Refurbishing Raw Denim to Reduce Bacterial Populations

by

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Abstract

Raw denim jeans are an emerging trend where a unique pattern of fading is created as the jeans mold to the wearer's body. Raw denim differs from traditional denim as it has not been treated or washed before being sold to consumers. Brand new raw denim has been suggested to be worn continuously without washing (at least six months) to preserve the customized fades and marks. Minimal washing thereafter is suggested to reduce the effects of degradation from laundering. As a result, practices to care and maintain raw denim jeans are recommended on raw denim websites, one of which includes freezing the jeans in order to reduce bacteria and unpleasant odour that may develop as a result of minimal washing. However, the efficacy of freezing at reducing bacteria is unsubstantiated. Other alternative refurbishing methods that may reduce bacteria are ironing and exposure to UV radiation (sunlight). The purpose of this research was to examine three selected alternative refurbishing methods in comparison to washing raw denim fabrics. An *in vivo* method was used to collect bacteria on fabrics where human participants (n=6) wore raw denim fabrics on the posterior forearm for 24 hours. Worn fabrics were subjected to refurbishing treatments. Aerobic bacteria were extracted and plated on a non-selective microbiological media where viable bacteria were counted and compared with the baseline counts (i.e., viable bacteria obtained from fabrics immediately after wear). Findings show that the alternative refurbishing treatments were not as effective at reducing bacteria as washing. Immediately after the treatment (0 hours), washing had the highest bacterial reduction with all counts being below the limit of detection, and bacteria did not grow during the 24-hour period post-treatment. Freezing was initially more effective than ironing and UV radiation. However, over the additional 24-hour period post-treatment bacteria grew more rapidly on denim fabrics that had been frozen, than those that had been ironed or exposed to UV radiation. Despite the interest from raw denim enthusiasts to refresh their clothing without the use of the washing machine, washing was the most effective treatment for both initial and sustained bacterial removal.

Preface

This thesis is an original work by Kathryn Anne-Mariko Wakefield. No part of this thesis has been previously published. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, "Refurbishing raw denim to reduce bacterial populations", No. Pro00075979, April 18, 2018.

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List of Abbreviations

AATCC	American Association of Textile Chemists and Colorists
ANOVA	Analysis of Variance
CFU/mL	Colony Forming Units per millilitre
CGSB	Canada General Standards Board
df	degrees of freedom
g	grams
h	hours
min	minutes
mL	millilitre
mm	millimetre
nm	nanometre
R.H.	Relative Humidity
°C	Degrees Celsius
P	P value
%	percentage
>	greater than
μL	microlitre

Chapter 1

Introduction

1.1 Background

Jeans are seen and worn almost everywhere and are a staple of many wardrobes; truly a global textile (Miller & Woodward, 2012; Su & Tong, 2016; Wu & Delong, 2006). Consumers have sought out jeans not only for their functional and utilitarian properties (Miller & Woodward, 2007) but also for their fashionable and ever-changing appearance (Card, Moore, & Ankeny, 2006; Rahman, 2015). While often referred to as an iconic American garment (Sullivan, 2007; Wu & Delong, 2006), ethnographic researchers have otherwise observed jeans commonly worn among pedestrians in public spaces as diverse as London (Miller, 2010) and Shanghai (Wu & Delong, 2006). Over time, the appearance of denim has been modified greatly as designers are able to creatively alter denim garments, appealing to consumers through various applications and treatments, adhering to emerging trends (Memon, 2014).

Denim treatments and finishes can drastically transform the appearance and feeling of the original fabric. Many of these treatments were initially intended to soften the inherent stiffness of denim to increase comfort and mobility for the wearer. Treatment methods are often chemical or mechanical, evolving with the advancement of technology and desired creative effects as well as the availability of required resources (DeLong, Koh, Nelson, & Ingvoldstad, 1998; Midha, Kumar, & Kumar, 2017). Common chemical treatments include, but are not limited to, bleaching (discoloration of sections or the entire garment) (DeLong et al., 1998) and enzyme washing (creating the appearance of texture on specific areas without degrading or reducing the tensile strength of the fibres) (Tarhan & Sarusk, 2009). Mechanical treatment methods include sand blasting, stone washing and grinding (Kan, 2015), which can fade the garment in unique patterns. Acid washing is another popular treatment, combining chemical and mechanical techniques (Kan, 2015). No acid is actually used; this process involves washing the garment with the addition of pumice stones and bleach to reduce the stiffness of denim, providing a softer feel. Prior to final distribution, traditional denim is often washed in a process known as sanforization (Card et al., 2006; El-Ghezal, Babay, Dhouib, & Cheikhrouhou, 2009;

Kalaoglu & Paul, 2015). Sanforization can set the colour from wet fabric dyes, improve structural stability (including shrinking before construction) and relax the rigid fabric (Coe, 2011; McKay & McKay, 2014). Notwithstanding the continued consumption and popularity of jeans altered by the use of chemical or mechanical techniques, a recent trend has emerged in response, devoid of these applications; it is referred to as raw denim.

Primary characteristics that distinguish raw denim (from its traditional counterpart) are the coarse hand of the fabric as it is devoid of softening treatments (Coe, 2011), and the absence of any mechanical or chemical applications. A dedicated raw denim subculture has developed to discuss and enlighten consumers regarding raw denim (Coe, 2011; Stege Bojer, 2017). In particular, the topic of cleaning raw denim is widely debated. Though some raw denim owners wash and dry their garments as they would other laundry, others argue against this in support of alternative solutions (Anonymous, 2014; Chad, 2011; Maxwell, 2016). Because raw denim is not washed or treated during the production and assembly of the garment, the fabric is susceptible to shrinkage, colour transfer and fading in high friction areas when washed by the consumer (McKay & McKay, 2014). A unique garment is created as the jeans mold to the body shape of the wearer, being influenced by the care and washing procedures more so than traditional denim jeans. Additionally, minimal use of household washing machines has been encouraged by environmentally conscious researchers (Laitala, Klepp, & Boks, 2012; McQueen, Batcheller, Moran, Zhang, & Hooper, 2017) as a method to promote sustainable behaviours by reducing the consumption of energy and water. The environmental benefits of using fewer resources has been remarked by raw denim enthusiasts, as well, further reinforcing the justification for reduced raw denim washing.

Since denim jeans (especially raw denim) are highly susceptible to influences such as wet abrasion, heat, and friction produced by household machine washers and dryers, the pursuit of alternative methods to clean or refresh the garment could lead to extending the life and use of the garment. To minimize these effects, raw denim manufacturers and vendors have suggested alternative ways for care and refreshment after multiple wears. Some suggest that the first wash of raw denim should occur only after six months of continuous wear (Wolfe, 2015). Refraining from early washing also allows the wearer to control the fading of the garment,

based on how it is worn and stored, contributing to the uniqueness of worn raw denim. Users interested in maintaining their customized raw denim while being concerned with odour and bacterial build up are encouraged by online media to incorporate alternative refurbishing methods in place of conventional laundering practices (Anonymous, 2014; Chad, 2011). Should the jeans be washed, then some users propose drying them in the sun instead of tumble drying to protect against the heat and friction that may cause degradation ("Expert Denim Care," n.d.; Makers, 2015; McKay & McKay, 2014). Recently, sources within popular media have proposed freezing raw denim as an alternative refurbishing treatment in place of laundering (Chad, 2011; Levinson, 2013). However, there is some debate regarding the effectiveness of freezing raw denim, as the evidence is largely anecdotal. Based on some online reviews, freezing is unlikely to remove bacteria or odour (Philipkoski, 2011; Stege Bojer, 2017; Zielinski, 2011).

The practice of laundering clothing is part of the complex convention of cleanliness, which includes frequent and common household actions (Jack, 2013; Pakula & Stamminger, 2010). Laundering practices are a crucial factor contributing to care and maintenance, which can affect the longevity of clothing garments (Laitala et al., 2012; Pakula & Stamminger, 2010). Care of some garments can become complicated, as is the case with raw denim jeans (Craig, n.d.; O'Connor, 2016). The challenge of maintaining raw denim is exacerbated by the owner's desire for fresh and stain-free clothing (Klepp, 2003) as care directions discourage any laundering for considerable periods of time (up to 6 months) (Chad, 2011; O'Connor, 2016). Concerns with minimal laundering of garments after multiple wears may arise as stains, dirt, bacteria and odour may build up.

Increased bacterial reduction has been achieved by ironing hospital and medical uniforms where employees are required to home launder their garments (Bloomfield, Exner, Carlo, & Scott, 2013; Patel, Murray-Leonard, & Wilson, 2006). Ironing imposes little abrasion to raw denim, is low-cost, consumes few resources, and is widely available. Similarly, sunlight exposure has been mentioned on raw denim websites as both a method for drying as well as for reducing odour (Dylan, 2017; Fields, 2016). Focused ultraviolet (UV) light is frequently used for sterilization. Examples include medical tools (Benson, 2002) and raw foods (Cilliers et al., 2014). But its effectiveness on raw denim is unclear. Given the degrading effect of frequent laundering

of denim (McQueen et al., 2017), finding alternatives to the washing machine which are less abrasive are of high importance for raw denim owners.

1.2 Statement of problem and purpose

Because common washing practices are often accepted without question, they are repeated as part of a normal routine (Jack, 2013). While the practice of caring for delicate garments has been widely developed and accepted, the proper method of care for raw denim is widely debated. Arguments for avoiding laundering have been fuelled by considerations of sustainability (Newman, 2011) and garment preservation (Maxwell, 2016). However, suggested alternative methods of refurbishing such as freezing denim are unsubstantiated and even criticized by other online sources (Cox, 2014; Zielinski, 2011).

Evidence supporting these claims is primarily anecdotal with little to no scientific investigation carried out. As a result, consumers may be misled and believe that their raw denim jeans have been "cleaned" when following such procedures. Therefore, the focus of the research described herein was to explore the effectiveness of alternative refurbishing or "cleaning" procedures on raw denim, systematically compared with laundering.

1.3 Research questions

The research questions were:

1) How effective are three selected alternative refurbishing methods (freezing, ironing or UV) on reducing skin bacteria collected on denim when compared with conventional laundering procedures?

2) How effective are three selected alternative refurbishing methods on maintaining low bacterial populations on denim over time when compared with conventional laundering procedures?

The three alternative refurbishing methods were selected based on methods suggested within raw-denim popular culture as well as other procedures typically used for maintaining clothing. Since, in real-life situations, raw denim would likely be worn for many hours after the

refurbishing treatment, what occurs to bacterial populations over time was included as a variable to assess growth behaviour following the selected treatments.

1.4 Research hypotheses

The following null hypotheses have been posed:

H_{o1}: There are no significant differences among the four refurbishing methods in the reduction of skin bacteria.

H_{o2}: There are no significant differences between time 0 hours and 24 hours in the reduction of bacteria after each of the four refurbishing methods.

1.5 Limitations and delimitations

1.5.1 Limitations

Denim samples were only worn for twenty-four hours before the refurbishing treatment (denim swatches were clean and sterilized), whereas, realistically, jeans would be subjected to multiple wears over the course of many months.

The UV treatment was simulated using a machine that provided the specified amount of light exposure for five and a half hours but did not directly reflect exposure to outdoor sunlight which includes other factors such as air circulation from the wind. Additionally, the variability delivered by ironing manually (as opposed to the other treatments which used a standardized machine) may impact the consistency of the results.

1.6 Terms and definitions

In the context of this research, the following terms are defined:

Aerobic bacteria	bacteria able to survive and grow in oxygenated environments (Murray, 2005)
Anogenital region	relating to anal or genital regions of the body (Murray, 2005)

"Cleaning"	processes suggested to refresh or remove bacteria from raw denim by online media or raw denim enthusiasts
Cutaneous	relating to the skin
Gastrointestinal system	system of the body including the digestion of nutrients and excretion of waste products
Microflora	collective microorganisms (including bacteria) found within an ecosystem
Occluded	to block or cover an area
Percent change (% change)	a calculation used to quantify the variable gain or loss of results by comparing initial and final numerical observations
Refurbishing	a process to restore clothing to a like-new condition or appearance

Chapter 2

Review of Literature

2.1 Introduction

A recent trend among denim producers is to supply jeans for distribution that have not had any initial mechanical or chemical treatment. The primary objective of this research was to explore care and maintenance recommendations proposed by raw denim manufacturers and popular media using scientific methods. Many alternative refurbishing treatments, in particular freezing, have been recommended to replace the machine washer and dryer. In order to understand the various components of this experiment, this review will discuss each section of the research problem. Included is an examination of denim's composition, history and evolution as a fashion garment, followed by an analysis of selected cleaning and refurbishing treatments. This section will conclude with information related to skin bacteria and textiles.

2.2 Denim

2.2.1 Denim composition and structure

Historically, denim jeans have been composed of 100% cotton (including raw denim), however some modern jeans are comprised of a blend of other materials (Humphries, 2009; Midha, Kumar, & Kumar, 2017) including an elastic component for stretch and comfort (Kumar, Chatterjee, Padhye, & Nayak, 2016). While denim fabric can be used for other garments and textiles such as shirts, jackets and bedding, this study will focus on jeans and research specific to that garment. The fabric itself is warp dyed (leaving the weft yarns uncoloured) and can be a 2/1 or 3/1 twill weave (Humphries, 2009).

2.2.2 History

It is well understood that the original intent of denim jeans design was utilitarian in nature. The versatility and storied history of the garment has led to its lasting presence in household wardrobes and global fashion collections (Kumar et al., 2016; Sullivan, 2007). Although much of the research does not distinguish raw denim from treated denim, it is still

relevant to this review as it pertains to denim jeans as a garment and not specifically the treatments and fibres of the garment.

The history of denim jeans documents their evolution from a functional and utilitarian garment to an iconic fashion piece, prevalent in popular culture. Few other garments rival the longevity of denim jeans, especially on such a global level. The roots of denim originate in Europe (Solomon, 1986) but it was when Levi Strauss brought his ideas and the fabrics to the United States that the popularity of denim significantly increased (Sullivan, 2007). Denim jeans have also been known as a garment that has represented different symbols and values over time ("Driving increases in the global denim market," 2018), ranging from blue collar socioeconomic status (Sullivan, 2007) to rebellious youth (DeLong et al., 1998). Denim jeans were also seen as distinctly American, particularly by Europeans in the 1960's and 1970's as American tourists easily stood out to locals due to their use of blue jeans (the garment's availability and popularity was just beginning in Europe). During this time, one of the most common jeans styles available was designed with exaggerated flared legs, known as bell bottoms. Jeans have also symbolized political movements. The uneasy political temperature in the United States during the Korean War and Vietnam War of the 1960s led to protests that primarily included young adults (Sturken, 1997). Denim was closely associated with this movement (Solomon, 1986). At present, current reports suggest denim sales are not slowing down ("Driving increases in the global denim market," 2018) and designers are more than ever creating new trends, refurbishing old trends and looking for new ways to reinvent the garment (Friedman & Friedman, 2017).

2.2.3 Consuming denim

Denim has been a subject of interest not only in the fashion industry but for its cultural meanings, consumer behaviour and scientific research. From the popular culture representations of blue jeans (Comstock, 2011; Kalaoglu & Paul, 2015; Rahman, 2012), the expanding availability of the garment in street fashion in Asia, Europe, Africa and North America (DeLong et al., 1998; Keet, 2011; Miller, 2010) confirms the global ubiquity of jeans. Interestingly, the same features of denim can be perceived differently analyzed in two different cultures (Rahman, Jiang, & Liu, 2010). Research with post-secondary students in the United

States indicated that jeans strongly symbolize American cultural values and social expectations such as comfort, informality, and versatility (DeLong et al., 1998). Around the world, denim is visibly worn and highly consumed. While South America is one of the few areas of the world where denim jeans have not been widely adopted (Miller, 2010), it has been observed that, in Brazil, some have adopted specific denim styles and fits to enhance desirable parts of the body for special occasions (Mizrahi, 2011). In South Korea, clothing frequently observed in public spaces is not traditional but more casual including western brand jeans (Geum & DeLong, 1992). Vintage and rare American Levi jeans are coveted in Japan, with enthusiasts offering a premium price to secure a pair (Keet, 2011). Keet (2011) noted that denim in Japan may be worn both as a common garment as well as something that could stand out, based on modifications to the style, colour or fit. In London, Miller (2010) observed public sidewalks and saw the majority of people passing by wearing denim jeans. Miller noted that those passersby were wearing numerous different styles and fits of denim, including a large percentage of garments that were unbranded, only being distinguished as denim (Miller, 2010). Consumer behaviour research has focused on denim to analyze perceptions of the garment's quality and price, cross-cultural views of foreign brands, features and colours and the consumption habits of various demographics (Miller & Woodward, 2012; Rahman et al., 2010).

2.3 Refurbishing treatments

2.3.1 Laundering

Washing clothing is an important element in garment maintenance and comes from a cultural desire for cleanliness. Caring for clothing can be a time consuming process which requires energy and attention, necessary to not only maintain the integrity of garments over time (Laitala, Boks, & Klepp, 2011; Morris & Prato, 1982) but also to return the garment close to its original state (Nayak & Ratnapandian, 2018). The widespread adoption of the washing machine has led to this appliance becoming the primary source for cleaning clothing in North America (Pakula & Stamminger, 2010). The effectiveness of the washing machine can be broken down into factors known as the Sinner Circle (Alborzi, Schmitz, & Stamminger, 2017; Ferri et al., 2016; Jakobi, 1987) which includes temperature, chemical action, mechanical agitation, time

(Ferri et al., 2016) and water (Alborzi et al., 2017), whose combination creates an interactive system for cleaning clothing and textiles, removing bacteria, stains and soils as well as odour. It has been shown that the combination of these factors varies globally, where altering one (reducing cycle time, for example) can be made up by increasing another (using hotter water temperatures) (Alborzi et al., 2017; Bockmühl, 2017).

Effective bacterial removal from worn clothing by laundering can be challenging at the domestic level as bacterial presence is not easily detectable. Comparative laundering research has explored the effectiveness of different washing temperatures due to the trend of using colder wash temperatures as a more sustainable alternative (Lakdawala, Pham, Shah, & Holton, 2011; Smith, Neil, Davidson, & Davidson, 1987). Lower temperatures, however, are not always as effective as higher temperatures at removing bacteria. Recovery of *Staphylococcus aureus* and *Escherichia coli* was observed at 40 °C and not at 60 °C on washed cotton and polyester samples (Riley et al., 2017). Although *Staphylococcus aureus* was effectively removed from medical scrubs at 40 °C and 60 °C, gram-negative bacteria was recovered from scrubs washed at 40 °C. Also at 60 °C, *Staphylococcus aureus* was removed from pockets and washing ballasts but not at 40 °C (Patel et al., 2006). What was notable regarding the presence of gram-negative bacteria was that it was likely attributed to a build-up in the washing machine (Lakdawala et al., 2011), potentially contaminating subsequent loads (Gattlen, Amberg, Zinn, & Mauclaire, 2010; Munk, Johansen, Stahnke, & Adler-Nissen, 2001). The interaction of chemicals and temperature with bacterial contamination is discussed by Blaser et al. (1984) and can influence the efficacy of bacterial reduction (Jaska & Fredell, 1980). The effectiveness of laundering becomes of concern with worn medical related garments, which can act as a vehicle for the movement of microorganisms and may not be completely removed when domestically washed (Munoz-Price et al., 2012). Worn medical uniforms and coats have tested positive for *Staphylococcus aureus* (Munoz-Price et al., 2012; Wong, Nye, & Hollis, 1991), gram-negative bacilli (Babb, Davies, & Ayliffe, 1983) and enterococci (Munoz-Price et al., 2012) and can be dangerous to vulnerable patients when exposed (Munoz-Price et al., 2012). Harmful microorganisms can survive laundering at lower temperatures (compared with high temperatures used in industrial facilities) which may contaminate other clothing or the machine itself (Riley et al., 2017). The

addition of bleach was shown to provide additional bacterial reduction but can be harmful to the fabric's colour and any surface finishes over time (Mitchell, Spencer, & Edmiston, 2015). The use of detergent (both biological and non-biological) was more effective at reducing methicillin-resistant *Staphylococcus aureus* than water and mechanical agitation alone for both 40 °C and 60 °C temperatures (Lakdawala et al., 2012).

