Use of Ultra-Violet (UV) Light to Increase the Shelf Life of Raw Diced Beef

MRES – Master of Science by Research

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ABSTRACT

Ultra-violet light has gained recent attention for its potential use as a non-thermal, non-chemical decontamination tool to reduce microbial loads across various food products and food contact surfaces. Most commonly, the current method to reduce and control bacteria is by controlling environmental conditions such as temperature, pH, available water, preservatives and by constantly implementing successful hygiene protocols. This study aimed to investigate the effect of UV light irradiation on food contact surfaces and the subsequent effect on the shelf-life of the raw diced beef being processed within a red meat processing facility. It was anticipated that the shelf life of the final product will be increased to greater than pack + 10 days. The study consisted of determining the Total Viable Count (TVC) of four food contact surfaces involved in the processing of diced beef at retail level, and assessing the current shelf life of the product by analysing the visual properties of the finished pack and testing various indicator organisms including total viable counts*, enterobacteriaceae, pseudomonas, e.coli and salmonella* prior to the intervention. Food contact surface analysis included taking swabs at the start of production at 6am, the middle of production at 12pm and at the end of production at 3pm. One swab was collected at each time slot and location twice per week, totalling 72 swabs during the pre-intervention. Finished pack analysis consisted of collecting 3 finished packs, 3 times per week for an external accredited laboratory to complete shelf life testing. On these testing days, the same amount of packs were collected and analysed for the visual properties and graded against a chart

by a team of trained panellists. After the installation of the UV light strobe, one of the four food contact surfaces (Conveyor 2) was treated continuously with UV light, and similar microbial tests were repeated post intervention and results compared to pre-trial. Overall the ultra-violet light did have a statistical significant effect ($p<0.05$) on the reduction of bacteria present on conveyor 2 with mean log reductions of 2.53 log cfu/cm^2 at 12pm and 1.78 log cfu/cm^2 at 3pm. However, no other surface tested had a significant difference between pre-intervention and post intervention. Finished pack analysis revealed that the decontamination of conveyor 2 had no impact on the microbiological counts post-intervention. There was no impact on the visual properties post intervention therefor the shelf life of final pack was not increased to greater than pack + 10 days.

Acknowledgments:

Firstly, I would like to thank my academic supervisor's Dr Jan Mei Soon, Dr Sarah Dennison and Dr Stephanie Dillon for both their patience and persistence throughout this previous academic year.

The knowledge and skills of which I have gathered from various parts of this project will help me for most if not all of my academic and practical career within the food industry for many years to come. I thank Dr Jan Soon for persevering with me over the full project, through encountering many expected practical issues during the planning and preparation of this study.

Further I thank Dr Sarah Dennison for providing not only technical assistance whenever needed but also being professionally approachable and vital time to ensure the practical side of the study ran smoothly.

Finally, I would like to personally thank the processing facility of which the study was carried out at including the technical team my direct manager Mark Waters for offering their support and guidance from start to finish of this project.

Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBRIEVIATIONS

'PDA' – Pret De Coupe

'TVC' – Total Viable Count

'ENT' – Enterobacteriaceae

'SAL' – Salmonella

'PSEU' – Pseudomonas

'HACCP' – Hazard Analysis Critical Control Plan

'FSA' – Food Safety Authority

'UV'- Ultra-violet

'MIG' – Meat Industry Guide

'EFSA' – European Food Safety Authority
'RTE' – Ready to Ea

CHAPTER 1 INTRODUCTION

In today's society the demands from the modern-day consumer are becoming more challenging, demanding healthy, organic, natural and fresh foods that are manufactured or produced in what's deemed to be a "green" setting (Guerrero-Beltr·n et al, 2015). Additional focus from the government and commercial customers to produce with little to no carbon footprints are all becoming more substantial (Bae et al, 2011). The negative reactions made by both the press and the public regarding the use of chemicals during food processing to increase shelf life are also on the rise. In order to address these new encounters that the food industry are now facing, alternative methods of food manufacturing and food processing to develop new, safe and more efficient ways to improve product shelf life and food safety need to be investigated.

Although substantial improvements have been made towards a greater understanding of bacterial pathogenicity and transmission in foods, the occurrence of food-borne reported illnesses associated with *raw beef* pathogens remains a significant issue within both the United Kingdom (UK) and globally GOV (2019). The contamination of both uncooked and unprocessed foods especially red meat, with potentially pathogenic bacteria is a cause for major concern and any method aimed to either eliminate or reduce such food contamination will have a major effect on the incidence of food related illnesses across the world (GOV, 2019). Within the red meat industry, the majority of this category could be classed as both uncooked and unprocessed. This term unprocessed defines a product that has not been altered and remains in its natural steak ie a beef steak,

vs a packet of crisps which has been largely altered (Montowska and Fornal 2017). The processing facility which was investigated in this study only produces uncooked (Raw) food. Further the economic burden to food manufacturers associated with products that have a short shelf life is ever increasing, due to the volume of product that may have to be disposed off if the use by date has expired (Thomas and Murray, 2014).

The use of Ultraviolet (UV) light has recently gained growing levels of interest to the food industry due to its use as a non-thermal and non-chemical method to increase the preservation and shelf life of fresh food product (Taoukis and Stoforos 2016). Relatively new developments in science and engineering of UV irradiation have previously demonstrated that treatments of UV-light has been successful in elimination or reduction of micro-organisms for liquid foods, post-processing measure for ready to eat (RTE) foods, and decontamination of food contact surfaces in cheese plants (Can et al, 2014).

Meat spoilage generally causes the food to alter its anticipated sensory properties to cause an undesirable appearance and poor odour defined as putrefaction (Mutwakil, 2011). The spoilage of red meat can differ over variable lengths of time depending on the initial contamination level of bacteria (Coombs et al 2017). The rate of spoilage can fluctuate between cuts of meat and is heavily dependent on the amount of time the product was handled and processed, meaning a product which is simply sliced, will have significantly lower levels of bacteria in comparison to a product that is sliced, diced and then marinated (Kamruzzaman, Makino and Oshita, 2015). The most common forms of bacterial

spoilage can include *pseudomonas, enterobacteriaceae, lactobacillus* and Total viable count (TVC) (Toldra, 2017).

Across the food industry, one of the most commonly used measures of food quality from a microbiological perspective is the level of *total viable count* in meat products (Hempel et al 2011). This measure analyses the total number of colony forming bacteria per gram, and can therefore be used as indication of food spoilage with a higher number of bacteria reflecting a greater level of food spoilage. Further, *total viable count* may also be used to establish how clean a food contact or non-food contact surface is within a food processing facility. Such test is important, to help validate specific hygiene and food safety protocols such as production clean downs at the end of a production day, to ensure that no bacteria remain on the surfaces to potentially cross contaminate the next days of production and therefor increase the rate of food spoilage. In relation to food contact surfaces, a lower number of *total viable count* on surface would reflect a cleaner area (Biranjia-Hurdoyal and Latouche 2016).

The shelf life of a product can be influenced by various conditions including but not limited to available water, pH, temperature and packaging. By controlling such conditions, an increase in the shelf life and the growth of spoilage bacteria may be inhibited (Wickreamasinghe et al 2019). Optimum conditions for beef comprise of chilled conditions 0-3°C and packaged in a skin pack tray which has been vacuum packed or modified atmosphere to prevent the growth of spoilage aerobic bacteria by limiting the percentage of oxygen. Across the food industry ways of increasing the shelf life of finished products have been extensively

investigated. For added value ambient foods such as spices and glazes, preservatives such as sulphites are added to inhibit the growth of bacteria, these glazes and spices are then used in added value meat products including burgers which further aids in the preservation of the product.

Although preservatives such as sulphites are highly effective at reducing the rate of food spoilage, there are many regulations that restrict the use of this preservative within the meat industry. The Food Standards Agency enforce legislation on additives within meat preparation (EC601/2014), which highlights that no additives or sulphites can be used in raw primal cuts of meat for example in a pack of diced beef. In addition, it could be suggested that consumer perception is everchanging, challenging the use of artificial preservatives such as sulphites being used in food products to extend shelf life (Lammarino et al 2012). This drive from consumers for products to only contain natural ingredients could be influenced by the health impact of artificial preservatives and additives. In addition to sulphites being a recognized declarable allergen that could cause anaphylaxis shock, a previous study conducted by Maddan et al (2009) reviewed the potential clinical effect of consuming this preservative. The study states that in sensitive individuals' exposure to sulphites has been reported to cause a range of effects from mild to severe including hypotension, dermatitis, urticaria, abdominal pain and diarrhoea.

Radha Krishnan et al (2014) investigated the effect of seasoning and glazing raw poultry on the quality and microbiological status of finished product. The antibacterial activity post glazing was suggested to have the most impact

against *L.Lactis*, further *Salmonella* Aromaticum. Further, additional preservation methods previously used can include the use of essential oils due to its antimicrobial and antioxidant activity to improve quality of processed meats. Barbosa et al (2014), suggested that *Ocimum basilicum* linn essential oil inhibited the growth of coliform bacteria of processed chicken sausage.

Most recently, the broiler industry has introduced the use of Ultra-violet light to treat finished whole chickens to reduce the prevalence of *Campylobacter*, however there has been limited published studies stating the use within the red meat industry (Keklik, Dermirci and Bock, 2011). Previously, UV-C light has been effective in controlling or reducing microbial counts within liquid mediums such as water and fruit juices (Water-research-net,2019). A survey of the relevant literature revealed that there is little data has been published on the decontamination of food contact surfaces within the red meat industry, specifically with the aim of increasing product shelf life as a result of a decontaminated surface.

In this study, the processing facility have identified TVC levels on the production conveyor belt of up to 6.47 log cfu/cm^2 by the end of a 12h full production day which impacts the finished product shelf life. Observations carried out before the implementation of the UV light at the testing facility, stated that average total viable counts on the conveyor belt at the beginning of production were between non-detectable levels and 3 Log cfu/cm^2. These levels were seen to significantly increase to between 6 – 6.47 Log cfu/cm^2 after a 12h period. However, few studies have yet determined if the overall level of total viable

counts increase throughout the production day on a continuous running diced beef production line conveyor belt, and if these levels may be reduced through the use of short wave UV-light which could have a positive impact on shelf life.

1.1 Research Aim and Objectives

The work here investigates a novel method of increasing the shelf life of raw diced beef via the use of UV light irradiation on food contact surfaces and the subsequent effect on the raw diced beef being processed on this line. The following study will introduce UV-light on the returning surface of an active conveyor belt which process, and transports diced beef, with the aim of reducing overall TVC levels on the food contact surface.

Objectives:

I. Install and introduce the use of short-wave ultraviolet light as an intervention to reduce total viable counts (TVC) on food contact surface.

II. Analyse the TVC of the food contact surface pre and post intervention.

III. Analyse and compare the indicator organisms of the diced beef pre and post intervention.

This study hypothesises that:

H1: Introduction of ultraviolet light will reduce overall TVC on the food contact surface over a 12h period

H2: A reduction of TVC on food contact surface can increase the shelf life of raw diced beef to greater than pack + 10 days.

CHAPTER 2 LITERATURE REVIEW

2.1 Factors affecting shelf life

Meat as a whole can be spoiled relatively quickly if stored at an incorrect temperature range. Specific bacteria species can multiply to harmful levels on fresh meat due to its chemical composition including, moisture, water activity and pH value. Growth of spoilage microorganisms cause sensory deviations including loss of colour, odour and poor taste that ultimately lead to spoilage of the meat (De filippis et al, 2013). The primal population of microbes on the meat is heavily dependent upon the animal's physiological state during slaughter and on the conditions of the environment in the slaughterhouse and packaging hall, which could cause cross contamination. Subsequent handling and slicing, later in the process coupled with exposure to food contact surfaces with poor hygiene practices enable the bacteria to grow further if not adequately controlled (Doulgeraki et al, 2012).

The growth of such bacteria that cause meat spoilage has been allocated into four separate influences (Bruckner et al, 2012):

A. **Intrinsic Factors:** an expression of what physical and chemical state the meat is in throughout processing including but not limited to pH value, water activity, structure and nutrients.

The physical composition of the meat can heavily influence the rate of spoilage. Taking into consideration pH, the typical pH of red meat is desired to be 5.6 post slaughter. Although this is slightly acidic, typically bacteria grow best around neutral pH values from 6.5-7.0 but they can also thrive in highly acidic environments therefor meat provides the correct pH for bacteria to grow. Although pH is a contributing factor to microbial growth, the most important intrinsic factor could be argued to be water activity. Sometimes previously described as water content, within red meat specifically the transition of waters from a high concentration to a low concentration outside of the cell can cause a microbe to become dormant therefor inhibiting growth. This rate of water activity can influence different bacteria in various ways, therefor influencing the growth rate (Hopkins et al 2014).

B. **Extrinsic Factors:** determined as the conditions in which the meat is processed from farm to fork including but not limited to exposure to poor hygiene practices, food contact surfaces, storage conditions, temperature, and atmosphere.

For this study, all extrinsic factors paly a role in the shelf life of the product. Typically, the ideal temperature to store red meat at is <3°c, storing red meat at this temperature prevents and inhibits the growth of mesophilic bacteria which only like to grow at moderate to warm temperatures such as 20-45°C. This cold

temperature, only allows psychrophiles to grow, although a large group of bacteria the optimum temperature is 10-20°C, however a minority of this group will grow below 20°c (Zhou et al 2017). Further the atmosphere for red meat includes what type of packaging is used throughout the process. For red meat there are mainly 3 types of packaging. Type one includes vacuum packaging for large primal and joints, which works in the same manner as type 2 skin packaging for small single or double steaks. This type of packaging completely removes the presence of oxygen which prevents the growth of all bacteria that requires oxygen to grow. Over long shelf life periods, the bacteria present on skin and vacuum packaged red meat products will consist of Lactic acid bacteria. The raw diced beef tested in this study is packaged within type 3 modified atmosphere packaging. This type of packaging involves the use of gases to create an atmosphere which inhibits spoilage but also maintains the desired colour of the meat. Industry guidelines recommend a gas mixture of 25% CO_2, which dissolves into carbonic acid within the meat and prevents bacteria from growing due to the harsh acidic environment within the meat. The second type of gas used is Oxygen, which is filled at 75%. The myoglobin within red meat absorbs the oxygen and remains the desired red colour (Lopacka et al 2016).

C. **Processing Factors:** detailing the methods at which the meat is treated i.e. cooking, cooling and chemical methods.

It is well known that cooking and cooling is the main method to ensure meat is safe to consume. Most commonly meat is deemed safe to consume when the internal temperature is reaches 72°C. This is recognised as the temperature at which all harmful bacteria will not be able to survive and therefore is safe to eat.

As previously mentioned and referenced within this study UV light has been reported to be used on the surface of red meat and food contact surfaces to reduce microbial counts the deactivation of DNA (Zhou et al 2017)).

D. **Implicit factors:** the relationship and reaction between coherent bacteria present on the same food or environment.

Linked in with environmental factors, this relates to the presence or absence of dominant bacteria on the meat itself. Depending on the conditions of storage, the growth of spoilage bacteria will influence what type of spoilage occurs (Mir et al 2017). Microorganism growth to higher levels is a prerequisite for meat spoilage, which could be considered an ecological phenomenon, incorporating multiple changes of readily accessible substrata during the proliferation of bacteria (Olusegun and Iniobong, 2011). Specifically, meat spoilage is the process describing the deterioration of red meat leading to the reduction of quality, where the meat is neither desirable nor consumable. The prevalence of microorganisms on the surface of the cut and meat foodstuffs determine the rate of meat spoilage depending on their interaction under optimal conditions (Casaburi et al, 2015). While there are multiple types of meat, the most common microbial populations that cause spoilage in beef are Enterobacteriaceae and *pseudomonas*. Psychrotrophic species including but not limited to *pseudomonas lundensis, P. fragi* and *P. fluorescens* most commonly occurs on fresh meat (Casaburi et al, 2015).

A previous study examined the visual and microbiological effects of *pseudomonas* on red meat over several days post packaging (Nychas et al, 2013).

The study concluded that population of *pseudomonas* reached levels of 10 log cfu/g after 7 days. The physical appearance of the meat with this attained level of *pseudomonas* caused a visual slime to develop on the meat with a poor odour. A further study, conducted by Pennacchia et al (2011), investigated 9 samples of raw sirloin beef, each sample size was 500g. The samples were stored in aerobic conditions at 4°C. The results after 15 days determined that bacteria including *pseudomonas*, *brochothorix thermosphacata, carnobacterium divergens* and *photobacterium* were present. The populace of *pseudomonas* after 15 days had reached 13 log cfu/g resulting in the sample not being edible. This study aims to focus on extrinsic factors, with the aim to reduce the build-up of microorganisms present on a production line that runs continuously over a 12h period.

