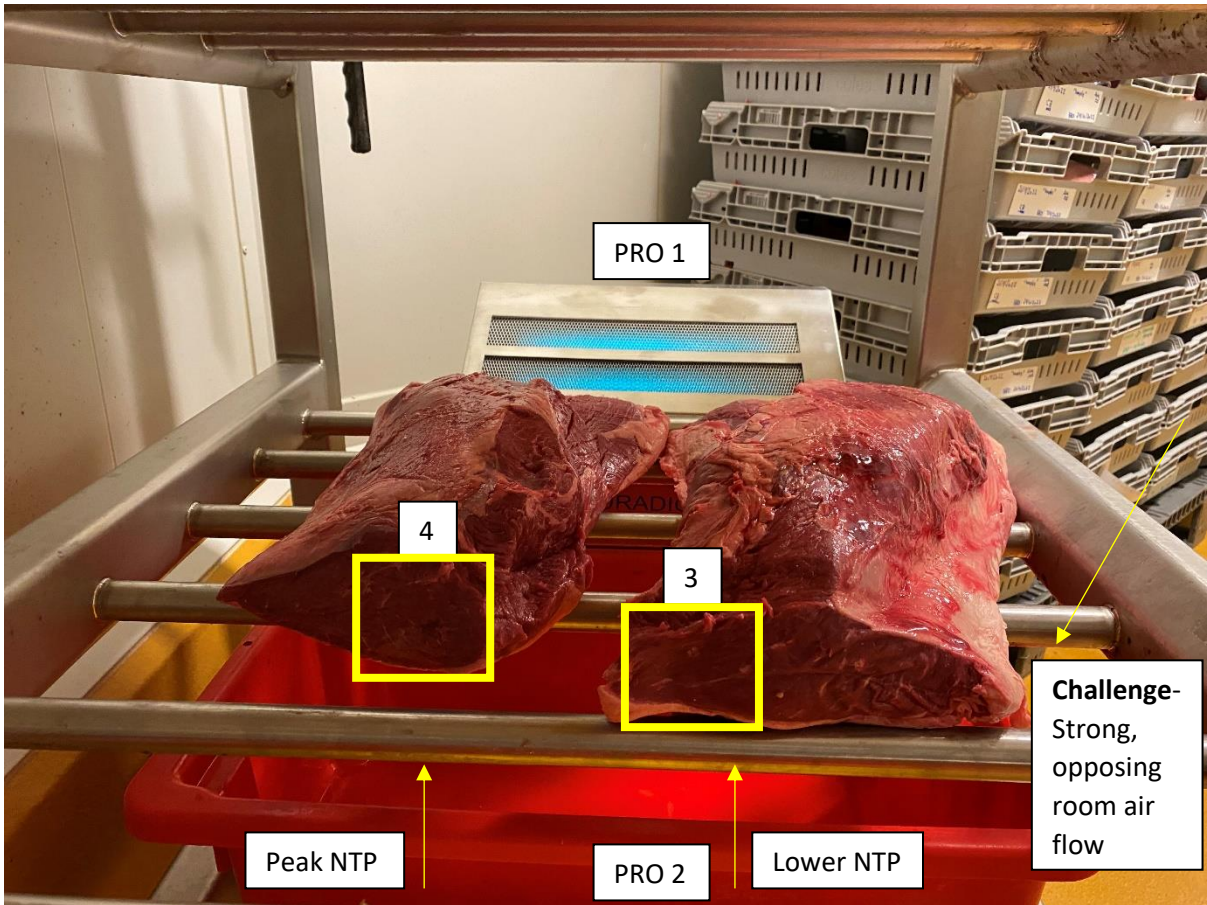


Check Ion Count (1000 ions/cm³) and Ozone levels (ppm) at T=0hrs Baseline (11th October, 2022)



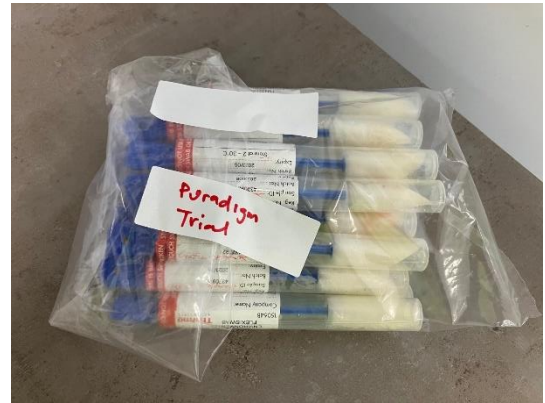
Swabs at T=0hrs, Baseline, in 10cm x 10cm surface regions (11th October, 2022)





Swabs collected from 0 to 144hrs






ALS Laboratory Submission Form – Test 4 – 0,2,4,6,8, 24hrs, 72hrs & 144hrs

FOOD MICROBIOLOGY - SAMPLE SUBMISSION FORM	
 <p>Office use only: Affix ALS Workorder label</p>	Company Name: Retail Ready Operations Australia Contact Person: Sheetal Maharaj Street Address: 54 Templar Road Erskine Park NSW 2759 Email Address: Sheetal.Maharaj@coles.com.au Phone: 0437 967 591
	<p>Print & Email to ALS</p> 
	<p>Office use only:</p> Date: Time: Condition: Opened by:
	<p>Purchase Order No. 11/10/2022</p>
	<p>Special Instructions:</p> <p>Puradigm 24-hour 2 hourly Reduction Trial on Primal Surface Environmental <u>FlexiSwab</u>.</p> <p>Separate invoice for this test.</p> <p>Limit of the tests as <10 cfu/swab</p> <p>Date Start: 11/10/2022 (T=0)</p> <p>T=0hr (11/10/2022) T=24hrs (12/10/2022) T=72hrs (14/10/2022)</p>

Sample Name	Testing Required (please tick tests required)																Other (please specify)	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
Sample 1. T=0hr		X																
Sample 2. T=0hr		X																
Sample 3. T=0hr		X																
Sample 4. T=0hr		x																
Sample 1. T=2hrs		X																
Sample 2. T=2hrs		X																
Sample 3. T=2hrs		X																
Sample 4. T=2hrs		x																
Sample 1. T=4hrs		X																
Sample 2. T=4hrs		X																
Sample 3. T=4hrs		X																
Sample 4. T=4hrs		x																
Sample 1. T=6hrs		X																
Sample 2. T=6hrs		X																
Sample 3. T=6hrs		X																
Sample 4. T=6hrs		x																
Sample 1. T=8hrs		X																
Sample 2. T=8hrs		X																
Sample 3. T=8hrs		X																
Sample 4. T=8hrs		x																
Sample 1. T=24hrs		X																
Sample 2. T=24hrs		X																
Sample 3. T=24hrs		X																
Sample 4. T=24hrs		x																

FOOD MICROBIOLOGY - SAMPLE SUBMISSION FORM			
 <p>ALS Food & Pharmaceutical 10/2-8 South St Rydalmere NSW 2116 T: +61 2 8832 7500 F: +61 2 9898 3472</p> <p>Office use only: Affix ALS Workorder label</p>	Company Name: Retail Ready Operations Australia		
	Contact Person: Sheetal Maharaj		
	Street Address: 54 Templar Road Erskine Park NSW 2759		
	Email Address: Sheetal.Maharaj@coles.com.au		
	Phone: 0437 967 591		
Purchase Order No. 14/10/2022		Special Instructions:	Paradigm 24-hour 2 hourly Reduction Trial on Primal Surface Environmental FlexiSwab. Limit of the tests as <10 cfu/swab Date Start: 11/10/2022 (T=0) T=0hr (11/10/2022) T=24hrs (12/10/2022) T=72hrs (14/10/2022)



Office use only:


Date:

Time:

Condition:

Opened by:

Sample Name	Testing Required (please tick tests required)																Other (please specify)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Sample 1. T=72hrs		X															
Sample 2. T=72hrs		X															
Sample 3. T=72hrs		X															
Sample 4. T=72hrs		x															

FOOD MICROBIOLOGY - SAMPLE SUBMISSION FORM			
 <p>ALS Food & Pharmaceutical 10/2-8 South St Rydalmere NSW 2116 T: +61 2 8832 7500 F: +61 2 9898 3472</p> <p>Office use only: Affix ALS Workorder label</p>	Company Name: Retail Ready Operations Australia		
	Contact Person: Sheetal Maharaj		
	Street Address: 54 Templar Road Erskine Park NSW 2759		
	Email Address: Sheetal.Maharaj@coles.com.au		
	Phone: 0437 967 591		
Purchase Order No. 17/10/2022		Special Instructions:	Paradigm 24-hour 2 hourly Reduction Trial on Primal Surface Environmental FlexiSwab. Limit of the tests as <10 cfu/swab Date Start: 11/10/2022 (T=0) T=144hrs (17/10/2022)



Office use only:

Date:

Time:

Condition:

Opened by:

Sample Name	Testing Required (please tick tests required)																Other (please specify)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Sample 1. T=144hrs		X															
Sample 2. T=144hrs		X															
Sample 3. T=144hrs		X															
Sample 4. T=144hrs		x															

Do you require a separate report for each sample: _____ Samples to be composited: _____

ALS Laboratory Microbiology Results – Test 4 – 0,2,4,6,8, 24hrs, 72hrs & 144hrs

For these **extremely high pathogen loads on 35 day aged primals, short duration** periods showed **no log reductions** in total plate count (TPC). In fact the first **2-4 hours typically resulted in an increase** in TPC. The **4-8 hour period had a gradual decline** of around 1 log reduction.