An additional concern for worn clothing is the presence of odour (Laitala et al., 2012) as its detection can prove to be embarrassing in public spaces. Sources of odour include the secretion of an individual's natural oils with cutaneous bacteria (Kanlayavattanakul & Lourith, 2011), exercise and sweat. Odour may also come from the environment such as cooking or smoking (Viessman, 1964) all of which may be unpleasant when detected. Thus, the pursuit of effective odour removal from clothing has been researched, often with the adjustment of various laundering factors, to monitor overall effectiveness. Lower washing temperatures have been suggested for sustainable or energy saving objectives, but are often not recommended for removing noticeable odours on clothing (Laitala et al., 2012). Furthermore, as lower temperatures have led to the presence of biofilm accumulation in washing machines, odour can result and transfer to laundered clothing as well (Munk et al., 2001). Exploration of odour removal on clothing has had mixed results when evaluating different washing liquids and combinations of lipase formulas on multiple fabrics using human sweat and sebum (Munk, Münch, Stahnke, Adler-Nissen, & Schieberle, 2000). Odour has been found to differ in intensity and ease of removal, and can depend on the fabric blend (Callewaert, Van Nevel, Kerckhof, Granitsiotis, & Boon, 2015). Evaluations of synthetic and treated fabrics present with human sweat and odour suggested further washing when compared to aired natural fabrics (Klepp, Buck, Laitala, & Kjeldsberg, 2016). Moreover, natural fabrics were found to have accumulated fewer odours than polyester after twenty wearings and washes (McQueen et al., 2014). Further comparison of these natural and synthetic fabrics have shown that polyester retains odour more readily than cotton after washing at 30 °C or 40 °C in different water hardness levels (Munk et al., 2001). Cotton fabrics have had odour more successfully removed from washing at 30 °C than polyester but, overall, hotter temperatures were recommended for odour reduction (Munk et al., 2001).

Visible soils on clothing may be considered socially unacceptable (Pink, 2005) and laundering clothing is often used to remove soils, making clothing visibly acceptable for the next wear (Yates & Evans, 2016). The composition of soils can vary and effective removal differs based on the nature of the composition and the detergent type (Bajpai & Tyagi, 2007). Detergents themselves are complicated (Bajpai & Tyagi, 2007) with ingredients added for enhanced brightness, stain removal and refreshing scent among many others. The effect of detergent type was evaluated by Laitala and Kjeldsberg (2012) who found that eco-friendly detergents were comparable to water alone, but not as effective as household detergents when evaluating stain removal at 40 °C. Some detergents are suited for the removal of stains from blood or lipids (Mukherjee, 2007), while others are suited for lower water temperatures to mimic the results often found from higher water temperatures (Choudhary, 2012). The efficacy of stain and soil removal using the washing machine has been evaluated by adjusting multiple combinations of detergent type and amount, water temperature, and cycle length to identify an effective combination. Visual stain removal was rated high when clothing is washed with a bleaching agent (Feather, Caselman, & Cooper, 1993), however bleach is not recommended for coloured or delicate textiles. Ultimately, at some point, visible stains that have accumulated over time and over numerous washes may not be removable (Murata, Hoshino, & Suzuki, 1992).

As mentioned from online media regarding raw denim, the washing machine may cause degradation to raw denim jeans, which is one of the main reasons for avoiding laundering as much as possible. Degradation is a concern for raw denim owners who may want to protect their clothing. Abrasion from laundering has been shown to contribute considerable damage to the garment (up to 50%) (Bresee, Annis, & Warnock, 1994). Furthermore, degradation effects on denim, researched by Card et al. (2006) saw pilling and edge abrasion. This further reinforces the interest in exploring alternative methods to protect garments. Laundering denim has also been shown to cause fibre loss, colour fading and reduced tensile strength (McQueen et al., 2017).

In the current study, 40 °C was the wash temperature chosen to explore the impact of numerous alternative treatments when compared with washing. While 40 °C temperatures may

not have been shown to be consistently effective in eliminating bacteria, the use of lower water temperatures was chosen because reduced resource consumption is valued by raw denim enthusiasts. The experimental design used separate canisters used for washing (not washing machines).

2.3.2 Freezing

The freezer has been promoted as an environmentally sustainable option among raw denim enthusiasts. Since the freezer is already in use, thus no additional energy or water will be consumed. Furthermore, the freezer does not facilitate the dye to bleed thereby retaining the unique appearance of the wearer's jeans. Many raw denim enthusiasts consider freezing to be a viable option for refurbishing their jeans because they believe it is a way to: 1) reduce bacteria; and 2) to reduce odour (Chad, 2011; Levinson, 2013; Lutz, 2015; Maxwell, 2016). Reportedly, when denim is put in the freezer, bacteria are then "shock[ed]" and the cold temperature "...kills all bacteria that may be accumulating..." (Chad, 2011). One raw denim owner, after expressing initial skepticism, acknowledged that after freezing her jeans, they seemed fresher and did not "...smell anymore" (Levinson, 2013). However, one shortcoming of freezing is that it its inability to remove surface stains and visible soiling which may accumulate after long-term continuous wear (Dachis, 2011).

Claims for freezing raw denim as a method for bacterial reduction from online sources are compelling. Since bacteria are indeed accumulated on the garment after continuous wear with no subsequent washing, there is a concern bacterial removal will alter the garment. Freezing is intentionally used in several applications including the preservation of bacteria so they remain viable once thawed. Examples include preservation of lactic acid bacteria for fermentation starters (Carvalho et al., 2004; Selmer-Olsen, Birkeland, & Sørhaug, 1999) and soil research to evaluate seasonal growth effects (Morley, Trofymow, Coleman, & Cambardella, 1983). Agricultural research benefits from bacterial freezing research as particular species of bacteria contribute to crop yield and health as well as overall soil productivity (Ehrlich, 1998; Hayat, Ali, Amara, Khalid, & Ahmed, 2010). Soil bacteria that are frozen over the winter season

thaw and survive during warmer seasons, where they are then able to begin reproducing in the soil.

In the food industry, freezing is used in a different way, to prevent food from perishing Spoilage which renders transportation and storage challenging and complicated (Archer, 2004). While the intention is to inhibit growth of bacteria that can breakdown foods, unfortunately, in some cases, harmful bacteria survive and can cause illness when ingested (Lund, Baird-Parker, & Gould, 2000). Outbreaks of disease-causing *E. coli* have been harmful to consumers, and some cases have been traced to frozen meats where the bacteria have survived (Ro, Ko, & Yoon, 2015). While bacterial counts are reduced once frozen (Morley et al., 1983; Skogland, Lomeland, & Goksøyr, 1988), sufficient viable bacterial counts are present to reproduce as the temperature increases.

Freezing is also used in textile conservation in an attempt to combat insect and bacterial growth (Peacock, 1999). Typically, a complex method of freezing, thawing and rapid refreezing has been developed to combat the growth of beetle larvae and eggs (a major concern for museum textile conservation) (Florian, 1986; Florian, 1987), ants and termites (Strang, 1992), and cellulose-digesting bacteria (Peacock, 2005) such as *Bacillus* or *Cellulomonas* (Gutarowska, Pietrzak, Machnowski, & Milczarek, 2016).

The accumulation of odour on worn clothing is a common concern that can be attributed to bacterial metabolism of bodily secretions (Abdul-Bari et al., 2018; Callewaert et al., 2014). Raw denim enthusiasts have suggested that freezing can reduce this odour and allow for multiple wearings without the wearer being self-conscious in public spaces (Levinson, 2013). Despite the anecdotal evidence that freezing can eliminate odours and presumably control bacteria (Chad, 2011; Karr, 2011; Levinson, 2013), there appears to be no scientific verification that freezing denim would be effective. Freezing has been used to preserve odour in studies investigating human body odours (Abdul-Bari et al., 2018; Lenochova, Roberts, & Havlicek, 2009; Roberts, Gosling, Carter, & Petrie, 2008; Singh & Bronstad, 2001; Sorokowska, Sorokowski, & Szmajke, 2012). In the study by Lenochova, Roberts, & Havlicek (2009), where researchers froze fabric samples with human sweat (with confirmed body odour) and subsequently thawed them for evaluation. The odour-assessing panel verified that body odour was present after the

freezing period (though the intensity had subsided in some cases), which may suggest that odour-causing bacteria survived the freezing process and subsequent thaw. To date, there have yet to be any studies carried out where skin bacteria on denim are frozen to evaluate the impact of this method on bacterial numbers.

2.3.3 Ironing

Often, steam and heat are used for sterilization of medical implements, via the use of an autoclave (Armstrong & Reinhardt, 2010; Garibaldi et al., 2017). Importantly, the successful use of the autoclave for sterilization includes the additional element of pressure (Boey & Lye, 1990; Koushyar, Alavi-Soltani, Minaie, & Violette, 2011) which would not be present with other appliances reliant on heat and steam, such as the household iron.

The handheld steam iron is a common domestic appliance used to create a smooth appearance by removing wrinkles and creases (Arild, Brusdal, Halvorsen-Gunnarsen, Terpstra, & Van Kessel, 2003; Pakula & Stamminger, 2010) with variable steam and temperature control settings. The energy used by household irons is considerably less than that of the household tumble dryer, using approximately one third of the kilowatts, usually for a short period of time (Porteous et al., 2012). The iron became available to consumers after being patented in 1882, facilitating pressed clothing for many as it was now more affordable and able to be conveniently performed at home (Bellis, 2017). Different fabrics require different temperatures; for example, the setting for cotton and linen is recommended to not exceed 200 °C which is higher than polyester or nylon, which should not exceed 150 °C as fabric damage can occur if higher heat is used (Nayak & Padhye, 2015). Damage and degradation from heat exposure to cotton has been documented, including yellowing after hours at 120 °C, breaking down at 150 °C and damage at 240 °C (only after a few minutes) (Cook, 2001).

The heat emitted from the flat surface of the iron is used not only for its ability to smoothen the appearance of fabrics, but as an additional sanitation method (Arild et al., 2003; Blaser, Smith, Cody, Wang, & LaForce, 1984; Eckert et al., 2012; Patel et al., 2006; Raymond, 2008). In Patel et al. (2006), results from washing, tumble drying and ironing revealed that viable bacterial counts were lower (0 CFU/mL) than with washing and air drying (1.9×10^9

CFU/mL) or washing and tumble drying (3.30 x 10^2 CFU/mL), however, they did not evaluate bacterial populations over time to determine if these methods had a lasting effect. An *in vitro* experiment by Eckert et al., (2012) showed bacterial reduction of *Staphylococcus aureus* after nine seconds at 120 °C and further reduction after three seconds and nine seconds at 120 °C with steam on inoculated cotton samples. While these results do suggest the benefits of ironing on bacterial reduction, these results were not further evaluated after twenty-four hours to determine if bacteria were able to replicate.

The addition of heat from the iron to garments already tumble dried showed increased bacterial reduction on contaminated uniforms (Bloomfield et al., 2013; Patel et al., 2006). It is worth noting that the use of ironing after drying garments is encouraged to further ensure cleanliness and bacteria removal of the garment (Arild et al., 2003; Bloomfield et al., 2013; Patel et al., 2006).

2.3.4 UV radiation

Hanging clothing outdoors is a common and economical option for drying clothes (Jiang Wu et al., 2012) compared with the tumble dryer (Labhard & Pedersen, 1989; Mirosa, Lawson, & Gnoth, 2013; Pedersen, Labhard, & Webb, 1988). For some, this may not be a viable option, as this practice is highly dependent on variable geographical conditions and seasonal limitations (Pink, Mackley, & Moroşanu, 2015). Research on drying clothing outdoors (often discussed in conjunction with indoor line-drying) is primarily focused on energy consumption (Pedersen et al., 1988) and usage frequency (Schmitz & Stamminger, 2014). Raw denim enthusiasts encourage outdoor drying (Yee, 2016), and highlight its cost effective and non-abrasive nature, unlike tumble drying (Muzquiz, 2018). However, drying in direct sunlight can weaken fabrics through photo-degradation as well as causing yellowing of white cotton fabrics (Fianu, Sallah, & Ayertey, 2005), which may be of concern if denim is dried inside out, exposing the white coloured weft yarns. Additionally, line-dried clothing may feel stiffer and show an increased appearance of wrinkles when compared to tumble-dried garments (Carver & Wylie, 1980; Morris, Prato, & White, 1984).

In the food industry, there has been considerable interest in developing methods for sterilization. One method used is UV radiation, which has been used on dairy products, fruit juices and water (Cilliers et al., 2014; Donaghy et al., 2009; Heinrich, Zunabovic, Varzakas, Bergmair, & Kneifel, 2016). UV radiation has the ability to damage the DNA structure of harmful bacteria enough to inhibit growth (Cilliers et al., 2014). Pulsed UV light has also been used for decontamination of food products and the prevention of spoilage (Heinrich et al., 2016).

Further uses of UV exposure include the sterilization of medical implements since some chemicals used to sterilize contaminated tools and surfaces can be potentially harmful for the environment, for patients and for professionals (Byrns et al., 2017). In medical operating theatres and outpatient rooms, the use of a stationary UV lamp has inactivated bacteria and was shown to have reduced bacteria levels overall (Byrns et al., 2017; Xu et al., 2003). UV radiation from the sun has been reported at 320-400 nm for UVA, at 290-320 nm for UVB (Braga, Flint, Miller, Anderson, & Roberts, 2001; Poon, Barnetson, & Halliday, 2005) and at 200-280 nm for UVC (Clingen et al., 1995) which may fluctuate depending on the geographic location (Schwartz & Hanchette, 2006). The lamp used in the previously mentioned research by Byrns et al. (2017) was reported to emit 254 nm (UVC) with an intensity of 2400 microwatts/cm^2; however the intensity did vary, lessoning by 30% from its peak after thirty minutes. Xu et al. (2003) used a lamp that ranged from 100-290 nm of UVC rays, where the majority of wavelengths were noted at 254 nm and the intensity was not disclosed.

UV lamps and wands can also be useful for sterilization in offices, in public spaces and in the home where surfaces such as doorknobs, keyboards and counters are touched often (Byrns et al., 2017). Notable limitations of UV wands include the possibility of photo-reactivation of bacteria from lower wavelengths of UV rays (Byrns et al., 2017; Kim, Petin, & Morozov, 2005; Riley & Kaufman, 1972), lower efficacy in higher humidity (Riley & Kaufman, 1972), potential harm to the user after prolonged use and uneven levels of light emitted (Byrns et al., 2017; Kim et al., 2005; Quek & Hu, 2008). Keeping this in mind, it is understood that UV light devices emit light at the subject directly; whereas air-drying denim may not be in direct sunlight for any or all of the time while outside. However, bacteria accumulated from normal

wear are likely less harmful to the user than the exposure of bacteria from patients in a medical setting.

2.4 Skin bacteria

2.4.1 Microflora

The human skin is a comprehensive and vast organ, serving as a protective barrier while hosting a diverse community of microorganisms (Edmonds-Wilson, Nurinova, Zapka, Fierer, & Wilson, 2015; Sullivan, Edlund, & Nord, 2001). Known as the skin microbiome, this ecosystem is comprised of a complex combination of fungus, bacteria, mites and viruses (Grice et al., 2009; Kong, 2011), and plays host to billions of organisms (Chen & Tsao, 2013). Colony diversity and population densities vary considerably throughout, as regions of the body vary in moisture (from natural fluids such as sweat or oil secreted from the body), light, oxygen exposure and temperature (Fredricks, 2001) and a blend of both anaerobic and aerobic bacteria (Grice et al., 2009; Kolde et al., 2018).

Regions of the body relevant to wearing jeans include moist and dry surfaces, whose inherent differences influence both the population and diversity of cutaneous microorganisms (Grice et al., 2009). Differences between moist and dry areas of the body include the diversity of microbial populations. Dry areas host higher diversities of cutaneous flora, represented by high levels of gram-negative bacteria (Kong & Segre, 2012). Moist surfaces include the anogenital region, gluteal crease, inner thighs and popliteal fossa (behind the knees). The most common bacteria identified on the lower body include gram-positive aerobic bacteria such as corynebacteria (near the hips, lower torso and gluteus crease), staphylococci (found on the hips, toe web, gluteal crease, buttocks and knee area) and propionibacteria (buttocks, gluteal crease and back) (Gregersen, 1978; Grice & Segre, 2011). Although, the presence of gram-negative bacteria are abundant among the skin microflora, they are not as prevalent in these lower body regions (Grice & Segre, 2011). In the anogenital region, additional microorganisms from the gastrointestinal system are likely to be present as well. Primarily for the anogenital region, research has focused on bacterial populations in humans with specific illnesses (Swidsinski et al., 2005; Wollina et al., 2012), and infections among children (Halbert & Chan, 2002; Myhre,

Bevanger, Berntzen, & Bratlid, 2002) and addressing particular situational symptoms (Chudáčková et al., 2010), and less towards skin bacteria topography in relatively healthy adults. However, McBride, Duncan and Knox, (1977) sampled this region in healthy adults and found consistent populations of staphylococci, micrococci and aerobic diphtheroids (or corynebacteria).

The gluteal crease, a moist region shows a predominance of unnamed gram-positive bacteria, *Staphylococcus aureus* and propionibacteria (Grice & Segre, 2011). The moist region behind the knees was dominated by staphylococci and additional unnamed gram-positive bacteria (Grice & Segre, 2011). The dry areas of the body covered by denim jeans include the buttocks, which was composed of various gram-negative proteobacteria as well as corynebacteria, and lower representations of propionibacteria, staphylococci and other gram-positive bacteria (Grice & Segre, 2011). In general, there has been little research examining the microbiome of the thigh and calf regions which are large areas that are in contact with denim when worn. However, a small scale and near full body topography of skin microflora was performed comparing two individuals (one male, relatively healthy; one female with atopic dermatitis) where populations and microbial presence differed significantly (Bibel & Lovell, 1976). From this research, *Staphylococcus aureus* was the most abundant for the thighs, behind the knees and both sides of the forearms for the female, while the male showed higher populations of *Staphylococcus saprophyticus* as well as small and large colony diptheroids on the thighs, calves and upper legs. *Staphylococcus aureus* was not reported for the male (Bibel & Lovell, 1976). In Leeming, Notman, and Holland (1989), a fungus known as *Malasserzia furfur* (synonymous with *Pityrosporum ovale* and *Pityrosporum orbiculare*) was identified with higher populations in the upper thighs in females when compared with other sampled body sites such as the hand or axilla. As well, a lower (but notable) presence of *Malasserzia furfur* in the calf region for males and females was also reported. Elsewhere, bacterial populations of legs were reported but it was unclear as to the specific physical site of the body (Kloos & Musselwhite, 1975), which is important as moist or dry influences microflora populations and species. This study noted that the levels of staphylococci, micrococci and coryneforms were dominant on the legs and similar in the population of cutaneous bacteria recovered (Kloos & Musselwhite, 1975).

The forearm has been a site for collecting cutaneous bacteria for *in vivo* studies involving fabrics as it was considered to be a convenient body site for testing (Walter et al., 2014). Walter et al. (2014) collected skin bacteria on nylon fabric samples by covering the top (posterior) of the forearm, in close proximity to the wrist (a relatively dry location). They used *Staphylococcus* selective media and a non-selective media for collecting aerobic bacteria including corynebacteria. Bacteria species were not further identified (Walter et al., 2014). Other studies have evaluated areas near the posterior forearm. However, research has focused primarily on moist sites which differ from the dry sites used in the Walter and colleagues (2014) study (i.e., the inner elbow and palms of hands (Grice & Segre, 2011), and dorsal forearm (Gao, Tseng, Pei, & Blaser, 2007; Kong & Segre, 2012). Leeming et al. (1989) did observe the fungus *Malassezia furjiur* on the posterior forearm of both male and female participants. The diversity of bacteria (including corynebacteria, streptococci, staphylococci, and propionibacteria) was similar when evaluating the forearm and the foreleg in one exploration (Cundell, 2018), however precise physical locations were not disclosed.

2.4.2 Bacteria on textiles

The ability for bacteria to survive and grow on textiles is well documented. The detection and overall survival of microorganisms on textiles has been the subject of extensive research not only in medical settings but also related to domestic and household matters. Bacterial survival on upholstery (Lankford et al., 2006), towels (Blaser et al., 1984), drapes (Whyte, Hodgson, Bailey, & Graham, 1978) and clothing (Teufel, Pipal, Schuster, Staudinger, & Redl, 2010) indicate that not only has this topic drawn considerable interest, but it can have major implications for minimizing healthcare related risk. Bacteria can come from the individual wearing the clothing, from the environment or contact with others (Al-Benna, 2010; Whyte et al., 1978). In medical settings, bacteria can attach to medical uniforms (Bloomfield et al., 2013; Patel, Murray-Leonard, & Wilson, 2012), ties (Weber, Khan, Fader, & Weber, 2012) and identification lanyards (Kotsanas, Scott, Gillespie, Korman, & Stuart, 2008). They can then detach or become airborne, potentially infecting vulnerable patients. Garment design and fabric comparisons have been evaluated (Al-Benna, 2010; Patel et al., 2012), as well as washing

procedures (Bloomfield et al., 2013; Patel et al., 2012) in an effort to mitigate potential risk (as discussed in the laundering section 2.3.1).