2.2 Microbiological Criteria – Regulations and Guidelines

Within the United Kingdom, current legislation aims to ensure that the food is produced in a way that guarantees the product is safe for human consumption (Food.gov.uk, 2019). This may be accomplished by identifying and controlling food-borne hazards effectively with the correct implementation of a successful Hazard analysis critical control plan (HACCP). The meat industry guide 2018 (MIG) (Food.gov.uk, 2018) outlines and combines current legislation to establish an accepted version of the safety criteria in regards to the processing of meat, specifically the tolerability to the presence or absence of pathogenic bacteria that forms part of every HACCP plan.

Due to bacteria not being visible to the naked eye during processing or a post-mortem inspection, testing against a microbiological criterion delivers a method

of determining how successful operators have controlled the product during slaughter, dressing and packaging to reduce cross contamination. In addition, the results of such testing may be utilised to validate the HACCP procedures on sites are successful in the controlling of food safety and quality structures and to ensure they are being applied and implemented correctly. From a legal stand point, current European Legislation 2073/2005 (Legislation.gov.uk, 2019) 2.1 defines the microbiological criteria for whole carcasses testing post dressing, minced meat and meat preparations which must be complied with. Food manufacturers are required to implement general hygiene measures which are specified in Article 4 of EC Regulation 852/2004. There are two separate criteria's stated in the regulation 2073/2005, respectively the food safety criteria which should be used to examine the safety of the food (Table 1) and the process hygiene criteria which should be used to determine the efficiency of the production (Table 2). The most significant difference is the additional action required when such food safety criteria has not been achieved. Due to a failed result, an investigation must concur to identify the root cause and provide full corrective actions to rectify the issue within the production chain. The microbiological criteria for finished pack standards, such as steaks and added value products are agreed between customer and supplier and can include limits for TVC, *Enterobacteriaceae* and specific pathogens.

2.2.1 Food Safety Criteria

The food safety criteria outlined in MIG (2018) and detailed in the sub-sections 1.4 to 9 of Annex 1 2073/2005, states that depending on the product and physical

state of the food (Meat) there should be an absence of *Salmonella*, specifically *salmonella* typhimurium and *salmonella enteritidis* in the below samples. For example, Table 1 indicates that salmonella should be absent in the samples taken from the meat preparation and minced meat (Table 1).

Table 1. Mandatory Meat Industry Food Safety Criteria (MIG,2018)

Meat Product	**Number of Samples**	**Weight of Sample (g)**
Meat prep and minced meat from any species intended to be consumed raw	5	25g
Meat prep and minced meat from poultry intended to be consumed raw	5	25g
Meat prep and minced meat from red meat intended to be eaten raw	5	25g
Mechanically separated meat	5	10g
Meat products intended to be eaten raw	5	25g
Meat from poultry intended to be eaten cooked	5	25g
Fresh poultry	n/a	25g

If such criteria is not achieved, the food business operator is solely responsible and will be required to remove the food from the market place as referred to in (EC) regulation 178/2002. Within the United Kingdom – the Food Standards Agency (FSA) are required to collect significant due diligence and investigate any outbreaks that occurs to ensure that the adequate corrective actions and preventative actions have been implemented at the facility.

2.2.2 Process Hygiene Criteria

This criterion has been designed to ensure maximum efficiency in relation to groups of indicator organisms containing pathogens and overall quality of the meat. If such criteria is not achieved, the product does not have to be removed from sales but instead is suggested to demand a full investigation to enquire about any insufficient practices during manufacturing leading to failed microbiological results. **Table 2** shows the guidelines for acceptable or unacceptable levels of microorganisms from cattle and sheep carcasses. (Food.gov.uk ,2018).

Table 2 Process Hygiene Criteria for Cattle and Sheep Carcasses* (MIG,2018)

Mean Log Tolerance	**Total Viable Count** (TVC)	**Enterobacteriaceae** (ENT)	***Salmonella*** (Sal)
Unacceptable >Mean Log	5.0	2.5	2/50
Acceptable <Mean Log	5.0	2.5	
Satisfactory ≤Mean Log	3.5	1.5	2/50

Note: 5 carcasses are to be sampled per sampling session, 1 sample per carcass

2.3 Spoilage Microorganisms in Beef

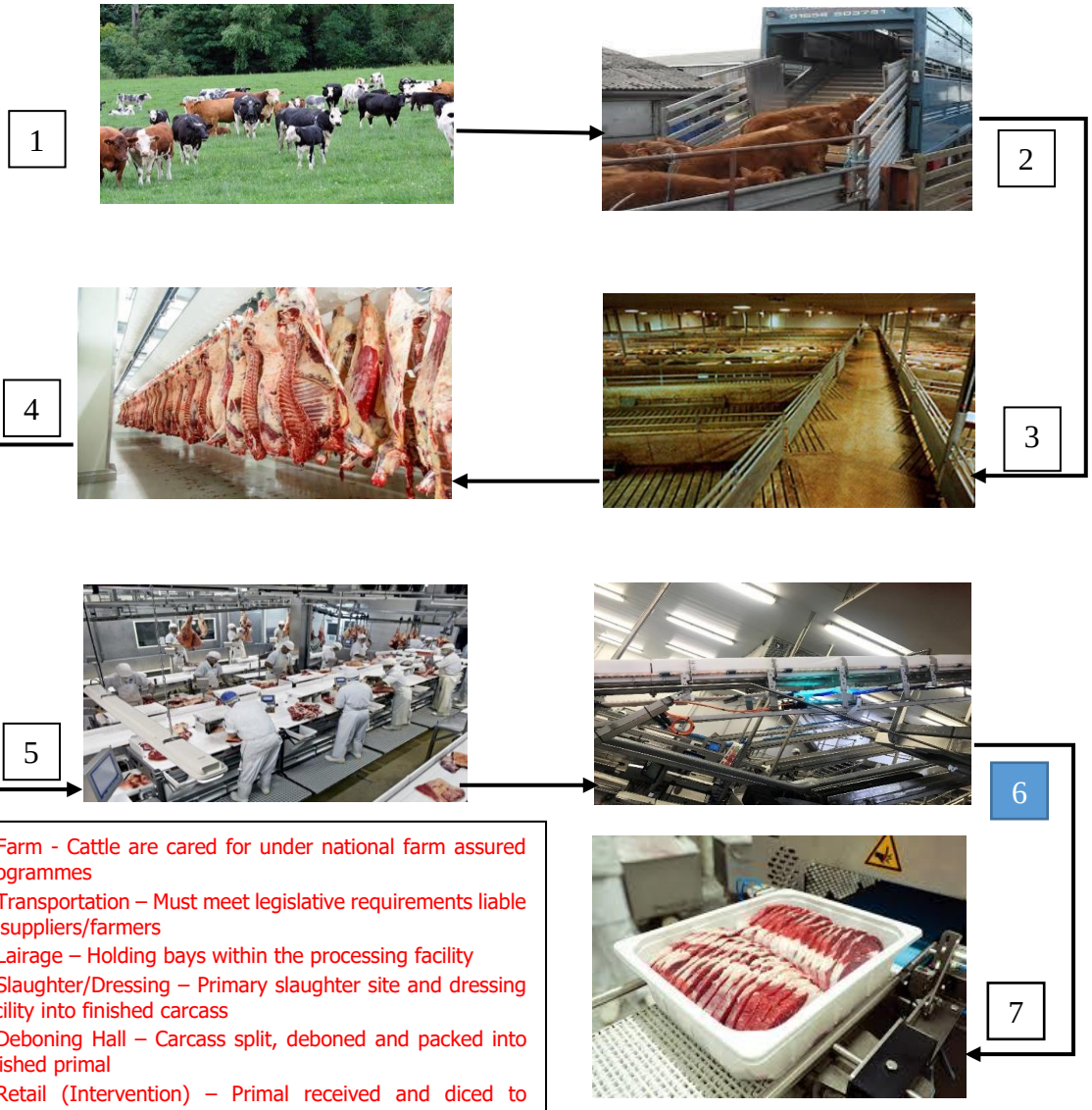
Each genus of bacteria has optimum conditions in which it may thrive and multiply within for beef and red meat specifically. Typically, bacteria that are able to withstand chilled temperatures between 0-5°C most commonly become the dominant microorganism in a chilled processing environment and within beef processing due to the common handling environments utilised within beef

processing. Fresh meat that is processed and stored within chilled temperatures will most frequently develop a flora containing the primary species including but not limited to *pseudomonas* with significant fractions of additional aerobic species including but not limited to *enterobacteriaceae* and *brochothorix thermosphacta*. Such aerobic spoilage bacteria grow by utilising low molecular weight and soluble complex nutrients on the surface of meat (Gram et al, 2002).

The most rapid growing species will fail to be the most dominant microorganism present if greater initial contamination with a slower growing contender occurs, due to an inadequate level of time for the faster growing microorganism to become dominant due to microbial competition (Jay, 2003). Unusually, not all of the initial microorganism contamination load will ultimately contribute to final spoilage. Simply only a negligible group of populations of initial microbiota will multiply to cause spoilage, due to storage conditions and competition, such organisms are known as ephemeral bacteria. Therefore, the conception of succession contamination and the development of spoilage organisms in addition to the importance of initial contamination plays a vital role in the final microbial make up of a finished product (Gram et al, 2002).

Nevertheless, initial contamination of the meat is a vital point influencing the rate of spoilage dynamics associated with diced beef. Throughout the production process, numerous sources of contamination can be acknowledged. Potential contamination with endogenous microbiota takes place during slaughter and dressing of contaminating carcasses (Petruzzelli et al, 2016). The significance of such contamination depends heavily on the hygiene protocols from the

farmhouse, the overall conditions during transport from farm to abattoir and the decontamination and hygiene practises of the slaughterhouse and processing facility (Buncic et al, 2014). Environmental contamination may also occur from subsequent slicing and handling arising from surfaces and tools utilised throughout the operation. Figure 1 displays the process flow and possible routes of contamination for diced beef from farm to finished pack analysed within this study.



1)Farm - Cattle are cared for under national farm assured programmes
2)Transportation – Must meet legislative requirements liable to suppliers/farmers
3)Lairage – Holding bays within the processing facility
4)Slaughter/Dressing – Primary slaughter site and dressing facility into finished carcass
5)Deboning Hall – Carcass split, deboned and packed into finished primal
6)Retail (Intervention) – Primal received and diced to specification and deposited into pack
7)Retail Packing – Diced beef pack labelled and stored as finished pack

Figure 1 Process Flow and processing zone for raw diced beef at the testing facility. (*UV intervention is carried out at Retail level)

Some of the most common spoilage microorganisms in red meat are *pseudomonas* spp. and *enterobacteriaceae*. *pseudomonas* are aerobic bacteria, meaning that they grow in the presence of oxygen. *Pseudomonas* growth rate within red meat may also be inhibited by the concentration of carbon dioxide, resulting in the choice of packaging being a vital component in the preservation of the final product. Within this study, the raw diced beef were packaged in a modified atmosphere packaging (MAP) consisting of 75% oxygen and 25% carbon dioxide. A previous study conducted by Hilgarth et al (2019), concluded that raw beef steaks stored under aerobic conditions in refrigeration environments <5°C, *pseudomonas* grew significantly from <1 log cfu/g to >5 log cfu/g after four days in storage. Furthermore, after an additional two days of storage, *pseudomonas* reached microbial levels of >7.47 cfu/g. At the end of the study, all of the meat samples analysed showed considerable signs of physical spoilage including sensory defects. The microbial load for *pseudomonas* at this stage after fourteen days of storage were as high as >9 log cfu/g.

Cohering with this study, previous research (Rouger, Tresse and Zagourec, 2017) determined that the higher the number of *Pseudomonas* present in the raw material prior to packaging, the shorter the time it takes for spoilage to occur. Further to this Hilgarth et al (2019) disclosed that it took a period of thirteen days to develop sensory defects including the development of slime and rancid odour when the initial bacterial load was <1 log cfu/g. However, this was

significantly less when the initial bacterial load was 3.3 cfu/g taking respectively only eleven days (Hilgarth et al 2019). When microbial loads of *pseudomonas* reach 10,000,000 cfu/g, sensory degradations start to appear and include but not limited to poor odour, development of slime and visible changes in appearance from a bright red to a dark green colour. The mechanism by which *pseudomonas* cause spoilage is via the transgression of glucose. During the process, when glucose levels become insufficient, the bacteria substitutes to the expenditure of amino acids, which results in the formation of sulphides, esters and amines that ultimately lead to the creation of organoleptic deficiencies such as putrid odours and rotten taste (Ercolini et al 2006).

In conclusion, the concept of meat spoilage is a very complex process. The range of bacteria found in beef can vary from pack to pack due to the various stages involved throughout the production process. Overall total viable count encompasses all living bacteria and provides the greatest understanding of how contaminated and the microbiological status of a product.

2.4 Total Viable Count and Relationship with Spoilage

The term total viable count (TVC) in the food industry is defined as a test used to determine and estimate the total amounts of viable (living), singular microorganisms that are present within a fixed volume sample. TVC is not microorganism specific, but instead may include a range of bacteria, mould species and yeasts that may arise from the sample when cultured in the correct environment and conditions (Pennacchia et al 2011). The main function of utilising this test is most commonly to gauge the microbial quality and

organoleptic acceptability, which may also indicate the factories commitment and compliance to good manufacturing practices (GMP) (Lagerstedt et al, 2011).

Total viable counts may not be used as an indicator of food safety, as they cannot specifically show a relationship or correlate to the amount of toxins or pathogens present. A small TVC result does not quantify if the sample is pathogen free, as the test does not identify individual microorganisms (Nel et al, 2004). However, if a food or sample display disproportionately or unusually high microbial counts it could be assumed that there is a high chance that the bacteria may contain pathogens, dependent upon pathogen testing. Analysis of the TVC result must consider the initial food sample, as each food will be expected to harbour significantly different TVC results (Nørrung and Buncic et al, 2008).

Previous research has utilised this test to assess the quality of many different foods. Significantly larger microbial counts could highlight that there may be a possible issue with sanitation during processing and production or indicate issues with poor hygiene practices (Kaur et al, 2017). Quality guidelines that define specifications can frequently be applied to raw materials and finished packs to ensure that the food has maintained the required standard for optimum quality. Specifically, for meat, TVC can be used to evaluate the quality and condition of incoming carcasses to analyse suppliers who have high counts indicating potential poor practices (Mansur et al, 2019). TVC may also be used to establish the quality of the finished product or to ensure the production process does not hinder quality. This test may also be utilised as a validation tool, to

provide clear evidence that individual procedures are complete correctly for example desensitisation or clean downs.

Previous research conducted by Stopforth et al (2006), examined the microbiological status of fresh beef cuts. In total, 1022 raw beef samples were collected from two separate processing facilities between July and December. Each sample consisted of 60g portions and was analysed for total viable counts and *escherichia coli*. The results revealed that the beef samples yielded 4.0 to 6.8 log cfu/g and 5.8 to 7.1 log cfu/g in each respective processing facility, however no substantial link was established between the level of TVC and incidence with *e. coli*. In addition, a microbial survey conducted by Eisel et al (2003) was utilised to assess the quality of incoming goods and overall production practices within a meat processing facility including retail cuts, boxed beef, ground beef and key food contact and non-food contact surfaces. The study revealed that average TVC results varied from 4.2 log cfu/g for retail cuts to 8 log cfu/g for boxed beef which consisted of flank and pad cuts of meat. For minced meat and meat preparations which included most commonly trims, average log values were reported to be 5.3 cfu/g. These results suggest that meat which has been handled more frequently ultimately leads to greater level of contamination and higher TVC levels.

Further total viable count has previously been used as an assessment to examine the shelf life of a food product. Yang et al (2018), conducted a study to compare the shelf life of beef steaks under different packaging conditions. The steaks were stored at 4°C for 20 days, in 80% oxygen and 20% carbon dioxide.

The results displayed a positive correlation between storage time and rate of microbial growth. The original reported level of TVC was 4.09 log cfu/g, this steadily increased to 6 log cfu/g over 4 days of storage. After 12 days of storage the beef steaks reached levels as high as 8.2 log cfu/g which was over the requirements which ultimately caused an off odour and poor taste, meaning that the shelf life was deemed unsuccessful.