There was a **power outage** of PRO 1 between 8-24hrs. This resulted in a **very large increase in TPC** for Sample points 1 and 2 which are the points very close to PRO 1. Sample 3 also experienced very significant increases in TPC during that period despite PRO 2 remaining on. Sample 3 is 1.5m away from PRO 2, but it is in a location which sees a minimum of non-thermal plasma (NTP) while Sample 4 is in line with the peak NTP flow. The room has a very strong air flow, from the condenser fans, which blow against the PRO 2's NTP, thus sample points 3 & 4 tend to receive less NTP than sample points 1 & 2. **This increase in TPC during the shutdown, shows that the low temperature, high air flow speed and low humidity were not aiding in log reductions, the only cause of reductions is the Puradigm NTP.**

However **from the 24hr point, after power was resumed, till the 144hr point, 4-5 log reductions were experienced**, bringing the TPC down to very low amounts typically only seen after a solid 24hrs of uninterrupted NTP at close range.

It is clear from the results that there is still **significant log reductions on TPC experienced despite distance and strong counteracting room airflows**. However durations shorter than 4 hours under those **extreme conditions** do not show significant reductions, as it **takes time for the NTP** to work its way through the many layers of pathogens as the NTP works on the surface, **one molecular layer at a time**. With **normal pathogen loads it is expected reductions will be rapid**. The next trials will confirm this.

The recommendation would be to situate **Puradigm devices fairly close to target** objects for **very contaminated subjects**, and ensure the NTP is flowing with the room air flow rather than against it, or that air flow is at a minimum speed for **maximum speed reductions**. Puradigm devices can **handle very large spaces and will cause reductions, even for highly contaminated air and surfaces, but these will take longer**, the further the device is from the surface. For fresh product, which have **low pathogen loads**, the Puradigm device can be **situated at a distance and achieve fast reductions** given the charged water aerosol permeates the entire space.

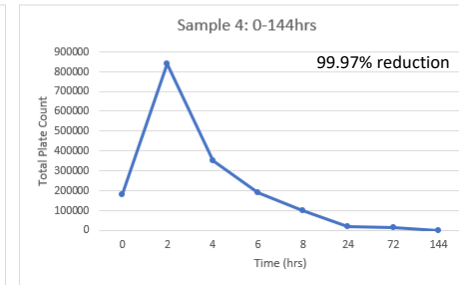
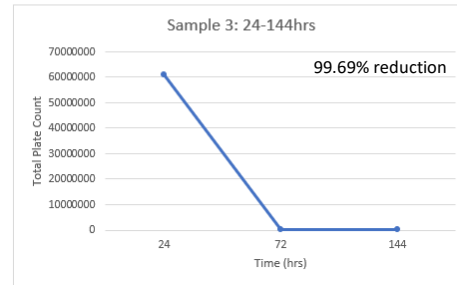
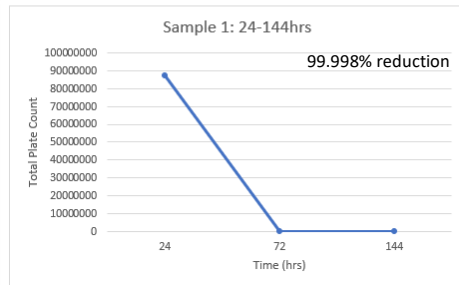
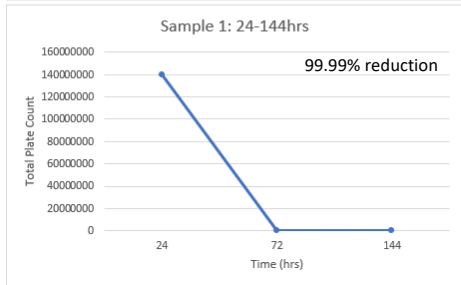
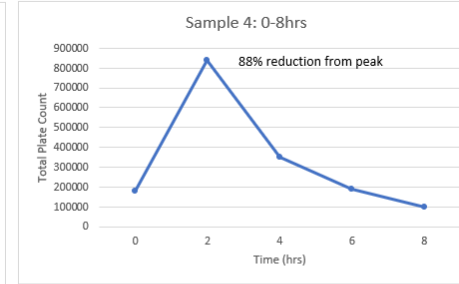
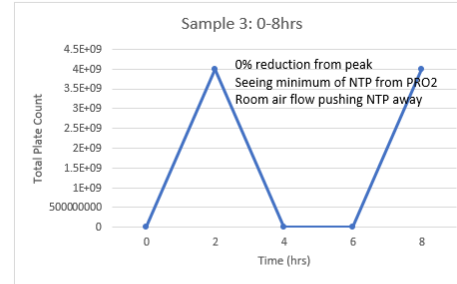
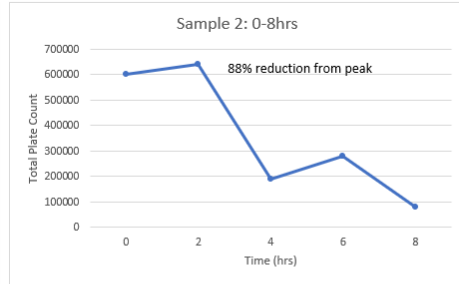
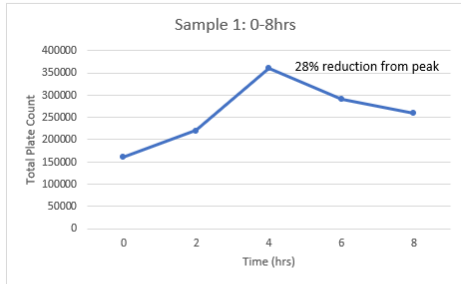
Additionally **significant reductions** on all surfaces that the product is in contact with, will benefit greatly from the **Puradigm NTP technology**, as will surfaces such as **drains, conveyor drive sections** which are **infrequently accessed** which may harbour traces of trapped product and other areas which could **harbour pathogens**. US secondary processors have reported listeria outbreaks every 2 weeks have been reduced to none after 6 months of exposure. **Further trials should be conducted to confirm these long term exposure benefits.**

It should be noted that these high levels of TPC are several orders of magnitude greater than those typical of fresh primals which are processed in the plant. The primals that have been tested are 35-day aged primals, and so their TPC is unusually high. Shorter term exposures, below 2 hours, could result in beneficial reductions when the TPC count is at normal levels, as the NTP doesn't have to work its way through so many layers of bacteria.

Total Plate Counts at each sampling time period 0 – 144hrs

Time (h)	Sample 1	Sample 2	Sample 3	Sample 4
0	160000	600000	340000	180000
2	220000	640000	4E+09	840000
4	360000	190000	2700000	350000
6	290000	280000	2300000	190000
8	260000	79000	4E+09	97000
24	1.4E+08	8.7E+07	61000000	16000
72	22000	60000	50000	12000
144	8400	1700	190000	220
Max Reduction	99.99%	99.998%	99.995%	99.97%
Reduction 0-8hrs	28%	88%	0%	88%
Reduction 24-72hrs	99.98%	99.93%	99.92%	98.57%
Reduction 24-144hrs	99.99%	99.998%	99.69%	99.97%

First 2-4 hours sees an increase in TPC. Moderate decrease from 4-8 hours. Interruption to power 8-24 hours saw a big increase except for Sample 4. 4-5 log reductions from 24-144hrs



**Overall Description: Puradigm 24-hour 2 hourly Reduction Trial on Primal Surface Environmental FlexiSwab
-Received 12/10/22; Arrival Temp:2.8C**

Our Ref No: FS2258092 Amendment 1

Your Ref: 11/10/2022

Project:

Report Date: 26 Oct 2022

Samples Received: 12 Oct 2022

Temperature when received: 2.8

Testing Commenced: 12 Oct 2022

This report cannot be reproduced except in full, without written approval from the laboratory.
Samples tested as received into the laboratory, unless the sampling was conducted by ALS.
This amended report replaces all earlier reports of the same number.