Bacterial survival has been explored on both synthetic and natural fabrics (Teufel et al., 2010). Sweat (dominated with *Enterobacteriaceae* and *Staphylococcus* among many other strains) was inoculated onto different fabrics. Results showed that polyester and polyamide exhibited the highest growth of bacteria when compared to lyocell and cotton, where *Bacillus* and *Pseudomonas* (microorganisms not highly represented in the original sweat) showed significant growth (Teufel et al., 2010).

Domestically, bacterial survival on clothing may not pose the same immediate risk of harm as clothing worn in healthcare environments, such as medical uniforms. However, illness from domestic laundry contamination is often not reported (or determined to be the cause) but can spread to others if bacterial pathogens attach to clothing from the same load of laundry (Bloomfield et al., 2013). Bloomfield et al. (2013) also remarked that the survival of *Escherichia coli*, *Salmonella* and strains of influenza can contaminate and remain on washed laundry, which is especially concerning to vulnerable individuals and older adults.

2.5 Summary

Bacteria play a highly influential role in textiles in addition to food and soil research. Additionally, the presence of bacteria has been shown to be beneficial in some situations and harmful in others. Since bacteria are able to survive and reproduce on textiles, harmful bacteria can transfer upon contact with other textiles. While care and maintenance methods differ for many textiles, the washing machine is one of the most common, which uses mechanical agitation, the addition of chemical detergent, warm washing temperatures, and time. The washing machine can be problematic for some raw denim owners, who wish to avoid washing their jeans to preserve unique markings from continuous wears. Concerned with an accumulation of bacteria over time, many raw denim enthusiasts have suggested alternative methods such as freezing, ironing or UV irradiation for refreshing their garments without compromising their uniqueness. To date, no comparative analysis of the efficacy of these methods has been performed on raw denim. In this thesis, a quantitative analysis of bacteria

was carried out to assess the effectiveness of each treatment method, both immediately after the treatment as well as twenty-four hours after the treatment.

Chapter 3

Methods

3.1 Fabric

Raw denim fabric (100% cotton) was purchased from Fabrictime Solutions Ltd, a fabric vendor in Vancouver, British Columbia. One of the distinct properties of raw denim is the lack of any washing or chemical treatments before the garment is constructed (discussed in Chapter 1) and wearers typically do not launder raw denim for many weeks or months after wear. Therefore, no laundering or stabilizing treatment was carried out on the fabric prior to testing.

The raw denim fabric was cut into 100 mm X 50 mm pieces. Fabrics were then sterilized in an autoclave at 118 °C for 30 minutes. Prior to attaching fabric on participants' arms, the denim fabrics were placed in a conditioned environment (65 ± 5 % R.H at 20 ± 2 °C) for 24 hours.

3.1.1 Fabric characterization

Characteristics of the raw denim fabric are shown in Table 3.1. Fabric mass (g/m^2) was determined using CAN/CGSB – 4.2, No. 5.1 – M90 (Canadian General Standards Board, 1997). Fabric count (yarns/cm) was determined using CAN/CGSB – 4.2, No. 6 – M89 (Canadian General Standards Board, 1989). Fabric Thickness (mm) was determined using CAN/CGSB – 4.2, No. 37 – 2002 (Canadian General Standards Board, 2002). A picture of the fabric used is shown in Figure 3.1.

Table 3.1 *Characteristics of denim fabric used*

Fibre content of fabric	100% Cotton
Fabric mass, g/m^2	431.22 ± 3.13
Fabric thickness, mm	0.88 ± 0.02
Weave	3/1 Twill
Fabric count, yarns/cm	Warp: 16 Weft: 15

a)

b)

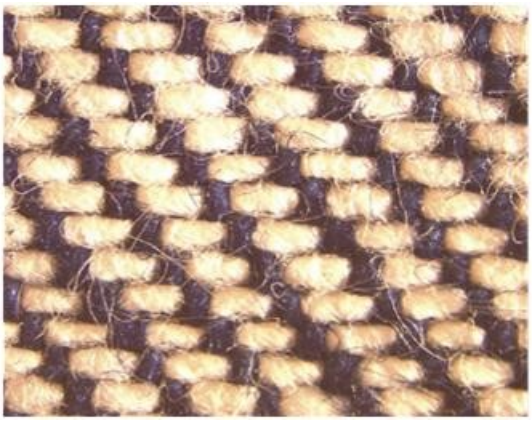

Figure 3.1 Fabric image at 35X magnification: a) fabric face; b) fabric back

3.2 Ethical requirements

Ethical approval was obtained before any research was conducted requiring human participants. The ethics were approved by the University of Alberta Human Research Ethics Board 2 on April 18, 2018. The information sheet and consent form are located in Appendix A

3.2.1 Participant screening

One hundred and twenty-two individuals responded to the participant recruitment posters and e-mail distributions and were contacted with the full study details. Fourteen individuals were able to commit to the full trial and were invited for the screening test. Of those that were invited, nine participants accepted the invitation for the screening test (seven females and two males). One male participant did not return for fabric removal. Individuals that were

selected were chosen based on bacterial counts recorded from the analysis of worn denim fabrics as well as the ability to commit to the full length of the study. After analysing all screening participants, the six with the highest bacterial counts and ability to commit to the full study were invited to participate.

3.3 Collection of skin bacteria on denim fabric

3.3.1 Participants

Six female students participated in this study. Participants were recruited from the University of Alberta campus. Prior to selection for inclusion in the study, interested individuals were screened to evaluate individual bacterial counts. The intention was to select participants who had bacterial counts with an order of magnitude of 10^4 CFU/mL as per the procedure described in Section 3.5.1. However, the lowest bacterial count from the selected participants was 3.62×10^3 CFU/mL. Male participants were not excluded from the study, however, during the recruitment phase, few males responded. Of the males who were screened during the recruitment phase, their bacterial counts were lower than the highest bacterial counts from all of the screening participants, resulting in rejecting male screening participants from the final study.

3.3.2 Protocol

Denim fabric swatches were placed and securely taped on the posterior surface of both forearms, approximately 30 mm from the prominent ulna joint, similar to the protocol outlined in Walter, McQueen, and Keelan (2014). Fabric specimens were positioned lengthwise (see Figure 3.2), covered with plastic film to increase humidity for bacterial growth (Hartmann, 1983) and secured using kinesiology tape (an elastic cotton strip with an acrylic adhesive). The specimens were placed directly on each participant's arms to simulate how denim would be worn on the body, with the face of the denim showing and the back of the denim against the skin. The arm was chosen based on results from preliminary testing of different areas of the body (Appendix B). Cloth bandages were then wrapped around the area to reduce the

likelihood of the plastic and tape moving from position. The fabrics were worn continuously for 24 hours.

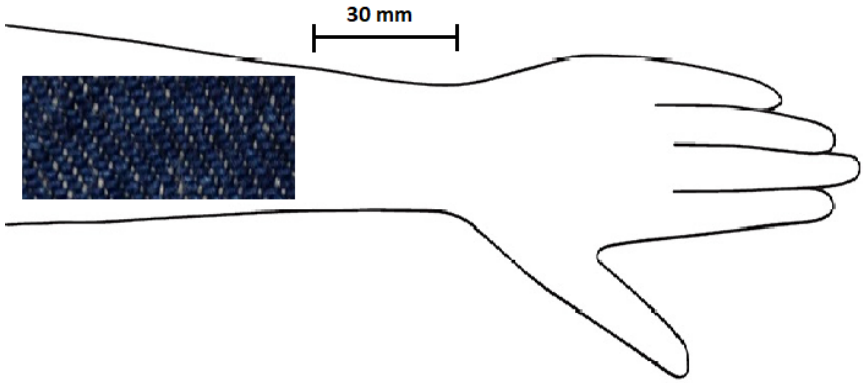

Figure 3.2 Placement of fabric on forearm

3.3.3 Test requirements

Participants were asked to wear assigned denim samples continuously for 24 hours. Participants were asked to not perform vigorous exercise while wearing samples to avoid the risk of dislodging tape or fabrics, although, light exercise was still permitted. Participants were allowed to bathe or shower during the 24-hour wear period but were instructed to cover the forearm with plastic wrap to ensure the fabric did not get wet.

Participants were asked to refrain from using anti-microbial soaps and exfoliating products during the length of the trial. Participants could not be taking antibiotic medication leading up to or during the trial. Participants were given a neutral bar soap (Dove®) to use for the duration of the trial.

3.4 Experimental design and procedure

3.4.1 Experimental design

The experimental design was a 4 x 2 factorial design. There were two independent variables in this study, four refurbishing treatments (freeze, iron, UV, wash) applied to the

fabrics after they were worn (Table 3.2) and two time periods post-treatment (0 h, 24 h). Immediately after the treatment, bacterial populations on specimens assigned to 0 hours were extracted; specimens assigned to the 24-hour time period were placed in a Petri dish in a conditioned environment (65 ± 5 % R.H at 20 ± 2 °C) for 24 hours before bacterial extraction.

Bacterial counts obtained from participants following refurbishing treatment (i.e., freeze, iron, UV, wash) and time (0 h or 24 h) were compared with their own baseline counts obtained from the worn fabrics prior to treatment because individual bacterial counts differed among participants and within the same participant on different days. The treatments were chosen as freezing and UV were suggested by raw denim enthusiasts as possible alternatives to laundering (Makers, 2015; McKay & McKay, 2014) and ironing was chosen as it had been shown to provide additional bacterial reduction (Patel et al., 2012). The first dependent variable was the percent change of bacterial counts for the treatment relative to the baseline bacterial counts prior to treatment. Additionally, a logarithmic calculation (the daily growth rate) was calculated to compare the results from 0 h and 24 h to analyse bacterial behaviour after the refurbishing treatments.

Table 3.2 *Description of Refurbishing Treatment*

Refurbishing treatment	Length	Equivalent to
Freeze	24 hours	n/a
Wash	9 minutes	1 wash cycle
Iron	15 seconds	n/a
UV	5.5 hours	8 hours of natural sunlight

3.4.2 Procedure

Participants were asked to return to the laboratory in order for the fabric samples to be removed (two fabrics per participant). Once the fabrics were received, each fabric swatch was cut into four specimens as per the sampling plan in Figure 3.3 using sterilized scissors. Individual specimens were then placed into designated Petri dishes. Two of the four pieces

from each swatch were designated for baseline counts (B1 and B2) and the other two were assigned a treatment (one for 0 h and one for 24 h).The fabrics returned on each test day were assigned to one of the refurbishing treatments (Table 3.2). As ironing and washing were more laborious treatments, they were not done on the same day. Therefore, the ironing treatment was done on the same day as freezing and the washing treatment was performed on the same day as the UV treatment.

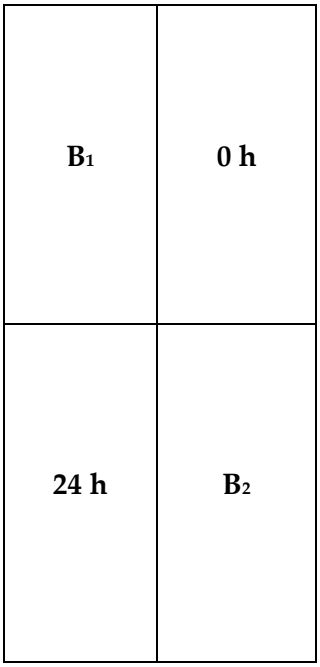

Figure 3.3 Sampling of fabric for microbiological analysis (B1= baseline 1; B2 = baseline 2; 0 h = analysis immediately after treatment; 24 h = analysis 24 hours after treatment)

As bacterial populations may fluctuate from day to day (Grice & Segre, 2011), using the baseline comparison within the same worn fabric swatch as the treatment was deemed an appropriate testing method to evaluate the effectiveness of the experimental treatment. In other words, if the bacteria levels collected from a participant on a given day were relatively low or high, it would affect both the treatment and the baseline portions of the fabric, and the relative change in bacteria would still be comparable.

3.4.3 Experimental treatments

Bacteria on fabrics were extracted over two different time periods. Firstly, as soon as possible after each refurbishing treatment (i.e., '0' hours following the treatment) and following 24 hours at room temperature (20 °C and 65% R.H.). This additional 24-hour period would simulate the behaviour of the owner storing denim after the garment had been laundered or alternatively refurbished before the next wear.

3.4.3.1 Machine wash and air dry

Fabric specimens were placed in a Launder-Ometer (Atlas Electric Devices Co.; Model B-5; Type LHD-EF) to simulate one washing cycle. The machine was run for one cycle (nine minutes) following the AATCC Standard 61, Colourfastness to Laundering: Accelerated (AATCC, 2010a), test category 1A using water at 40 °C. The specimens were dipped in distilled water for one second, three times then dried on three sterile paper towels under a 188 g weight for three minutes. Sterile paper towels were used to efficiently dry fabrics for immediate analysis for the 0 h time period.

3.4.3.2 Freezing

Fabric specimens were placed in separate Petri dishes, covered and secured with tape. The Petri dishes were then placed in a chest freezer (brand unknown) for 24 hours at -26 ± 2 °C. The chest freezer was chosen to mimic the equipment commonly found in a household, described by online media (Chad, 2011). The chosen length of time (24 hours) was used as it is a common length of time disclosed in online media (Chad, 2011).

3.4.3.3 Ironing

Fabric specimens were placed in between two cotton cloth fabrics for the ironing treatment. The cotton cloths were used to reduce any contamination from the iron when pressing multiple specimens during treatment application. An iron (Black & Decker; Model IR1040SC TYPE 1) was then passed over the cotton covering the fabric specimens using the steam setting for 15 seconds, as this would mimic household ironing practice by passing over

the garment briefly. The setting used for the iron was "cotton" (as the denim was 100% cotton) whose mean temperature is displayed in Figure 3.4 (using Omega HH502 Thermocouple).

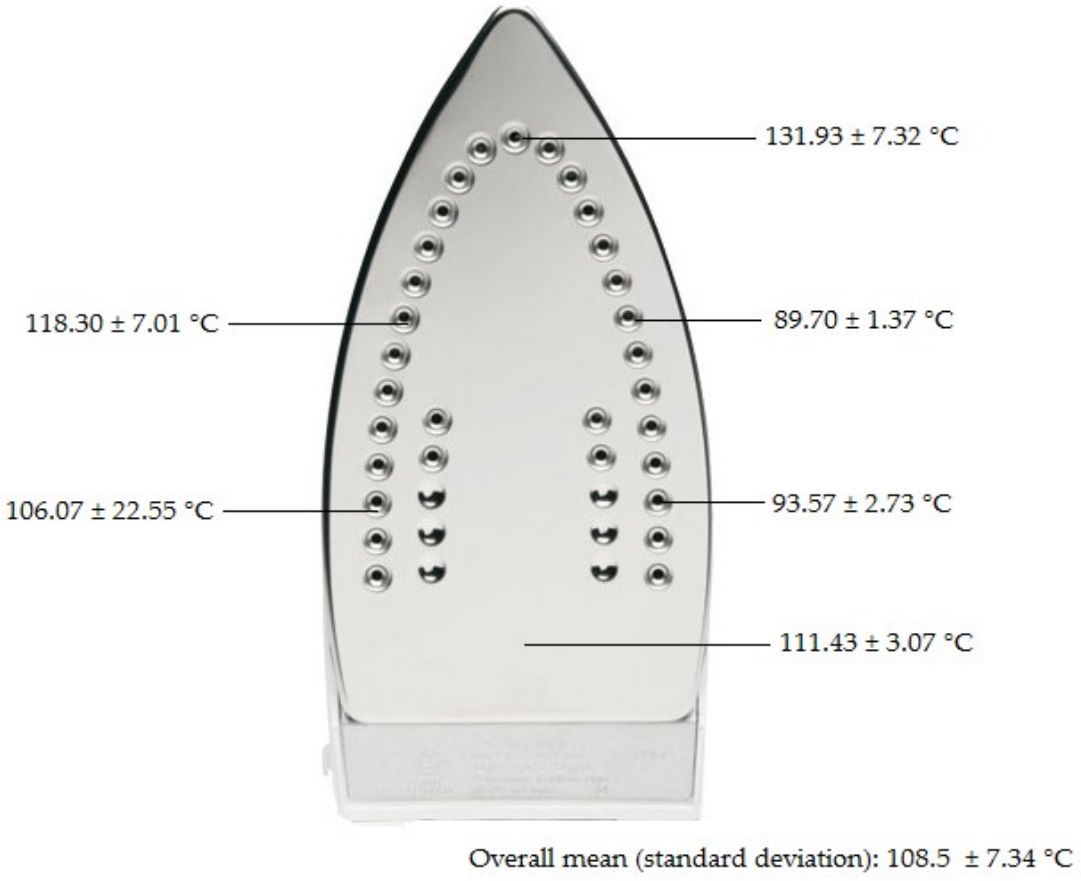

Figure 3.4 Distribution of iron temperature on cotton setting

3.4.3.4 UV treatment

Fabric specimens were placed in a Weather-Ometer (Model C135W), programmed to simulate 8 hours of sunlight following AATCC Test Method 111-2009, Weather Resistance of Textiles: Exposure to Daylight and Weather (AATCC, 2010c) displayed in Table 3.3. The Weather-Ometer was turned on for 5 hours in total to simulate eight hours of sunlight.

Table 3.3 *Weather-Ometer settings*

Black panel temperature	68 °C
Chamber air temperature	43 ± 2 °C
Conditioning water	43 °C
Wet bulb depression	12 °C
Wet bulb depression	20 °C
Dry bulb	32 °C
R.H.%	32%
AATCC Fading Units (AFU)	5
Irradiance @ 420 nm	1.1 W/m^2
Test interval	5 hours
Lamp wattage	3.9 kW

3.5 Bacterial extraction protocol and colony analysis

3.5.1 Bacterial extraction

After removal from the arm (B1 and B2) or after treatment (0 h and 24 h) the denim fabric specimens were placed in 50 mL conical tubes using sterile forceps containing 50 mL of saline phosphate buffer solution (PBS) amended with 0.05 % Tween 80 (PBS/T80) added to them. Sterile glass beads were then added to the tube (between 10-15 beads) and then the tube was vortexed for 60 seconds on the highest setting. A ten-fold dilution series was created from the shaken tube containing the buffer solution and 15 µL aliquots were drop-plated onto non-selective blood agar in triplicate on three different plates of agar (nine replicates in total) and incubated at 37 °C for 24 hours.

Non-selective blood agar (500 mL) was comprised of 19.75 g of blood agar base no. 2; 1.5 g of yeast extract; 1 g of glucose; 2.5 mL of Tween80; 25 mL of defibrinated horse blood. Biological media components were sourced from Fisher Scientific, Oxoid brand (Ottawa, ON), and horse blood was sourced from Dalynn Biological (Calgary, AB).

Visible bacterial colonies were counted and recorded after the twenty-four hour incubation time. Calculation for viable counts of observed bacterial colonies were expressed in colony forming units per millilitre (CFU/mL) and were calculated using the following equation:

$$average \quad no. \quad colonies \times \frac{1}{dilution} \times \frac{1000}{15} = cfu/mL$$

The viable bacterial counts were expressed as per volume of solution rather than as per unit of fabric as it was unknown to what extent the bacteria remained on the denim during the extraction process. The mean viable counts of bacterial colonies were taken for each specimen from each participant (both baseline and laundering treatment) which were used for comparison. The limit of detection was 7.41 x 10^1 CFU/mL.

The change in percent from the baseline measurements for each participant/fabric was calculated using the following equation:

$$\%\ change = \frac{X_t - X_b}{X_b} \times 100$$

where:

X_t = the bacteria in CFU/mL recovered from the treated test specimens at a specific time (t= 0 h or 24 h)

X_b = the bacteria in CFU/mL recovered from the baseline test specimens

3.5.2 Calculation of growth rate

A further calculation was used to compare the rate of growth over the 24-hour period among the refurbishing treatments. This was described as the daily growth rate. During the growth phase, bacteria grow logarithmically (Mahon, Lehman & Manuselis, 2007). Therefore, assuming that bacterial growth took the form $e^{\gamma t}$ the daily growth rate was estimated by:

$$\gamma = \log(X_{24}) - \log(X_0)$$

γ = the growth rate per day

X_{24} = the bacterial count in CFU/mL recovered from the treated test specimen 24 h after treatment

X_0 = the bacterial count in CFU/mL recovered from the treated test specimen 0 h after treatment

3.6 Statistical analysis

Two pieces from the removed worn swatches were assigned as the baseline and were analysed without any treatment application. The average between the calculated CFU/mL results observed from baseline samples was taken to account to assess the differences between results and establish a reference point for comparison with treated samples.