The relationship between the spoilage of beef and total viable count does not define a generic type of spoilage unlike lactic acid bacteria which outlines a specific set of declines in the sensory properties including but not limited to sour tasting and green meat (Pothakos et al, 2015). Total Viable count may be used a measurement to assess the overall microbial contamination of product in various ways. The test could be used to evaluate the initial level of bacteria (Start of Life) of a product or be used as indicator to determine the maximum shelf life of a food product (End of Life). In addition, total viable count can also be used as a validation tool, to validate individual procedures such as hygiene protocols or interventions. Further, total viable count can be utilised as measurement which defines if a surface is clean or dirty by defining the total level of bacteria present.

2.5 Hygiene Indicators in Beef

The commonly referred to term 'Hygiene Indicators' has previously been used interchangeably to mean index organisms, relating to gauges of both sanitation and hygiene on environmental surfaces and equipment of process and production controls or areas, finished product quality and of spoilage rate potential (Barco et al, 2015). Classically, the most common microorganisms tested and used as

indicators within the food industry are total viable counts, *enterobacteriaceae, escherichia coli* and coliform bacteria as they include both pathogenic and non-pathogenic genus which could be harmful to human health if consumed in significant quantities (Meat Industry Guide, 2018). Through analysing indicator organisms on a food contact surface or food product, results may deliver reliable and rapid information about the overall safety of the product for human consumption and apparent failures within processing, post processing environmental contamination and overall hygiene status of the process flow.

If specific pathogenic bacteria do contaminate carcasses during slaughter, they most commonly are only present in low levels and only in small areas of the carcass. Meaning if specific pathogenic testing does occur, a negative result would not validate an absence of harmful bacteria (Da Silva et al, 2016). For specific pathogenic testing to be successful and validated, a significantly large portion of the carcase must be tested to portray a statistically effective measure, which is neither feasible nor economically achievable for many manufacturers. Instead, the most popular form of validation for process control can be best accomplished through creating microbiological criteria for indicator organism's which contain pathogenic bacteria such as *enterobacteriaceae* in combination with total viable counts (Williams et al, 2017).

Further to measuring the quality of carcasses and production operations within the processing chain. Hygiene indicators can be assessed as performance measures to evaluate the efficiency and food safety status of a product and process. Williams, Ebel and Golden (2017), investigate the prevalence of hygiene

indicators across beef carcass and the correlation between pathogens and *Total viable count*. Carcasses were assessed at both de-hiding and pre-chilling phases throughout production. Overall at de-hiding 91.9% of carcasses achieved greater than 10 cfu/ml, significantly more than at pre-chilling stage where only 81.1% of carcasses achieved levels greater than 10 cfu/ml. The study concluded that it is more effective to establish a criteria of log reduction for *total viable count* instead of trying to achieve a maximum level of bacteria required. It is well known that the majority of bacteria is present on the hide of the carcass therefore the highest level of bacteria recorded is typically prior to de-hiding (Kaur et al, 2017). As a result of this well-known phenomena, the study concluded that a 2 log reduction in the level of bacteria prior to chilling post de-hiding would conclude that the process was effective.

In addition to carcass analysis, indicator organisms are also assessed for retail packs and products to ensure product safety and quality. Säde et al (2013), investigate *enterobacteriaceae* on modified atmosphere packed diced beef and poultry products. In total 54 samples were collected for diced beef and tested over the products full shelf life of 9 days. At the start of shelf life pack + 2 days, overall all samples contained >4 log cfu/g suggesting poor handling during processing. This significantly increased to 7.2 log cfu/g on pack + 8 days. In addition, this study suggested that primal packs which attained higher initial levels of contamination resulted in higher finished pack microbial contamination highlighting the need for decontamination tools to reduce the initial microbial levels and extend shelf life by reducing the growth rate.

2.6 Pathogenic Bacteria and Food-borne Disease associated with Red Meat

Raw meat due to its physical nature, can often be vehicles for the transportation of pathogenic bacteria which is carried by various animals whose primary purpose is for human consumption. Previous literature suggests that the majority of human illnesses as a result of foods are not typically reported (European food safety authority, 2019), further the level of investigation into the detail of each foodborne disease case identifying the causative agent and root of transmission varies significantly (Hoffman, 2017). Estimates concluded from reported illnesses, pathogenic bacteria in foods and surveys conducted on the occurrence of food borne disease highlights that the occurrence of pathogen related illness varies greatly from country to country (Gill et al, 2018). However, it may be concluded that for the majority of regions, raw meat from livestock play a vital role in the transmission of food borne illness globally (Painter et al, 2013).

The occurrence and re-emergence of communal disease as a result of pathogenic bacteria is still a major cause for concern for public health authorities. Potential food poisoning within the red meat industry although remains significant is arguably a low risk product due to the majority of products requiring adequate cooking which kills and eradicates harmful bacteria. However, the most repeated pathogenic bacteria present within red meat can be identified as *staphylococcus aureus, campylobacter, salmonella* and *escherichia coli* (Omer et al, 2018). With salmonellosis being one of the leading causes of food borne disease in the world, and recognised to form colonies in the digestion tract of the animal but lacking visible and physical symptoms (EFSA, 2017), carcasses are at high risk of

contamination during the time of slaughter and therefore could lead to further contamination of finished pack products such as diced beef (Hoffmann et al, 2017). Meyer et al (2010), sampled a total of 4170 raw meat samples from beef obtained from several slaughterhouses. The results revealed that the inclusive total amount of positive samples was 2.9% suggesting that only 120 samples contained *salmonella*. The most affected part of the carcass was the tongue, this could be attributed to the cow chewing and processing any food/grass of which contains significantly high bacteria prior to being slaughtered. Further, commonly after the point of kill the carcass is hung by its hind leg after bleeding, resulting in the tongue occasionally dragging/contacting the abattoir floor depending on the size of the carcass.

A recent study conducted by the Food Standards Agency (FSA, 2018) published results based on a three-year evaluation of the incidence of antimicrobial resistant *e. coli* present across British retail beef (FSA, 2018). The last year of the research conducted between 2017-2018 assessed 313 retail packs of beef which were sold at various retail premises across the United Kingdom. In summary the results obtained that less than 1% of total samples were positive for antimicrobial resistant *e. coli.* The implications of antimicrobial resistant bacteria can have significant impacts to public health. Contaminated red meat with resistant bacteria can transmit such bacteria to humans via direct contact or consumption, if contracted infections can cause more severe longer illnesses and greater incidence of hospitalisation. Some resistant bacteria, have developed

mechanisms of which are resilient to most of modern day medications leading to increased prevalence of death (World Health Organisation, 2018)

Regarding foodborne illness related to red meat, outbreaks across Europe were most commonly caused by *E.coli* O157 and *Salmonella* (**Table 3**).

Table 3. Foodborne disease outbreaks caused by *E. coli* and *Salmonella* between (1980-2015) in red meat products

Pathogens	Number of outbreaks	Cases (confirmed cases)	Hospitalisations	Deaths	References
***Escherichia coli* O157:H7**	33	1543	476	32	Omer et al (2018)
Salmonella	21	1891	94	7	Omer et al (2018)

The entire number of *e.coli* outbreaks were caused by *e.coli* O157 except for four cases which were O26:H11, O111:H8, O111 and O103:H25. Although *e.coli* and *salmonella* were the most common causes of disease, other pathogenic bacteria also caused illness between 1980-2015. The pathogens linked to red meat included but not limited to *listeria monocytogenes, staphylococcus aureus, clostridium botulinum, clostridium perfringens* and *bacillus cereus*. Such previous reported outbreaks highlight the implications and burdens of outbreaks associated with red meat that is faced on a global scale by all of the meat industry to increase food safety and reduce microbial pathogens in food.

2.7 Contamination of Food Contact Surfaces

Various factors during processing influence the capability of microorganisms to be transported from one surface to another. Specific inherent factors can be classified as the type of microorganism present and the physiological characteristic of such bacteria, the significance of attachment and the bacteria ability to form biofilms and clusters (Wang, 2019). Environmental factors include but not limited to the moisture content and surfaces roughness of both the contaminated surface and receiving material or product, in addition to the contact period between dirty and clean surface (Leadley, 2016). In food processing in general cross contamination is a consistent risk across all sectors food industry. Wang and Ryser (2019), highlighted that contact period plays a vital role in the impact of cross contamination from food contact surface to food product. The study investigated the effect of mechanical slicing has on the cross-contamination rate of tomatoes. The research found that slice thickness, processing temperature did not affect the microbial load, however slicing rate did correlate with overall microbial count. Suggesting that as contact period increased so did the levels of microbial bacteria. Within the red meat industry specifically, the dynamic of *Pseudomonas* and biofilm formation was investigated, to assess the risk of cross contamination (Wang et al, 2018). The study concluded that after 10 minutes of contact period between meat and stainless steel surface that up to 4.5 log cfu/cm^2, after 5 hours of contact period, the level of bacteria present increase to 5.5 log cfu/cm^2. Biofilm formation occurred over longer period of contact time at 7 hours.

The environmental surfaces harbouring microorganisms in food processing facilities have repeatedly been associated as sources of contamination that potentially affect the quality and shelf life of the meat (Hultman et al, 2015). Previous studies have established that bacteria present across food processing stages are repeatedly found on processing tools and surfaces including conveyor belts (Table 4) (Bokulich et al, 2013; Cunningham et al, 2011; Haughton et al, 2011; Schlegelova et al, 2010), highlighting the importance of good manufacturing practice (GMP) and adequate hygiene protocols. In meat production facilities, the prevalence of resident bacteria that have the potential to contribute to the rate and speed of spoilage, often lead to economic losses and occasionally are a cause for product recalls causing infections and diseases (Stellato et al, 2015). Various sources of contamination have been identified including chopping boards, knives, production belts, cloths and other operator's tools (De fillipis et al, 2013). A previous study conducted by Eisel et al (2003), conducted a survey of incoming raw beef products and environmental sources of contamination in a red meat processing facility. The study concluded that TVC counts were significantly higher for meat samples in comparison to environmental swabs, often 3-4 log cfu greater. On average total microbial counts for floors were typically seen between 3-3.6 log cfu/cm^2, with the highest reported counts isolated from the beef chiller, suggested to be due to the continuous defrosting of raw beef throughout the day, resulting in leaking blood from boxes onto the floor. In addition, relatively low TVC counts were seen on production room walls <1 log cfu/cm^2, apart from in the incoming goods area which in comparison was

significantly higher at 3 log cfu/cm^2. Overall, various food contact surfaces were sampled including production lines, conveyor belts, depositors, chopping boards and slicing machinery. As expected, these samples reported the highest total viable count levels at 3.7-4.2 log cfu/cm^2 being a significant risk of cross contamination to the finished product.

Table 4. Contamination of food contact surfaces with TVC

Type of food contact surfaces	Microbial count range (Log cfu/cm^2)	Meat Product	References
Knife	>4	Pork Sausage	(Gounadaki et al, 2013)
Conveyor Belt	7	Chicken	(Haughton et al, 2011)
Food Scoop	5.9	'RTE' Roast Beef	(Beccalli et al, 2019)
Cutting Boards	6.2	Retail Beef	(Cunningham et al, 2011)
Knife	6.4	Processed Meat	(Fratamico, Annous and Guenther, 2009)
Conveyor Belt	6.3	Beef	(Schlegelova et al,2010)

Contradicting Eisel's study, Beccalli et al (2019) examined the residential microbiological status of a roast beef production plant over 6 months. In total 55 environmental samples were assessed to recognise the main bacterial populations across the processing facility. Overall surfaces which were not in direct contact with food established much greater mean counts for aerobic bacteria, up to 5.97 log CFU/cm^2 in contrast to anaerobic bacteria at 4.5, *enterobacteriaceae* 1.9 log CFU/cm^2 and *e.coli* at 0.88 CFU/cm^2. The surfaces

classed as the highest risk of causing cross contamination was the drain reporting 5.98 log cfu/cm^2, followed by the floor 3.90 log cfu/cm^2 with the conveyor belt obtaining the 3rd highest total viable counts out of 9 locations samples at 2.29 cfu/cm^2. This highlights the demand for control measures to reduce cross contamination risk during production to lower bacterial counts across the facility.

Wang et al. (2018) reported the growth and biofilm formation of *P. fluorescens* on stainless steel surfaces. The biofilm contained more than 5.0 log CFU/cm^2 cells after 24-hour incubation. With the application of UV treatment, the formation of biofilm can potentially be reduced as TVC levels are reduced on the production line. Bacterial biofilms thrive in food processing environments due to the surplus of nutrients and moisture available provided from the raw material. Such formation of individual biofilms may act as a source of contamination due to the speed at which they may bind to the surface which becomes the first process in the formation of biofilms described as reversible attachment. Succeeding this step the biofilm then enters an irreversible attachment phase, where the production of micro colonies occurs, the structure then transforms to a three dimensional formation creating a diverse ecosystem ready for the dispersion and contamination of microorganisms. Include an explanation and implication of the 3-dimensional biofilm which could cross contaminate or re-contaminate other parts of the processing equipment and / or food products.

Within the red meat industry, the food safety risk associated with the formation of biofilms depends on the contents of each biofilm. Potentially, the contents could contain pathogenic strains including but not limited to *e. coli* and

s*almonella.* Iibuchi et al. (2010) examined the survival of *salmonella* strain biofilms on plastic discs over 175 days. *salmonella* strains continued to be over 3 log cfu/cm^2 after the full testing period, suggesting that plastic surfaces may increase the resistance of biofilms as they prevent the bacterial cells from drying up, therefore decreasing environmental stresses making it significantly difficult to eradicate them from the food chain. Meanwhile, Li et al. (2017) conducted a study examining the effect of meat juices (pork and chicken) as a marginally processed food model to analyse its effect on the rate and formation of biofilms. The juice from each primal meat was collected via the freeze and thaw process collecting the drip loss. A total of 96 plates were supplemented with 25% meat juice which led to a significant increase in the formation of biofilms. Throughout the first stage of biofilm formation, abiotic surfaces were treated with meat juices which enabled the growth and progress of *campylobacter* and *salmonella* under both flow and static conditions. This study shows the survival mechanism of these bacteria and highlights the resistance to the surrounding environment, exposing the need for decontamination and hygiene protocols to clean meat residues during production.

In addition, although traditional cleaning methods have been successful at reducing environmental bacteria, Samapundo et al (2019) conducted a study examining presence of psychotropic bacteria post clean down within a poultry processing facility. The study concluded although typical cleaning methods do result in significant reductions, the bacteria still remain a cross contamination risk post clean down with food contact surfaces obtaining mean 3.54 log CFU/cm^2

across cutting boards, leg hooks and slicing equipment. Specifically, conveyor belts utilised for the transportation of poultry meat into packaging trays revealed 3.50 log CFU/cm^2 post clean down highlighting the demand for more efficient decontamination tools within the industry to reduce the risk of cross contamination.

2.8 Mechanisms of microbial inactivation by UV light

A numerous amount of different light-based technologies has been explored as the most effective measure to disinfect food contact and food surfaces. Such technologies include but not limited to pulsed ultra-violet light, continuous ultra-violet light and light emitting diodes (LED). With each method utilising a separate type of light which may be emitted or generated from a different source or form (Koutchma, 2016).

Pulsed ultraviolet light also referred to as PL, is best known for its ability to decontaminate surface by utilising short high energy burst of light of an intense wavelength (Gómez-López et al, 2007). The elimination of microbial loads has been accredited to the DNA damage that happens similarly to the continuous version of UV light, although mostly the damage which occurs to cell walls, internal structures and cell membranes is most active when treated with this form of Pulsed UV light (Cheigh et al 2012). In comparison continuous ultra-violet light is defined as the continuous treatment by emitting ultra-violet light over a period of time, which harbours greater damage to the specific DNA within the bacteria (Haughton et al 2011).

A previous study conducted by Donskey et al (2019), compared the effectiveness of pulsed ultra-violet light to continuous ultra-violet light as a disinfection tool against pathogenic bacteria on surfaces. The study ran the light for the same time frame at the same distance and compared results post treatment. The results displayed unexpectedly low pathogenic kill percentages for the pulsed ultra-violet device, with mean 0.5 log reductions for both *clostridium difficile* and Methicillin-Resistant *staphylococcus aureus* (MRSA). However, the continuous ultra-violet light device clearly established much greater reduction for the same pathogens from the same treatment period, suggesting that continuous ultra-violet light may be more effective at reducing pathogens on surfaces than pulsed ultra-violet light.