Sample Details: Test Description	Results	Units	Site
001 Sample 1. T=0 (NATA Accredited)			
FM0010	Aerobic Plate Count	160,000	cfu/swab. NSW
002 Sample 2. T=0 (NATA Accredited)			
FM0010	Aerobic Plate Count	~600,000	cfu/swab. NSW
003 Sample 3. T=0 (NATA Accredited)			
FM0010	Aerobic Plate Count	~340,000	cfu/swab. NSW
004 Sample 4. T=0 (NATA Accredited)			
FM0010	Aerobic Plate Count	180,000	cfu/swab. NSW
005 Sample 1. T=2h (NATA Accredited)			
FM0010	Aerobic Plate Count	~220,000	cfu/swab. NSW
006 Sample 2. T=2h (NATA Accredited)			
FM0010	Aerobic Plate Count	~640,000	cfu/swab. NSW
007 Sample 3. T=2h (NATA Accredited)			
FM0010	Aerobic Plate Count	~400,000,000	cfu/swab. NSW
008 Sample 4. T=2h (NATA Accredited)			
FM0010	Aerobic Plate Count	~840,000	cfu/swab. NSW
009 Sample 1. T=4h (NATA Accredited)			
FM0010	Aerobic Plate Count	~360,000	cfu/swab. NSW
010 Sample 2. T=4h (NATA Accredited)			
FM0010	Aerobic Plate Count	190,000	cfu/swab. NSW
011 Sample 3. T=4h (NATA Accredited)			
FM0010	Aerobic Plate Count	2,700,000	cfu/swab. NSW
012 Sample 4. T=4h (NATA Accredited)			
FM0010	Aerobic Plate Count	~350,000	cfu/swab. NSW
013 Sample 1. T=6h (NATA Accredited)			
FM0010	Aerobic Plate Count	290,000	cfu/swab. NSW
014 Sample 2. T=6h (NATA Accredited)			
FM0010	Aerobic Plate Count	~280,000	cfu/swab. NSW

015 Sample 3. T=6h (NATA Accredited)				
FM0010	Aerobic Plate Count		2,300,000	cfu/swab. NSW
016 Sample 4. T=6h (NATA Accredited)				
FM0010	Aerobic Plate Count		190,000	cfu/swab. NSW
017 Sample 1. T=8h (NATA Accredited)				
FM0010	Aerobic Plate Count		~260,000	cfu/swab. NSW
018 Sample 2. T=8hrs (NATA Accredited)				
FM0010	Aerobic Plate Count		79,000	cfu/swab. NSW
019 Sample 3. T=8hrs (NATA Accredited)				
FM0010	Aerobic Plate Count		~400,000,000	cfu/swab. NSW
020 Sample 4. T=8hrs (NATA Accredited)				
FM0010	Aerobic Plate Count		97,000	cfu/swab. NSW
021 Sample 1. T=24hrs (NATA Accredited)				
FM0010	Aerobic Plate Count		140,000,000	cfu/swab. NSW
022 Sample 2. T=24hrs (NATA Accredited)				
FM0010	Aerobic Plate Count		87,000,000	cfu/swab. NSW
023 Sample 3. T=24hrs (NATA Accredited)				
FM0010	Aerobic Plate Count		61,000,000	cfu/swab. NSW
024 Sample 4. T=24hrs (NATA Accredited)				
FM0010	Aerobic Plate Count		16,000	cfu/swab. NSW



Certificate of Analysis

Retail Ready Operations Australia Pty Ltd
PO Box 97
St Clair NSW, 2759

Attention: Sheetal Maharaj

Overall Description: Paradigm 24-hour 2 hourly Reduction Trial on Primal Surface Environmental FlexiSwab. - Received: 14/10/2022

Our Ref No: FS2258810

Your Ref: 14/10/2022

Project:

Report Date: 19 Oct 2022

Samples Received: 14 Oct 2022

Temperature when received: 2.8

Testing Commenced: 14 Oct 2022

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Sample Details: Test Description	Results	Units	Site
001 Sample 1. T=72hrs (NATA Accredited)			
FM0010	Aerobic Plate Count	22,000	cfu/swab. NSW
002 Sample 2. T=72hrs (NATA Accredited)			
FM0010	Aerobic Plate Count	~60,000	cfu/swab. NSW
003 Sample 3. T=72hrs (NATA Accredited)			
FM0010	Aerobic Plate Count	~50,000	cfu/swab. NSW
004 Sample 4. T=72hrs (NATA Accredited)			
FM0010	Aerobic Plate Count	12,000	cfu/swab. NSW

*NATA accreditation does not cover the performance of this service. Measurement of Uncertainty values for your compliance are available at <https://www.alsglobal.com/au/services-and-products/food-safety/laboratory-downloads/client-downloads>.

Signatories

This document has been electronically signed by the authorized signatories indicated below. Electronic signing has been carried out in compliance with procedures specified in 21 CFR Part 11.





Certificate of Analysis

Retail Ready Operations Australia Pty Ltd
 PO Box 97
 St Clair NSW, 2759

Attention: **Sheetal Maharaj**

Overall Description: Puradigm 24-hour 2 hourly Reduction Trial on Primal Surface Environmental FlexiSwab.
Date start: 11/10/2022 (T=0)

Our Ref No: FS2259055

Your Ref: 14/10/2022

Project:

Report Date: 21 Oct 2022

Samples Received: 17 Oct 2022

Temperature when received: 2.8

Testing Commenced: 17 Oct 2022

This report cannot be reproduced except in full, without written approval from the laboratory.
 Samples tested as received into the laboratory, unless the sampling was conducted by ALS.

Sample Details: Test Description	Results	Units	Site
001 Sample 1. T=144hrs (NATA Accredited)			
FM0010	Aerobic Plate Count	8,400	cfu/swab. NSW
002 Sample 2. T=144hrs (NATA Accredited)			
FM0010	Aerobic Plate Count	1,700	cfu/swab. NSW
003 Sample 3. T=144hrs (NATA Accredited)			
FM0010	Aerobic Plate Count	~190,000	cfu/swab. NSW
004 Sample 4. T=144hrs (NATA Accredited)			
FM0010	Aerobic Plate Count	220	cfu/swab. NSW

*NATA accreditation does not cover the performance of this service.
 Measurement of Uncertainty values for your compliance are available at <https://www.alsglobal.com/au/services-and-products/food-safety/laboratory-downloads/client-downloads>.

Signatories

This document has been electronically signed by the authorized signatories indicated below. Electronic signing has been carried out in compliance with procedures specified in 21 CFR Part 11.



6 Milestone 4

Milestone 4 focuses on the implementation through practical trials and initial testing in a production environment, including:

Install trial units in RROA production HVAC Air Handling Unit (AHU) locations.

Collect and analyse test samples, including:

- Microbiological reductions on surfaces within the HVAC environment
- Mould before and after photos

Continue shelf-life trials

- Microbiological reductions on the surface of fresh primals focusing on speed of reduction for short duration exposures

6.1 HVAC AHU – Analysis of Environmental Conditions and Microbiological Reduction Trials

The RR11 red meat Air Handling Unit (AHU) was thoroughly chemically cleaned as part of its usual 3-monthly cleaning cycle.

The previously installed Paradigm HVAC units were powered on to start their cleaning cycle.

Photos of surfaces were taken as the baseline after the chemical clean. These will be compared to surface photos taken at intervals from this baseline until the next chemical cleaning cycle.

The expectation is that the walls exposed to the Paradigm non-thermal plasma output will have a reduction in mould compared to that expected after 3-months.

Before Chemical Clean – Large quantities of mould on surfaces

The mould build up shown below is typical after 3 months between chemical cleans. It is expected that the Paradigm units will reduce this mould build up and thereby reduce the amount of chemical cleaning either in frequency ie extend to more than 3 months, or in amount of chemicals required to clean all AHU surfaces.