Descriptive statistics were calculated from bacterial counts for worn samples (i.e., mean, standard deviation (SD), minimum and maximum values). Since the percent change data (transformed and non-transformed) did not meet the assumptions of normality and/or equal variance, non-parametric statistics were conducted to determine whether there were significant differences among treatments and time. Non-parametric analysis of the data was used with a confidence level of $p<0.05$ for hypothesis testing. Friedman's test was used to analyse the difference among treatments for both time frames (0 h and 24 h). The Wilcoxon signed-rank test was used to compare differences among treatment pairs (when significant differences by Friedman's test were found) and also for each treatment with respect to time (i.e., 0 h vs. 24 h).

Since the growth rate data met the assumptions of normality and equal variance, a one-way ANOVA followed by Tukey's HSD tests were used to determine significant differences among refurbishing treatments for growth rate. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS), version 25.

Chapter 4

Results

4.1 Bacterial counts

4.1.1 Bacterial counts on raw denim immediately after wear (baseline)

Bacterial counts for all replicates are displayed in Appendix C, Table C.1. The range of bacterial colony densities observed on the worn fabrics immediately after wear (baseline counts) varied among participants. Participants 1, 2 and 3 had lower baseline counts overall than Participants 4, 5 and 6. In general, Participant 6 consistently had the highest baseline counts and Participant 3 had the lowest counts. For example, the mean (± SD) counts for Participant 6 were $1.30 \times 10^4 \pm 2.80 \times 10^3$ CFU/mL and ranged from 6.78×10^3 CFU/mL to 1.61×10^4 CFU/mL; Participant 3 mean baseline counts were $5.61 \times 10^3 \pm 2.16 \times 10^3$ CFU/mL and ranged from 2.67×10^3 CFU/mL to 8.93×10^3 CFU/mL.

4.1.2 Bacterial counts on raw denim following refurbishing treatments

Table 4.1 shows a summary of bacterial counts obtained from denim fabrics before and after refurbishing treatment. The bacterial counts obtained from the fabrics following the refurbishing treatment appeared to differ depending on the treatment applied. It also appeared that the delay after the treatment (0 h or 24 h) influenced the quantity of bacterial populations on the fabrics. The washing treatment resulted in the lowest bacterial numbers with some bacterial counts being below the limit of detection ($< 7.41 \times 10^1$ CFU/mL). Bacterial counts obtained on fabrics 24 hours following washing also had the lowest bacterial counts. The treatment with the next lowest bacterial counts of 0 h was obtained on fabrics following freezing: the mean value immediately after treatment was 7.25×10^2 CFU/mL ($\pm 3.17 \times 10^2$). The bacterial counts ranged from 1.48×10^2 to 1.33×10^3 CFU/mL (Participant 5 and Participant 2 respectively, see Table C.1). However, twenty-four hours after freezing, bacterial counts had increased, with a mean value of 7.17×10^3 CFU/mL ($\pm 2.38 \times 10^3$) and ranging from 3.33×10^3 to 1.10×10^4 CFU/mL (Participant 3 and Participant 6 respectively). The ironing treatment had the next highest bacterial counts, with a mean of 1.72×10^3 CFU/mL ($\pm 6.14 \times 10^2$) ranging from 6.67

x 10^2 CFU/mL to 2.74 x 10^3 CFU/mL (Participant 3 and Participant 1 respectively). The UV treatment results were similar to the ironing treatment 0 h after treatment with a mean value of 3.72 x 10^3 CFU/mL (± 2.23 x 10^3). The ranges of bacterial counts were 1.33 x 10^3 CFU/mL from Participant 3 to 8.00 x 10^3 CFU/mL from Participant 6. Twenty-four hours following the refurbishment treatment the denim fabrics that had been ironed had a mean value of 6.85 x 10^3 CFU/mL (± 1.82 x 10^3); and those exposed to UV light 7.46 x 10^3 CFU/mL (± 4.07 x 10^3).

Table 4.1 *Summary of bacterial counts obtained from denim fabrics before and after treatment*

		Bacterial Counts (CFU/mL)			
		Mean	SD	min	max
Freeze	Baseline	9.44 x 10^3	2.67 x 10^3	5.81 x 10^3	1.44 x 10^4
	0 h	7.25 x 10^2	3.17 x 10^2	1.48 x 10^2	1.33 x 10^3
	24 h	7.17 x 10^3	2.38 x 10^3	3.33 x 10^3	1.10 x 10^4
Iron	Baseline	6.99 x 10^3	2.48 x 10^3	3.52 x 10^3	1.22 x 10^4
	0 h	1.72 x 10^3	6.14 x 10^2	6.67 x 10^2	2.74 x 10^3
	24 h	6.85 x 10^3	1.82 x 10^3	4.59 x 10^3	1.02 x 10^4
UV	Baseline	9.85 x 10^3	3.85 x 10^3	5.78 x 10^3	1.69 x 10^4
	0 h	3.72 x 10^3	2.23 x 10^3	1.33 x 10^3	8.00 x 10^3
	24 h	7.46 x 10^3	4.07 x 10^3	2.00 x 10^3	1.59 x 10^4
Wash	Baseline	8.60 x 10^3	3.71 x 10^3	2.67 x 10^3	1.40 x 10^4
	0 h	< 7.41 x 10^1	0.00	< 7.41 x 10^1	< 7.41 x 10^1
	24 h	< 7.41 x 10^1	0.00	< 7.41 x 10^1	< 7.41 x 10^1

4.2 Relative percent change in bacterial counts following treatment

Both the refurbishing treatment and the amount of time following the treatment had an impact on the relative change in bacterial counts compared with the baseline. This is shown as a percent change in bacterial counts, displayed in Table 4.2 and Figure 4.1. The washing treatment resulted in the greatest percent change with a reduction of bacterial counts at both the 0 h and 24 h following treatment. The percent change following washing ranged from -99.47% for Participant 6 to -97.22% for Participant 3, at both 0 and 24 h. The mean percent change (and

standard deviation) in bacterial counts for washing at both 0 and 24 hours was -98.88% (± 0.68%).

Table 4.2 *Percent change in bacterial counts from baseline 0 h and 24 h after treatment*

		Fabric bacterial counts (CFU/mL) per treatment							
		Freeze		Iron		UV		Washing	
Participant	Rep	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
1	1	-93.10	-23.28	-51.63	49.02	-61.82	-28.18	-98.75	-98.75
	2	-87.26	-42.68	-83.20	-30.40	-62.04	-21.30	-98.79	-98.79
2	1	-93.75	28.75	-50.00	48.39	-67.59	-75.00	-98.84	-98.84
	2	-86.31	-61.98	-76.96	-40.09	-74.39	-24.39	-98.81	-98.81
3	1	-83.15	-26.97	-81.05	62.11	-76.92	-64.10	-97.22	-97.22
	2	-90.04	-13.69	-86.74	-18.78	-69.79	5.21	-97.94	-97.94
4	1	-94.07	-23.70	-68.12	60.87	-23.58	3.77	-99.39	-99.39
	2	-91.81	-24.91	-86.21	-26.72	-79.31	-28.74	-99.38	-99.38
5	1	-98.39	-32.53	-49.61	100.00	-58.77	-19.30	-99.22	-99.22
	2	-94.25	-0.38	-75.21	-33.33	-72.69	-15.87	-99.29	-99.29
6	1	-94.36	-23.59	-74.86	50.82	-50.41	-1.74	-99.47	-99.47
	2	-95.05	-28.57	-87.27	-62.42	-64.89	-43.51	-99.42	-99.42

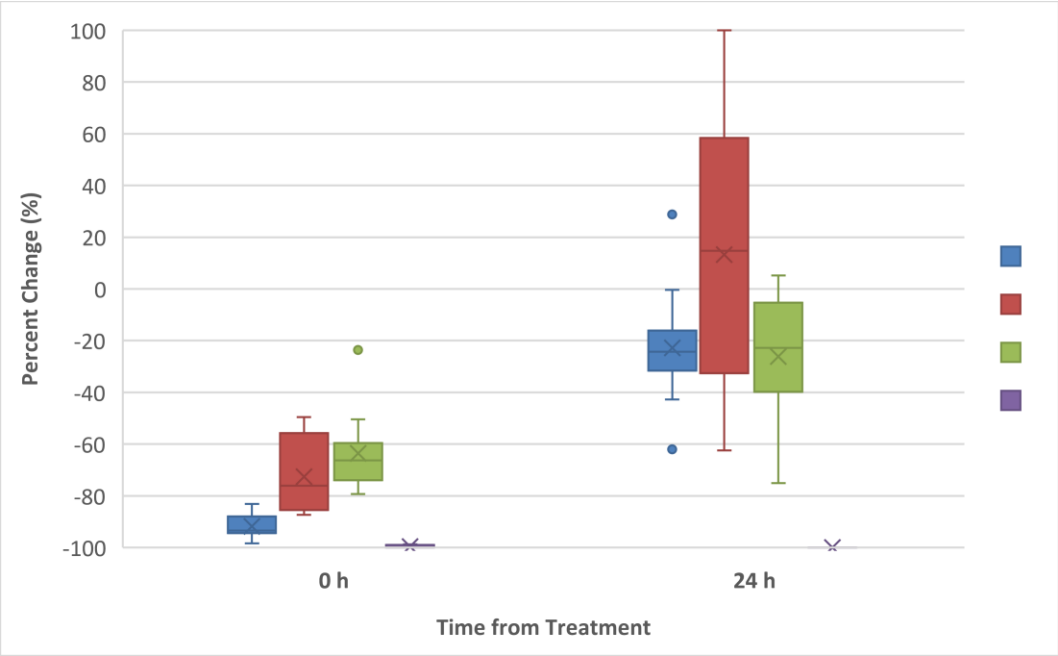

Figure 4.1 Percent change of bacterial counts from baseline to post-treatment

The percent change in bacterial counts for the freezing treatment showed a high reduction in bacterial counts 0 h after the treatment with a mean percent change of -91.80% (± 4.33) and ranging from -98.39% (Participant 5) to -83.15% (Participant 3) (Figure 4.1 and Table 4.2 respectively). Twenty-four hours after freezing, bacterial counts were noticeably increased, with a mean percent change in bacterial counts of -22.79% (± 22.00) ranging from -61.98% for Participant 2 to 28.75% for Participant 2. There was considerable variability in percent change in bacterial counts for specimens that had been exposed to the ironing treatment. The percent change ranged from -87.27% to -49.61% (Participant 6 and Participant 5 respectively) at 0 h. Twenty-four hours after ironing, growths of bacterial colonies were observed. The percent change in bacterial counts ranged from -62.42% (reduction from baseline) for Participant 6 to 100.00% (growth) for Participant 5. The mean percent change at 0 hours was -72.57% (± 14.50%) and increased to 13.29% (± 53.41%) after 24 hours. Bacterial counts obtained from denim specimens exposed to UV radiation ranged from -79.31% for Participant 4 to -23.58% for Participant 4 at 0 h following treatment. The bacterial counts on denim specimens 24 h after the UV treatment varied from Participant 2 (-75.00%) to Participant 3 (5.21%). The mean percent change at 0 hours was -63.52% (± 15.03%) and increased to -26.10% (± 24.85%) after 24 hours.

As the data did not follow a normal distribution or adhere to the assumptions of equal variance required for parametric statistical analysis, non-parametric tests were performed to determine significance among treatments and time. As it was clear from the results, washing was significantly different from all other treatments at 0 h and 24 h, and was excluded from further analysis. The Friedman test was used to determine if there was a significant difference among the three alternative refurbishing treatments at each time variable (see Table 4.3). Additionally, the Wilcoxon test was used to determine any significant difference among each treatment in pairs. At 0 h, there was a significant difference among the percent change for the three treatments ($\chi^2 = 19.500$, $p = 0.002$). Freezing was significantly different from the iron and UV treatments (Freeze-Iron: $Z = -3.059$, $p = 0.002$; Freeze-UV: $Z = -3.059$, $p = 0.002$). However, the iron and UV treatments did not differ significantly (Iron-UV: $Z = -1.569$, $p = 0.117$). At 24 h, there was no significant difference among the three treatments ($\chi^2 = 0.558$, $p = 0.558$).

Table 4.3 *Effect of time on bacterial counts after treatments*

	Mean Rank* Refurbishment Treatment			Friedman test results			
	Freeze	Iron	UV	N	χ^2	df	Sig.
0 hours	1.00[a]	2.25[b]	2.75[b]	12	19.500	2	0.002
24 hours	1.83[a]	2.25[a]	1.92[a]	12	1.167	2	0.558

* means with the same letter along a row are not significantly different from one another at $p < 0.05$

4.3 Effect of time on bacterial counts for each treatment

There were noticeable differences in bacterial counts after 24 h compared with counts at 0 h for the three alternative refurbishing treatments (i.e., freeze, iron, UV) (see Tables 4.1, 4.2 and Figure 4.1). However, there were no differences in bacterial counts obtained from the washed denim fabrics over time, with bacterial counts being consistently below the limit of detection at both 0 h and 24 h.

Following the freezing treatment, mean bacterial counts were 7.25×10^2 CFU/mL at 0 h and this increased to 7.17×10^3 CFU/mL at 24 h (see Table 4.1). This resulted in a mean percent change from -91.80% at 0 h to -22.79% at 24 h. The mean bacterial counts also increased for both ironed specimens and specimens exposed to UV radiation, although the differences between 0 and 24 hours were not as large. For the ironing treatment, mean bacterial counts were 1.72×10^3 CFU/mL at 0 h and this increased to 6.85×10^3 CFU/mL at 24 h (mean percent change: -72.57% at 0 h; 13.29% at 24 h). For the UV treatment, mean bacterial counts were 3.72×10^3 CFU/mL at 0 h and increased to 7.46×10^3 CFU/mL at 24 h (mean percent change: -63.52% at 0 h; -26.10% at 24 h).

When analysing the difference between time periods for each treatment the Wilcoxon signed test was conducted. The results indicated that the difference in bacterial counts between both 0 h and 24 h were significant for all three alternative refurbishing treatments: freezing ($Z = -3.059$, $p = 0.002$), ironing ($Z = -3.059$, $p = 0.002$), and UV ($Z = -2.598$, $p = 0.003$).

4.4 Change in growth rate from 0 hours to 24 hours

There was a clear difference in the rate of growth from 0 h to 24 h for the four treatments as shown in Figure 4.2. The growth rate was expressed as log differences and therefore is unitless. As mentioned previously, there was no change in the bacterial counts for the washed fabrics over time; therefore, the daily growth rate was calculated as 0.00. For the specimens that had been frozen, the mean daily growth rate was 1.02 (± 0.33). The mean daily growth rate for ironed samples was 0.62 (± 0.17) and for UV exposed samples was 0.30 (± 0.19).

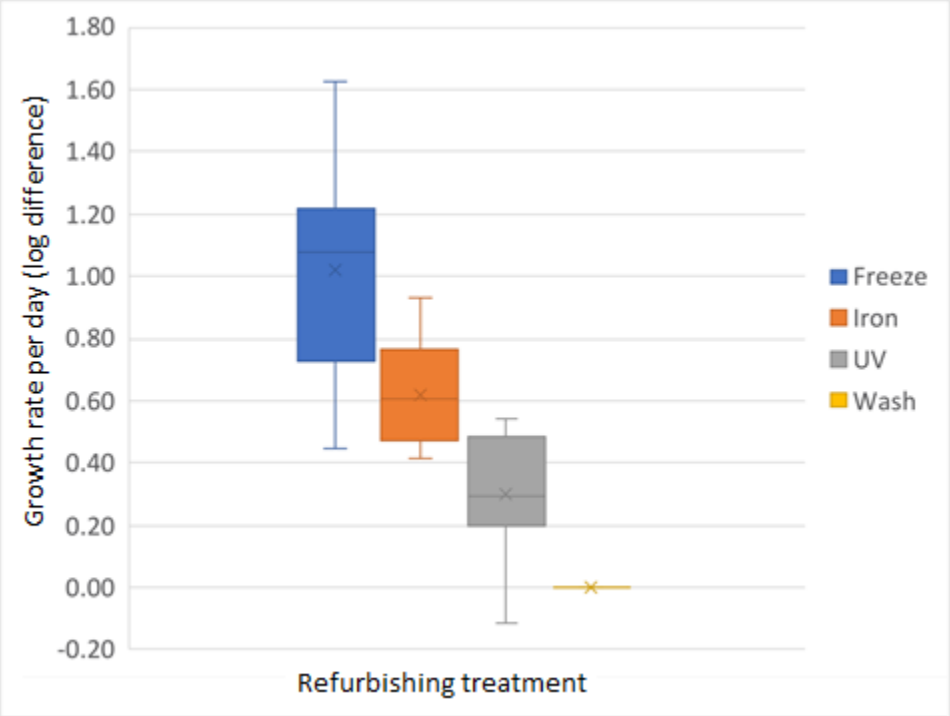

Figure 4.2 Bacterial growth rate (log10) per day on raw denim after refurbishing treatments

Again, from Figure 4.2 it is clear that washed samples differed significantly from the other refurbishing treatments. Therefore, in order to determine whether there were significant differences among the three alternative refurbishing treatments, the analysis was carried out without the washed data. From the one-way ANOVA data presented in Table 4.4 it was evident that there was a significant difference among the three refurbishing treatments ($F_{2,35} = 27.532$, $p <$

0.001). The daily growth rate for freezing was significantly different than both ironing and UV; ironing and UV also significantly differed from one another (see Table 4.5).

Table 4.4 *One-way ANOVA comparison of daily growth rate for three alternative refurbishing treatments*

Source of Variation	d.f.	SS	MS	F	p-value
Between Groups	2	3.110	1.555	27.532	0.000
Within Groups	33	1.864	0.057		
Total	35	4.974			

Table 4.5 *Difference in daily growth rate using Tukey's test for significant differences*

Groups	Difference	Test Statistic	p-value
Freeze vs Iron	0.403	5.875	0.0006
Freeze vs UV	0.718	10.468	0.0000
Iron vs UV	0.315	4.593	0.0074

Chapter 5

Discussion

5.1 Effect of treatments on reduction of bacteria

An overall reduction in viable bacterial counts was observed on all denim specimens immediately after the refurbishing treatment. However, during the 24-hour period following the treatment, the bacterial populations increased on some fabrics. At times this even resulted in higher populations than the baseline counts.

The following null hypothesis concerning the effect of the selected treatments on bacterial reduction was tested:

H_{o1}: There are no significant differences among the four refurbishing methods in the reduction of skin bacteria.

Reject: The statistical analysis (described in Section 4.2) regarding the results obtained from each treatment was used to determine any statistical difference among the four refurbishing treatments. Washing was significantly different from the three alternative refurbishing treatments at both 0 h and 24 h time periods. Freezing was significantly different from the ironing and UV treatments at 0 h; ironing and UV did not differ significantly at 0 h. At 24 h, there were no significant differences among freezing, ironing and UV treatments.

Of the four refurbishing methods, washing was the most effective at reducing viable skin bacteria on denim fabrics, with greater than 97% reduction of bacterial populations immediately after washing (Table 4.2) as bacterial colonies were below the limit of detection. This was not unexpected as laundering is a common method for removing soils, odour and other contaminants from clothing (Shove, 2003). For the other refurbishing treatments, freezing had the next highest percent reduction, followed by the UV and the ironing treatment (both of which were not significantly different from one another).