Further supporting this claim, a study completed by Luo (2014) compared the sterilisation proficiency between pulsed and continuous ultraviolet light at different frequency ranges at reducing food related pathogenic bacteria. At frequency 0 the UV light was classed as continuous, then compared to 2, 4, 6, 8 Hz representing pulsed UV light at different frequencies. The pathogenic parameters used for this study were *e.coli* and *salmonella*, for *e.coli* the frequency of 8 Hz showed slightly higher reductions compared to the continuous treatment however no significant difference was observed. In addition, for the surface decontamination of *salmonella*, continuous UV light was found to be more effective gaining greater log reductions in comparison to pulsed UV light at low frequencies. However, at a frequency of 6 Hz, pulsed UV light had slightly higher log reductions than continuous although no significant difference occurred. When

focusing on *e.coli*, primary populaces were recorded at 8.32 log CFU/g which reduced to 4.28 log cfu/g post treatment with continuous UV light. Similar results were achieved for *salmonella* with a 4.4 log cfu/g reduction being accomplished. Although the two separate UV light techniques differ in rate of pulse and frequencies of light, the majority of previous literature (Pala et al, 2011; Sommers et al 2010) concludes that the most effective wavelength used to eradicate bacteria is 254nm due to its germicidal effect, and rate of absorption by microbial bacteria.

2.9 Short wave UV-Light Irradiation as a Decontamination Tool

The effect of UV-Light on microorganisms may vary from species to species and is dependent upon growth rate, stage of culture, density of the microorganism and other characteristics like type of food (Koutchma et al, 2009). The absorption of radiation by the bacteria DNA may stop cell replication and potentially lead to cell death, hence the reduction in overall microbial counts (Keyser et al, 2008). UV-Light intervention has been proven most successful at a wavelength of 254nm, as at this concentration the bacteria absorbs most of the UV light and therefore is classified as germicidal (Pala et al, 2011). The UV radiation has specific advantages in that it does not produce chemical residues or chemical contamination, meaning that production lines or surfaces do not need to be cleaned down as much but still gain the same result of a clean/decontaminated area (Chia et al, 2012). The non-thermal capability of UV-decontamination makes this form of light an accessible and cheap system of sanitisation, which

can be utilised without slowing down the speed of production and processing due to its absence of chemicals or heat (Chun et al, 2010).

Previous literature states that the most common application of this technology has been for the sterilization of air, liquids and packaging materials within the food industry (Parmegiani et al, 2010; Chen et al, 2015; Mahendran et al, 2019). The disinfection of packaging materials (Bolton et al, 2012), by UV-Light has been proven successful on multiple materials including bottle caps, wrappers, foil caps and cartons for liquid products (Tran et al, 2004). For example, Haughton et al (2011), examined the effect of UV light on food contact and packaging surface materials with contamination from *e. coli*, TVC and *enterobacteriaceae*. Succeeding direct treatment with UV light, significant reductions in overall counts were observed. Respectively, 3.97, 4.50 and 4.20 log cfu/cm^2 reductions were seen on plastic surface materials. However, UV-Light has also been utilised across other industries such as hotels, restaurants, schools and hospitals (Chia et al, 2012). Processing equipment and medical devices have also been sterilized by UV-light with successful reduction in microbial counts being observed. Previous research within the medical industry examined the efficacy of UV-light on the decontamination of surfaces. A study conducted by Chitnis et al (2008), used shortwave ultraviolet light to disinfect both steel and plastic surfaces. The study concluded that UV-light was effective up to 5 feet away from the surface with an exposure time of up to 20 minutes. After a period of 10 minutes, TVC reduced to 0.2 x 10^4, after 15 minutes 2.6 x 10^4 and after 20 minutes a reduction of 3.5 x 10^4 was observed.

Within the meat industry Haughton et al, (2010) examined the effect of short-wave UV-Light treatment on raw poultry. The study revealed that following the most intense dose of up to 0.192 J/cm^2, significant reductions were achieved for *E.coli, Salmonella* and total viable counts of up to 1.29 log cfu/g in skinless chicken breasts. In addition, no significant difference was observed in the reduction of microbial counts by increasing the dosage of UV-light from 0.048 to 0.192 J/cm^2. Furthermore, Chun et al. (2009) claimed that after UV-Light treatment, 0.7 – 0.8 log cfu/g reductions in overall total viable counts were also seen in broiler chicken breasts. Haugen et al. (2017) investigated the effect of UV-C light on bacterial reductions of chicken fillets. Exposure to UV-C light range from 0.5 – 2J/cm^2 was effective as a decontamination tool for many bacteria including *c. divergens, s. aureus, pseudomonas, s. enteritidis* and *e.coli*. The most significant reduction throughout the experiment was seen in *e.coli*, a 2.9 log reduction as a result of UV-C light.

Within the United States of America, the Food and Drug Administration (FDA, 2000) have approved the use of ultraviolet light as decontamination method within the food processing industry. Keklik (2010), investigated the effect of ultraviolet light on the reduction of microorganisms on both raw boneless chicken breasts and whole chicken carcasses that were vacuum packed. Firstly, the outer most surface of the chicken breast was artificially inoculated with an antibiotic resistant *salmonella typhimurium* strain. The chicken breasts were then packed in vacuum packaging and exposed to UV-light from 5-60 seconds at a distance of 13cm. On average, the treatment resulted in 2 log cfu/cm^2 reductions.

The second segment of the study evaluated the effectiveness of whole carcass decontamination on a continuous conveyor belt. Each chicken carcass was inoculated with *escherichia coli* prior to UV treatment. Together with chicken breast, whole carcasses encompassed significant log reductions ranging from 0.87 to 1.43 cfu/cm^2 post treatment.

Further Sommers et al (2010), investigated the effect of Ultraviolet light at a wavelength of 254 nm on both food contact surfaces and raw meat and poultry itself. Pathogenic bacteria was analysed post treatment to assess the UV lights ability to increase food safety in a production environment. A decrease in bacteria was observed across all treated samples, specifically red meat and poultry seeing 0.5 log reductions per gram of meat. No pathogenic bacteria was cultivated from the stainless steel food contact surface post treatment, suggesting that UV light is an effective application to decrease pathogenic bacteria and increase food safety for the consumer. Moreover, a study conducted by Rajkovic et al (2010) supports the hypothesis that UV light may be used as a non-thermal decontamination tool for food contact surfaces. The study focused on the decontamination of a frequently used slicing knife fabricated out of stainless steel within a meat processing facility. The bacteria challenged were *listeria monocytogenes* and *e. coli,* after treatment with UV light at 3 J/cm^2. The results established a significant reduction of both *l. monocytogens* and *e. coli*, however suggested that the type of meat matrices (e.g. pork meat or sausages with different protein and fat content) and the time between contamination and treatment dramatically affected the rate of disinfection. The results displayed that

the largest log reduction of bacteria 6.5 cfu/cm^2 was obtained when the knife had been in contact with the meat sources consisting of lower fat and reduced protein content highlighting the possible variation of bacteria between alternate cuts of meat. Due to the knife being part of the production chain, these results portray the effectiveness of UV light as a decontamination intervention to control and reduce the sources of contamination within a processing facility.

As displayed in Table 5, UV light has been utilized as a decontamination tool across the food industry for various food products and food contact surfaces. The variance in appliances, show the capability of the UV-light to eradicate various types of bacteria across totally different conditions and sub-species. Table 5 highlights the effectiveness as a decontamination tool due to the separately treated food products or food contact surfaces containing different bacteria.

Table 5. Summary of UV-Light treatment utilised as a decontamination tool

Raw material / surfaces	**Method**	**Microbial count Log Reductions**	**References**
Apple Face	*UV-C*	*3.0 log CFU*	*(Manzocco et al, 2009)*
Egg Shell	*UV-C*	*5.0 log CFU*	*(Lasagabaster et al, 2011)*
Chicken Breast	*UV-C*	*3.0 log CFU*	*(Mcleod et al, 2017)*
Post Packaged Solid Foods	*UV-C*	*3.2 Log CFU*	*(Heinrich et al, 2015)*
Sliced Ham	*UV-C*	*2.6 Log CFU*	*(Chun et al, 2009)*
Conveyor Belt	*UV-C*	*5.3 Log CFU*	*(Morey et al, 2010)*

Within the red meat and beef industry specifically, the application of Ultraviolet light has slowly begun to be introduced at various points of the production process due to its low cost effectiveness. The main function of the UV light treatment within the red meat industry is within the abattoir, where whole primal cuts may be treated with UV light during the dry aging process to maintain reduced bacterial counts (Dashdorj et al, 2016). However little studies have been published on the decontamination of production lines during processing, specifically a continuous food processing line with aim of increasing the shelf life of raw diced beef by reducing total viable counts on food contact surfaces.

2.10 Visual Specifications of Raw Beef Products

In conjunction with food safety, the visual appearance of the finished product also greatly influences the overall shelf life of the pack. The desired look for raw beef products includes but not limited to a deep bright red colour that attains its saturation for the entire shelf life of the product. This colour occurs due to the activity of myoglobin found within all muscle tissues, when in the presence of air containing both carbon dioxide and oxygen, myoglobin has the ability to bind to oxygen creating the desired bright red colour oxymyoglobin (MbO_2) which is associated with the product being 'Fresh' and ready to consume (Ramanathan et al, 2019). However, an abundance of oxidation causes the formation of metmyoglobin resulting in the meat turning a brown colour associated with the product being unpleasant. There for it is vital that the colour is maintained in addition to correct microbial levels when aiming to increase final shelf life.

Previous research conducted by Kerry and Walsh (2012), investigated the effect of modified atmosphere packaging on the rate of colour change in diced steak. The results suggested that after 10 days of storage in chilled conditions (<7 °C) a negative colour change indicated that the steak started to alter its sensory properties. On day 14, the steaks were declared unsatisfactory and turned an off brown colour. Correlating with this study, although a slightly different product, previous reports by Jayasingh et al (2002) found that minced beef packaged in the presence of high oxygen, preserved its desired vibrant red colour up to 10 days. Further John et al (2005) revealed that sliced rump steak packaged in high oxygen sustained the required red colour for only 7 days; however, browning started to be apparent by the 12th day with the steaks turning completely unappetizing by day 21. Microbial contamination showed a positive correlation with the rate of discolouration, suggesting that as meat became further discoloured the growth rate of spoilage bacteria also increased. Further to this process, Djenane et al (2001), discusses the limitations of treating the beef directly with UV light. The study claims that when the beef itself is over exposed to the UV light ,the chemical reactions within the beef speed up the rate of reaction, therefore leading to further oxidation of myoglobin causing premature discolouration. In food matrices, oxidation can occur due to direct reaction of UV radiation on nutrients such as proteins, lipids and micronutrients. Lipid oxidation is of primary concern as the reaction could lead to modification of sensory properties and development of rancidity (Hinds et al., 2019; Wambura and Verghese, 2011).

In addition to the colour defects associated with the presence or absence of oxygen, not all defects originate from this process but instead may be attributed to microbial contamination. Some species of bacteria, such as *pseudomonas fluorescens,* can alter the meats appearance by producing yellow pigments within the meat itself defined as siderophore, which is a molecule that is commonly utilised used to transports iron (Cornellis, 2010). Further, blue pigment has also been previously reported within beef (Andreani et al, 2015). In addition to *pseudomonas,* the discolouration of meat leading to a "greening" effect has been linked occasionally to *lactic acid* bacteria (Woraprayote et al, 2016) although true links were not observed. Include a couple of sentences here to conclude the studies on visual specifications of raw beef products and link it to your study.

CHAPTER 3 MATERIALS AND METHODS

3.1 Ethical Approval and Health and Safety

This study was approved by the Science, Technology, Engineering, Medicine and Health (STEMH) ethical committee at the University of Central Lancashire preceeding instigation of research. Prior to any research task, extensive laboratory and health and safety risk assessments were completed in line with university protocols and legislation (Appendix 1). Biological safety and Control of Substances Hazardous to Health (COSHH0 applications were also reviewed and accepted prior to commencing laboratory work.

3.2 Experimental Design

This study consisted of a three-week pre intervention phase to determine the current TVC levels and microbiological status of the raw diced beef and food contact surface prior to installing the UV-C decontamination equipment. The UV-C equipment was then installed for a period of 3 weeks due to the limited availability and timeline to test the equipment from the supplier. Results were then analysed and compared to identify if the UV-C treatment has been successful in decreasing TVC levels of the food contact surface therefore increasing the shelf life of the raw diced beef. Visual assessments were completed during the intervention phase to categorise the sensory acceptability of the product to the consumer over the extended shelf life of the product. The meat and swab samples were collected from a red meat processing facility within Preston and analysed at the University of Central Lancashire.

3.3 Ultra-Violet Light

3.3.1 Ultra-Violet Light Installation

The Ultraviolet light equipment (Steril Air, T2018, Switzerland) was installed on the lower returning side of the white conveyor belt between the dicing machine and the secondary conveyor belt due to this surface having the longest contact period with the diced beef. The equipment was placed as close to the dicing machine as possible to reduce possible cross contamination prior to transportation of the diced beef at (Figure 2). The equipment was installed and provided by Cutting Edge Services, Chorley, UK in agreement with manufacturer's instructions and the researcher's advice, at a distance of 7-10 cm away from the conveyor belt surface (Mcleod et al, 2017). The UV light has been installed on conveyor 2, due to the rate at which this conveyor belt moves. As this is the largest conveyor on the line, it moves the slowest and therefore means the longest contact period of UV light on the surface. A longer contact period has been previously stated to have the greatest affect at decontaminating the surface. The equipment was installed half way up the conveyor belt 2 (Figure 2 and 4). The health and safety guidelines and risk assessment (Appendix 1) detailed that there should be no direct eye contact visible to the naked eye, otherwise harm may be caused. Therefore, it was decided that the unit would be placed slightly above eye level, which would prevent and reduce the risk associated with eye contact from the UV light (Steril Air, 2019).

3.3.2 Ultra-Violet Light Surface Decontamination

The application of the UV light was installed solely to treat the conveyor belt surface. The light will remain on from the start of the shift at 6am to the end of shift at 3pm with no calibration period, meaning as production start at 6am the UV light will start to treat the belt. The speed of the belt was set to slow to allow the dicing and depositing of diced beef, meaning the immediate contact period will range from 45 seconds to two minutes depending on the rate of packaging. The UV-light in the C spectrum with a wavelength of 253.7 nanometres was utilised for this study. Due to the slight flex within the conveyor belt the distance between the UV-light and the conveyor belt varied with a minimum and maximum distance being 7cm and 10cm (Mcleod et al, 2017).

3.4 Food Contact Sample Points Protocol

All food contact surface swabs were intended to determine the overall microorganism level present on each surface sampled. A total of 3 swabs per day were collected from four different food contact surfaces across the production line of which the raw diced beef is processed on. Swabs were collected before and after the UV-C equipment throughout both pre and post-intervention with ultra-violet light (Figure2).

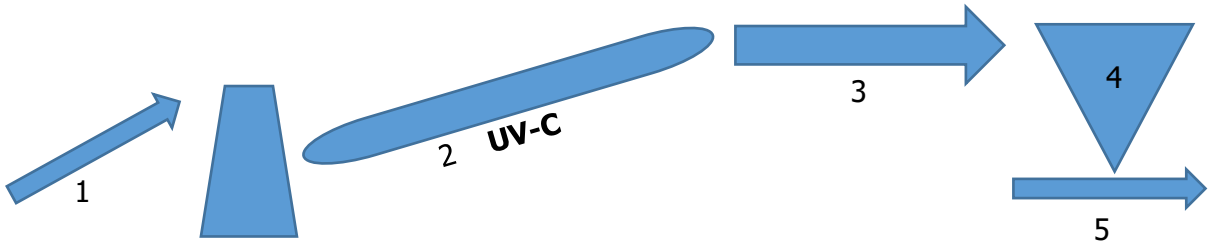


Figure 2. Flow Diagram showing swabbing points across production

Food contact sample points (No. 1 – 4)

1. Conveyor 1 (Conveyor into dicer)
2. White Conveyor 2 (UV-C Intervention)
3. Conveyor 3
4. Ishida (Depositor)
5. Finished Pack (Diced Beef)

3.5 Swabbing Procedure

The swabs utilised for this study were sterile polyester tip and polystyrene shaft swabs in a sterile pouch, which were sealed in 10ml of neutralising buffer (Technical Services, Lancashire TS/5-42). A swabbing template was utilised to ensure a 10 cm^2 surface area was consistently swabbed throughout the entire study (Technical Services, Lancashire, TS/15-T). The swab was then removed from the pouch and held between the third finger and fourth finger. Whilst holding the open tube in one hand, the area were then swabbed utilising 3 different planes (vertical, horizontal and diagonal) covering the full 10 cm^2 area. While swabbing the tip was fully rotated to ensure maximum surface contact has occurred. When swabbing was concluded, the tip was returned into the solution, shook vigorously for 5 minutes using a vortex (Thermo Scientific/UK) and tightly sealed and labelled. Samples were stored in a chiller at <7°C until they were transferred to the laboratory in chilled ice box for testing. Swabs

were collected over a full day's production (6 am, 12pm and 4 pm) twice per week (Table 6). A total of 144 swabs were collected over the pre and post-intervention period.