After Chemical Clean – all mould removed





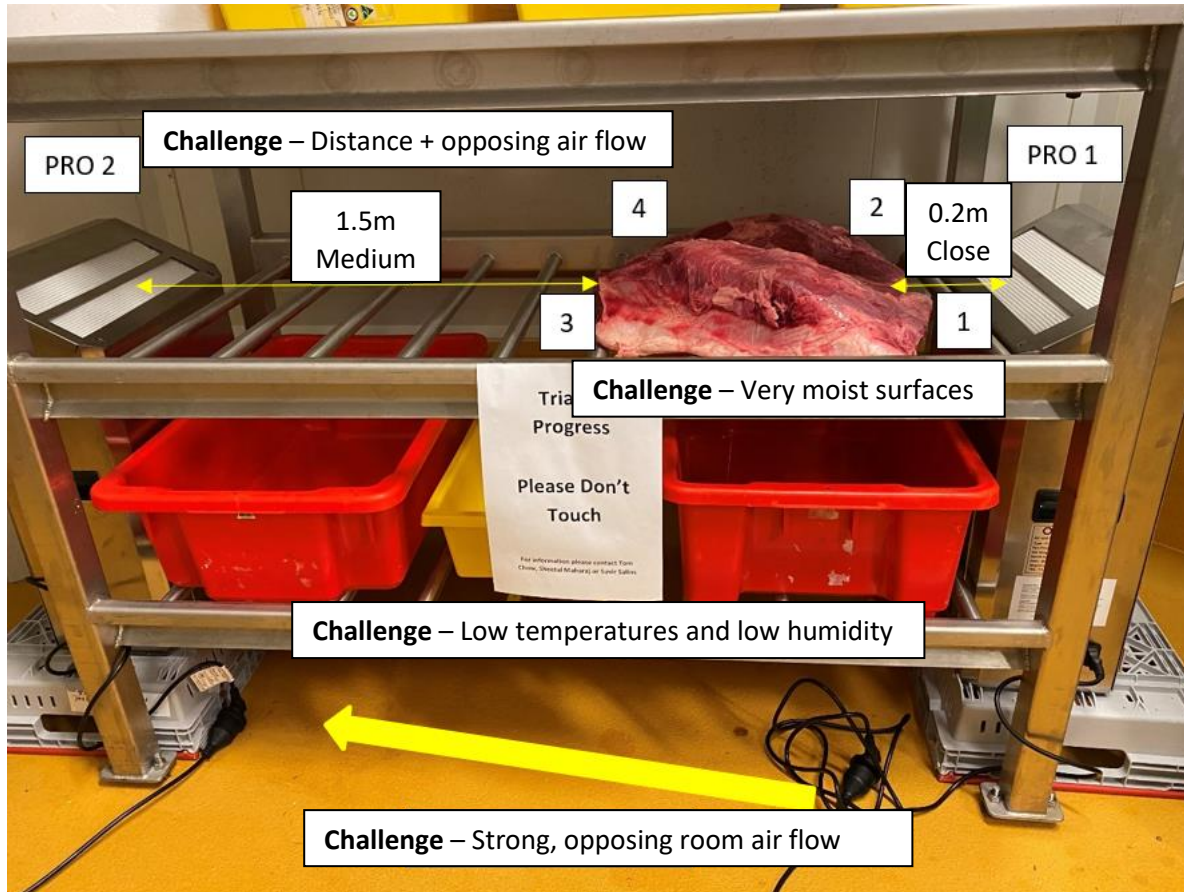




6.2 Shelf-Life Room – Microbiological Reduction for Short Exposure Times

In Milestone 3 red meat primals were exposed for long periods of time, up to 24, 96 and 144hrs. This trial was conducted in the QA Retention Chiller Room where samples are stored for analysis. The temperature of the room is 4°C. These red meat *primals had been aged for 35 days*.

In this Milestone 4, the *speed of reduction is calculated for short term durations*. These reductions can then be used to determine how quickly fresh primals can have their surfaces sterilised.



The *fastest rates of reduction* for each test surface 1, 2, 3 and 4 during the Milestone 3 trials were as follows:

Primal Surface 1: 810 counts per second

Primal Surface 2: 503 counts per second

Primal Surface 3: 353 counts per second

Primal Surface 4: 68 counts per second

Fresh primals at RROA from previous shelf life studies typically have a plate count of 1000 which means that the surfaces would have the following reduction times to achieve a zero plate count.

Primal Surface 1: $1000 / 810 = 1.23$ s

Primal Surface 2: $1000 / 503 = 1.99$ s

Primal Surface 3: $1000 / 353 = 2.83$ s

Primal Surface 4: $1000 / 68 = 14.71$ s

These reduction times suggest that fresh primals can be decontaminated in very short periods of time with close proximity, between 0.2m to 1.5m, direct exposure to Puradigm NTP. Even with 10x the amount of plate counts, the **exposure times will be no more than 2-3 minutes for complete decontamination.**

6.3 Shelf-Life Room – Microbiological Reduction for combined Denba and Puradigm exposures

Puradigm NTP deactivates or kills viruses and bacteria on surfaces. Denba+ inhibits the growth of bacteria throughout the product for produce which has sufficient quantities of moisture. This trial intends to quantify the extent of the shelf life extension using the combination of these two technologies. Baselines for reductions experienced using Puradigm alone and Denba alone have been conducted in previous milestones within this project, or in the Co-Innovation program's milestones.

Fresh primals will be exposed to 3 minutes of Puradigm NTP on all surfaces and then placed within the Denba freezer at a target temperature of -1.5 to -2C as the test subjects. The Control will be fresh primals which are not exposed to Puradigm NTP or Denba, and placed in a freezer at -1.5C to -2C.

Denba and Control Freezers





These trials are ongoing and the results will be reported in a separate project which has been specifically launched to review the benefits of combining the Puradigm and Denba technologies.

7 Milestone 5

Practical trials in production environment, including:

- Final results for HVAC trials in Air Handling Units
- Microbiological reduction final report on effectiveness in various conditions and timeframes.
- Packaging decontamination final report on effectiveness

7.1 HVAC AHU –Microbiological Reduction Trials in real-world environmental conditions

This final milestone marks the end of the HVAC Air Handling Unit (AHU) two month trial. Usually this point would show mould developing on most of the surfaces in the ductwork across the AHU.

However as is evidenced through the following photos, no mould is visible on any of the surfaces, which remain in pristine condition.

A typical non-chemical method of attacking mould is using direct UV light exposure. As can be seen from the chart below, stachybotrys chartarum commonly known as black mould, requires one of the highest exposure times and intensity levels to achieve even the tiny reduction of 0.41%. This chart shows the relative ease with which UV can cause microbial reductions when exposed to different viruses and bacteria. Mould is one of the hardest pathogens. Given this understanding, Puradigm’s results of keeping the black mould from forming on the HVAC AHU surfaces, as is typical every few months, shows that the Puradigm technology is capable of handling bacteria and viruses of all types with a similar, if not even greater efficacy.