The outcome from washing raw denim aligns to some degree with previous laundering research where bacterial populations were evaluated after treatment. Bacteria were not recovered from inoculated samples when laundered at 60 °C and 90 °C (without detergent), however, some gram-negative bacteria were recovered at 40 °C (likely due to a build-up of

biofilms from the machine itself) (Lakdawala et al., 2011). In the same study, worn uniforms, washed at a 40 °C temperature also showed bacterial recovery of gram-negative bacilli likely attributed to contamination from laundering equipment used (Lakdawala et al., 2011). Elsewhere, 40 °C and 60 °C wash water temperatures were used to remove bacterial colonies from fabrics. Gram-negative bacilli was again recovered and attributed to the washing machine and not the inoculated broth (Patel et al., 2006). Where the current study diverges from Lakdawala et al. (2011) and Patel et al. (2006) was that denim samples were laundered in separate canisters, using 100% cotton raw denim (uniforms were a cotton and polyester blend). This may also explain why bacteria were not recovered from washed samples in this study as fabrics were not in direct contact with biofilms that may exist in the washing machine. Additionally, skin bacteria were gathered directly from healthy skin in the current study and not from an *in vitro* inoculation of specific microorganisms or exposure to medical settings with bodily fluids and potentially infectious microorganisms (an additional experiment performed by Lakdawala et al. (2011)). It should be noted that these previous studies involved the laundering of hospital and medical textiles, exposed often to multiple ill or vulnerable patients, whose nosocomial bacteria types and levels may be harmful to others. In the current study, the accumulation of skin bacteria on raw denim may not be as critical as microorganisms on medical garments as raw denim is more likely worn in non-medical circumstances. What may prove valuable as further research, is the exploration of laundering raw denim under lower washing temperatures as these conditions have likely led to cross-contamination, as suggested by Lakdawala et al. (2011) and Patel et al. (2006).

While less effective than washing, freezing was more effective than either the ironing or the UV treatment at reducing skin bacteria. The percent change ranged from –98.39% to –83.15% immediately after removal from the freezer. Bacterial structures are often damaged or greatly altered by freezing (Skogland et al., 1988), but over time, surviving bacteria repopulate once a growth-favourable temperature is achieved. In the current study bacteria were subjected to a large temperature change when the raw denim specimens were placed in the freezer. This destroyed many but not all of them. This also has been shown in soil research, where bacterial levels are reduced significantly after freezing (Morley et al., 1983). At different stages of the

freeze-thaw process, the structure of bacteria is altered depending upon strain types, final freezing temperature and time frame (Fonseca, Béal, & Corrieu, 2001). Freezing may cause cell death or irreversible damage and occasionally cause the cell to undergo some form of hibernation (Walker, Palmer, & Voordouw, 2006). As a result, a large number of viable bacterial colonies are eliminated upon analysis but those that survive freezing can sustain growth and reproduction once thawed. It is suspected that similar bacterial effects occurred to skin bacteria obtained on denim fabrics in the current study.

Reductions of bacterial population on denim specimens following ironing were not as large as that for washing and freezing. A percent change for ironed fabrics at 0 hours ranged from -87.27% to -49.61%. The iron has been recommended as an additional hygienic method for removing bacteria but is often supplemental to laundered samples that have also been put through a cycle in the tumble dryer (Bloomfield et al., 2013; Church & Loosli, 1953). The combination of heat and steam are common elements used in methods for bacterial reduction. Standard practice for disposal of medical waste includes a decontaminating step, often with an autoclave (Rutala, Stiegel, & Sarubbi, 1982) which may produce larger quantities of steam, use higher temperatures and create a pressurized atmosphere (which is not present with the household iron). Dental implements benefit from steam sterilization over other manual and chemical methods for eliminating bacteria (Eldik, Zilm, Rogers, & Marin, 2004) as many tools are reused. Increasingly, heat and steam have been adopted as methods for soil sterilization as the use of chemical pesticides and fumigants have been shown to be hazardous (Tanaka et al., 2003). With respect to the iron and bacterial reduction on raw denim, the length of time, temperature, and amount of steam was considerably lower than that of the previous methods mentioned, likely contributing to some but not complete bacterial elimination.

The use of sunlight has been recommended by online bloggers as a form of bacterial reduction and means of refreshing the garment. It is a non-abrasive, natural, sustainable and inexpensive method (Muzquiz, 2018; Yee, 2016). Applying UV light to the denim samples also resulted in a reduction of bacteria, however, like ironing, it was also less effective than washing and freezing immediately after exposure. Based on the results, the percent change in bacterial population ranged from -79.31% to -23.58% at 0 hours. Exposure to concentrated UV light

explored by Mitoraj et al. (2007) indicates that this type of treatment can be effective in bacterial reduction. However, in the case for the current study, the UV light used may not have had adequate intensity or perhaps there was insufficient exposure time to reduce large numbers of bacteria. Elsewhere, UV light is relied upon for sterilization purposes. Equipment emitting highly concentrated UV light, requiring the user to be protected by safety glasses, or tinted windows, are used for the elimination of bacteria in laboratories (Meechan & Wilson, 2006). These types of lamps have been used to reduce the bacteria on fruits responsible for accelerated deterioration. By emitting UVC rays of approximately 254 nm (Erkan, Wang, & Krizek, 2001) the formation of mold is reduced (Forges et al., 2018). The difference between these two studies and the current study was that fruits were subjected to multiple exposure periods to UV light, whereas, for the raw denim, only a one-time exposure treatment was carried out. Additionally, the thickness of raw denim is different than the skin of the fruits tested (pear and strawberries) and UV light may not be as effective on raw denim as fruit skin. Sterilization from UV light is also widely used for medical devices (Dempsey & Thirucote, 1988; Kylián & Rossi, 2009). These UV lights emit pulsed or concentrated light between 200-300 nm which lead to inactivation of microorganisms or permanently altering their DNA rendering bacteria incapable of reproducing (Kylián & Rossi, 2009). Sunlight as a disinfectant for drinking water is often implemented when other means are not accessible (Davies, Roser, Feitz, & Ashbolt, 2009). For example, harmful bacteria found in fully oxygenated water (*Escherichia coli* and *Enterococcus faecalis*) have been shown to be inactivated using sunlight (Reed, 1997), although there is much variability in light intensity and other environmental considerations (Davies et al., 2009; Gates, 1966).

Immediately after UV treatment (at 0 hours), it did appear that the treatment reduced viable bacteria, but they were not completely eliminated and showed growth after 24 hours. With raw denim, the interlacing of the warp and weft yarns may have had an impact, as exposure to UV light may not have penetrated the underlapping yarns where bacteria may have inhabited. Bacteria on the surface were exposed to the UV light, but within the layers of the raw denim, bacteria may have been protected by the yarns within the fabric structure and been able to persist.

5.2 Effect of treatment on bacterial reduction 24 hours after treatment

Twenty-four hours after the refurbishing treatments bacteria grew on the refurbished denim specimens for all of the treatments except for the washed specimens (Figure 4.1). The percent change from the baseline counts did not differ among the three alternative refurbishing treatments (i.e., freeze, iron and UV) at the 24-hour period post-treatment. However, there was wide variability among counts for specimens that were ironed, with many specimens having counts higher than the baseline (particularly notable among many ironed specimens).

The following null hypothesis concerning the effect of the selected treatments on bacterial reduction was tested:

H_{O2}: There are no significant differences between time 0 hours and 24 hours in the reduction of bacteria after each of the four refurbishing methods.

Reject: Based on the statistical analysis from Section 4.2, there were significant differences between both time periods for freezing, ironing and UV but not for the washing treatment.

While analysing washed samples, there were no visible bacterial colonies observed, however, bacteria may have been present, below the limit of detection. This result is not entirely surprising, since the removal of bacteria and soils from clothing items after they have been worn is the intention of laundering (Sams, 2001; Shove, 2004). As previously mentioned, the combination of detergent, mechanical agitation, water and heat, contribute to the successful elimination of bacteria when compared with the other three treatments. Furthermore, because the laundering procedure is successful at removing not only bacteria but also other contaminants (e.g., oils and skin cells) that could act as nutrient sources for bacterial metabolism, bacterial growth did not appear to occur during that 24-hour period.

Interestingly, although freezing was shown to be more effective at killing bacteria compared to the other two alternative refurbishing methods immediately after treatment, there were no significant differences in bacterial counts after 24 hours, indicating bacteria re-populated within the time period. Compared to the results for UV and ironing, the greatest amount of growth during the 24-hour period occurred for denim samples that had been frozen (see Figure 4.2). One explanation for this more rapid increase in growth on frozen samples

could be related to the moisture levels in denim samples after thawing. When denim swatches were removed from the arm, the fabrics were moist due to the humidity created by the plastic barrier. As the fabrics were immediately frozen after removal from the body, the thawed fabrics were still moist and this may have been favourable for bacterial growth during the 24-hour thawing process, once the fabric had increased to room temperature. Although bacterial counts of frozen fabrics were initially much lower suggesting some merit to the freezing method, upon thawing, freezing may not be the best alternative "cleaning" solution due to the rapid rate of bacterial growth.

Twenty-four hours after ironing, bacteria not only grew to counts close to the baseline numbers, but in many specimens exceeded baseline counts. The initial heat and steam appeared to have some impact on reducing bacterial levels, but over time, was not lasting as bacteria displayed some growth. It could be possible that due to the increase of heat on the fabric from the treatment, the environment was more favourable for bacterial growth, which could have continued when the samples were placed in ambient temperatures. These environmental conditions may have been more favourable than those of the UV samples, thereby experiencing a longer period of favourable growth conditions, possibly causing increased growth. What was also notable regarding the iron was the variability of bacterial populations. In a realistic setting, the variability could be even more pronounced as the iron is generally passed over garments rapidly, not directly remaining on the garment for extended periods of time (which may cause damage). Additionally, jeans have numerous layers of denim fabric, where bacteria in these areas may not have full contact with the iron and be less likely to be damage by the heat and steam.

The rate of growth for bacteria on denim specimens exposed to UV light during that 24-hour period was much lower than for freezing. What this means is that from what had remained of the bacteria that survived following the UV treatment, bacteria did not replicate as quickly as those that had survived freezing and many of the ironing samples. A potential reason for this lower rate of growth may be due to fabric drying out during the UV treatment (unlike the samples removed from the freezer, which were moist) before being placed in ambient temperatures for 24 hours. The surface of the raw denim would not have been as favourable for

additional bacterial growth as when the samples were moist and warm, ideal for any remaining bacteria on the fabrics to replicate over time.

5.3 Analysis of findings

In this study the alternative methods for reducing bacteria on denim were evaluated and compared to laundering which is the most common type of refurbishing method carried out on garments. The results showed that laundering was indeed the most effective solution for reducing bacterial populations both immediately after the treatment as well as over time. Since laundering is the principal method for refreshing and cleaning clothing, this study confirmed that this is a reliable domestic choice. However, this method poses problems for raw denim owners as laundering causes some of the degradation of the garment (Kan & Yuen, 2009; McQueen et al., 2017). This can lead to alteration of custom fading patterns and creases unique to the individual and change in size or fit. Moreover, the washing machine and dryer has been shown to consume high levels of energy and water (Laitala et al., 2012), which are not considered sustainable practices. As a result, there has been discussion about reducing the use of these machines or altering the settings (lower temperatures with longer cycles or using fuller loads (Laitala et al., 2012)) to consume fewer resources.

The heat of the iron, with the addition of steam was effective in reducing some bacterial colonies. Further reduction could potentially occur if time and/or temperature were increased as a relationship between temperature and length of time in effectiveness of bacterial elimination exists among many methods. Increasing the temperature (or time for direct contact) may be hazardous as it may result in burning or damage to the cotton fibres. Since the purpose of this study was to explore alternative methods for denim refurbishing while avoiding any garment degradation, extending the contact time with a hot iron pressed on denim may not be ideal. Previous research has suggested the use of the iron to provide increased hygienic benefits, one method disclosed the temperature reached 350 °C (Church & Loosli, 1953), another had no reference to temperature in the method at all (Patel et al., 2006); so an increase in the temperature of the iron may have resulted in greater reduction in percentage of bacteria in the current study. In addition, Church & Loosli (1953), Eckert et al. (2014) and Patel et al. (2006) all

found significant bacterial reduction with the iron after the use of the tumble dryer, which was not performed in this study. Also, small samples of denim were used, not the entire garment. It may not be practical for complete ironing of the garment which may have multiple layers of fabric and complicated stitching where the heat from the iron may not penetrate. Having said that, there may be increased effectiveness in increasing the temperature and / or contact time before the damage point, which would be worth further investigation.

The use of sunlight is a common method of drying clothing for many. Anecdotal reports of a refreshed outdoor smell contribute to its popularity (Jack, 2013) as well as its low consumption of energy. One notable barrier to outdoor drying includes geography and climate of the region which contributes to the seasonal popularity of this method and geographical reports (Pink et al., 2015). Although there was some reduction in bacterial colonies immediately after the UV treatment, bacterial growth was still observed over time. After the treatment, bacteria were able to replicate, therefore this was not an effective treatment. The use of the Weather-Ometer is limiting as it does not completely simulate the realistic conditions of having the garment exposed to sunlight outside. The length of time may be another factor to consider as extended exposure time may lead to increased initial bacterial reduction. This recommendation may not be practical in a realistic situation as the level of sunlight on a given day could be highly variable although it may still be worth exploring in a laboratory setting. Also, a pair of raw denim jeans is not the same as a small sample of fabric; the multiple layers of fabrics and design features (such as pockets) are unlikely to be penetrated by sunlight.

While showing early promise, the use of the freezer to kill bacteria behaved along the same lines as research purposefully using this method to conserve bacteria (Morley et al., 1983). What may be worth pursuing further would be to experiment with worn denim that is completely dry before placing into the freezer, potentially reducing the amount of moisture when thawed that may encourage bacterial growth. Or, perhaps, a combination of freeze-thaw-freeze, which has been used in textile conservation research?

5.4 Research limitations

As mentioned previously, denim specimens were worn for twenty-four hours. This time frame may have impacted the population density of cutaneous flora on the samples which was relatively low upon analysis of worn baseline samples in this current study. Additionally, only one type of denim was used, which may not be indicative of other denim fabrics or other materials. Other forms of denim may be treated with numerous chemicals or are comprised of a fabric blend which could influence bacterial behaviour differently.

Odour was not analyzed, even though this has been mentioned as an important factor when avoiding washing raw denim, and could have provided additional insight into the effect of the treatments.

The type of agar used may not have enhanced the growth of particular microorganisms, which may have influenced the visible colonies observed during analysis.

It is important to qualify conclusions drawn from this study as denim samples were placed on the arm and only worn for twenty-four hours before removal. The efficacy of the selected treatments may differ from a build-up of microorganisms over the course of consecutive wears, which would more closely reflect raw denim wear patterns.

Chapter 6

Summary, conclusions and recommendations

6.1 Summary

The purpose of this study was to determine the impact of selected alternative refurbishing treatments (freezing, ironing, UV light) on skin bacterial count growth on raw denim and how they compare to results from washing. A human wear trial was employed to collect skin bacteria on raw denim fabrics. Female participants (n=6) were recruited and wore fabrics attached to the skin with a plastic barrier on the posterior of both forearms for twenty-four hours. Counts of bacteria extracted from worn fabrics were conducted before refurbishing treatments (baseline counts), immediately after the refurbishing treatment (0 hour counts) and 24 hours after the refurbishing treatment (24 hour counts) were taken. Bacterial density varied among participants (and to some extent within participants). Participants' baseline counts were used to compare the percent change in bacterial populations for each refurbishing treatment and time period. Fabrics that were washed had the highest reduction of bacteria, both immediately after the treatment as well as 24 hours following treatment. For the other three refurbishing treatments, freezing had the highest bacterial reduction immediately after treatment; ironing and UV treatments were less effective at reducing bacteria than freezing. However, during the 24-hour period after treatment there was a greater rate of bacterial growth in the specimens that had been frozen. Lower bacterial growth occurred between the 0-24 hours for specimens that had been exposed to the UV and ironing.

6.2 Conclusions

Raw denim has recently emerged as a popular style of jeans, sparking a growing community of enthusiasts. The unique properties of raw denim paired with the considerable cost for some have inspired numerous online media and aficionados to find alternative methods for maintaining the garment without the use of traditional laundering methods. This is due to not only sustainable endeavours but also to avoid the negative effects from degradation associated with laundering denim. As such, this study sought to evaluate the viability of

suggested alternative methods for reducing the build-up of bacteria on denim and refresh them for continuous wears.

Washing effectively removed skin bacteria to the point that growth did not reoccur during the 24-hour period after refurbishing. Mechanical agitation, detergent, and warm water are likely the reasons for the significant and sustained bacterial reduction in this experiment. Laundering clothing is the most common method used to clean and refresh clothing, but has also been identified as a method to avoid for raw denim according to raw denim blogs and enthusiasts, posing a challenging dilemma for the care and maintenance of these garments.

Not one of the alternative refurbishing treatments was effective in eliminating skin bacteria from raw denim fabrics. Freezing greatly reduced bacterial populations, likely because the sub-zero temperatures caused cell damage to some of the bacteria (more specifically, bacterial membrane and/or DNA structure). This suggested that freezing may be more effective at reducing bacteria in denim jeans than either ironing or UV light exposure. However, while the sample thaws, surviving bacteria can rapidly multiply and any initial effectiveness due to freezing may make little difference by the time the jeans are ready to be worn again. As an accessible and low-cost alternative, sun drying garments is common and sometimes relied upon by some who do not have access to a machine dryer. While this method may be useful for drying clothing and consumes fewer resources, it was not an effective method for bacterial reduction. Another low-cost method for drying, smoothing and finishing clothing is the handheld steam iron, which uses less energy than the tumble dryer and overall, very little water (Porteous et al., 2012). Similar to UV light exposure, the iron was not effective at reducing bacteria.

The desire to preserve the unique markings developed on worn denim, the colour intensity and the fit makes this garment a challenge to maintain. As the results from this study indicate, bacteria reduction is not sustained over time from treatments other than washing and further research is required to determine the viability of alternatives.

6.3 Recommendations

The following recommendations are proposed for further evaluation of possible alternative refurbishing methods to not only further clarify issues raised in this thesis, but to extend the test method herein to better understand the practices involved:

1. Carry out an experiment allowing for the accumulation of bacteria within fabrics to occur over multiple wears in order to increase bacterial populations which would better simulate raw denim wearer's behaviour.
2. Include male participants in future research to provide further insight into cutaneous bacteria with respect to alternative refurbishing treatments on raw denim.
3. Incorporate supplemental methods to further explore alternatives to laundering for denim refurbishing such as (but not limited to) the oven, exposure to direct sunlight, use of the dryer and iron, a garment steamer, a UV wand or denim cleaning spray.
4. Conduct further research incorporating human participants where denim is in contact with the lower body to provide increased understanding of skin bacteria in this region.
5. Extend the time period on worn samples post-treatment beyond twenty-four hours as a means to observe bacterial growth and reduction behaviour for longer periods of time.
6. Explore the effect of body temperature on raw denim post-treatment. This could simulate wearing the garment after treatment with an increase in temperature from the wearer's body which may affect bacterial growth on raw denim.
7. Prior to freezing, drying raw denim will reduce the moisture (which can be favourable for bacterial growth).
8. Evaluate different lengths of time for freezing raw denim, to explore any impact this variable may have.

References

American Association of Textile Chemists and Colorists. (2010a). *AATCC Test Method 61-2009 Colorfastness to laundering: Accelerated* (Technical Manual). Research Triangle Park.

American Association of Textile Chemists and Colorists. (2010b). *AATCC Test Method 100-2004 Antibacterial finishes on textile materials: Assessment of* (Technical Manual). Research Triangle Park.

American Association of Textile Chemists and Colorists. (2010c). *AATCC Test Method 111-2009 Weather resistance of Textiles: Exposure to daylight and weather* (Technical Manual). Research Triangle Park.

Abdul-Bari, M. M., McQueen, R. H., Nguyen, H., Wismer, W. V., de la Mata, A. P., & Harynuk, J. J. (2018). Synthetic Clothing and the Problem With Odor: Comparison of Nylon and Polyester Fabrics. *Clothing and Textiles Research Journal*, *36*(4), 251–266. https://doi-org.login.ezproxy.library.ualberta.ca/10.1177/0887302X18772099

Al-Benna, S. (2010). Laundering of theatre scrubs at home. *Journal of Perioperative Practice*, *20*(11), 392–396. https://doi.org/10.1177/175045891002001101

Alborzi, F., Schmitz, A., & Stamminger, R. (2017). Long wash cycle duration as a potential for saving energy in laundry washing. *Energy Efficiency*, *10*(4), 823–838. https://doi.org/10.1007/s12053-016-9486-z

Anonymous. (2014, July 16). The No Wash Club. Retrieved December 8, 2018, from https://medium.com/makers-mavericks/the-no-wash-club-dc0597b0239f#.r2rtydt7f

Archer, D. L. (2004). Freezing: An underutilized food safety technology? *International Journal of Food Microbiology*, *90*(2), 127–138. https://doi.org/10.1016/S0168-1605(03)00215-0

Arild, A.-H., Brusdal, R., Halvorsen-Gunnarsen, J., Terpstra, P. M., & Van Kessel, I. A. (2003). *An investigation of domestic laundry in Europe: Habits, hygiene and technical performance* (No. 1–2003). Oslo: National Institute for Consumer Research.