Table 6. Swabbing Schedule and Locations

Time	**Locations (Sample No.)**			
	Conveyor 1	**Conveyor 2 (UV-C)**	**Conveyor 3**	**Ishida**
6am	1	2	3	4
12pm	5	6	7	8
3pm	9	10	11	12

Note: Pre-intervention: 12 swabs were collected twice a week over 3-week period; Post-intervention: 12 swabs were collected twice a week over 3-week period.

Collecting swabs over different time periods in both the pre and post intervention sampling schedule, shows the change in number of bacteria present on the line from the start to the end of the shift. Swabs were collected from different location to determine how the intervention potentially affects the samples in Conveyor 3 and the Ishida line. The same number of samples in both pre and post intervention was collected to allow for a clear comparison to establish if the UV light was successful in reducing microbial contamination.

3.6 Total Viable Count Procedure - Environmental Swabs

The total viable count were tested both prior to the ultra-violet light installation and after treatment with the UV light to assess the impact on the food contact surface. Swabs were kept in a chilled box (< 4°C) and transported to the laboratory immediately after 3pm (end of sample collection).

3.6.1 Preparation of Nutrient Agar and Plate Pouring

Nutrient agar (Oxoid, Thermoscientific) was made as manufacturer's instructions, autoclaved (Prestige Medical, UK), at 121°C for 15 minutes then cooled in a water bath set at 50 °C before being poured into triple vented petri dishes in a biological safety class II cabinet (Nuaire, UK). All nutrient agar plates were dried in a biological safety class II cabinet before being stored at 4 °C. Prior to inoculation the plates were incubated a room temperature. Nutrient agar plates were poured and prepared within a biological safety cabinet, which was first sterilized using Virkon. Triple ventilated sterile petri-dishes (Thermoscientific, UK) measuring 100mm x 15mm were aseptically removed from the packaging within the cabinet and evenly spread out on the surface. The nutrient agar was then poured into the plates and swirled accordingly to ensure the full surface of the plate was covered. Nutrient agar plates were then allowed to dry entirely prior to inoculation.

3.6.2 Preparation of Nutrient Broth

Nutrient broth (Oxoid, Basingstoke, UK) was made according to manufacturer's instructions, 9 ml was then aliquoted into individual glass universals before being autoclaved at 121 °C for 15 minutes. After sterilisation the broth was then cooled to 25 °C and stored at room temperature.

3.6.3 Dilution Ratio

For consistency, a series of tenfold dilutions were utilised for this study. Within a biological safety cabinet, 1ml of each swab solution was added to 9 ml of nutrient

broth to create the basic 10^{-1} dilution ratio. This was then further diluted up to 10^{-6} to establish the total count of the bacteria present from each swab.

3.6.4 Inoculation and Incubation

Aliquots (100 µL) of each dilution (1/10 (10^1) to (10^6)) were surface spread using an L-spreader (Petrifilm, 3M) separately onto the nutrient agar plates and labelled. The inoculated agar plates were dried in the biological safety class II cabinet until the solution was completely absorbed into the agar. These plates were then incubated (Thermoscientific, UK) at 37 °C for 24 hours.

3.6.5 Calculation of Colony Forming Units

After 24h incubation, plates were removed from the incubator, each individual plate was examined and plates containing approximately 30-300 colonies were counted using a colony counter (Thermoscientific, UK). The number of colonies were recorded for each of the dilution plates and the following equation was applied (Formula 1) to indicate the Total Viable count in colony forming units (CFU) per ml. The results were then converted to cfu/cm2 (Formula 2) and reported in log values. These experiments were repeated in triplicate, meaning each swab was tested 3 times following good microbiological practice.

Calculation of Colony Forming unit (CFU/ml)

$$CFU/ML = \frac{Number\ of\ colonies\ per\ Ml\ Plated}{\text{Total dilution Factor}}$$

Calculation of Colony Forming unit (CFU/cm^2)

$$CFU/CM = \frac{Total\ CFU\ per\ Ml}{10\text{cm2}}$$

Formula 1. Calculation of colony forming unit (CFU/cm^2)

3.6 Finished Pack Analysis

The finished packs were sampled and tested both pre and post UV intervention. The raw material used to create the finished pack, diced beef consisted of 'Pret A decoupe' PAD cuts of beef which consisted of the front quarter of the carcass cuts to produce 98% visual lean beef ([5]-Figure 2). The finished pack samples were collected from the end of the production line, when the diced beef has been packaged and labelled in accordance with standard operating procedures. All finished packs were packed into in to modified atmosphere packaging (Quinn, UK) containing 75% oxygen and 25% carbon dioxide, and kept in chilled ice box and transported to the testing facility's external laboratory. The finished packs were analysed for both start of life (SOL) and end of life (EOL) microbial levels on the same day as the environmental swabs and tested for Total Viable Count, *enterobacteriaceae, pseudomonas* and *e. coli* in compliance with FDA, BAM Detection and Enumeration Method (2017) (Table 7). Note that the current shelf-life of the raw diced beef product produced at the testing facility (prior to the intervention) was pack + 10 days. The sampling size consisted of 1 finished pack sample which was collected at the end of the production shift, every day over a three day period. This was then repeated over a 3 week period for both pre and post intervention phase. The samples were stored in a refrigerator, following on pack guidelines to replicate a consumers fridge at <4°C. In Modified atmosphere packaging the most relevant bacterial groups that cause spoilage in red meat are total viable count, *enterobacteriaceae, pseudomonas.* This could be due to the presence of such bacteria in the gastrointestinal tract of cattle which are shed

during slaughter and hence can contaminate beef carcasses (Lenahan et al 2010). In addition to the risk of contamination during slaughter, the finished pack conditions of oxygen and carbon dioxide with a chilled environment result in these groups of bacteria becoming the most dominant (Wickramasinghe et al 2019). The relevance of *e.coli* within this study provides a scale of how safe the food is to consumer. As *e.coli* is one of the pathogens which cause human illness, and is typically associated with red meat, the absence or presence of such bacteria in this study will act as a food safety parameter. The term Total Viable Count is defined as the total amount of living cells such as bacteria present within a sample.

Table 7. Finished Pack and Raw Material Sample Schedule

Product Status	Time	Tested On (Pack + Days)
Finished Pack (SOL)	3pm	1
Finished Pack (EOL)	3pm	9, 10, 11, 12

Note: *SOL – Start of Life; EOL – End of Life. Samples were collected once per day – 3 x per testing week.*

3.7 Finished Pack Analysis (Lab Method)

For sample testing for groups of organisms such has *E.coli* and *Pseudomonas*, 10g samples were aseptically and diluted with 90ml buffered peptone water (BPW) (Oxoid, Basingstoke, UK). Samples were then placed in a stomacher which uses paddles to manipulate the bag from the outside to breakdown the meat, mixing it with the BPW and allow the bacteria to 'dislodge' from the product. After this pipettes with sterile disposable straws were used to measure out 0.5ml or 1ml quantities from the bag containing the diluted

sample – this volume depends on the specific test requirements and were added to the petri dishes. Either 0.5ml were added to a plate which already contains set agar or 1.0ml were pipetted into an empty petri dish and molten agar were then added and incubated at 46ºc in accordance with FDA, BAM Detection and Enumeration Method (2017)

3.7 Visual Assessment

The finished pack containing raw diced beef was assessed against specific criteria to ensure the product meets the high-quality standard required by customers. The assessment occurred over the duration of the anticipated maximum shelf life and was graded on a scale of 1 to 5 with the lowest number being the highest quality. The grading criteria was created in line with commercial customer requests (Table 8.) Samples were collected at the start of each testing week and visually assessed on pack + 5, 8, 9, 10, 11 and 12 days. Over a three day period, mirroring the finished pack analysis schedule, finished packs from the end of a production day were taken and visually assed over the full shelf life of the product. Meaning that one pack was analysed on each shelf life day, resulting in a total of 108 samples were collected over both the pre and post-trial period. Four trained colleagues within the technical department with knowledge of food safety participated in the visual assessment. The assessment consisted of grading each finished pack on each shelf life day, against the below scoring criteria. The score was then given and then analysed using SPSS.

Table 8. Visual Specification Grading Criteria of Raw Diced Beef

Visual Assessment - Scoring Criteria		Acceptance rate / Action taken
5	Excellent appearance, no issues.	Accepted
4	Colour of product beginning to dull.	Accepted
3	Discolouration <50% - dehydration - residual fluid begging to appear	Rejected
2	Discolouration >50% - extensive dehydration - residual fluid 10mm to 20mm at	Rejected
1	100% non-contact discolouration - Excess fluid > 25mm at 45 degree angle	Rejected

Note: Traffic light assessment was used in conjunction with the grading criteria where red indicates unacceptable samples.

3.8 Statistical Analyses

All data has been evaluated using the statistical software (SPSS 23, IBM, New York). The statistical tests carried out for the individual pre and post intervention data consisted of one-way repeated measures ANOVA to compare the differences between time frames/ shelf life periods. A paired samples t-test was used to compare the differences between means from pre to post intervention data to determine if the intervention was successful. T value measures the size of the difference in the pre- and post-intervention samples. The greater the t value, the greater the evidence against the null hypothesis. Results are expressed as mean ± standard deviation.

CHAPTER 4 RESULTS

4.0 Pre-Intervention: Microbial profile of food contact surfaces and finished packs

4.1 Microbial profile of Food contact Surfaces – Pre-Intervention

Four individual locations across the raw diced beef production line were swabbed at points throughout each testing day over a three-week period. The results were reported in log cfu/cm^2 (Table 9).

Table 9. Pre-Intervention: Total Viable Count from Food contact Swab Surfaces of Raw Diced Beef Production Line

Time of Swab	Conveyor 1	Conveyor 2	Conveyor 3	Ishida
6am	1.79^{a}±1.061	1.85^{a}±1.04	1.99^{a}±1.02	2.92^{a}±0.60
12pm	5.72^{b}±0.59	5.91^{b}±0.30	6.06^{b}±0.17	5.95^{b}±0.18
3pm	6.36^{c}±0.15	6.2°±0.06	6.38^{c}±0.06	6.35^{C}±0.12

Note -^{abc} values with different superscripts in the same column indicate significant difference (P<0.05) for each swab.

Combining all the results from each location, it is clear to see that the overall amount of total viable count increases over time. A clear increase in the level of TVC on each food contact surface is displayed in (Table 9). A statistical significant difference p<0.05 occurred between 6am and 3pm on every surface. Most commonly, at 3pm the level of total viable count would reach level higher than 6 log cfu/cm^2.

4.2 Microbial profile of Finished Pack – Pre-Intervention

In total, 45 finished packs were sent to the lab for analysis prior to the installation of the ultraviolet light. The finished packs were tested for Total viable count, *Pseudomonas, Enterobacteriaceae, E. coli* and *Salmonella*, the results are displayed below (Table 10).

Table 10. Pre-intervention: Microbiological Profile of Finished Pack Raw Diced Beef

Shelf Life (Pack + Day)	**TVC (cfu/g)**	***Pseudomonas* (cfu/g)**	***Enterobacteriaceae* (cfu/g)**
1	$4.29^{a}\pm0.42$	$2.74^{a}\pm0.59$	$1.98^{a}\pm0.31$
9	$5.58^{b}\pm0.83$	$2.77^{a}\pm0.56$	$2.09^{a}\pm1.13$
10	$5.95^{b}\pm0.70$	$2.80^{a}\pm0.60$	$1.60^{a}\pm0.64$
11	$6.33^{b}\pm0.93$	$3.19^{a}\pm1.44$	$1.76^{a}\pm0.79$
12	$6.42^{b}\pm1.09$	$3.36^{a}\pm0.36$	$1.93^{a}\pm1.11$

^{ab} values with different superscripts in the same column indicate significant difference ($P<0.05$) for each shelf life (pack + day)

4.2.1 Total Viable Count

Total viable count was assessed over the full shelf life period for the proposed 12 days. A Mauchly's test of sphericity specified that the assumption was violated ($P<0.05$). Therefore, a one-way analysis of variance repeated measures was completed, and a Greenhouse-Geisser correction was utilised. The results revealed a statistical significant difference between different shelf life periods for total viable counts ($F(4/32)=13.413$, $P=<0.01$).

A pairwise comparison Bonferroni test revealed a significant difference between the level of total viable count on pack + 1 day ($p<0.05$) and other shelf life periods respectively pack + 9, 10, 11 and 12 days. However, no statistical significant difference was observed between the level of total viable counts from shell period pack + 9 days to pack + 12 days. Together these results suggest that the overall microbial load of the finished pack raw diced beef significantly increased from initial packaging on day 1 to day 9 and slowed over time.

4.2.2 *Pseudomonas and Enterobacteriaceae*

When examining the results from Table 10. The overall level of both *pseudomonas* and *enterobacteriaceae* did not statistically significantly increase from 6am to 3pm $p<0.05$.

4.2.4 *Escherichia coli*

Overall, every sample of finished pack was tested for *e. coli* over the full testing period. Each sample provided a negative result for the bacteria, suggesting that all samples were absent of *e. coli.* Therefore, no statistical analysis was completed for this specific bacteria.

4.3 Visual Assessment of Finished Pack

The visual properties of the finished pack were scored against the chart detailed in the method, on a scale of 1 – 5, of which 1 represents a spoiled poor-quality finished pack and 5 representing a high-quality desired pack. The mean score (Mean) on pack + 5 days was 5.00 indicating high-quality and desired visual properties. This then decreased further to Mean=4.60 on pack + 8 days, and Mean=3.80 on pack + 9 days. On the last day of the current shelf life of the

product (pack + 10 days), the visual assessment determined that the average score was (Mean=3.20), which further decreased to Mean=2.60 on pack + 11 days. On the final day of testing, the visual assessment scores reduced to Mean=2.00 on pack + 12 days indicating discolouration at >50% and extensive dehydration. These results suggest that the raw diced beef starts to deteriorate from day pack + 8 onwards, of which by pack + 10 days the beef has discoloured to an undesirable level.

4.4 Post-Intervention: Microbial analysis of environmental surfaces and finished packs

4.5 Microbial Analysis of Environmental Surfaces - Post Intervention

Post intervention with ultraviolet light, additional swabs were sampled to assess the level of total viable count across the food contact surfaces on the raw diced beef production line post trial (Table 11).

Table 11. Post-Intervention – Total Viable Count from Environmental Swab Surfaces of Raw Diced Beef Production Line

Time of Swab	Conveyor 1	Conveyor 2 (UV treated)	Conveyor 3	Ishida
6am	$0.90^{a}\pm0.83$	$1.66^{a}\pm0.89$	$1.53^{a}\pm0.92$	$2.21^{a}\pm0.72$
12pm	$6.02^{b}\pm0.17$	$3.38^{b}\pm0.29$	$5.86^{b}\pm0.11$	$5.95^{b}\pm0.26$
3pm	$6.45^{c}\pm0.11$	$4.43^{c}\pm0.61$	$6.25^{c}\pm0.18$	$6.29^{c}\pm0.14$

^{abc} values with different superscripts in the same column indicate significant difference ($P<0.05$) for each swab.

Similar to the pre intervention results, Table 11 display's a statistically significant difference p<0.05 from 6am to 3pm across each swab location, suggesting that TVC increases over time.