BIO-CONTAMINANT DISTRUCTION RATES WHEN EXPOSED TO UVC - Weakest to Strongest							1 SECOND @	
Contaminant	Name	<i>kin AIR or Surf</i>		D 90%	Type	micron	UV Dose= $\mu\text{m}^2/\text{cm}^2$	
		Custom unit	SI unit				1,000	
							DISINFECTON	
Mycobacterium tuberculosis		0.4721 m2/J	0.4721 m2/J	0.49	bacteria	0.637	95.11%	
Legionella pneumophila		0.44613 m2/J	0.44613 m2/J	0.52	bacteria	0.52	98.35%	
Coronavirus (SARS)		0.377 m2/J	0.377 m2/J	0.61	virus	0.113	97.69%	
Proteus mirabilis		0.289 m2/J	0.289 m2/J	0.80	bacteria	0.494	94.44%	
Mycoplasma pneumoniae		0.2791 m2/J	0.2791 m2/J	0.83	bacteria	0.177	93.86%	
Listeria monocytogenes		0.2303 m2/J	0.2303 m2/J	1.00	bacteria	0.707	90.0%	
Salmonella		0.221 m2/J	0.221 m2/J	1.04	bacteria	0.800	89.03%	
Aeromonas		0.2031 m2/J	0.2031 m2/J	1.13	bacteria	2.098	86.88%	
Rickettsia prowazekii		0.176 m2/J	0.176 m2/J	1.31	bacteria	0.600	82.80%	
Staphylococcus epidermis		0.1621 m2/J	0.1621 m2/J	1.42	bacteria	0.866	80.23%	
E. coli		0.15611 m2/J	0.15611 m2/J	1.48	bacteria	0.500	79.01%	
Yersinia enterocolitica		0.15351 m2/J	0.15351 m2/J	1.50	bacteria	0.707	78.46%	
Coxiella burnetii		0.1535 m2/J	0.1535 m2/J	1.50	bacteria	0.283	78.45%	
Lactobacillus reuteri		0.1535 m2/J	0.1535 m2/J	1.50	bacteria	0.307	78.45%	
Vaccinia virus		0.153 m2/J	0.153 m2/J	1.51	virus	0.307	78.3%	
smallpox		0.001528 cm2/ μ	0.1528 m2/J	1.51	virus	0.212	76.31%	
Newcastle disease		0.144000 m2/J	0.144 m2/J	1.60	virus	0.212	76.31%	
Acinetobacter baumannii		0.128 m2/J	0.128 m2/J	1.80	bacteria	1.225	72.20%	
influenza A virus		0.119 m2/J	0.119 m2/J	1.94	virus	0.098	69.58%	
MRSA		0.113 m2/J	0.113 m2/J	2.04	bacteria	0.866	67.70%	
Coxsackievirus		0.111 m2/J	0.111 m2/J	2.07	virus	0.027	67.04%	
Avian influenza virus		0.106 m2/J	0.106 m2/J	2.17	virus	0.098	65.35%	
Measle virus		0.1051 m2/J	0.1051 m2/J	2.19	virus	0.319	65.04%	
Pseudomonas aeruginosa		0.1047 m2/J	0.1047 m2/J	2.20	bacteria	0.494	64.90%	
Serratia marcescens		0.095 m2/J	0.095 m2/J	2.42	bacteria	0.632	61.33%	
Parvovirus H-1		0.092 m2/J	0.092 m2/J	2.50	virus	0.022	60.15%	
Proteus vulgaris/mirabilis		0.07875 m2/J	0.07875 m2/J	3.00	bacteria	0.291	55.58%	
Corynebacterium diptheriae		0.0701 m2/J	0.0701 m2/J	3.29	bacteria	0.698	50.39%	
Ustilago zeae		0.0658 m2/J	0.0658 m2/J	3.50	fungi sp	5.916	48.21%	
Streptococcus pyogenes		0.06161 m2/J	0.06161 m2/J	3.74	bacteria	0.894	46.00%	
Haemophilus influenza		0.0599 m2/J	0.0599 m2/J	3.84	bacteria	0.285	45.06%	
Yeast		0.05356 m2/J	0.05356 m2/J	4.00	VegY	0.000	43.78%	
Klebsiella pneumoniae		0.04919 m2/J	0.04919 m2/J	4.20	bacteria	0.671	42.19%	
Neisseria catarrhalis/meningitidis		0.05233 m2/J	0.05233 m2/J	4.40	bacteria	0.177	40.74%	
Clostridium tetani		0.04699 m2/J	0.04699 m2/J	4.90	fungi sp	5.000	37.49%	
VRE		0.0419 m2/J	0.0419 m2/J	5.50	virus	0.065	34.23%	
Burkholderia cenocepacia		0.03956 m2/J	0.03956 m2/J	5.82	bacteria	0.707	32.67%	
Adenovirus		0.039 m2/J	0.039 m2/J	5.91	virus	0.079	32.29%	
Enterobacter cloacae		0.03598 m2/J	0.03598 m2/J	6.40	bacteria	1.414	30.22%	
Reovirus		0.03358 m2/J	0.03358 m2/J	6.86	virus	0.075	28.52%	
Norwalk virus		0.0304 m2/J	0.0304 m2/J	7.58	virus	0.029	26.21%	
Echovirus		0.217 m2/J	0.219 m2/J	10.52	virus	0.024	19.67%	
Bacillus Anthracis		0.0167 m2/J	0.0167 m2/J	13.79	bacteria	1.118	15.38%	
Cryptococcus neoformans		0.0419 m2/J	0.0167 m2/J	13.79	fungi sp	4.899	15.38%	
Blastomyces dermatidis		0.0419 m2/J	0.01645 m2/J	14.00	VegY	11.000	15.17%	
Histoplasma capsulatum		0.0419 m2/J	0.01645 m2/J	14.00	fungi sp	2.550	15.17%	
Mucor spores		0.01645 m2/J	0.01645 m2/J	14.00	fungi	7.070	15.17%	
Bacillus subtilis spores		0.0155 m2/J	0.0155 m2/J	14.86	bacteria	1.120	14.36%	
Francisella Tularensis		0.144000 m2/J	0.0147 m2/J	15.62	virus	0.200	13.71%	
Fusarium oxysporum		0.0142 m2/J	0.0142 m2/J	16.22	fungi sp	11.225	13.24%	
Powdery Mildew		0.013 m2/J	0.013 m2/J	17.72	bacteria	0.707	12.19%	
Botrytis cinerea		0.0092 m2/J	0.0092 m2/J	25.03	fungi sp	11.180	8.79%	
Rhizopus nigricans		0.00861 m2/J	0.00861 m2/J	26.75	fungi sp	6.928	8.25%	
Nocardia asteroides		0.00822 m2/J	0.00822 m2/J	28.02	bacteria	1.118	7.89%	
Penicillium digitatum		0.00718 m2/J	0.00718 m2/J	32.08	fungi sp	3.262	6.93%	
Bacillus Cereus spores		0.00564 m2/J	0.00564 m2/J	40.83	fungi sp	1.118	5.48%	
Algae blue-green		0.00512 m2/J	0.00512 m2/J	44.98	algae	5.000	4.99%	
Penicillium chrysogenum		0.00434 m2/J	0.00434 m2/J	53.06	fungi sp	3.262	4.25%	
Trichophyton rubrum		0.00411 m2/J	0.00411 m2/J	56.03	fungi sp	4.899	4.03%	
Candida albicans		0.00407 m2/J	0.00407 m2/J	56.58	VegY	4.899	3.99%	
Candida albicans		0.00407 m2/J	0.00407 m2/J	56.58	VegY	4.899	3.99%	
Mucor mucedo		0.00399 m2/J	0.00399 m2/J	57.72	fungi sp	7.071	3.91%	
C.diff.sp		0.00346 m2/J	0.00346 m2/J	59.86	bacteria	0.060	3.77%	
Cladosporium herbarum		0.0037 m2/J	0.0037 m2/J	62.24	fungi sp	8.062	3.63%	
Scopulariopsis brevicaulis		0.00344 m2/J	0.00344 m2/J	66.95	fungi sp	5.916	3.38%	
Bacillus Anthracis spores		0.0031 m2/J	0.0031 m2/J	74.29	bacteria	1.118	3.05%	
Aspergillus fumigatus spores		0.00103 m2/J	0.00103 m2/J	223.59	fungi sp	2.640	1.02%	
Aspergillus niger spores		0.00058 m2/J	0.00058 m2/J	397.07	fungi	3.354	0.58%	
Cladosporium wemecki		0.00051 m2/J	0.00051 m2/J	451.57	fungi sp	8.062	0.51%	
stachybotrys chartarum		0.00041 m2/J	0.00041 m2/J	561.71	fungi sp	5.623	0.41%	
Moraxella		0.00022 m2/J	0.00022 m2/J	1046.82	bacteria	1.225	0.22%	

Photos of the Red Meat HVAC Air Handling Unit RR11 After Two Month's Exposure to the Paradigm

No Black Mould has grown on any of the many surfaces of the AHU ductwork in the two months of exposure to the Paradigm technology.

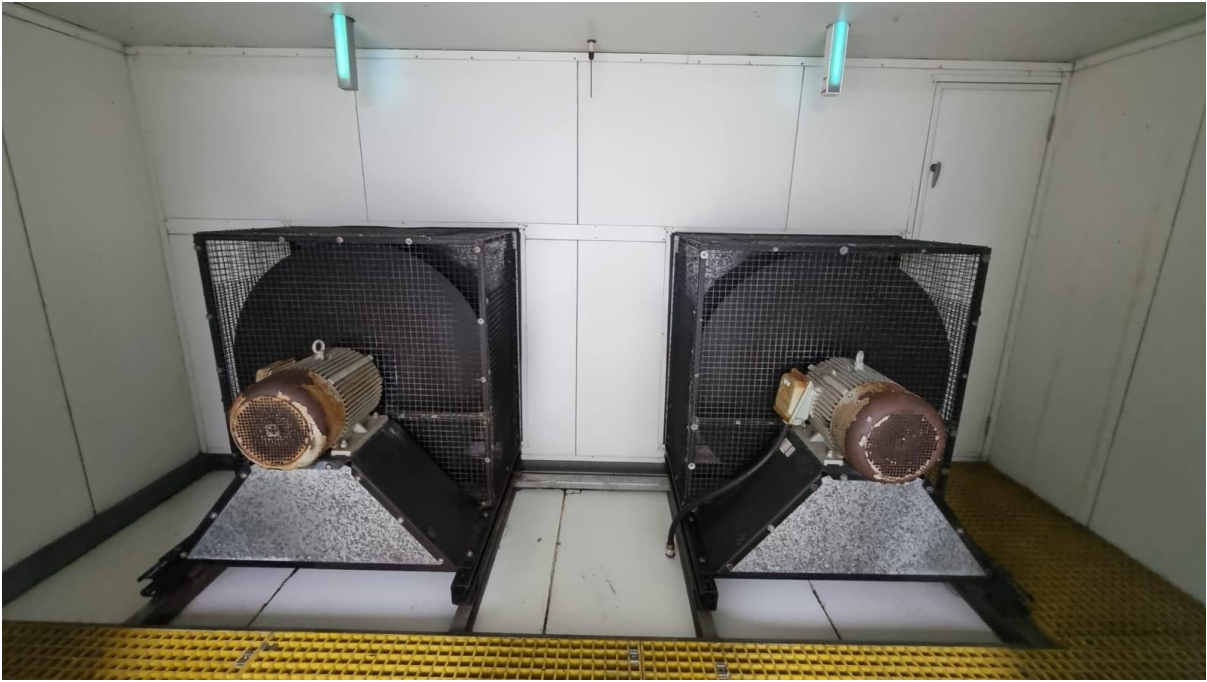














It is expected that the **black mould will continue to be limited in the air and on surfaces within the Air Handling Unit**. As such the frequency of cleaning can be reduced and thus the **use of chemicals and their associated labour and material costs will be reduced** while retaining the high hygiene standards that is typical of Coles manufacturing environments.

The next steps would be to roll out these Puradigm HVAC units into all of the plant AHUs.

7.2 Fresh primals –Microbiological Reduction Trials over short time frames

Having shown the significant microbial reductions, on the surface of 35 day aged primals, in previous milestones, with 2-4 log reductions achieved over periods of 8hrs or more in very cold and high, opposing air flow environments, this final milestone will focus on **fresh primals, aged for 10 days** or less. The estimated speed of reduction in the previous milestone, using the fastest reduction speeds measured for the very contaminated primals, showed fresh primals should be able to be **decontaminated in the order of several minutes**.

The environment is once again a 4C, cool room, with high, opposing air flow conditions.

One Puradigm PRO was utilised for this trial, whose output was measured at 300K ions/cc near the unit as shown below.



A fresh primal of Cube roll was supplied on the Thursday and the test was run on the following Monday morning. **The primal is aged 10 days.**

The cube roll was cut open and immediately swabbed at Time = 0 minutes at two locations on the face of the roll as shown below.