Armstrong, B. A., & Reinhardt, P. A. (2010). Managing laboratory biomedical waste using a large on-site autoclave–shredder. *Journal of Chemical Health and Safety*, *17*(6), 33–39. https://doi.org/10.1016/j.jchas.2010.02.002

Babb, J., Davies, J., & Ayliffe, G. (1983). Contamination of protective clothing and nurses' uniforms in an isolation ward. *Journal of Hospital Infection*, *4*(2), 149–157.

Bajpai, D., & Tyagi, V. K. (2007). Laundry detergents: An overview. *Journal of Oleo Science*, *56*(7), 327–340. https://doi.org/10.5650/jos.56.327

Bellis, M. (2017, April 13). Who Can We Thank for Inventing the Electric Iron? Retrieved September 21, 2018, from https://www.thoughtco.com/who-invented-the-iron-1991675

Benson, R. S. (2002). Use of radiation in biomaterials science. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, *191*(1), 752–757. https://doi.org/10.1016/S0168-583X(02)00647-X

Bibel, D. J., & Lovell, D. J. (1976). Skin flora maps: A tool in the study of cutaneous ecology. *Journal of Investigative Dermatology*, *67*(2), 265–269. https://doi.org/10.1111/1523-1747.ep12513459

Blaser, M. J., Smith, P. F., Cody, H. J., Wang, W.-L. L., & LaForce, F. M. (1984). Killing of fabric-associated bacteria in hospital laundry by low-temperature washing. *The Journal of Infectious Diseases*, *149*(1), 48–57.

Bloomfield, P. S. F., Exner, P. M., Carlo, P., & Scott, D. E. A. (2013). Effectiveness of laundering processes used in domestic (home) settings. *International Scientific Forum on Home Hygiene*, 62.

Bockmühl, D. (2017). Laundry hygiene—how to get more than clean. *Journal of Applied Microbiology*, *122*(5), 1124–1133.

Boey, F. Y. C., & Lye, S. W. (1990). Effects of vacuum and pressure in an autoclave curing process for a thermosetting fibre-reinforced composite. *Journal of Materials Processing Technology*, *23*(2), 121–131. https://doi.org/10.1016/0924-0136(90)90152-K

Braga, G. U. L., Flint, S. D., Miller, C. D., Anderson, A. J., & Roberts, D. W. (2001). Both solar UVA and UVB radiation impair conidial culturability and delay germination in the entomopathogenic fungus metarhizium anisopliae¶. *Photochemistry and Photobiology*, *74*(5), 734–739. https://doi.org/10.1562/0031-8655(2001)0740734BSUAUR2.0.CO2

Bresee, R. R., Annis, P. A., & Warnock, M. M. (1994). Comparing actual fabric wear with laboratory abrasion and laundering. *Textile Chemist & Colorist*, *26*(1), 17–23.

Byrns, G., Barham, B., Yang, L., Webster, K., Rutherford, G., Steiner, G., … Scannell, M. (2017). The uses and limitations of a hand-held germicidal ultraviolet wand for surface disinfection. *Journal of Occupational and Environmental Hygiene*, *14*(10), 749–757. https://doi.org/10.1080/15459624.2017.1328106

Callewaert, C., De Maeseneire, E., Kerckhof, F.-M., Verliefde, A., Van de Wiele, T., & Boon, N. (2014). Microbial odor profile of polyester and cotton clothes after a fitness session. *Applied and Environmental Microbiology*, *80*(21), 6611–6619. https://doi.org/10.1128/AEM.01422-14

Callewaert, C., Van Nevel, S., Kerckhof, F.-M., Granitsiotis, M. S., & Boon, N. (2015). Bacterial exchange in household washing machines. *Frontiers in Microbiology*, *6*. https://doi.org/10.3389/fmicb.2015.01381

Canadian General Standards Board. (1989). *CAN/CGSB-4.2 No.6-M89 textile test methods:Woven fabrics – Construction – Methods of analysis – Part 2: Determination of number of threads per unit length.* Ottawa, ON:Author.

Canadian General Standards Board. (1997). *CAN/CGSB-4.2 No.5.1-M90 textile test methods: Unit mass of fabric.* Ottawa, ON:Author.

Canadian General Standards Board. (2002). *CAN/CGSB-4.2 No.37-2002 textile test methods: Fabric thickness.* Ottawa, ON:Author.

Card, A., Moore, M. A., & Ankeny, M. (2006). Garment washed jeans: Impact of launderings on physical properties. *International Journal of Clothing Science and Technology*, *18*(1), 23–52.

Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2004). Relevant factors for the preparation of freeze-dried lactic acid bacteria. *International Dairy Journal*, *14*(10), 835–847. https://doi.org/10.1016/j.idairyj.2004.02.001

Carver, M. N., & Wylie, M. J. (1980). Fabric whiteness as affected by selected laundry treatments and environmental factors. *Home Economics Research Journal*, *9*(2), 96–104.

Chad. (2011, April 5). Cleaning Via Freezing – Controversial But Effective. Retrieved from https://www.heddels.com/2011/04/cleaning-via-freezing-controversial-but-effective/

Chen, Y. E., & Tsao, H. (2013). The skin microbiome: Current perspectives and future challenges. *Journal of the American Academy of Dermatology*, *69*(1), 143–155.

Choudhary, V. (2012). Compatibility with commercial detergents and stain removal capability of Aspergillus versicolor protease. *J Acad Indus Res*, *1*, 301–305.

Chudáčková, E., Geigerová, L., Hrabák, J., Bergerová, T., Liška, V., & Scharfen, J. (2010). Seven isolates of actinomyces turicensis from patients with surgical infections of the anogenital area in a Czech hospital. *Journal of Clinical Microbiology*, *48*(7), 2660–2661. https://doi.org/10.1128/JCM.00548-10

Church, B. D., & Loosli, C. G. (1953). The role of the laundry in the recontamination of washed bedding. *The Journal of Infectious Diseases*, *93*(1), 65–74.

Cilliers, F. P., Gouws, P. A., Koutchma, T., Engelbrecht, Y., Adriaanse, C., & Swart, P. (2014). A microbiological, biochemical and sensory characterisation of bovine milk treated by heat and ultraviolet (UV) light for manufacturing cheddar cheese. *Innovative Food Science & Emerging Technologies*, *23*, 94–106. https://doi.org/10.1016/j.ifset.2014.03.005

Clingen, P. H., Arlett, C. F., Roza, L., Mori, T., Nikaido, O., & Green, M. H. L. (1995). Induction of cyclobutane pyrimidine dimers, pyrimidine(6-4)pyrimidone photoproducts, and dewar valence isomers by natural sunlight in normal human mononuclear cells. *Cancer Research*, *55*(11), 2245–2248.

Coe, N. (2011, September 12). The Essential Raw Denim Breakdown – Our 100th Article. Retrieved March 29, 2017, from https://www.heddels.com/2011/09/the-essential-raw-denim-breakdown-our-100th-article/

Comstock, S. C. (2011). The making of an American icon: The transformation of blue jeans during the great depression. In D. Miller & S. Woodward (Eds.), *Global Denim* (pp. 23–50). Oxford, England: Berg.

Cook, J. (2001). *Handbook of Textile Fibres* (Vol. 1-Natural Fibres). Cambridge, England: Woodhead Publishing Limited.

Cox, C. (2014, May 22). The CEO of Levi Wants You to Freeze Your Jeans, but Does Science? Retrieved December 17, 2018, from https://www.themarysue.com/freezing-jean-science/

Craig, A. (n.d.). The Best (and Worst) Ways to Wash Raw Denim. Retrieved January 20, 2018, from ://www.bespokepost.com/the-post/the-best-and-worst-ways-to-wash-raw-denim

Cundell, A. M. (2018). Microbial ecology of the human skin. *Microbial Ecology*, *76*(1), 113–120. https://doi-org:10.1007/978-1-4615-8222-9_6

Dachis, A. (2011, April 5). Freeze Your Jeans to Keep Them Clean and the Denim Intact. Retrieved October 21, 2018, from https://lifehacker.com/5788793/freeze-your-jeans-to-keep-them-clean-and-the-denim-intact

Davies, C. M., Roser, D. J., Feitz, A. J., & Ashbolt, N. J. (2009). Solar radiation disinfection of drinking water at temperate latitudes: Inactivation rates for an optimised reactor configuration. *Water Research*, *43*(3), 643–652. https://doi.org/10.1016/j.watres.2008.11.016

DeLong, M., Koh, A., Nelson, N., & Ingvoldstad, A. (1998). Jeans: A Comparison of Perceptions of Meaning in Korea and The United States. *Clothing and Textiles Research Journal*, *16*(3), 116–125. https://doi.org/10.1177/0887302X9801600302

Dempsey, D. J., & Thirucote, R. R. (1988). Sterilization of medical devices: A review. *Journal of Biomaterials Applications*, *3*(3), 454–523. https://doi.org/10.1177/088532828800300303

Donaghy, J., Keyser, M., Johnston, J., Cilliers, F., Gouws, P., & Rowe, M. (2009). Inactivation of Mycobacterium avium ssp. paratuberculosis in milk by UV treatment. *Letters in Applied Microbiology*, *49*(2), 217–221. https://doi-org:/10.1111/j.1472-765X.2009.02644.x

Driving increases in the global denim market. (2018, July 23). Retrieved August 23, 2018, from https://lifestylemonitor.cottoninc.com/innovation-culture/

Dylan. (2017, February 9). A Guide on How to Break In Raw Denim the Right Way. Retrieved November 20, 2018, from https://www.parcelpal.com/2017/02/09/break-raw-denim/

Eckert, A., Booten, Y., Jarchow, M., Lucassen, R., Nemitz, L., & Bockmühl, D. (2012). *Reduction of microorganisms by ironing*. Presented at the Poster presentation 2012, University of Applied Sciences.

Edmonds-Wilson, S. L., Nurinova, N. I., Zapka, C. A., Fierer, N., & Wilson, M. (2015). Review of human hand microbiome research. *Journal of Dermatological Science*, *80*(1), 3–12.

Ehrlich, H. L. (1998). Geomicrobiology: Its significance for geology. *Earth-Science Reviews*, *45*(1), 45–60. https://doi.org/10.1016/S0012-8252(98)00034-8

Eldik, D. V., Zilm, P., Rogers, A., & Marin, P. (2004). Microbiological evaluation of endodontic files after cleaning and steam sterilization procedures. *Australian Dental Journal*, *49*(3), 122–127. https://doi-org.login.ezproxy.library.ualberta.ca/10.1111/j.1834-7819.2004.tb00060.x

El-Ghezal, S., Babay, A., Dhouib, S., & Cheikhrouhou, M. (2009). Study of the impact of elastane's ratio and finishing process on the mechanical properties of stretch denim. *The Journal of The Textile Institute*, *100*(3), 245–253. https://doi.org/10.1080/00405000701757925

Erkan, M., Wang, C. Y., & Krizek, D. T. (2001). UV-C irradiation reduces microbial populations and deterioration in Cucurbitapepo fruit tissue. *Environmental and Experimental Botany*, *45*(1), 1–9. https://doi.org/10.1016/S0098-8472(00)00073-3

Expert Denim Care: Getting The Most From Your Jeans. (n.d.). Retrieved November 20, 2018, from https://www.garmentcare.com/blog/dos-and-donts-to-get-the-most-from-your-denim

Feather, B., Caselman, M., & Cooper, J. (1993). Home laundry: Get the results you want. University Extension.

Ferri, A., Osset, M., Abeliotis, K., Amberg, C., Candan, C., Owens, J., & Stamminger, R. (2016). Laundry performance: Effect of detergent and additives on consumer satisfaction. *Tenside Surfactants Detergents*, *53*(4), 375–386. https://doi.org/10.3139/113.110451

Fianu, D. A. G., Sallah, R. A., & Ayertey, A. (2005). The effect of sunlight and drying methods on the strength of Ghanaian Real Wax printed fabrics. *International Journal of Consumer Studies*, *29*(1), 34–40. https://doi.org/10.1111/j.1470-6431.2005.00371.x

Fields, J. (2016, October 27). Stop ruining your jeans: The right way to wash your denim. Retrieved November 20, 2018, from https://www.usatoday.com/story/tech/reviewedcom/2016/10/27/stop-ruining-your-jeans-right-way-wash-your-denim/92855130/

Florian, M. (1986). The freezing process-effects on insects and artifact materials. *Leather Conservation News*, *3*(1), 1–13.

Florian, M.-L. (1987). *The effect on artifact materials of the fumigant ethylene oxide and freezing used in insect control*. na.

Fonseca, F., Béal, C., & Corrieu, G. (2001). Operating conditions that affect the resistance of lactic acid bacteria to freezing and frozsn Storage. *Cryobiology*, *43*(3), 189–198. https://doi.org/10.1006/cryo.2001.2343

Forges, M., Vàsquez, H., Charles, F., Sari, D. C., Urban, L., Lizzi, Y., … Aarrouf, J. (2018). Impact of UV-C radiation on the sensitivity of three strawberry plant cultivars (Fragaria x ananassa) against Botrytis cinerea. *Scientia Horticulturae*, *240*, 603–613. https://doi.org/10.1016/j.scienta.2018.06.063

Fredricks, D. N. (2001). Microbial ecology of human skin in health and disease. *Journal of Investigative Dermatology Symposium Proceedings*, *6*(3), 167–169. https://doi.org/10.1046/j.0022-202x.2001.00039.x

Friedman, A., & Friedman, A. (2017, October 26). New Denim Fiber and Fabric Innovations to Increase Jeans' Appeal. Retrieved August 23, 2018, from https://sourcingjournal.com/topics/raw-materials/new-denim-fibers-and-fabrics-innovation-71724/

Gao, Z., Tseng, C. -h., Pei, Z., & Blaser, M. J. (2007). Molecular analysis of human forearm superficial skin bacterial biota. *Proceedings of the National Academy of Sciences*, *104*(8), 2927–2932. https://doi.org/10.1073/pnas.0607077104

Garibaldi, B. T., Reimers, M., Ernst, N., Bova, G., Nowakowski, E., Bukowski, J., … Parrish, N. M. (2017). Validation of autoclave protocols for successful decontamination of category a

medical waste generated from care of patients with serious communicable diseases. *Journal of Clinical Microbiology*, *55*(2), 545–551. https://doi.org/10.1128/JCM.02161-16

Gates, D. M. (1966). Spectral distribution of solar radiation at the earth's surface. *Science*, *151*(3710), 523–529.

Gattlen, J., Amberg, C., Zinn, M., & Mauclaire, L. (2010). Biofilms isolated from washing machines from three continents and their tolerance to a standard detergent. *Biofouling*, *26*(8), 873–882. https://doi.org/10.1080/08927014.2010.524297

Geum, K.-S., & DeLong, M. R. (1992). Korean traditional dress as an expression of heritage. *Dress*, *19*, 57–68.

Gregersen, T. (1978). Rapid method for distinction of gram-negative from gram-positive bacteria. *European Journal of Applied Microbiology and Biotechnology*, *5*(2), 123–127. https://doi.org/10.1007/BF00498806

Grice, E. A., Kong, H. H., Conlan, S., Deming, C. B., Davis, J., Young, A. C., … Segre, J. A. (2009). Topographical and temporal diversity of the human skin microbiome. *Science*, *324*(5931), 1190–1192.

Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature Reviews Microbiology*, *9*(4), 244. https://doi.org/10.1038/nrmicro2537

Guide to Apparel and Textile Care Symbols. (2004). Industry Canada's Office of Consumer Affairs.

Gutarowska, B., Pietrzak, K., Machnowski, W., & Milczarek, J. M. (2016). Historical textiles – A review of microbial deterioration analysis and disinfection methods. *Textile Research Journal*, *87*(19), 2388–2406. https://doi.org/10.1177/0040517516669076

Halbert, A. R., & Chan, J. J. (2002). Anogenital and buttock ulceration in infancy. *Australasian Journal of Dermatology*, *43*(1), 1–8. https://doi.org/10.1046/j.1440-0960.2002.zx563.x

Hartmann, A. (1983). Effect of occlusion on resident flora, skin-moisture and skin-pH. *Archives of Dermatological Research*, *275*(4), 251–254.

Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: A review. *Annals of Microbiology*, *60*(4), 579–598. https://doi.org/10.1007/s13213-010-0117-1

Heinrich, V., Zunabovic, M., Varzakas, T., Bergmair, J., & Kneifel, W. (2016). Pulsed light treatment of different food types with a special focus on meat: A critical review. *Critical Reviews in Food Science and Nutrition*, *56*(4), 591–613. https://doi.org/10.1080/10408398.2013.826174

Humphries, M. (2009). *Fabric glossary* (4th ed.). Upper Saddle River, N.J. : Pearson/Prentice Hall,.

Jack, T. (2013a). Laundry routine and resource consumption in Australia. *International Journal of Consumer Studies*, *37*(6), 666–674. https://doi.org/10.1111/ijcs.12048

Jack, T. (2013b). Nobody was dirty: Intervening in inconspicuous consumption of laundry routines. *Journal of Consumer Culture*, *13*(3), 406–421. https://doi.org/10.1177/1469540513485272

Jakobi, G. (1987). Detergents and textile washing. *VCH GmbH*.

Jaska, J. M., & Fredell, D. L. (1980). Impact of detergent systems on bacterial survival on laundered fabrics. *Applied and Environmental Microbiology*, *39*(4), 743–748.

Kalaoglu, F., & Paul, R. (2015). 14 - Finishing of jeans and quality control. In R. Paul (Ed.), *Denim* (pp. 425–459). Woodhead Publishing. https://doi.org/10.1016/B978-0-85709-843-6.00014-7

Kan, C. W. (2015). 11 - Washing techniques for denim jeans. In R. Paul (Ed.), *Denim* (pp. 313–356). Woodhead Publishing. https://doi.org/10.1016/B978-0-85709-843-6.00011-1

Kan, C. W., & Yuen, C. W. M. (2009). Evaluation of the performance of stretch denim fabric under the effect of repeated home laundering processes. *International Journal of Fashion Design, Technology and Education*, *2*(2–3), 71–79. https://doi.org/10.1080/17543260903302329

Kanlayavattanakul, M., & Lourith, N. (2011). Body malodours and their topical treatment agents. *International Journal of Cosmetic Science*, *33*(4), 298–311. https://doi.org/10.1111/j.1468-2494.2011.00649.x

Karr, A. (2011, November 11). Quick tip: Freeze your jeans. *Flare Magazine*. Retrieved from https://www.flare.com/fashion/quick-tip-freeze-your-jeans/

Keet, P. (2011). Making new vintage jeans in Japan: Relocating authenticity. *TEXTILE*, *9*(1), 44–61. https://doi.org/10.2752/175183511X12949158771437

Kim, J., Petin, V., & Morozov, I. (2005). Quantitative evaluation of the parameters of bacterial photoreactivation after exposure to ultraviolet light and ionizing radiation. *International Journal of Radiation Biology*, *81*(1), 55–62. https://doi-org.10.1080/09553000500092244

Klepp, I. (2003). Clothes and cleanliness :Why we still spend as much time on laundry. *Ethnologia Scandinavica*, *33 (2003)*, 61–73.

Klepp, I. G., Buck, M., Laitala, K., & Kjeldsberg, M. (2016). What's the problem? Odor-control and the smell of sweat in sportswear: Fashion practice: *Fashion Practice*, *8*(2), 296–317. https://doi-org.10.1080/17569370.2016.1215117

Kloos, W. E., & Musselwhite, M. S. (1975). Distribution and persistence bof Staphylococcus and Micrococcus species and other aerobic acteria on human skin. *Journal of Applied Microbiology*, *30*(3), 381–395.

Kolde, R., Franzosa, E. A., Rahnavard, G., Hall, A. B., Vlamakis, H., Stevens, C., … Huttenhower, C. (2018). Host genetic variation and its microbiome interactions within the Human Microbiome Project. *Genome Medicine*, *10*(1), 2–18.

Kong, H. H. (2011). Skin microbiome: Genomics-based insights into the diversity and role of skin microbes. *Trends in Molecular Medicine*, *17*(6), 320–328.

Kong, H. H., & Segre, J. A. (2012). Skin microbiome: Looking back to move forward. *Journal of Investigative Dermatology*, *132*(3, Part 2), 933–939. https://doi.org/10.1038/jid.2011.417

Kotsanas, D., Scott, C., Gillespie, E. E., Korman, T. M., & Stuart, R. L. (2008). What's hanging around your neck? Pathogenic bacteria on identity badges and lanyards. *Medical Journal of Australia; Pyrmont*, *188*(1), 5–8.