4.6 Microbial Analysis of Finished Pack – Post Intervention

Post intervention with the ultraviolet light, an additional 45 packs were sent to the laboratory to analyse total viable count*, pseudomonas, enterobacteriaceae, e. coli and salmonella*. The overall results are reported below (Table 12)

Table 12. Post-Intervention: Microbiological Profile of finished Pack Diced Beef

Shelf Life (Pack + Day)	Total Viable Count (cfu/g)	*Pseudomonas* (cfu/g)	*Enterobacteriaceae* (cfu/g)
1	$4.33^{a}\pm0.39$	$1.93^{a}\pm0.63$	$1.94^{a}\pm0.49$
9	$5.69^{b}\pm0.97$	$2.51^{b}\pm0.55$	$2.00^{a}\pm0.56$
10	$6.22^{b}\pm0.62$	$2.67^{b}\pm0.62$	$1.85^{a}\pm0.34$
11	$6.64^{b}\pm0.66$	$3.02^{b}\pm0.29$	$2.17^{a}\pm0.86$
12	$6.86^{b}\pm0.68$	$3.32^{b}\pm0.39$	$2.44^{a}\pm0.72$

^{ab}values with different superscripts in the same column indicate significant difference ($P<0.05$) for each shelf life (pack + day)

4.6.1 Total Viable Count

Assessing the level of total viable count of the finished pack post-trial, a Mauchly's test of sphericity was non-significant ($p<0.05$). A repeated measures one way analysis revealed a statistical significant difference between shelf life periods ($F(4,32)=20.90$, $p<0.01$). Further a pairwise Bonferroni comparison test stated that there was a statistical significant difference between shelf life period pack + 1 day and pack + 9, 10, 11 and 12 days. However, there was no statistical significant difference ($p>0.05$) from pack + 9 days to pack + 12 days. Similar to pre-trial, the results suggest that total viable counts increase initially but plateau from pack + 9 days onwards.

4.6.2 *Pseudomonas*

The results revealed that a Mauchly's test of sphericity was violated ($P<0.05$) therefore a Greenhouse-Geisser correction was used. A repeated measures one way analysis stated a statistical significant difference between shelf life periods ($F(2.06,16.48)=17.08$, $p<0.01$). In addition, a pairwise Bonferroni test determined that there was a statistical significant difference between shelf life periods pack + 1 days and pack +9, 10 ,11 and 12 days. However similar to total viable counts, there was no statistical significant difference in the level of *Pseudomonas* post-trial between shelf life periods pack + 9 days to pack + 12 days.

4.6.3 *Enterobacteriaceae*

Examining the results from post-trial *enterobacteriaceae,* a Mauchly's test of sphericity was not violated ($P<0.05$). A repeated measures one way analysis revealed no statistical significant difference between shelf life periods ($F4,32)=1.21$, $P=0.325$). Therefore, no further statistical analysis was carried out.

4.6.4 *Escherichia coli* and *Salmonella*

Post-trial, there was also no *e. coli* and *salmonella* detected in any of the finished pack, raw diced beef throughout the whole trial. Therefore, no statistical analysis was carried out.

4.7 Microbial Analysis of Environmental Surfaces Pre and Post Intervention

The overall effect of the ultraviolet light had on the decontamination of the food contact surfaces was measured using a paired samples t-test, comparing the

level of total viable count from each sampled surface of both pre and post-trial data (Table 13).

Table 13. Pre and Post intervention – Paired Sample T-test of Total Viable Count Levels from Food Contact Swab Surfaces of Raw Diced Beef Production Line

Time of Swab	TVC (Pre intervention)	TVC (Post intervention)	T-value	Location of Swab
6am	1.79[a] ± 1.06	0.90[a] ± 0.83	2.15	Conveyor 1
12pm	5.72[a] ± 0.59	6.02[a] ± 0.17	-1.00	
3pm	6.36[a] ± 0.15	6.45[a] ± 0.11	-1.03	
6am	1.85[a] ± 1.04	1.66[a] ± 0.89	0.36	Conveyor 2 (UV-Treated)
12pm	**5.90[a] ± 0.30**	**3.38[b] ± 0.29**	18.76	
3pm	**6.22[a] ± 0.66**	**4.40[b] ± 0.92**	4.48	
6am	1.99[a] ± 1.02	1.53[a] ± 0.92	1.04	Conveyor 3
12pm	**6.06[a] ± 0.17**	**5.86[b] ± 0.11**	2.76	
3pm	6.38[a] ± 0.63	6.25[a] ± 0.18	1.74	
6am	**2.92[a] ± 0.60**	**2.21[b] ± 0.72**	2.68	Ishida
12pm	5.95[a] ± 0.18	5.96[a] ± 0.26	-0.06	
3pm	6.35[a] ± 0.12	6.29[a] ± 0.14	1.28	

[ab]values with different superscripts in the same row indicate significant difference ($P<0.05$) for each swab.

When analysing all these results together, the ultraviolet light disinfection had no effect on the overall level of total viable count build up across the untreated belts, however individual inconsistent statistical significant reductions were observed at 12pm on conveyor 3 and 6am on the ishida ($p<0.05$) . However, statistically significant difference $p<0.05$ was observed between pre and post intervention levels of TVC on the ultraviolet light treated white conveyor 2. A 1.6 – 2.5 log reduction was seen across both 12pm and 3pm when the light was actively treating the belt (Figure 3)

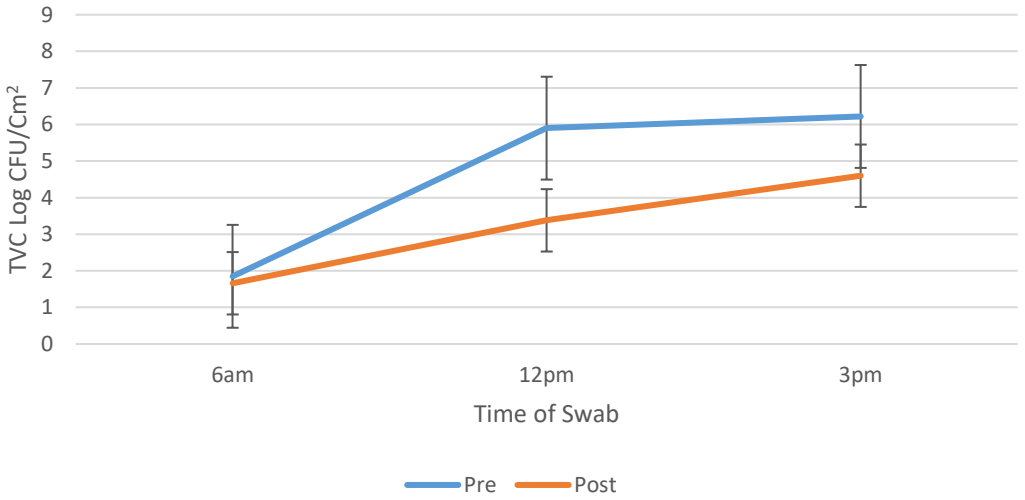


Figure 3. Pre and Post Intervention: Total Viable Count levels on Conveyor 2 (UV treated) of Raw Diced Beef Production Line

4.8 Visual Assessment of Finished Pack – Post Intervention

Post-trial, additional finished packs were analysed for the visual properties after the decontamination of conveyor 2 with ultraviolet light. The results revealed the same score as pre-trial for shelf life period pack + 5 days with a mean score of Mean=5.00 indicating excellent appearance. A small reduction in the visual properties on pack + 8 days, decreasing to Mean=4.30 with a 0.30 difference in comparison to pre-trial. Further, deteriorations were observed on pack + 9 days (Mean=3.80) and pack +10 days (Mean=2.90), which is the current shelf life of the product. Finally, on the proposed shelf life of the product pack + 11 days, the visual appearances further decreased to Mean=2.60 and pack + 12 days to Mean=2.10, suggesting that the quality of the pack hat deteriorated to below the desired standard from day pack + 10 onwards.

4.9 Microbiological Count of Finished Pack Diced Beef – Pre and Post Intervention

The effectiveness of the UV light treatment was assessed by comparing the level of total viable count, *pseudomonas* and *enterobacteriaceae* pre- and post-treatment with ultraviolet light, across each shelf life period using a paired sample t-test. *E. coli* were not analysed as no samples tested positive for the detection of either bacteria (Table 14)

Table 14. Microbiological Count of Finished Pack Diced Beef – Pre and Post Intervention

Shelf Life (Pack + Days)	**TVC Log (cfu/g)**			***Pseudomonas* Log (cfu/g)**			***Enterobacteriaceae* Log (cfu/g)**		
	Pre	Post	T-value	Pre	Post	T-value	Pre	Post	T-value
1	4.29[a] ± 0.42	4.33[a] ± 0.39	0.28	2.74[a] ± 0.59	1.93[a] ± 0.63	-2.29	1.97 [a] ± 0.31	1.94[a] ± 0.49	-0.16
9	5.58 [a] ± 0.83	5.69 [a] ± 0.97	0.96	**2.76[b] ± 0.56**	**2.51[a] ± 0.55**	-3.33	2.09 [a] ± 1.13	2.0[a] ± 0.56	-0.034
10	5.95 [a] ± 0.70	6.22 [a] ± 0.62	1.80	2.80[a] ± 0.60	2.67[a] ± 0.62	-1.63	1.60 [a] ± 0.64	1.85[a] ± 0.34	1.06
11	6.32 [a] ± 0.93	6.64 [a] ± 0.66	1.76	3.64[a] ± 1.44	3.02[a] ± 0.29	-1.38	1.76 [a] ± 0.79	2.17[a] ± 0.86	1.82
12	6.42 [a] ± 1.09	6.86 [a] ± 0.68	1.49	3.35[a] ± 0.36	3.32[a] ± 0.39	-0.65	**1.93[b] ± 1.11**	**2.44[a] ± 0.72**	2.58

[ab]values with different superscripts in the same row indicate significant difference ($P<0.05$) for each shelf life (pack + day).

Although the results displayed no statistically significant difference for the level of total viable counts of the raw diced beef finished back between pre and post-trial, across each shelf life period, all levels of TVC demonstrated slight log reduction ranging from 0.04 to 0.32 log cfu/g. Only pack + 9 days witnessed a significant difference in the level of *pseudomonas* out of the full testing period, however this difference had a negative impact as 0.25 log cfu/g increase was observed. *Enterobacteriaceae* count revealed a statistically significant microbial decline in pack + 12 days with a 0.54 log cfu/g reduction.

CHAPTER 5 DISCUSSION

The current study has many key aims. Firstly, the study aimed to investigate and install an ultra-violet light unit as a decontamination tool on a vital food contact surface used throughout the production of raw diced beef. Secondly, the study aimed to examine the effect of UV light intervention on the level of total viable count of food contact surfaces along the production line. The third aim was to determine if the decontamination of the production line would increase the shelf life of the raw diced beef from pack + 10 days to > 10 + days by investigating the microbial load and visual properties of the finished packs.

5.1 Ultraviolet Light Installation

Ultra-violet light has been used to decontaminate various surfaces across the food industry. The application of UV light has been shown to have a significant effect on the decontamination of selected non-food and food applications (D'Souza et al, 2015). The UV light is characterised as a non-thermal, chemical free form of disinfection hence allowing the method to be used on food contact surfaces including but not limited to knives, processing machines, chillers and conveyor belts (John and Ramaswamy,2018).

The first aim of the study was to install the ultra-violet light on the reverse side of the conveyor belt, on the raw diced beef production line at the most efficient and practical place possible to decontaminate the surface itself without hindering production or affecting the raw diced beef. Out of the full production line, the unit was installed on conveyor 2 (Figure 4) as the diced beef was in contact with this surface for the longest period time (up to 2 minutes) out of all

the food contact surfaces involved. This meant that the beef would be exposed to a potentially clean surface for a longer period, instead of a dirty surface. This could potentially result in a reduced risk of cross contamination on the product from the belt. Supporting this claim, Miranda and Shaffner, (2016) conducted a study investigating the effect that contact period has on the level of cross contamination from surface to food product. The results revealed a highly statistically significant result in the difference between contact period and rate of transfer, suggesting that the longer the contact period, the greater the rate of microbial cross contamination. Therefore, this highlights the importance of decontaminating the belt which has the longest contact period with the diced beef within this study.

Similarly, Dantas et al (2018), investigated the rate of cross contamination and biofilm formation from poultry to various food contact surfaces. Various cutting boards made of either plastic, wood or glass were utilised to process poultry carcasses, after processing cucumbers were put in contact with the dirty surfaces under different conditions. The results revealed that surfaces of which contained biofilms attained 100% recovery of *Salmonella* strains after cleaning down, with plastic surfaces enabling the recovery of 40% of *Salmonella*. Further, results suggested that cucumbers left in contact with the surfaces for the longest period resulted in the most significant recovery of bacteria, suggesting the greatest rate of cross contamination.

Figure 4. Installation of UV light on raw diced beef production line

However, although the unit was placed above eye level it was decided that in line with previous studies and manual instructions that the unit should be placed as close as possible (Pedros-Garrido et al., 2018; Steril Air, 2019) to the dicing machine to reduce the risk of drip contamination. There is potential for drip contamination to occur from the beef products that were transported on the belt above. The composition of the raw diced beef being in its natural state means that there could be large amounts of blood and muscle residue as a result of the dicing process. Due to the raw diced beef leaving the conveyor belt at the top of the line, most of the drip loss from the product would occur at the top of the belt as the conveyor turns over to return to the dicer. Therefore the closer the UV light is to the dicer, this enables most of the meat residue and any potential drip to occur before the conveyor reaches the UV light again. This helps to minimise drip contamination on the UV light. The installation near to the dicing machine

also reduced potential for air contamination prior to receiving fresh product (Steril Air, 2019). Pedros-Garrido et al (2018), measured the effectiveness of ultraviolet light at a range of distances including 6 cm, 16 cm and 26 cm on the decontamination of food contact surfaces and the surface of salmon. The results suggested that maximum log reduction were observed for the closest distance at 6cm, however statistical significant log reductions ($P<0.05$) were witnessed for all three distances tested.

Further supporting the distance between the uv unit and the conveyor belt 2 within this study, Cassar et al (2019) investigated the effect of ultra-violet light at various distances as a microbial decontamination device for boneless chicken thigh meat. The study concluded that there was no significant difference in the log reduction of bacteria present on the meat between the range of distances tested (8 and 13cm). This point further supports the results from this study, as the distance from the UV light to the conveyor belt within this study also varies due to the slack in the belt when returning to pick up more meat. As the belt contains slack, the distance ranges from 7-10cm.

Food contact surface disinfection was selected for this study as ultra-violet light has been shown to compromise a products quality from a sensory perspective. Wambura and Verghese (2011), analysed the effect ultra-violet light has on the finished organoleptic properties of sliced ham. The study concluded that although microbial counts were significant lower post treatment with uv light ($p<0.05$), the colour and texture of the ham had been adversely affected turning darker post treatment. Further supporting the evidence that ultra-violet light

works at various distances and also negatively impacts organoleptic properties. Demirci and Ozer (2005) evaluated the effect of ultra-violet light on raw salmon fillets. The results revealed that significant microbial log reductions were observed at each distance tested (3,5 and 8cm), however at 3 and 5cm some fillets obtained visual burn marks which altered the appearance of the product making it undesirable. Concluding these studies, the risk associated with treating the actual raw diced beef would be to high, and could result in significant volume of meat being disposed of due to changes in its visual appearance including colour.

5.2 Environmental Food Contact Surfaces

The BRC Global Standard Food Safety Issue 8 state in section 4.11.1 (BRC.UK, 2019), that it is essential that every production site must frequently clean all equipment to an appropriate standard, to prevent or reduce the risk of cross contamination. Further, section 4.11.3, states that acceptable limits for food contact surfaces must be determined based on the potential hazard which relates to the product, which in this case would be defined as the total viable count limit on conveyor 2 of the raw diced beef production line, which possess a threat to the quality and safety of the raw diced beef.

In addition to the installation of the ultra-violet light equipment to reduce the level of bacteria on the production line itself, it could also potentially improve the quality of the finished product by reducing the risk of cross contamination of spoilage bacteria. Before the UV unit was installed, the results from this study showed the variance in the level of bacteria present prior to production initiating

across all surfaces, results ranged from non-detected to 3.56 log cfu/cm^2 at 6am which suggests that the production surface is commonly unclean prior to starting. This suggests that the facility need to improve the accuracy and efficiency of the overnight hygiene protocols to ensure that that there is no risk of cross contamination from pervious products or shift.

Moreover, further to inadequate cleaning procedures at the facility, if biofilms have formed on the food contact surface, often they can form resistance to various chemicals and cleaning methods also, which could further explain the results from 6am within this study. Fagerlund et al (2017), examined the microbial make up of food contact surfaces within a meat processing plant after cleaning and disinfection. The results suggested that across two separate meat processing plants, conveyor belts which have been extensively cleaned and disinfected harboured 121 isolates from 22 individual genera of bacteria. Most commonly, *pseudomonas* were reported to be the most prevalent across all food contact surfaces. Further to this, Jessen and Lammert (2003) highlighted that visually clean surfaces can still harbour significant levels of bacteria. The study concluded that after efficient cleaning and sanitation food contact surfaces obtained total viable count levels up to 4.56 log cfu/cm^2. Ineffective or lack of cleaning measures of food contact surfaces may pose a threat to food safety due to the accumulation of food debris, moisture and microorganisms leading to multiplication of spoilage microorganisms and pathogens which could lead to the formation of biofilms.