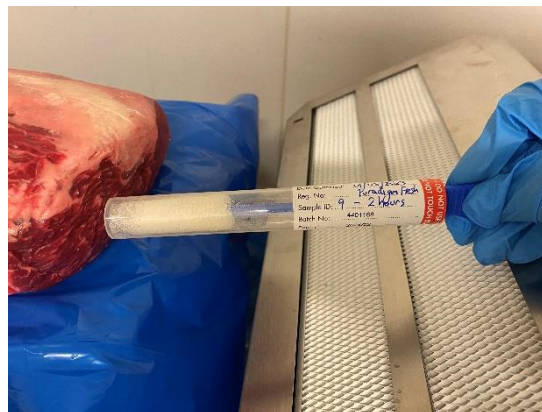
The following sample schedule was the followed for the next set of sample swabs at the same locations on the cube roll face.

Sample Left	Sample Right	Time (mins)
1	0	0
3	2	5
5	4	10
7	6	15
9	8	30
11	10	120
13	12	240
15	14	360

Right sample swab location



Left sample swab location




Location of Trial Equipment within the Cool room




Sample Swabs after Trial



ALS Lab Submission

FOOD MICROBIOLOGY – SAMPLE SUBMISSION FORM																				
	Company Name:		Retail Ready Operations Australia																	
	Contact:		Sheetal Maharaj																	
	Street Address:		54 Templar Road Erskine Park NSW 2759																	
	Email Address:		Sheetal.Maharaj@coles.com.au																	
	Phone:		0437 967 591																	
Purchase Order No.		2/05/23		Special Instructions:		Paradigm Fresh Test on Primal Surface Environmental Flexi Swabs Limits of test as <10cfu/swab Date start: 01/05/2023														
Sample Name			Testing Required (please tick tests required)																	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Other (please specify)	
Sample 0. T= 0min			X																	
Sample 1. T= 0min			X																	
Sample 2. T= 5mins			X																	
Sample 3. T= 5mins			X																	
Sample 4. T= 10mins			X																	
Sample 5. T= 10mins			X																	
Sample 6. T= 15mins			X																	
Sample 7. T= 15mins			X																	
Sample 8. T= 30mins			X																	
Sample 9. T= 30mins			X																	
Sample 10. T= 2hrs			X																	
Sample 11. T= 2hrs			X																	
Sample 12. T= 4hrs			X																	
Sample 13. T= 4 hrs			X																	
Sample 14. T= 6hrs			X																	
Sample 15. T=6hrs			X																	
Do you require a separate report for each sample:										Samples to be composited:										

Print & Email to ALS



Office use only:

Date:

Time:

Condition:

Test No.	Test Description	Test No.	Test Description	Test No.	Test Description
1	Standard Plate Count - food and dairy	7	E.coli – PETRIFILM <10	13	Enterobacteriaceae Enumeration
2	Standard Plate Count - Raw Meat and Fish	8	Coliforms - PETRIFILM <10	14	Coagulase Positive Staph - Enumeration
3	Yeast & Mould Enumeration	9	Coliforms Enumeration	15	Coagulase Positive Staph - Presence / Absence
4	Listeria Detection ELISA	10	Salmonella Detection ELISA	16	Clostridium Perfringens Enumeration
5	E.coli - MPN <3	11	Salmonella Detection AS		

ALS Microbiological Results



Certificate of Analysis

Retail Ready Operations Australia Pty Ltd
PO Box 97
St Clair NSW, 2759



Accreditation No. 1247
Accredited for compliance with
ISO/IEC 17025 - Testing

Attention: **Sheetal Maharaj**

Overall Description: Pradigm Fresh Test on Primal Surface environmental Flexi Swabs, Date Start: 01/05/23

Our Ref No: FS2323774

Your Ref: 2/05/23

Project:

Report Date: 09 May 2023

Samples Received: 02 May 2023

Temperature when received: 2.8°C

Testing Commenced: 03 May 2023

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Sample Details: Test Description	Results	Units	Site
001 Sample 0. T= 0min (NATA Accredited)			
FM0010 Aerobic Plate Count	~90	CFU/swab	NSW
002 Sample 1. T= 0min (NATA Accredited)			
FM0010 Aerobic Plate Count	360	CFU/swab	NSW
003 Sample 2. T= 5mins (NATA Accredited)			
FM0010 Aerobic Plate Count	~30	CFU/swab	NSW
004 Sample 3. T= 5mins (NATA Accredited)			
FM0010 Aerobic Plate Count	~20	CFU/swab	NSW
005 Sample 4. T= 10mins (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	CFU/swab	NSW
006 Sample 5. T= 10mins (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	CFU/swab	NSW
007 Sample 6. T= 15mins (NATA Accredited)			
010 Sample 9. T= 30mins (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	CFU/swab	NSW
011 Sample 10. T= 2hrs (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	CFU/swab	NSW
012 Sample 11. T= 2hrs (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	CFU/swab	NSW
013 Sample 12. T= 4hrs (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	CFU/swab	NSW
014 Sample 13. T= 4 hrs (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	CFU/swab	NSW
015 Sample 14. T= 6hrs (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	CFU/swab	NSW

NATA: NSW - 1247 (site: 2040) VIC - 1247 (sites: 1240, 16438) QLD - 1247 (site: 25153)
TGA: NSW - MI- 2012 - LI - 05733 - 3 VIC - MI-2012 - LI - 06776 - 3
APVMA: NSW - 6179 VIC - 6181

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Our Ref No : FS2323774
Report Date : 09 May 2023

Your Ref: 2/05/23

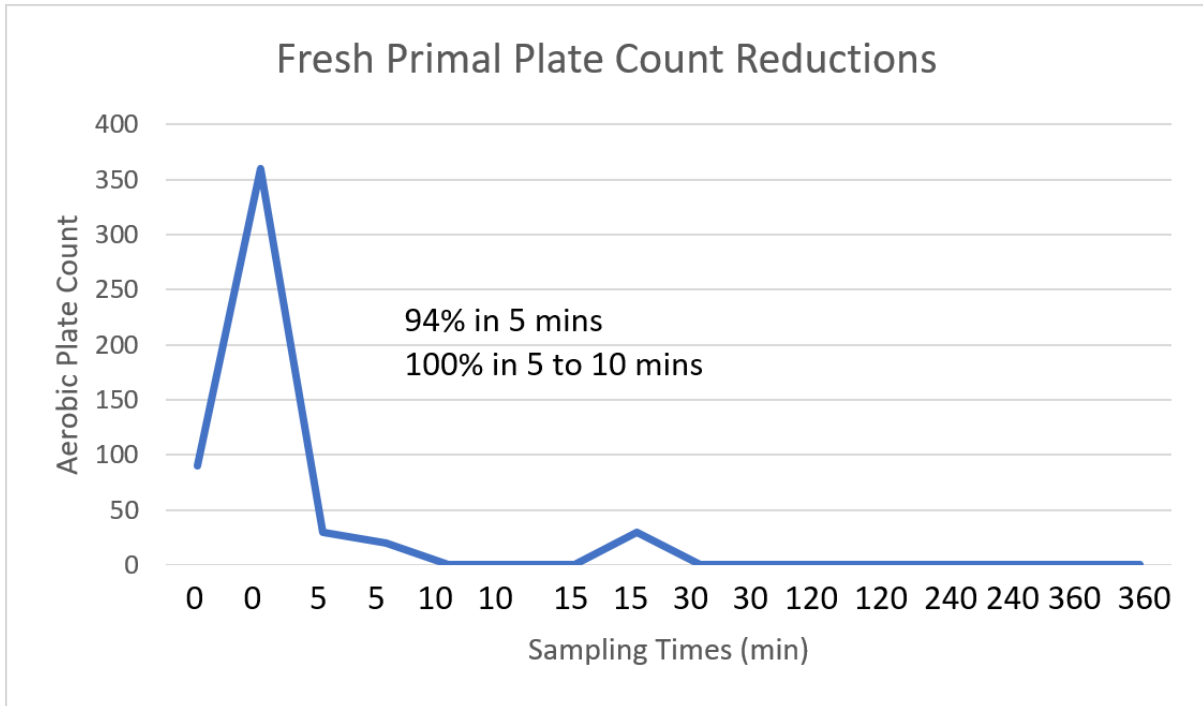
016 Sample 15. T=6hrs (NATA Accredited)			
FM0010 Aerobic Plate Count	<10	CFU/swab	NSW

*NATA accreditation does not cover the performance of this service. Measurement of Uncertainty values for your compliance are available at <https://www.alsglobal.com/muvalues>.

Signatories

This document has been electronically signed by the authorized signatories indicated below. Electronic signing has been carried out in compliance with procedures specified in 21 CFR Part 11.