Koushyar, H., Alavi-Soltani, S., Minaie, B., & Violette, M. (2011). Effects of variation in autoclave pressure, temperature, and vacuum-application time on porosity and mechanical properties of a carbon fiber/epoxy composite. *Journal of Composite Materials*, *46*(16), 1985–2004. https://doi.org/10.1177/0021998311429618

Kumar, S., Chatterjee, K., Padhye, R., & Nayak, R. (2016). Designing and development of denim fabrics: Part 1 - Study the effect of fabric parameters on the fabric characteristics for women's wear. *Journal of Textile Science and Engineering*, *6*(4), 1–5. https://doi.org/10.4172/2165-8064.1000265

Kylián, O., & Rossi, F. (2009). Sterilization and decontamination of medical instruments by low-pressure plasma discharges: Application of Ar/O 2 /N 2 ternary mixture. *Journal of Physics D: Applied Physics*, *42*(8), 085207. https://doi.org/10.1088/0022-3727/42/8/085207

Labhard, L. A., & Pedersen, E. L. (1989). Laundry practices: Line-drying and load characteristics. *Journal of Consumer Studies & Home Economics*, *13*(4), 307–312. https://doi.org/10.1111/j.1470-6431.1989.tb00026.x

Laitala, K, Klepp, I., & Boks, C. (2012). Changing laundry habits in Norway. *International Journal of Consumer Studies*, *36*(2). https://doi.org/10.1111/j.1470-6431.2011.01081.x

Laitala, Kirsi, Boks, C., & Klepp, I. G. (2011). Potential for environmental improvements in laundering. *International Journal of Consumer Studies*, *35*(2), 254–264. https://doi-org.login.ezproxy.library.ualberta.ca/10.1111/j.1470-6431.2010.00968.x

Laitala, Kirsi, & Kjeldsberg, M. (2012). Cleaning effect of alternative laundry products. A comparison of soap nuts, laundry balls, washing pellets, laundry magnets, water and regular detergent. *Household and Personal Care Today*, *7*, 53–56.

Lakdawala, N., Pham, J., Shah, M., & Holton, J. (2011a). Effectiveness of low-temperature domestic laundry on the decontamination of healthcare workers' uniforms. *Infection Control & Hospital Epidemiology*, *32*(11), 1103–1108. https://doi.org/10.1086/662183

Lakdawala, N., Pham, J., Shah, M., & Holton, J. (2011b). Effectiveness of low-temperature domestic laundry on the decontamination of healthcare workers' uniforms. *Infection Control & Hospital Epidemiology*, *32*(11), 1103–1108. https://doi.org/10.1086/662183

Lankford, M. G., Collins, S., Youngberg, L., Rooney, D. M., Warren, J. R., & Noskin, G. A. (2006). Assessment of materials commonly utilized in health care: Implications for bacterial survival and transmission. *American Journal of Infection Control*, *34*(5), 258–263. https://doi.org/10.1016/j.ajic.2005.10.008

Leeming, J. P., Notman, F. H., & Holland, K. T. (1989). The distribution and ecology of Malassezia furfur and cutaneous bacteria on human skin. *Journal of Applied Bacteriology*, *67*(1), 47–52. https://doi.org/10.1111/j.1365-2672.1989.tb04953.x

Lenochova, P., Roberts, S. C., & Havlicek, J. (2009). Methods of human body odor sampling: The effect of freezing. *Chemical Senses*, *34*(2), 127–138. https://doi.org/10.1093/chemse/bjn067

Levinson, L. (2013, November 13). Why You Should Store Your Jeans in the Freezer. Retrieved January 20, 2017, from https://www.elle.com/news/fashion-style/levis-jeans-in-bag

Lund, B. M., Baird-Parker, T. C., & Gould, G. W. (2000). *Microbiological safety and quality of food*. Aspen Publishers.

Lutz, A. (2015, October 20). Levi's CEO doesn't wash his jeans — and he says you shouldn't either. Retrieved September 17, 2018, from https://www.businessinsider.com/why-you-shouldnt-wash-your-jeans

Makers, Y. (2015, May 25). Maker of $695 Jeans: Here's the Right Way to Wash Your Denim. Retrieved November 20, 2018, from https://www.yahoo.com/makers/finally-how-to-wash-your-jeans-the-right-way-118894362110.html

Maxwell, R. (2016, September 1). How To Clean Your Jeans Without Water (In the Freezer!). Retrieved September 17, 2018, from https://www.apartmenttherapy.com/how-to-clean-your-jeans-without-water-freezer-109266

McBride, M. E., Duncan, W. C., & Knox, J. (1977). The environment and the microbial ecology of human skin. *Applied and Environmental Microbiology*, *33*(3), 603–608.

McKay, B., & McKay, K. (2014, April 22). Your Grandpa's Jeans: A Primer on Raw and Selvedge Denim. Retrieved August 12, 2017, from https://www.artofmanliness.com/articles/raw-selvedge-denim-introduction/

McQueen, R., Batcheller, J., Moran, L., Zhang, H., & Hooper, P. (2017). Reducing laundering frequency to prolong the life of denim jeans. *International Journal of Consumer Studies*, *41*(1), 36–45. https://doi-org.login.ezproxy.library.ualberta.ca/10.1111/ijcs.12311

Meechan, P. J., & Wilson, C. (2006). Use of ultraviolet lights in biological safety cabinets: A contrarian view. *Applied Biosafety*, *11*(4), 222–227. https://doi.org/10.1177/153567600601100412

Memon, N. A. (2014). Versatile denim fabrics enjoy unabated global popularity. *Pakistan Textile Journal*, *63*(6).

Midha, V., Kumar, S., & Kumar, M. (2017). Investigation on permeability and moisture management properties of different denim fabrics after repeated laundering. *The Journal of The Textile Institute*, *108*(1), 71–77. https://doi.org/10.1080/00405000.2016.1153873

Miller, D. (2010). Anthropology in blue jeans. *American Ethnologist*, *37*(3), 415–428. https://doi.org/10.1111/j.1548-1425.2010.01263.x

Miller, D., & Woodward, S. (2007). Manifesto for a study of denim. *Social Anthropology*, *15*(3), 335–351.

Miller, D., & Woodward, S. (2012). *Blue Jeans: The Art of the Ordinary*. Oakland: University of California Press.

Mirosa, M., Lawson, R., & Gnoth, D. (2013). Linking personal values to energy-efficient behaviors in the home. *Environment and Behavior*, *45*(4), 455–475. https://doi.org/10.1177/0013916511432332

Mitchell, A., Spencer, M., & Edmiston, C. (2015). Role of healthcare apparel and other healthcare textiles in the transmission of pathogens: a review of the literature. *Journal of Hospital Infection*, *90*(4), 285–292. https://doi.org/10.1016/j.jhin.2015.02.017

Mitoraj, D., Jańczyk, A., Strus, M., Kisch, H., Stochel, G., Heczko, P. B., & Macyk, W. (2007). Visible light inactivation of bacteria and fungi by modified titanium dioxide. *Photochemical & Photobiological Sciences*, *6*(6), 642–648. https://doi.org/10.1039/B617043A

Mizrahi, M. (2011). Brazilian jeans: materiality, body and seduction at a Rio de Janeiro's Funk Ball. In *Global Denim* (pp. 103–126). London: Oxford.

Morley, C. R., Trofymow, J. A., Coleman, D. C., & Cambardella, C. (1983). Effects of freeze-thaw stress on bacterial populations in soil microcosms. *Microbial Ecology*, *9*(4), 329–340. https://doi.org/10.1007/BF02019022

Morris, M.A., & Prato, H. H. (1982). The Effect of Wash Temperature on Removal of Particulate and Oily Soil from Fabrics of Varying Fiber Content. *Textile Research Journal*, *52*(4), 280–286. https://doi.org/10.1177/004051758205200409

Morris, Mary Ann, Prato, H. H., & White, N. L. (1984). Line-dried vs. machine-dried fabrics: Comparison of appearance, hand, and consumer acceptance. *Home Economics Research Journal*, *13*(1), 27–35. https://doi-org.login.ezproxy.library.ualberta.ca/10.1177/1077727X8401300105

Mukherjee, A. K. (2007). Potential application of cyclic lipopeptide biosurfactants produced by Bacillus subtilis strains in laundry detergent formulations. *Letters in Applied Microbiology*, *45*(3), 330–335. https://doi.org/10.1111/j.1472-765X.2007.02197.x

Munk, S., Johansen, C., Stahnke, L. H., & Adler-Nissen, J. (2001). Microbial survival and odor in laundry. *Journal of Surfactants and Detergents*, *4*(4), 385–394. https://doi.org/10.1007/s11743-001-0192-2

Munk, S., Münch, P., Stahnke, L., Adler-Nissen, J., & Schieberle, P. (2000). Primary odorants of laundry soiled with sweat/sebum: Influence of lipase on the odor profile. *Journal of Surfactants and Detergents*, *3*(4), 505–515. https://doi.org/10.1007/s11743-000-0150-z

Munoz-Price, L. S., Arheart, K. L., Mills, J. P., Cleary, T., DePascale, D., Jimenez, A., … Lubarsky, D. A. (2012). Associations between bacterial contamination of health care workers' hands and contamination of white coats and scrubs. *American Journal of Infection Control*, *40*(9), e245–e248. https://doi.org/10.1016/j.ajic.2012.03.032

Murata, M., Hoshino, E., & Suzuki, A. (1992). Importance of the removal of sebum soil from cotton undershirts in Japanese home laundry. *Journal of Japan Oil Chemists' Society*, *41*(6), 472–479. https://doi.org/10.5650/jos1956.41.472

Murray, P. R. (2005). *Medical microbiology*. (K. S. Rosenthal & M. A. Pfaller, Eds.) (5th ed.). Philadelphia: Elsevier Mosby,.

Muzquiz, A. (2018, February 8). To Freeze or Not to Freeze Raw Denim Jeans? (Spoiler: Don't Freeze). Retrieved November 21, 2018, from https://www.heddels.com/2018/02/to-freeze-or-not-to-freeze-denim-jeans-spoiler-dont-freeze/

Myhre, A. K., Bevanger, L. S., Berntzen, K., & Bratlid, D. (2002). Anogenital bacteriology in non-abused preschool children: A descriptive study of the aerobic genital flora and the isolation of anogenital Gardnerella vaginalis. *Acta Paediatrica*, *91*(8), 885–891. https://doi.org/10.1111/j.1651-2227.2002.tb02850.x

Nayak, R, & Padhye, R. (2015). The Care of Apparel Products. In *Textiles and Fashion* (pp. 799–822). Boca Raton: Elsevier. https://doi.org/10.1016/B978-1-84569-931-4.00031-3

Nayak, Rajkishore, & Ratnapandian, S. (2018). *Care and Maintenance of Textile Products Including Apparel and Protective Clothing: Apparel and Protective Clothing*. CRC Press.

Newman, A. (2011, July 27). Wanna Save the World? Don't Wash Your Jeans | SciJourner. Retrieved December 24, 2018, from http://www.scijourner.org/2011/07/27/wanna-save-the-world-dont-wash-your-jeans/

O'Connor, R. (2016, February 18). Machine-washing a good pair of jeans can actually cause damage to the material. Retrieved from https://www.independent.co.uk/life-style/fashion/levis-ceo-explains-why-you-should-never-wash-your-jeans-a6881031.html

Pakula, C., & Stamminger, R. (2010). Electricity and water consumption for laundry washing by washing machine worldwide. *Energy Efficiency*, *3*(4), 365–382. https://doi.org/10.1007/s12053-009-9072-8

Patel, S., Murray-Leonard, J., & Wilson, A. (2006). Laundering of hospital staff uniforms at home. *Journal of Hospital Infection*, *62*(1), 89–93.

Patel, S., Murray-Leonard, J., & Wilson, A. (2012). Laundering of hospital staff uniforms at home. *Journal of Hospital Infection*, *62*(1), 89–93.

Peacock, E. E. (1999). A note on the effect of multiple freeze-thaw treatment on natural fibre fabrics. *Studies in Conservation*, *44*(1), 12–18. https://doi-org.login.ezproxy.library.ualberta.ca/10.1179/sic.1999.44.1.12

Peacock, E. E. (2005). The biodeterioration of textile fibres in wet archaeological contexts with implications for conservation choices. *Intrecci Vegetali e Fibre Tessili Da Ambiente Umido: Analisi Conservazione e Restauro: Atti (Trento, 28–30 Maggio 2003), Incontri Di Restauro*, *4*, 46–61. https://doi-org.login.ezproxy.library.ualberta.ca/10.1179/sic.1999.44.1.12

Pedersen, E., Labhard, L., & Webb, J. (1988). Line-drying versus machine-drying: Energy consumption comparison. *Journal of Consumer Studies & Home Economics*, *12*(4), 349–360. https://doi-org.login.ezproxy.library.ualberta.ca/10.1111/j.1470-6431.1988.tb00490.x

Philipkoski, K. (2011, November 8). Sorry, Freezing Your Jeans Will Not Ungross Them. Retrieved November 20, 2018, from https://gizmodo.com/5857596/sorry-levis-freezing-your-jeans-will-not-ungross-them

Pink, S. (2005). Dirty laundry. Everyday practice, sensory engagement and the constitution of identity. *Social Anthropology*, *13*(3), 275–290. https://doi.org/10.1017/S0964028205001540

Pink, S., Mackley, K. L., & Moroşanu, R. (2015). Hanging out at home: Laundry as a thread and texture of everyday life. *International Journal of Cultural Studies*, *18*(2), 209–224. https://doi.org/10.1177/1367877913508461

Poon, T. S. C., Barnetson, R. S. C., & Halliday, G. M. (2005). Sunlight-induced immunosuppression in humans is initially because of UVB, then UVA, followed by interactive effects. *Journal of Investigative Dermatology*, *125*(4), 840–846. https://doi.org/10.1111/j.0022-202X.2005.23894.x

Porteous, C. D. A., Sharpe, T. R., Menon, R. A., Shearer, D., Musa, H., Baker, P. H., … Markopoulos, A. (2012). Energy and environmental appraisal of domestic laundering appliances. *Building Research & Information*, *40*(6), 679–699. https://doi.org/10.1080/09613218.2012.708599

Quek, P., & Hu, J. (2008). Influence of photoreactivating light intensity and incubation temperature on photoreactivation of Escherichia coli following LP and MP UV disinfection. *Journal of Applied Microbiology*, *105*(1), 124–133. https://doi-org.login.ezproxy.library.ualberta.ca/10.1111/j.1365-2672.2008.03723.x

Rahman, O. (2012). The influence of visual and tactile inputs on denim jeans evaluation. *International Journal of Design*, *6*(1), 11–25.

Rahman, O. (2015). Denim jeans: A qualitative study of product cues, body type, and appropriateness of use: *Fashion Practice*, *7*(1), 53–74. https://doi.org/10.2752/175693815X14182200335691

Rahman, O., Jiang, Y., & Liu, W. (2010). Evaluative criteria of denim jeans: A cross-national study of functional and aesthetic aspects. *The Design Journal*, *13*(3), 291–311. https://doi.org/10.2752/146069210X12766130824894

Raymond, J. (2008). *Streamlined Irons*. Streamline Press.

Reed, R. H. (1997). Solar inactivation of faecal bacteria in water : The critical role of oxygen. *Letters in Applied Microbiology*, *24*(4), 276–280. https://doi.org/10.1046/j.1472-765X.1997.00130.x

Riley, K., Williams, J., Owen, L., Shen, J., Davies, A., & Laird, K. (2017). The effect of low-temperature laundering and detergents on the survival of Escherichia coli and

Staphylococcus aureus on textiles used in healthcare uniforms. *Journal of Applied Microbiology*, *123*(1), 280–286.

Riley, R. L., & Kaufman, J. (1972). Effect of relative humidity on the inactivation of airborne Serratia marcescens by ultraviolet radiation. *Applied Microbiology*, *23*(6), 1113–1120.

Ro, E. Y., Ko, Y. M., & Yoon, K. S. (2015). Survival of pathogenic enterohemorrhagic Escherichia coli (EHEC) and control with calcium oxide in frozen meat products. *Food Microbiology*, *49*, 203–210. https://doi.org/10.1016/j.fm.2015.02.010

Roberts, S. C., Gosling, L. M., Carter, V., & Petrie, M. (2008). MHC-correlated odour preferences in humans and the use of oral contraceptives. *Proceedings of the Royal Society of London B: Biological Sciences*, *275*(1652), 2715–2722. https://doi.org/10.1098/rspb.2008.0825

Rutala, W. A., Stiegel, M. M., & Sarubbi, F. (1982). Decontamination of laboratory microbiological waste by steam sterilization. *Applied and Environmental Microbiology*, *43*(6), 1311–1316.

Sams, P. (2001). Clothes care-sending the right signals'. Presented at the International Appliance Technical Conference, Columbus, Ohio, 27th March.

Schmitz, A., & Stamminger, R. (2014). Usage behaviour and related energy consumption of European consumers for washing and drying. *Energy Efficiency*, *7*(6), 937–954. https://doi.org/10.1007/s12053-014-9268-4

Schwartz, G. G., & Hanchette, C. L. (2006). UV, latitude, and spatial trends in prostate cancer mortality: All sunlight is not the same (United States). *Cancer Causes & Control*, *17*(8), 1091–1101. https://doi.org/10.1007/s10552-006-0050-6

Selmer-Olsen, E., Birkeland, S.-E., & Sørhaug, T. (1999). Effect of protective solutes on leakage from and survival of immobilized Lactobacillus subjected to drying, storage and rehydration. *Journal of Applied Microbiology*, *87*(3), 429–437. https://doi.org/10.1046/j.1365-2672.1999.00839.x

Shove, E. (2003). Converging conventions of comfort, cleanliness and convenience. *Journal of Consumer Policy*, *26*(4), 395–418. https://doi.org/10.1023/A:1026362829781

Shove, E. (2004). *Comfort, Cleanliness and Convenience: The Social Organization of Normality (New Technologies/New Cultures)*. {Berg Publishers}.

Singh, D., & Bronstad, P. M. (2001). Female body odour is a potential cue to ovulation. *Proceedings of the Royal Society of London B: Biological Sciences*, *268*(1469), 797–801. https://doi.org/10.1098/rspb.2001.1589

Skogland, T., Lomeland, S., & Goksøyr, J. (1988). Respiratory burst after freezing and thawing of soil: Experiments with soil bacteria. *Soil Biology and Biochemistry*, *20*(6), 851–856. https://doi.org/10.1016/0038-0717(88)90092-2

Smith, J. A., Neil, K. R., Davidson, C. G., & Davidson, R. W. (1987). Effect of water temperature on bacterial killing in laundry. *Infection Control*, *8*(5), 204–209. https://doi.org/10.1017/S0195941700065954

Solomon, M. R. (1986). Deep-Seated Materialism: the Case of Levi's 501 Jeans. *ACR North American Advances*, *NA-13*. Retrieved from http://acrwebsite.org/volumes/6566/volumes/v13/NA-13

Sorokowska, A., Sorokowski, P., & Szmajke, A. (2012). Does personality smell? Accuracy of personality assessments based on body odour. *European Journal of Personality*, *26*(5), 496–503. https://doi-org.login.ezproxy.library.ualberta.ca/10.1002/per.848

Stege Bojer, T. (2017, July 28). 3 Popular Raw Denim Care Myths and Why They're Totally Busted. Retrieved November 20, 2018, from https://medium.com/@tsbojer/3-popular-raw-denim-care-myths-and-why-theyre-totally-busted-47fc0bb69921

Strang, T. (1992). A review of published temperatures for the control of pest insects in museums (Vol. 8, pp. 41–67). Presented at the Collection Forum, Ottawa.

Sturken, M. (1997). *Tangled Memories: The Vietnam War, the AIDS Epidemic, and the Politics of Remembering*. Oakland: University of California Press.

Su, J., & Tong, X. (2016). Brand personality, consumer satisfaction, and loyalty: A perspective from denim jeans brands. *Family and Consumer Sciences Research Journal*, *44*(4), 427–446. https://doi-org.login.ezproxy.library.ualberta.ca/10.1111/fcsr.12171

Sullivan, Å., Edlund, C., & Nord, C. E. (2001). Effect of antimicrobial agents on the ecological balance of human microflora. *The Lancet Infectious Diseases*, *1*(2), 101–114. https://doi.org/10.1016/S1473-3099(01)00066-4

Sullivan, J. (2007). *Jeans : A cultural history of an American icon.* New York: Avery.