Nyati, (2006) suggested that microorganisms present on wet surfaces have an increased ability to proliferate and produce micro-colonies that lead to the construction of biofilms. When biofilms are created on food contact surfaces, generally they contain an outer protective layer of which prevent sanitisers and disinfection chemicals from killing the bacteria, therefore highlighting the importance of proper cleaning procedures and the potential use of alternate technologies such as ultra-violet light. Further, due to inconsistent cleaning, the rate of biofilm formation may be significantly increased (Bridier et al, 2015). Due to the availability of microorganisms (i.e. TVC mean levels ranged from 1.79 – 2.92 log CFU/cm^2) highlighted in the 6am results, it is has been previously shown that the bonding of biofilms may form within a few hours of contact. Multiple studies have proven that the irreversible attachment phase of biofilm formation may take 20 minutes to a maximum of 4 hours at 4C (Henriques and Fraqueza 2017, Galie et al 2018, Garrett Bhakoo and Zhang 2008). This reiterates how vital proper decontamination of food contact surfaces occurs overnight prior to the next shift. At this stage of biofilm, the removal of such cells becomes extremely difficult, hence the application of either heat, specific enzymes or ultra-violet light may be the only mechanism of eradicating the biofilm (Dantas et al,2018).

This study has concluded that prior to the installation of ultra-violet light over a full day's production of up to 9 hour shift, the total viable count levels significantly increase from the start of production to the end ($p<0.05$) reaching a maximum of 6.47 log cfu/cm^2 which relates to 3,780,000 cfu/cm^2 on the surface of which the raw beef is transported across from dicer to conveyor. Levels this

high were seen frequently at 3pm on all surfaces prior to the intervention. Bacteria is at the highest at 3pm due to the exponential growth which can be categorised as geometric, due to the division and growth of bacteria observed from 6am. Due to the environmental conditions across each food contact surface within this study, the continuous flow of raw beef and endless supply of oxygen provides optimum conditions to enable the growth of bacteria to obtain levels as high as >6 log cfu/cm^2 (EFSA,2016).

Although these levels are pointedly high, similar results were seen in a small-scale meat processing plant previously as reported by Gounadaki et al, (2013). The authors assessed the microbial floral across different environmental sampling points in a small-scale meat factory. The results displayed various outcomes which were heavily dependent on location of samples, food contact surfaces including, but not limited to conveyor belts and weighing scales, ranged from 1.3 log cfu/cm^2 to 7.43 log cfu/cm^2. Higher levels of bacteria were constantly reported across mincing machines, with the lowest bacteria reported against knives indicating that the greater the volume of meat processed the higher the level of bacteria harboured. In addition, supporting the results from this study, Zailani et al (2016) conducted an evaluation of the microbial loads of critical surfaces within various red meat abattoirs. In total 6 individual abattoirs were sampled, and key food contact surfaces were analysed including cutting instruments such as knives and slicers and transport mechanisms such as conveyor belts and tables. Overall the results stated that the highest total viable counts of all the meat contact surfaces was the table at 7.8 log cfu/cm^2, with the

lowest achieving 6.4 log cfu/cm^2. It could be argued that the table achieved the highest log result due to the volume of meat processed over this contact surface, a greater volume of meat processed would result in a higher level of nutrients provided and possible increased risk of cross contamination. In addition, transport mechanisms such as conveyor belts obtained total viable count levels ranging from 6.7 to 7.6 log cfu/cm^2.

As shown in Table 9 of this study, there is little to no variance in the level of bacteria between locations pre-trial, this could be due to the volume of meat of which is processed across each location being concisely the same within the same conditions, therefore the growth rate of bacteria is mirrored on each surface (Rajkovic et al, 2010). The testing facility used in this study processed 5.5 tonne of raw diced beef per day during the pre- and post- intervention period.

When comparing the non-treated food contact surfaces across the raw diced beef production from pre-trial to post trial, there was no statistically significant difference $p>0.05$ in the level of total viable count present. This result was to be expected as conveyor 1, conveyor 3 and the ishida were not directly treated with UV light and did not alter or reduce microbial levels significantly. However, when examining the results from conveyor 2, there was no statistically significant difference observed at 6am.

The lack of significant microbial reduction at 6am could be linked to the start of production time and decontamination of the surface. The UV-light strobe was only switched on when the production line started running with no calibration period prior to the beef being processed. Typically, the swab was collected at the

same time as production starting, therefor indicating that the amount of treatment time to decontaminate conveyor 2 was significantly limited, hence reducing the likelihood of total viable counts falling. Potentially the conveyor belt may have only been running for a few seconds prior to the swab being taken, Pedros-Garrido et al (2018) suggested that the longer the contact period of uv light the greater the log reduction of bacteria. Therefore highlighting that the minimum contact period at 6am observed within this study would support no significant reduction.

At both 12pm and 3pm, a significant reduction of 2.52 log cfu/cm^2 and 1.79 cfu/cm^2 was witnessed after ultra-violet light treatment. Together these results suggest that through the application of Ultra-violet light, dramatic microbial deactivation has occurred through reducing cell replication. Across both time frames, a mean log reduction of 2.15 log cfu/cm^2 was observed at both 12pm and 3pm. Similar results were portrayed by Calle et al (2017), who examined the effect of UV-C light on the surface of chicken breast and food contact surfaces. Statistically significant reductions were observed after treatment with UV-light of up to 3 minutes. Calle et al (2017) suggested a positive correlation was seen between treatment time and log reduction of bacteria on the food contact surfaces, with >2 log cfu/cm^2 reductions seen with a longer treatment period. Similarly, a study conducted by Lim and Harrison et al (2016), investigated the effect of continuous ultra-violet light on *Salmonella* on various food contact surfaces across a single facility with treatment periods ranged from 5 seconds to 30 seconds. UV treatment of 3.3J/cm^2 on plastic Polyvinyl Chloride

(PVC) surfaces which were replicated from the same material as common conveyor belts, witnessed log reductions of 2.39 and 1.39 log cfu/cm^2. On stainless steel surfaces, the greatest log reduction was seen at 2.75 log cfu/cm^2 post UV treatment, indicating that the effectiveness of UV light disinfection may possibly vary between surface material.

The results from this study further support Haughton et al (2010) who examined the effect of UV light on the microbial load of packaging materials and food contact surfaces of a broiler processing industry. Statistically significant reductions were observed $p<0.05$ across all materials tested. For bacteria specific *Campylobacter*, recorded levels were reduced to less than the detectable limit (<0.4cfu/cm^2) excluding on polyethylene-polypropylene surfaces of which obtained levels (<0.76 cfu/cm^2).

5.3 Microbiological Analysis of Finished Pack and Visual Analysis

Due to the nutrient density of meat being an ideal environment itself for microorganisms to not only survive but also thrive defines its fragile nature. Although some microorganisms are a threat to human health, many microorganisms purely cause spoilage to the product. Through both cases, this results in meat being disposed of from the food chain frequently, not only becoming an environmental burden but also an economical issue to food manufacturers. Up to 20% of the meat produced globally is either discarded due to spoilage or contamination with pathogens (Saucier, 2016).

Many groups of bacteria have members of which may cause spoilage even though the product could be stored within the correct conditions to prevent

microbial growth. This relationship between food and bacteria makes the spoilage of raw meat an extremely complex process resulting in spoilage being difficult to avoid (Jääskeläinen et al, 2016). However, prior to the installation of the UV equipment, the finished pack analyses revealed initial total viable count levels to be 4.29 log cfu/g after 1 day of packing and no visible spoilage was noticeable. This result was to be expected as good manufacturing practices coupled with the optimum storage conditions at <5C and modified atmosphere packaging would ultimately prevent or control the growth of spoilage bacteria (Zhou, Xu and Lie, 2010). The initial level of *pseudomonas* and *enterobacteriaceae* were also considerably low at 2.74 and 1.98 log cfu/g respectively. Kim and Yim (2016), similarly revealed initial microbial loads that replicate this study, suggesting that retail packaged beef typically contain total viable count levels as high as 4.81 log cfu/g. When looking at the post intervention results of this study, no significant reduction was observed ($p>0.05$) in the TVC levels. This finding was surprising and suggests that the decontamination of conveyor 2 had no impact on the microbial load of the raw diced beef.

The raw material used to produce the finished pack diced beef consisted of front quarter cuts with all external fat trimmed including but not limited to chuck tender, brisket, salmon muscle, knuckles and rump tails etc. The quality and microbiological status of this meat could be influenced by the production process. In comparison to primal steaks such as sirloin or ribeye, the diced beef would have been handled significantly more therefore increasing the risk of cross contamination prior to arriving at retail to be packaged and processed into diced

beef. For diced beef, the raw material would have gone through an extensive butchery method to achieve the specification for retail use such as slaughter, dressing, de-boning, trimming fat, trimming muscle, and vacuum packaged. All of the primal raw material has a process date of kill + 21 days, of which within this period the raw material must be used to create diced beef therefore could influence the initial microbial levels reported in this study (Mean=4.29 log cfu/g). Supporting this study, Li et al (2013) investigated the effect vacuum packaging primal cuts of beef has on the level of bacteria over various periods of time. The results displayed total viable count levels to be 2.9 log cfu/g after 14 days. In addition, Hauge et al (2015) observed similar total viable counts for primal beef products after chilling. Overall Hauge et al (2015) analysed the microbial make up of beef products through the production chain, the results revealed the highest contamination risk to be on the hides (Mean=7.2 Log cfu/cm^2) of the carcass prior to de-hiding. This statistically significantly reduced ($p<0.05$) to 4.3 log cfu/g, correlating with this study for raw material.

It could be argued that the decontamination of conveyor 2 had no impact on the quality of the finished product, due to the depth at which the diced beef sits on conveyor 2 being greater than 10cm. Although this is against company procedure to prevent meat from piling, only the meat of which is directly in contact with the belt would benefit from the decontaminated surface. Further, the raw diced production line consisted of four individual transportation systems including conveyor 1, conveyor 2 (UV treated), conveyor 3 and the ishida (Figure 2). It is therefore likely that two addition possible routes of cross contamination

occurred as conveyor 3 and the ishida harboured total viable count levels at greater than 6 log cfu/cm^2, which could potentially increase the microbial load of the raw diced beef and reduce quality.

Across each conveyor system, it could be argued that although processing the same diced beef, they may harbour different levels of TVC as displayed within this study. The difference in result could be attributed to the length of time that the diced beef is in contact with the belt for, with the longer belts having the higher level of TVC. Further to this, the difference in the level of TVC across each belt may vary due to the hygiene practices at night. If all of the belts were not effectively cleaned, this could result in cross contamination to the belt and increase TVC level. With this variance in mind, in future studies the entire processing line must be decontaminated to see the full effect on the diced beef. Without treating the full line with UV light, the later conveyor belts may act as a source of contamination.

In addition, the decontamination has no impact on the microbial levels of the finished pack. It could suggest that the source of microbial flora is not influenced by the retail environment but instead is influence by the abattoir and carcass dressing hygiene. The main source of contamination could be argued to be the point at which the hide is removed from the carcass as this contains a greater variety of bacteria due to the presence of faecal matter (Kebede et al 2016). If good hygiene is not followed at this point of production, cross contamination could occur from the hide to the carcass and result in greater levels of TVC. Further, room temperature was not included as one of the tested

variables in this study. However, the facility temperature were kept below 2°C which is a pre requisite for the HACCP plan within this study.

On pack + 9 days pre-trial, total viable count significantly increased to 5.58 log cfu/g, this was repeated for post-trial total viable counts which resulted in no significant difference between pre and post-trial with UV light. This increase from pack +1 day to pack + 9 days harvested the largest increase in total viable count between shelf life days, this could reflect the longest time period of 8 days between testing but also signify the previously reported expected growth curve of microbial flora associated with beef stored under modified atmosphere conditions. The increase in total viable counts reflected in this study, agrees with a previous study conducted by Koutsoumanis et al (2006), which investigate the development of microbial flora associated with beef under temperature controls. The results revealed initial total viable counts at 0 hour after packing were 4.5 log cfu/g, which increased to 6.7 log cfu/g after 220 hours equating to 9 days. Further, there was no significant reduction between pre and post-trial of *pseudomonas* further signifying that the ultraviolet light food contact surface disinfection had no impact on the spoilage microorganisms of the finished product. In this study, the level of *pseudomonas* had only slightly increased from pack + 1 day to pack + 9 days. This could be due to the composition of gas within the pack which contained 25% carbon dioxide. As a result of this gas combination, the presence of carbon dioxide greatly inhibits the growth of the bacteria due to it being a strictly aerobic gram-negative bacterium. Meaning that

although this ubiquitous microorganism will survive most environments, it prefers conditions of which are rich in oxygen (Yim, Jin and Hur, 2019).

In addition to modified gas composition, a different form of packaging that can be used within red meat processing is skin packaging. This form of packaging removes oxygen from the pack via a vacuum. Through removing oxygen, the level of aerobic bacteria is greatly inhibited and therefor can improve overall shelf life. However due to the product itself, this type of packaging was not suitable (Polkinghorne et al 2018).

On day 9, post-trial, the visual assessment exposed that the ultraviolet light had no effect on the visual properties of the finished raw diced beef, evidentially due to an absent change in the microbial make up it could be argued that this result could be expected. Overall 3 out of 9 packs tested on this day failed the visual assessment, with a mean score of 3.80.

Moving to the current shelf life of the product at pack + 10 days, the microbial load had not significantly reduced after treatment with ultra-violet light. Total viable count levels slightly increased to on average 1.53 log cfu/g greater than the previous day at both pre and post-trial. Overall TVC bacteria levels were as high as 6.94 log cfu/g, which could include various species of spoilage bacteria including but not limited to *brochotorix, lactobacilli, total viable count , and enterobacter* (Saucier 2016; Rodrigues et al 2018). The visual properties of the finished pack raw diced beef at + 10 days reflected the results from the microbiological analyses with a mean score of 2.90. At this point, most of the diced beef packs tested had started to show discolouration with extensive

dehydration (**Figure 5**). It can thus be suggested that the meat did not meet the correct quality standards desired by customer expectations.

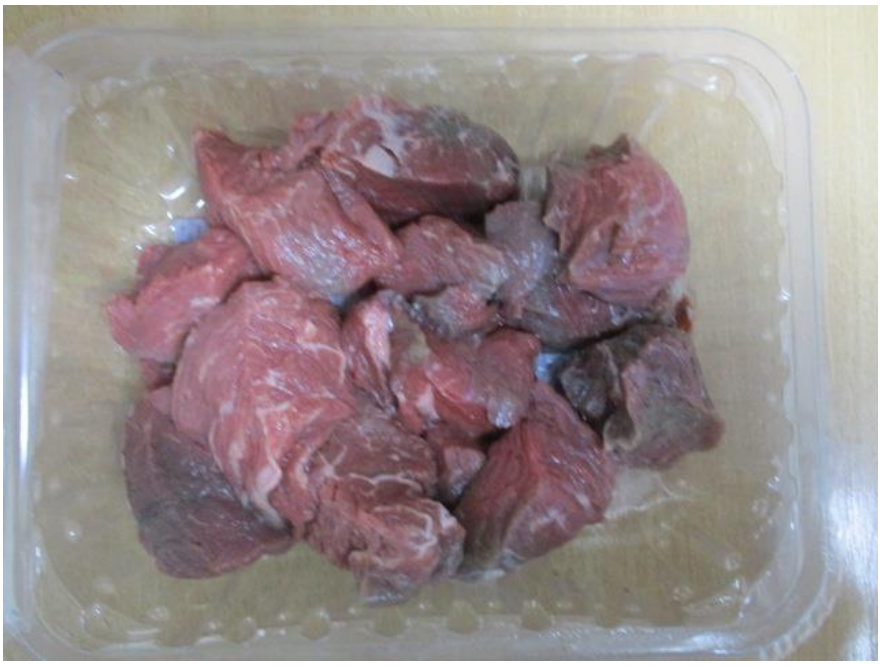


Figure 5: Raw Diced Beef Pack + 10 Days

Further on the remaining days including pack + 11 and 12 days of shelf life, total viable counts reached a maximum of 6.53 and 6.97 log cfu/g. This level of bacteria caused visible sensory degradation of the diced beef. The mean score reported in the visual assessment on both days was respectively 2.60 and 2.10 and included the greying of meat including production of putrid odours. These results are consistent with Nollet et al (2012), who examined the distribution of spoilage bacteria associated with beef over various time periods. The study revealed that over a ten-day period, beef harbours total viable count levels as high as 7-8 log cfu/g, which carries significant organoleptic defects including change in colour, altered appearance such as slime development and rancid taste. According to the European Commission (2005), meat with greater than

log 7 cfu/g must not be sold internationally due to the food safety risk and the unacceptable sensory properties associated with meat containing bacteria levels this high.