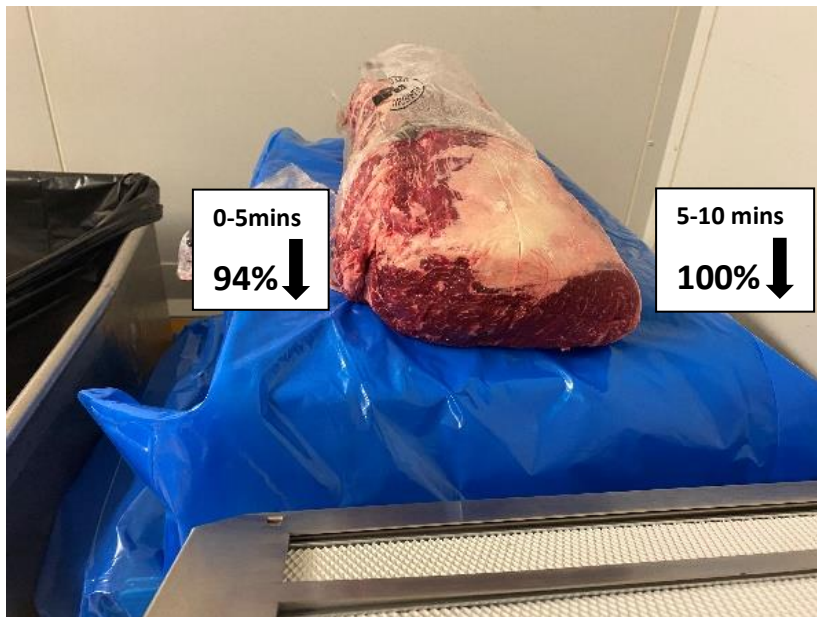
Signatories	Position	Accreditation Category
Sema Boduk	Microbiologist	Food Microbiology



Conclusion of Results

The fresh cube roll primal was tested at 10 days aged. The baseline aerobic plate count at 0 minutes on the left and right sides of the primal facing the Puradigm unit were measured at 360 and 90 counts.

After **5 minutes** the plate count had reduced **67-94% (1 Log reduction)** and after **10 minutes** the plate counts were undetectable ie **100% reduction** and remained so at 15, 30, 120, 240 and 360 minutes.



Practical Applications

This fresh primal trial demonstrates how the Puradigm technology can be applied in **several practical ways at both primary and secondary processing facilities**.

Primary processors currently use chemical washes to **clean carcasses**. However while the carcass is hanging in the cool rooms, being tested and then portioned, there are numerous points pathogens are introduced to the surface of the carcass and its portions as the products is being handled. Utilising the Puradigm technology can **reduce these pathogens as they are being introduced** and avoid them getting a foothold from which they multiply into significant numbers.

Secondary processing have very clean equipment and tools with which they process the incoming primals into the finished goods. However if the incoming primal surfaces are covered in bacteria after several days of aging, these bacteria will be introduced into each portion through the cutting and other processing actions and these bacteria will eventually multiply despite the packaging environments and temperatures that the finished goods are transported and stored within. The Puradigm technology can be utilised to **minimise the surface bacteria from debagging through portioning and into packaging**, which will **extend the shelf life** of the finished product.

Since the Puradigm output is **a natural intervention, containing only water and charged ions**, this intervention **doesn't damage the DNA** of the meat and thus doesn't change the taste of the product.

There is no excessive drying out of meat above that which is already occurring due to the air flow in the cool rooms while the carcasses are hanging. During portioning at either primary or secondary processing, it is only short duration exposures so there is no significant drying of the primals. Additionally the use of dry fog humidifier sprays will not only maximise the effect of the Puradigm output but will also ensure the meat is kept from drying out.

Primals –Microbiological Reduction Trial Summary

Puradigm's natural intervention was shown to generate surface plate count reductions of **2 log (99%) in 24hrs and 4 log (99.99%) in excess of 24hrs** for heavy micro-loads in **35 day aged primals** with starting (baseline) plate counts over 100 million.

Primal Age	2-24hr (Log Reduction)	2-24hr (% Reduction)	24-144hr (Log Reduction)	24-144hr (% Reduction)
35 days	2	99	4	99.99

For carcasses hanging in cool rooms for 24hrs or more, or primals and portions exposed for 24hrs or more, these results are very significant, given their starting bacterial loads will be more in the order of a **few hundred plate counts**, which Milestone 6's results show are able to be significantly **reduced in a matter of 5-10 minutes**.

7.3 Packaging Decontamination – Final report on effectiveness

Puradigm's air and surface purification was shown to be **unsuitable for pathogens submerged in water**. This is a known limitation. Thus packaging, which is possibly contaminated but wet, will not experience significant reductions in micro-load when exposed to Puradigm's technology during short term exposures.

However for dry packaging, this technology is suitable, as can be seen through the primal reduction trials. It should be noted that primals are a greater challenge when compared to dry packaging, given primals are moist and contain nutrients that promote bacterial growth.

Puradigm's units are being deployed to the Dry Packaging Decontamination room at Chef Fresh.

It should be noted that only exposed surfaces will be purified, and thus for packaging to be adequately decontaminated, each packaging bag should be placed on racks which allow air to circulate fully around the bag, and be left in the room for at least 10 minutes, with best results obtained for exposures 4hrs or more, depending on how contaminated the surfaces have become.

7.4 Puradigm and Denba – Next Steps

A separate MLA MDC has been submitted to run the investigation of the combination of Puradigm and Denba technologies as a separate project. It is expected that both technologies being ***natural technologies***, with ***Puradigm removing surface pathogens and Denba slowing internal pathogens, shelf-life of the product will be maximised*** without changing the texture or taste. Both technologies have extremely low power requirements, and in fact can significantly reduce the energy required for frozen applications, and are thus not only natural and safe, but also sustainable.

7.5 Next Steps

Trials in supermarkets and primary processing to investigate optimal implementation strategies for all the benefits this technology has shown to provide, such as shelf-life extension, odour reduction, continuous and safe purification of air and surfaces for the protection of people and products.

Further research aimed at incorporating the technology into ***MAP packaging, odour control in waste management, equipment cleaning, transportation of fresh produce, transportation sanitation for mould control, and combined applications with Denba*** to provide additional microbial reductions/control for enhanced shelf-life of products, reduced energy consumption for frozen products and enhance frozen products to fresh or sashimi-grade on defrosting, should be conducted.

This project has confirmed that Puradigm represents a paradigm shift in purification, promising a wide range of benefits to all industries, including the red meat industry.

8 Success in meeting the Milestone

8.1 Milestone 1

Milestone 1 has been successfully completed through the development of the design and detailed work testing schedule. The initial phase will evaluate the effectiveness of the air and surface purification technology through pilot testing against various microbial loads in the Chef Fresh packaging material decontamination rooms (ozone rooms), the RROA HVAC Air Handling Units and RROA Primal shelf-life trials.

This milestone 1 has involved the design of trials & testing methodologies, evaluation of safety aspects and development of operating protocols for the purification technology. Test units were procured from the supplier. A detailed project testing schedule was developed. The project steering group was formed. The progress report on design and detailed project schedule was submitted to the project steering group & approved by the MLA. The outcomes of the pilot studies will be used to inform and develop detailed work plans for the next commercial proving phases.

8.2 Milestone 2

Milestone 2 has been successfully completed through practical trials and initial testing in a production environment, including:

- Microbiological reductions on packaging material within old ozone rooms
- Analysis of environmental conditions and design to maximise Ion efficiencies in new and old ozone rooms
- Preparation of HVAC tests through the installation of HVAC devices within the air handling units
- Preparation of the Shelf-life tests through 24hr microbiological reduction tests for beef primal surfaces

8.3 Milestone 3

Milestone 3 has been successfully completed through practical trials and initial testing in a production environment, including:

- Shelf-life tests through 24hr, 72hr, 96hr, 144hr and 0-24hr at 2hr interval, microbiological reduction tests for beef primal surfaces at varying distances, under 4C temperatures and strong room air flows.

8.4 Milestone 4

Milestone 4 has been successfully completed through:

- Microbiological reduction speed tests for fresh, non-aged beef primal surfaces, under 4C temperatures and strong room air flows.
- HVAC trials in Air Handling Units, in extremely high speed air currents
- Puradigm and Denba combined shelf life extension trials

8.5 Milestone 5

Milestone 5 has been successfully completed through:

- Microbiological reduction final report on effectiveness in various conditions and timeframes.
- Results for HVAC trials in Air Handling Units, for extremely high speed air currents
- Puradigm and Denba combined shelf life extension trials
- Packaging decontamination final report on effectiveness

9 Conclusions & Recommendations

9.1 Conclusions

Milestone 1, 2, 3, 4 & 5 have been successfully completed through the development of the design and detailed work testing schedule and implementation in a production environment at RROA's HVAC AHUs and Quality testing rooms for shelf-life trials with red meat primals.

9.2 Recommendations

The project proposes to accept this final public report for Milestone 5 and publish on the MLA portal for wider industry benefit.

10 APPENDIX - Supporting Documents

10.1 Appendix 1 Coles Group - Vision, Purpose & Strategy



10.2 Appendix 2 – Certifications

Organic Certification – Washington State Department of Agriculture



PO Box 42560, Olympia, WA 98504-2560 Ph (360) 902-1805
In accordance with Chapter 15.86 RCW and Chapter 16-160 WAC

**Washington State
Department of Agriculture**

**MATERIAL REGISTRATION
CERTIFICATE**

is issued to:

Puradigm LLC
11440 W. Bernardo Court
San Diego, CA 92127
United States

The products listed below have been verified to comply with the USDA National Organic Standards (7 CFR Part 205):

#	Product Name	Sub-Type	Type	Annotation
3314	PURADIGM GROW	Hydrogen Peroxide	DPC	Preventative practices must be implemented prior to use.

Types: CPA - Crop Production Aid, DPC - Disease and Pest Control, FSA - Fertilizer and Soil Amendment, LPA - Livestock Production Aid, PH - Processing and Handling

WSDA Registered Company #: 945
Issue Date: 09/08/2020
Registration valid through 10/31/2021



Brenda Book
Organic Program Manager
DEPARTMENT OF AGRICULTURE
AGR 2291 (R/3/16)



REGISTERED MATERIAL
For Use In
Organic Agriculture
Washington State Dept. of Agriculture

Ozone Safety

TEST CERTIFICATE

ISSUED BY THE FOOD SCIENCE INSTITUTE, KANSAS STATE UNIVERSITY



OZONE SAFETY TEST FOR THE PURDIGM AIR HVAC 14 INCH AIR PURIFICATION SYSTEM

Manufacturer: Puradigm, LLC
720 S. 7th St.
3rd Floor
Las Vegas, NV 89101

Model or Type Identification: Puradigm Air HVAC 14 inch

Sample Delivery Date: 9th October 2012

Tests Conducted Between: 17th and 19th October 2012

The Food Science Institute at Kansas State University has tested the above Puradigm Air HVAC 14 inch duct mounted advanced oxidation air purification system, evaluating ozone levels in a room environment with the output set per manufacturer's instructions. Test results showed maximum equilibrium concentration of ozone below 0.05 ppm.

The National Ambient Air Quality Standard, established by the U.S. Environmental Protection Agency (EPA) is .08 ppm for outdoor ozone. National Institute of Occupational Safety and Health recommendations and Occupational Safety and Health Administration regulations both establish ozone levels of 0.10 ppm as the safety threshold for workers on the job. The most stringent standard, those of the U.S. Food and Drug Administration for indoor medical devices, specifies that ozone output be no more than 0.05 ppm.

Based upon tests performed and international safe standards for ozone exposure, the ozone levels produced by the tested purification system pose no risk to building occupants.

A handwritten signature in black ink, appearing to read 'James L. Marsden'.

James L. Marsden, Ph.D.
Distinguished Professor
Food Safety & Security

Dated 22nd October 2012

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Page 1 of 1

Certificate No: FSI 58D215

Food Science Institute
Kansas State University, 216 Call Hall
Manhattan, KS 66506
Phone: (785) 532-4057
Fax: (785) 532-5861

Hydrogen Peroxide Safety

TEST CERTIFICATE

ISSUED BY THE FOOD SCIENCE INSTITUTE, KANSAS STATE UNIVERSITY



HYDROGEN PEROXIDE TEST FOR THE PURDIGM AIR HVAC 14 INCH AIR PURIFICATION SYSTEM

Manufacturer: Puradigm, LLC
720 S. 7th St.
3rd Floor
Las Vegas, NV 89101

Model or Type Identification: Puradigm Air HVAC 14 inch

Sample Delivery Date: 9th October 2012

Tests Conducted Between: 17th and 19th October 2012

The Food Science Institute at Kansas State University has tested the above Puradigm Air HVAC 14 inch duct mounted advanced oxidation air purification system, evaluating gaseous Hydrogen Peroxide (H₂O₂) levels produced by the purifier in a room environment with the output set per manufacturer's instructions. Test results showed hydrogen peroxide levels remained below 0.05 ppm.

OSHA's permissible exposure limit (PEL) for gaseous hydrogen peroxide is 1.0 ppm (continuous) over 8-h work shifts.

The gaseous hydrogen peroxide levels produced by the purification system are over 20 times below the permissible exposure limit. Based upon tests performed and international safe standards, the gaseous hydrogen peroxide produced by the tested purification system pose no risk to building occupants.

A handwritten signature in black ink, appearing to read 'James L. Marsden'.

James L. Marsden, Ph.D.
Distinguished Professor
Food Safety & Security

Dated 22nd October 2012

This certificate may only be reproduced in its entirety and without change

Page 1 of 1

Certificate No: FSI 58D211

Food Science Institute
Kansas State University, 216 Call Hall
Manhattan, KS 66506
Phone: (785) 532-4057
Fax: (785) 532-5861

Summary of Test Results

Puradigm Test Results Summary



Surface Reductions		
Pathogen	Reduction	Testing Organization
Acinetobacter baumannii	98.22%	Ankara Oncology Hospital
Aspergillus brasiliensis	84.15%	UC Colorado Hospital
Bacillus atrophaeus	99.81%	Kansas State University Food Science Institute
Bacteria	99.45%	Institut Jantung Negara PICU Malaysia
Bacteria	99.98%	Quadrants Scientific Inc - Golds Gym
Bacteria	99.63%	Quadrants Scientific Inc - Nail Salon
C. Diff	99.53%	NSF international Lab
Candida albicans	99.99%	Kansas State University Food Science Institute
CRE	99.98%	Kansas State University Food Science Institute
Coronavirus 229E	99.00%	Central Michigan University College of Medicine
COVID 19 (SARS-CoV-2)	97.70%	University of Florida College of Medicine
COVID 19 (DELTA Variant)	99.78%	Australia Government Lab
Dengue virus type 2	99.00%	Central Michigan University College of Medicine
E. coli	99.59%	Kansas State University Food Science Institute
E. coli	99.99%	Ministry of Healthcare of Ukraine
E. coli O157:H7	99.41%	Kansas State University Food Science Institute
Enterococcus faecalis	99.99%	Ministry of Healthcare of Ukraine
Fungus	99.99%	Ministry of Healthcare of Ukraine
H1N1	99.60%	Guangdong Detection Center of Microbiology
Legionella	99.99%	Kansas State University Food Science Institute
Listeria monocytogenes	99.87%	Kansas State University Food Science Institute
Mold	89.00%	Ultimate Labs
MRSA	99.24%	Kansas State University Food Science Institute
Pseudomonas aeruginosa	74.88%	UC Colorado Hospital
Pseudomonas aeruginosa	99.54%	Kansas State University Food Science Institute
Stachybotrys chartarum	99.99%	Kansas State University Food Science Institute
Staphylococcus aureus	99.99%	Ankara Cancer Education and Research Clinic Turkey
Staphylococcus aureus	99.15%	Kansas State University Food Science Institute
Staphylococcus aureus	99.90%	Pontiac General Hospital
Staphylococcus aureus	74.88%	UC Colorado Hospital
Staphylococcus epidermidis	99.99%	Ministry of Healthcare of Ukraine
Streptococcus pneumoniae	98.83%	Kansas State University Food Science Institute
Total Aerobic Count	66.49%	Meat Production Plant
Total Coliforms	93.88%	Meat Production Plant
VRE	98.22%	Ankara Oncology Hospital

Puradigm Test Results Summary



Air Reductions		
VOC	Reduction	Testing Organization
Acetaldehyde	96.00%	Hye-sung Environment Inc. Korea
Acetone	97.81%	Ministry of Healthcare of Ukraine
Acetone	89.75%	Atmospheric Analysis-Consulting
Ammonia	82.90%	Guangdong Detection Center of Microbiology
Ammonia	93.00%	Hye-sung Environment Inc. Korea
Ammonia	97.57%	Ministry of Healthcare of Ukraine
Bacteria	95.50%	Ultimate Labs
Benzene	80.00%	Guangdong Detection Center of Microbiology
Butanone	97.00%	Hye-sung Environment Inc. Korea
Butyraldehyde	87.00%	Hye-sung Environment Inc. Korea
Carbon Disulfide	99.99%	Atmospheric Analysis-Consulting
COVID 19 (BETA Variant)	98.70%	University of Florida College of Medicine
Dimethyl Disulfide	71.00%	Hye-sung Environment Inc. Korea
Dimethyl Sulfide	87.00%	Hye-sung Environment Inc. Korea
Formaldehyde	81.10%	Guangdong Detection Center of Microbiology
Hydrogen Sulfide	97.00%	Hye-sung Environment Inc. Korea
i-Valeric Acid	94.00%	Hye-sung Environment Inc. Korea
Methyl isobutyl ketone	99.99%	Hye-sung Environment Inc. Korea
Mold	99.99%	Ultimate Labs
n-Butyric Acid	98.00%	Hye-sung Environment Inc. Korea
n-Valeric Acid	69.00%	Hye-sung Environment Inc. Korea
Propanol (IPA)	60.69%	Atmospheric Analysis-Consulting
Propene	99.99%	Atmospheric Analysis-Consulting
Propionaldehyde	92.00%	Hye-sung Environment Inc. Korea
Propionic Acid	75.00%	Hye-sung Environment Inc. Korea
Styrene	86.00%	Hye-sung Environment Inc. Korea
Toluene	99.00%	Hye-sung Environment Inc. Korea
Toluene	99.99%	Atmospheric Analysis-Consulting
Total VOC	83.20%	Guangdong Detection Center of Microbiology
Trimethylamine	93.00%	Hye-sung Environment Inc. Korea
Xylene	95.00%	Hye-sung Environment Inc. Korea