Although there is no statistically significant difference in the level of TVC between finished pack pre and post intervention Table 14 highlights that there was a small increase in the level of bacteria after the intervention. This small increase in TVC could be attributed to poor handling and GMP practices. During the post intervention, it could be suggested that there may have been a poorer level of dressing immediately after slaughter resulting in a greater level of TVC than pre intervention. If the initial microbial load of the raw material used into the diced beef was higher than the pre intervention raw material, a higher level of bacteria would result in the finished pack.

Overall *Enterobacteriaceae* encompasses a wide group of gram negative, facultative anaerobic bacteria that can be present across various food products. Within red meat specifically, enterobacteria has the ability to replicate and grow through the respiration using oxygen however can also survive in oxygen depleted environments through fermentation. Typically within the red meat industry, it is known that cross contamination with this bacteria is commonly found on the hide of the carcass and is brought into the production zone via the live carcass (Alvseike et al, 2019). Although there was no statistical significant decrease in the level of Enterobacteriaceae from pre-post trial, a small reduction of this bacteria was observed. This could indicate that the decontamination of the food contact surface may have slightly reduced the risk

of cross contamination and therefore reduced the level of Enterobacteriaceae present on the finished product (McEvoy et al, 2004). However, initial contamination of the raw diced beef pre trial (M=1.98) and post trial (M=1.94) is rather low, therefor indicating that operational hygiene measures and evisceration procedures were effective and good. Although a small increase in the level of bacteria was observed over the shelf life of the product, Crowley et al (2005) supports the results from this study through conducting a study which investigate the level of bacteria present in retail cuts of beef across Ireland. The study concluded that carcass samples reported Enterobacteriaceae to be m=1.63 log cfu/g, which increased significantly $p<0.05$ for retail packaged beef obtaining levels ranging from 2.2-4.64 log cfu/g.

5.4 Limitations

The main limitation to this study consisted of resourcing (this is a self-funded study) due to the expense of consumables to carry out microbiological testing of additional samples. Due to limited expenses, the study was restricted in terms of volume of samples which could be tested to verify the results, with additional funding/resources a longer period of testing could of provided further information regarding the success rate of the decontamination. In addition, the availability of the ultra-violet light also created severe difficulty as the researcher and the testing facility needed to source for suitable suppliers of UV light strobes. Additionally, the unit had to be gifted on a trial basis without incurring cost, therefore greatly limiting the supplier of which could be used. Furthermore, the unit itself had to be supplied from a BRC accredited supplier which meets the

company policy of the site where the testing was carried out, therefore further reducing the range of supplier which could be used. The sourcing and organisation of the installation of the UV light was significantly delayed for the reasons above. Due to the resourcing difficulties in relation to the UV unit, a greater time period with the UV unit would of aloud the study to examine a range of alternate location so decontaminate either the product or production line. For example, with more time with the UV unit the study could of compared the effect of contaminating the raw material itself against the conveyor belt. Further, due to the design of the production line, a slack within the conveyor 2 belt exists, hence the distance of which the UV unit could be installed resulted in varying distance of 7cm to 10cm. Due to the variation, it is important to bear in mind that some inconsistencies between treatments could have affected the results. Nevertheless, practical steps have been taken to ensure that the optimal and safest location was identified to install the UV light. This reiterates the practical challenges of installing novel, decontamination units on existing production facilities. In addition, the difficulty to obtain completely clean surfaces overnight in between production shifts has been highlighted throughout this study due to the frequently reported dirty surfaces at 6am. Although all staff have been trained sufficiently, the versatility of local bacteria to remain persistent throughout the production chain has proved a challenge to eradicate all bacteria.

Further to this, due to the speed of the line the limitation on the amount of contact period between UV light and contact surface (Conveyor 2) was greatly inhibited due to the speed of production. With only a short treatment time on the

surface of the belt, it could be argued that the UV light has less time to sanitize the belt and therefore had less impact on the shelf life of the finished product (Usaga et al 2016). In addition, the UV equipment utilized within this study only encompassed one wavelength, the study was limited to therefor only treating the belt at 253nm. Finally, due to the size and shape of the UV light source, the study was limited as to where the equipment called be installed on. Starting on conveyor 1, the raw material was passed up this conveyor in large blocks and there for was deemed not achievable. Conveyor 3 and the ishida were to small, with the equipment being a long thin light, botch sections of the line had no acceptable attachment point.

5.5 Recommendations for further studies

This study has revealed many questions in need for further investigation. It would be interesting to assess the effects of UV light on a single conveyor belt since this study identified other possible pathways of contamination from multiple conveyor belts.

This way the product would only be in contact with the UV treated belt and could see a greater effect as a result of the decontaminated food contact surface. Further experimental investigations are needed to estimate the optimal distance and contact time needed to decontaminate food contact surfaces in real food production facilities. Within the red meat industry, there is limited studies investigating the direct UV treatment of raw diced beef and the effects on sensory properties and the microbial load of the finished product.

Lastly, it could be recommended to instead of treating the conveyor belt, the UV intervention could be used to treat the surface of the meat whilst as a whole primal on the carcass. At this stage, after slaughter the meat remains the most susceptible to cross contamination. If UV light were introduced at this stage, the intervention could potentially decrease the level of microbes present and therefore increase the shelf life of raw diced beef.

5.6 Conclusion

This study has shown that the overnight hygiene practices were poor as consistently unclean surfaces were recorded at 6am. Further the microbial load of each food contact surfaces reached high mean levels of 6.46 log cfu/cm^2, levels this high could pose a threat to the quality of raw diced beef by cross contaminating during processing. One of the more significant findings to emerge from this study is that the ultra-violet light was deemed successful in the decontamination of conveyor 2, with significant reductions of 1.8 – 2.5 log being observed at both 12pm and 3pm. hence supporting Hypothesis 1 that the uv light will decontaminate the conveyor by reducing the overall microbial count. However, this log reduction had no physical effect on the microbial or visual properties of the finished pack. Microbiologically, the raw diced beef from this study had failed on pack + 10 days, with high TVC levels measuring 7.92 log cfu/g which caused dramatic sensory deteriorations including off smell and grey colour. Therefore, hypothesis 2 was rejected as the reduction of TVC levels on food contact surfaces did not increase the shelf life of raw diced beef.

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RISK ASSESSMENT FORM

Risk Assessment For	Assessment Undertaken By	Assessment Reviewed
Service / School: Sport and Well being	**Name: Rhys Pugsley**	**Name:**
Location of Activity: Darwin Microbiology lab	**Date: 20/03/19**	**Date:**
Activity: Tissue culture training Date of activity: **REF: 20/03/19**	**Signed by Dean of School, Head of Service or nominee:** **Date**	

List significant hazards here:	List groups of people who are at risk:	List existing controls, or refer to safety procedures etc.	For risks, which are not adequately controlled, list the action needed.	Remaining level of risk: high, med or low
Working with cell cultures and potential pathogenic bacteria - Infection	Staff, students	Wear adequate PPE with nitrile disposable gloves Use a biological safety cabinet Complete COSHH risk assessment		Low

List significant hazards here:	List groups of people who are at risk:	List existing controls, or refer to safety procedures etc.	For risks, which are not adequately controlled, list the action needed.	Remaining level of risk: high, med or low
		Operative trained and familiar with Appendix 3 of SHE 067 Biological and GMO Safety Rev 1 Procedure for the use of Biological Safety Cabinet in conjunction with good microbiological techniques Treat all microorganisms as potentially pathogenic		
Spillages	Staff, Students	Wear appropriate PPE Complete autoclave procedure to correctly disinfect surfaces and prevent contamination		Low
Glass breakage	Staff, Students	Ensure glass wear is in the correct condition for use Ensure correct storage of glass wear to prevent breakage		Low

List significant hazards here:	List groups of people who are at risk:	List existing controls, or refer to safety procedures etc.	For risks, which are not adequately controlled, list the action needed.	Remaining level of risk: high, med or low
Lone working – Potential injury or accident	Student	All lab work will be conducted under supervision of Principal supervisor in compliance with FM SHE 011 Lone Worker Guidance		Low

CoSHH RISK ASSESSMENT FORM. (*Page 1 of 2)*

School/Service	Assessors Name(s)	Job Title/Position	☎
Sport and Well Being	Rhys Pugsley	Student Researcher	07555377620

Briefly describe the task/process. (description, use, users)

Enumeration of *total viable count* from an environmental swab

Substances (used or produced as by-products or wastes)	Quantity	Hazard Class	WEL	Exposure Route(s)	Frequency and Duration of Exposure	Known Health Effects:
Class 2 pathogens Aerobes	Variable -if detected positive, quantity can be greater than 100CFU/g	Harmful risk Class 2 Biological hazard	NA	Ingestion Inhalation Absorb through skin Eye contact	5 hours per week over a 4-week period	Harmful Xn R 22 S 36 RG2 Possibility of infection.
Results of Relevant Health Surveillance				Results of Exposure Monitoring		

Control Measures				
☐ Elimination	☐ Substitution	☐ Reduction	☐ Isolation	√ Eng. Control
Details	*Details*	*Details* The number of samples analysed reduced	*Details (glovebox)*	*Details(LEV, fumehood)* Biological Safety Cabinet Class 2
Further Details (if required) All research is completed in compliance with Good Microbiological Practice section 6.2 of SHE 067 Biological and GMO Safety Rev 1 Correct Hygiene practice, with sufficient hand wash facilities are available Operative trained and familiar with Appendix 3 of SHE 067 Biological and GMO Safety Rev 1 Procedure for the use of Biological Safety Cabinet in conjunction with good microbiological techniques				
Personal Protective Equipment				
X Gloves	X Eye protection	X Coverall/lab coat	X Foot protection	☐ Respiratory protection
Details Nitrile Disposable	*Details* Safety Glasses	*Details* Lab coat worn throughout testing	*Details* Closed toe shoes worn through testing	*Details:* N/A
☐ Health Surveillance required No		☐ Exposure monitoring required no		

Emergency Arrangements

First Aid:	
Eyes	Flush eyes and face directly for approximately 15 minutes at the eyewash station within the lab
Skin	For areas of intact skin, wash with soap and water for a minimum of 15 minutes. In the event of an open wound or sore wash with both soap and water for a minimum of 20 minutes and rinse sufficiently. Remove contaminated clothing and shoes

Ingestion	If ingestion occurs rinse mouth thoroughly with water provided person is conscious and consult a physician
Inhalation	Move to fresh air and contact a physician
Fire: Extinguisher Type	

X Water	X Foam	X Powder	X CO_2

Spillage/release:

Biological Safety Cabinet

Keep the cabinet on and running

Inform all people in surrounding area

Cover an area twice the size of the spill, with paper towels soaked in disinfectant (1% virkon or 70% methylated spirits) as per the label's directions

Allow for a contact period for the duration of 20-minutes

Continue to wipe down any potential contaminated equipment of furniture using disinfectant

Use brush or tongs to remove any sharps or broken glass that may have occurred and place in sharps container

Remove the paper towels and re-clean entire area with disinfectant

Decontaminate via the autoclave procedure all equipment used to clean up a spillage

Inform all other laboratory colleagues and personal that clean up is complete

If the spill has resulted in material entering the catch basin bellow the work surface, ensure that disinfectant is added at an equal volume to the quantity in the basin and allow to soak for 20 minutes with paper towels.

Once complete – ensure cabinet runs for a duration of 10 – 20 minutes before work is resumed

If a major spill or loss of contaminant occurs – contact the BSO and SHE directly. Spill kit located in DB 305

Waste Disposal procedure

Plates will be placed in biohazard autoclaveable bags and will be identified as safe to handle before being removed from the laboratory via the autoclaving procedure. Each waste bag will be clearly labelled stating the type of waste and the responsible researcher who created it. The responsible researcher will dispose of the waste directly via the designated and suitable routes as per Uclan's Health and Safety regulations.

1% virkon will be added to liquid cultures and left for 24 hours before following standard disposal proceedures in line with thee BSO.

Persons likely to be exposed

X Staff	X Student	☐ Visitor	☐ Contractor
☐Public	☐ Other (specify)		

Additional risks: for example circumstances where work will involve exposure to more than one substance hazardous to health, consider the risk presented by exposure to such substances in combination. Also, non-routine maintenance may present additional risk of exposure.
All samples will be labelled in compliance with section 6.1 Signage and Labelling of SHE 067 Biological and GMO Safety Rev 1 Treat all microorganisms as potential pathogens. All lab work will be conducted under supervision of Principal supervisor in compliance with FM SHE 011 Lone Worker Guidance

Signed by Dean of School, Head of Service or nominee:		Review date due:	
Date:			

Notes:

<u>Hierarchy of control</u>

Change the task or process so that the hazardous substance is not required or generated.
Replace the substances with a safer alternative.
Totally isolate or enclose the process.
Partially enclose the process and use local exhaust ventilation.
Ensure good general ventilation.
Use a system of work that minimises the chance and degree of exposure.
Provide personal protective equipment (PPE).
Train and inform staff in the safe system of work and risks.
Additional supervision.

Examination, testing and maintenance of engineering controls and/or PPE.
Monitoring of exposure.
Health Surveillance.
Other (specify).

Paired Samples T-Test – Significant difference on Conveyor 2 (UV Intervention)

```
Your license renewal date has passed. This product will stop working if a new license is not installed soon.
T-TEST PAIRS=PRE_12 PRE_3 WITH POST_12 POST_3 (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

[DataSet0]

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	PRE_12	5.9083	6	.30149	.12308
	POST_12	3.3817	6	.29822	.12175
Pair 2	PRE_3	6.2200	6	.06633	.02708
	POST_3	4.4083	6	.57701	.23556

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	PRE_12 & POST_12	6	.395	.438
Pair 2	PRE_3 & POST_3	6	.642	.169

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	PRE_12 - POST_12	2.52667	.32989	.13468	2.18047	2.87286	18.761	5	.000
Pair 2	PRE_3 - POST_3	1.81167	.53682	.21916	1.24831	2.37503	8.267	5	.000

Paired Samples T-Test – Significant difference on Conveyor 3

```
T-TEST PAIRS=PRE_12 WITH POST_12 (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	PRE_12	6.0600	6	.17978	.07339
	POST_12	5.8600	6	.11696	.04775

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	PRE_12 & POST_12	6	.348	.499

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	PRE_12 - POST_12	.20000	.17709	.07230	.01416	.38584	2.766	5	.040

Paired Samples T-Test – Significant difference on Ishida

```
T-TEST PAIRS=PRE_6 WITH POST_6 (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	PRE_6	2.9283	6	.60314	.24623
	POST_6	2.2150	6	.72660	.29663

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	PRE_6 & POST_6	6	.533	.276

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	PRE_6 - POST_6	.71333	.65173	.26607	.02939	1.39728	2.681	5	.044

Paired Samples T-Test – Significant difference Pseudomonas Pack + 9

```
T-TEST PAIRS=Pre_Pseu_9 WITH Post_Pseu_9 (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pre_Pseu_9	2.5111	9	.55777	.18592
	Post_Pseu_9	2.7667	9	.56347	.18782

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Pre_Pseu_9 & Post_Pseu_9	9	.916	.001

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Pre_Pseu_9 - Post_Pseu_9	-.25556	.22973	.07658	-.43214	-.07897	-3.337	8	.010

Paired Samples T-Test – Significant difference Enterobacteriaceae Pack + 12

```
T-TEST PAIRS=Pre_Ent_12 WITH Post_Ent_12 (PAIRED)
  /CRITERIA=CI(.9500)
  /MISSING=ANALYSIS.
```

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Pre_Ent_12	2.4422	9	.72952	.24317
	Post_Ent_12	1.9322	9	1.11939	.37313

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Pre_Ent_12 & Post_Ent_12	9	.879	.002

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Pre_Ent_12 - Post_Ent_12	.51000	.59121	.19707	.05556	.96444	2.588	8	.032