Swidsinski, A., Mendling, W., Loening-Baucke, V., Ladhoff, A., Swidsinski, S., Hale, L. P., & Lochs, H. (2005). Adherent biofilms in bacterial vaginosis. *Obstetrics & Gynecology*, *106*(5), 1013–1023.

Tanaka, S., Kobayashi, T., Iwasaki, K., Yamane, S., Maeda, K., & Sakurai, K. (2003). Properties and metabolic diversity of microbial communities in soils treated with steam sterilization compared with methyl bromide and chloropicrin fumigations. *Soil Science and Plant Nutrition*, *49*(4), 603–610. https://doi-org.login.ezproxy.library.ualberta.ca/10.1007/s11743-999-0073-8

Tarhan, M., & Sarusk, M. (2009). A comparison among performance characteristics of various denim fading processes, *79*(4), 301–309. https://doi-org.login.ezproxy.library.ualberta.ca/10.1177/0040517508090889

Teufel, L., Pipal, A., Schuster, K. C., Staudinger, T., & Redl, B. (2010). Material-dependent growth of human skin bacteria on textiles investigated using challenge tests and DNA genotyping. *Journal of Applied Microbiology*, *108*(2), 450–461. https://doi.org/10.1111/j.1365-2672.2009.04434.x

Viessman, W. (1964). Ventilation control of odor. *Annals of the New York Academy of Sciences*, *116*(2), 630–637. https://doi.org/10.1111/j.1749-6632.1964.tb45096.x

Walker, V. K., Palmer, G. R., & Voordouw, G. (2006). Freeze-thaw tolerance and clues to the winter survival of a soil community. *Applied and Environmental Microbiology*, *72*(3), 1784–1792. https://doi.org/10.1128/AEM.72.3.1784-1792.2006

Walter, N., McQueen, R., & Keelan, M. (2014). In vivo assessment of antimicrobial-treated textiles on skin microflora. *International Journal of Clothing Science and Technology*, *26*(4), 330–342. https://doi.org/10.1108/IJCST-12-2012-0078

Weber, R., Khan, P., Fader, R., & Weber, R. (2012). Prospective study on the effect of shirt sleeves and ties on the transmission of bacteria to patients. *Journal of Hospital Infection*, *80*(3), 252–254. https://doi.org/10.1016/j.jhin.2011.12.012

Whyte, W., Hodgson, R., Bailey, P. V., & Graham, J. (1978). The reduction of bacteria in the operation room through the use of non-woven clothing. *British Journal of Surgery*, *65*(7), 469–474. https://doi.org/10.1002/bjs.1800650708

Wolfe, J. (2015, May 6). How to Break In Your Denim According to Three Experts. Retrieved February 23, 2016, from https://www.gq.com/story/raw-denim-guide-experts

Wollina, U., Tilp, M., Meseg, A., Schönlebe, J., Heinig, B., & Nowak, A. (2012). Management of Severe Anogenital Acne Inversa (Hidradenitis Suppurativa). *Dermatologic Surgery*, *38*(1), 110–117. https://doi.org/10.1111/j.1524-4725.2011.02157.x

Wong, D., Nye, K., & Hollis, P. (1991). Microbial flora on doctors' white coats. *British Medical Journal*, *303*(6817), 1602–1604. https://doi.org/10.1136/bmj.303.6817.1602

Wu, Jiang, Zhao, L., Xie, N., Gao, L., Gao, W., Dai, X., & Zhang, J. (2012). Research on the characteristics of a novel solar drying system and its application. *Energy Procedia*, *14*, 399–404. https://doi.org/10.1016/j.egypro.2011.12.949

Wu, Juanjuan, & Delong, M. (2006). Chinese perceptions of western-branded denim jeans: A Shanghai case study. *Journal of Fashion Marketing and Management: An International Journal*, *10*(2), 238–250. https://doi.org/10.1108/13612020610667531

Xu, P., Peccia, J., Fabian, P., Martyny, J. W., Fennelly, K. P., Hernandez, M., & Miller, S. L. (2003). Efficacy of ultraviolet germicidal irradiation of upper-room air in inactivating airborne bacterial spores and mycobacteria in full-scale studies. *Atmospheric Environment*, *37*(3), 405–419. https://doi.org/10.1016/S1352-2310(02)00825-7

Yates, L., & Evans, D. (2016). Dirtying Linen: Re-evaluating the sustainability of domestic laundry. *Environmental Policy and Governance*, *26*(2), 101–115. https://doi.org/10.1002/eet.1704

Yee, T. (2016, July 19). smoothly polished: Taking Care of Raw Denim: The Sun. Retrieved November 21, 2018, from https://smoothlypolished.blogspot.com/2016/07/taking-care-of-raw-denim-sun.html

Zielinski, S. (2011, November 7). The Myth of the Frozen Jeans. Retrieved September 17, 2018, from https://www.smithsonianmag.com/science-nature/the-myth-of-the-frozen-jeans-129092730/

Zielinski, S. (n.d.). The Myth of the Frozen Jeans. Retrieved October 22, 2018, from https://www.smithsonianmag.com/science-nature/the-myth-of-the-frozen-jeans-129092730/

Appendix A

Ethics Documents

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EFFECTIVENESS OF ALTERNATIVE REFURBISHMENT TECHNIQUES IN CONTROLLING SKIN BACTERIA ON DENIM FABRICS

Purpose: The purpose of this study is to evaluate the effect of selected refurbishing treatments on bacterial growth collected on denim fabric. We are looking for individuals to wear denim fabric patches on their forearm for multiple 24-hour cycles, with weekly drop-offs, over the course of two months. This study is a requirement as part of a MSc. program in the department of Human Ecology.

Participant Requirements: In order to qualify for the study, we ask that interested individuals meet the following criteria:

- must be at least 18 years of age
- must be free from skin conditions, open wounds or cuts or taking antibiotics
- must successfully complete a screening test to ensure adequate bacteria transfers onto fabrics for analysis (see below)
- must be prepared to commit to participate in up to 7 separate trials (which require wearing fabrics for 24 hours continuously per session) over the course of 7 weeks
- while fabrics are being worn, participants will be asked to refrain from strenuous exercise or movements, removal of fabrics (unless irritation or discomfort arise) and should be covered by a plastic wrap (demonstration will be provided) for bathing or showering

Screening procedure: In order determine if you qualify for the study, you will be asked to take part in a screening test which will involve the same procedure as the trial itself. Two denim patches (one for each arm) will be attached to your forearm (using Kinesiology tape) and worn for 24 hours. Upon completion, you will return to the researcher for removal of the denim fabric. Fabrics will then be analysed, by extracting the bacteria and growing them on bacteriological media for colony counting. You will be contacted with results and further instructions should they qualify. A $5 gift card will be given to all screening participants who complete the 24-hour wear period and return for removal of the fabrics.

Main research procedure: If you pass the screening test you will be invited to participate in the full study. This will involve the same requirements as the screening procedure (24 hour wearing of fabrics, on campus attachment / drop-off) but you will be asked to repeat the procedure a minimum of 5 times, up to a maximum number of 7 times. In other words, we will require your participation for a minimum of 5 weeks up to a maximum of 7 weeks. You will be provided with a soap to use during bathing over the course of the main research.

Confidentiality: All information collected in the study will remain strictly confidential. Your information will be coded so that personal identification is not possible. The data will be stored in a secure location (e.g., a locked file cabinet and secure computer) that is accessible only to the student researcher. The coded results will be used for the purposes of scientific research only. Research information is kept in secure storage at the University of Alberta for a period of five years and the information will be destroyed after this period.

Incentives: If you complete the trial sessions, you will receive a $60 supermarket gift card in acknowledgement of your time and contribution to the study. Partial completion of the study requirements will result in a pro-rated gift card.

Risks: There are no immediate risks associated with participating in this study. Please inform the student researcher if you have an allergy to latex or the adhesive in K-Tape, so that an alternative tape can be provided (Leukotape). Additionally, you may experience some discomfort during fabric removal, as the adhesive may remove some hairs or stretch skin in sensitive areas on the arm. The student researcher will remove the tape as carefully as possible to minimize any discomfort. If for any reason you experience irritation on the skin during the 24 hour wear period you should remove the fabric and tape immediately and contact the student researcher.

Withdrawal from the study: You can change your mind about being in this study at any time. You can withdraw from the study by informing any of the researchers by email, telephone or in person. If you wish to withdraw your data you can do so up to 2 days after completing the final completed wear and drop-off of fabrics.

Voluntary participation: You are under no obligation to participate in this study, it is completely voluntary. Even if you agree to be in the study you can change your mind and withdraw at any time up until 2 days after the final drop off of the fabrics. You are asked to let the researcher know you if you no longer want to participate in the remaining trial sessions in person or through email.

Questions or concerns: If you have any questions about your participation in the study, you can email the researcher at mariko@ualberta.ca. If you would like to contact the principal investigator's supervisor directly you can contact Dr. Rachel McQueen at 780-492-2045 or rachel.mcqueen@ualberta.ca. The plan for this study has been reviewed for its adherence to ethical guidelines by the University of Alberta's Research Ethics Board 2. If you have any questions regarding your rights as a research participant, you may contact the Research Ethics Office at 780-492-0459. This office has no affiliation with the study investigators.

Researchers:

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EFFECTIVENESS OF ALTERNATIVE REFURBISHING TECHNIQUES IN CONTROLLING SKIN BACTERIA ON DENIM FABRICS

Consent Form

Researchers
Mariko Wakefield mariko@ualberta.ca
Rachel McQueen rachel.mcqueen@ualberta.ca 780 492 2045

Please circle one:

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet?	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had an opportunity to ask questions and discuss this study?	Yes	No
Do you understand that your participation is voluntary?	Yes	No
Has the issue of confidentiality been explained to you?	Yes	No
Do you understand who will have access to your information?	Yes	No
Do you have any allergies to latex used in Kinesiology tape?	Yes	No

Participant name: _____ (please print)

Participant signature: _____ Date: _____

Witness name: _____ (please print)

Witness signature: _____ Date: _____

Appendix B

Preliminary testing

B.1 Procedure

Five preliminary trials were carried out using bacteria (Trial #1) or using human participants (n=2) (Trial #2, #3, #4, and #5) to explore suitable methods for the freezing treatment and bacterial growth techniques for the final trial. Test variables including freezing time, thawing time, fabric location on the body and bacterial extraction procedure were altered during the preliminary trials. The fabric specimens used for pretesting were cut from previously used jeans (which had been washed and worn several times), with a fibre content of 99% cotton and 1% elastane and were sterilized before each trial (in an autoclave, on a gravity cycle at 118 °C for 30 minutes). Pretesting fabric (which was not raw denim) was selected for its availability as raw denim required out of province sourcing which included an extended shipping timeframe. In pretesting trials using human participants, denim was occluded to the skin using medical tape, and covered by a plastic barrier. Additionally, fabrics were not placed in ambient conditions in Trial #1, #2, and #3 but were for #4 and #5.

Bacterial application (Trial #1) and extraction procedure (all trials) was adapted from the AATCC 100 – 2004 – Antibacterial Finishes on Textile Materials: Assessment of (AATCC 2010b) where bacteria was inoculated onto fabrics, vigorously shaken in a buffering solution and drop-plated onto nutrient agar plates.

B.2 Results from preliminary trials

B.2.1 Trial #1

Results from Trial #1 are displayed in Table B.1. Two different bacteria strains were used, *Staphylococcus aureus* (SA; ATCC #33591) and *Micrococcus luteus* (ML; unspecified strain) and were chosen as they are bacteria commonly found on the skin (Kloos & Musselwhite, 1975). Denim fabrics were inoculated with a cultured broth from one of the selected bacterial strains, were frozen for 24 hours (in a Petri dish) and were assigned to different thawing methods. After freezing, fabrics were thawed for either 6, 26 or 51 hours in either a room temperature space or

in an incubator (37 °C). Additionally, control fabrics (inoculated with bacteria and analysed without freezing), and unthawed fabrics (fabrics were analysed immediately after removal from freezer) were included in this trial to explore bacterial results with and without freezing.

Table B.1 *Results from Trial #1 with bacterial strains*

Plate #	Dilution	1	2	3	Average	CFU/mL
SA-CON-1	10^{-1}	39	43	30	37.33	2.49E+04
SA-CON-1	10^{-2}	6	7	12	8.33	5.56E+04
SA-CON-1	10^{-3}	1	2	2	1.67	1.11E+05
SA-CON-2	10^{-1}	38	31	46	38.33	2.56E+04
SA-CON-2	10^{-2}	8	8	15	10.33	6.89E+04
SA-CON-2	10^{-3}	1	0	0	0.33	2.22E+04
ML-CON-1	10^{-1}	16	13	17	15.33	1.02E+04
ML-CON-1	10^{-2}	4	0	1	1.67	1.11E+04
ML-CON-1	10^{-3}	0	0	0	0.00	0.00E+00
ML-CON-2	10^{-1}	1	1	2	1.33	8.89E+02
ML-CON-2	10^{-2}	2	0	0	0.67	4.44E+03
ML-CON-2	10^{-3}	0	0	0	0.00	0.00E+00
SA-NT-1	10^{-1}	9	1	0	3.33	2.22E+03
SA-NT-1	10^{-2}	2	1	0	1.00	6.67E+03
SA-NT-1	10^{-3}	0	0	0	0.00	0.00E+00
SA-NT-2	10^{-1}	3	2	0	1.67	1.11E+03
SA-NT-2	10^{-2}	1	1	0	0.67	4.44E+03
SA-NT-2	10^{-3}	2	0	0	0.67	4.44E+04
ML-NT-1	10^{-1}	2	0	0	0.67	4.44E+02
ML-NT-1	10^{-2}	2	0	0	0.67	4.44E+03
ML-NT-1	10^{-3}	0	0	0	0.00	0.00E+00
ML-NT-2	10^{-1}	0	0	0	0.00	0.00E+00
ML-NT-2	10^{-2}	0	0	0	0.00	0.00E+00
ML-NT-2	10^{-3}	0	0	0	0.00	0.00E+00
SA-I6-1	10^{-1}	28	32	29	29.67	1.98E+04
SA-I6-1	10^{-2}	20	16	20	18.67	1.24E+05
SA-I6-1	10^{-3}	4	5	3	4.00	2.67E+05
SA-I6-2	10^{-1}	19	30	26	25.00	1.67E+04
SA-I6-2	10^{-2}	17	20	20	19.00	1.27E+05
SA-I6-2	10^{-3}	2	1	4	2.33	1.56E+05
ML-I6-1	10^{-1}	1	2	5	2.67	1.78E+03
ML-I6-1	10^{-2}	1	0	0	0.33	2.22E+03
ML-I6-1	10^{-3}	0	0	0	0.00	0.00E+00
ML-I6-2	10^{-1}	2	2	2	2.00	1.33E+03
ML-I6-2	10^{-2}	1	1	2	1.33	8.89E+03
ML-I6-2	10^{-3}	0	0	0	0.00	0.00E+00
SA-RT6-1	10^{-1}	5	6	2	4.33	2.89E+03

SA-RT6-1	10^{-2}	1	0	0	0.33	2.22E+03
SA-RT6-1	10^{-3}	0	0	0	0.00	0.00E+00
SA-RT6-2	10^{-1}	10	8	8	8.67	5.78E+03
SA-RT6-2	10^{-2}	4	1	0	1.67	1.11E+04
SA-RT6-2	10^{-3}	1	1	0	0.67	4.44E+04
ML-RT6-1	10^{-1}	0	0	0	0.00	0.00E+00
ML-RT6-1	10^{-2}	0	0	0	0.00	0.00E+00
ML-RT6-1	10^{-3}	0	0	0	0.00	0.00E+00
ML-RT6-2	10^{-1}	1	1	0	0.67	4.44E+02
ML-RT6-2	10^{-2}	1	0	0	0.33	2.22E+03
ML-RT6-2	10^{-3}	0	0	0	0.00	0.00E+00
SA-RT26-1	10^{-1}	7	7	4	6.00	4.00E+03
SA-RT26-1	10^{-2}	1	3	2	2.00	1.33E+04
SA-RT26-1	10^{-3}	1	0	0	0.33	2.22E+04
SA-RT26-2	10^{-1}	TNTC	TNTC	TNTC	N/A	N/A
SA-RT26-2	10^{-2}	36	28	27	30.33	2.02E+05
SA-RT26-2	10^{-3}	9	19	16	14.67	9.78E+05
ML-RT26-1	10^{-1}	2	3	2	2.33	1.56E+03
ML-RT26-1	10^{-2}	1	3	4	2.67	1.78E+04
ML-RT26-1	10^{-3}	1	0	0	0.33	2.22E+04
ML-RT26-2	10^{-1}	1	0	0	0.33	2.22E+02
ML-RT26-2	10^{-2}	0	0	0	0.00	0.00E+00
ML-RT26-2	10^{-3}	0	0	0	0.00	0.00E+00
SA-I26-1	10^{-1}	TNTC	TNTC	TNTC	N/A	N/A
SA-I26-1	10^{-2}	30	22	21	24.33	1.62E+05
SA-I26-1	10^{-3}	4	7	3	4.67	3.11E+05
SA-I26-2	10^{-1}	4	6	10	6.67	4.44E+03
SA-I26-2	10^{-2}	3	3	31	12.33	8.22E+04
SA-I26-2	10^{-3}	1	0	1	0.67	4.44E+04
ML-I26-1	10^{-1}	2	1	0	1.00	6.67E+02
ML-I26-1	10^{-2}	0	0	0	0.00	0.00E+00
ML-I26-1	10^{-3}	0	0	0	0.00	0.00E+00
ML-I26-2	10^{-1}	1	0	0	0.33	2.22E+02
ML-I26-2	10^{-2}	0	0	0	0.00	0.00E+00
ML-I26-2	10^{-3}	0	0	0	0.00	0.00E+00
SA-RT51-1	10^{-1}	10	9	15	11.33	7.56E+03
SA-RT51-1	10^{-2}	2	1	1	1.33	8.89E+03
SA-RT51-1	10^{-3}	0	0	0	0.00	0.00E+00
SA-RT51-2	10^{-1}	6	8	10	8.00	5.33E+03
SA-RT51-2	10^{-2}	3	0	1	1.33	8.89E+03
SA-RT51-2	10^{-3}	0	0	0	0.00	0.00E+00

ML-RT51-1	10^{-1}	10	12	12	11.33	7.56E+03
ML-RT51-1	10^{-2}	2	3	1	2.00	1.33E+04
ML-RT51-1	10^{-3}	3	1	1	1.67	1.11E+05
ML-RT51-2	10^{-1}	4	6	2	4.00	2.67E+03
ML-RT51-2	10^{-2}	1	2	0	1.00	6.67E+03
ML-RT51-2	10^{-3}	0	0	0	0.00	0.00E+00

SA – Staphylococcus aureus
ML – Micrococcus luteus

CON – Control – fabrics were inoculated with bacteria and tested without freezing treatment
NT – No Thaw – bacteria was extracted from fabrics within 20 minutes of freezer removal
I6 – Fabrics were incubated for 6 hours after removal from the freezer
RT6 – Fabrics were put in a room temperature space for 6 hours after removal from the freezer
I26 – Fabrics were incubated for 26 hours after removal from the freezer
RT26 – Fabrics were put in a room temperature space for 26 hours after removal from the freezer
RT51 – Fabrics were put in a room temperature space for 51 hours after removal from the freezer

B.2.2 Results

Fabrics that were inoculated with SA and thawed at room temperature for 26 hours and incubated for 26 hours resulted in bacterial counts that were too numerous for the first serial dilution (10^{-1}). The lowest bacterial counts were observed on specimens that were not thawed as well as specimens that were thawed at room temperature for 6 hours.

The highest bacterial count results recovered from fabrics that were inoculated with ML were observed on fabrics that were thawed at room temperature for 51 hours. The lowest bacterial count results were observed on samples that were not thawed after freezing and room temperature thawed for 6 hours.

B.2.3 Trial #2

The purpose of Trial #2 was to introduce the inclusion of two human participants to collect skin bacteria, and in this case, fabrics were occluded to the inner thighs on both legs for 24 hours. With the exception of the "not frozen" assigned samples, fabrics were placed into the

freezer for 95.5 hours (in a Petri dish) after removal from participants. Fabrics were assigned different thawing times (0, 5 and 24 hours) and temperatures after freezing (room temperature or incubator) for comparison. Different thawing times served to simulate different lengths of time the owner may wait before wearing raw denim removed from the freezer. Different temperatures served to compare leaving frozen raw denim at room temperature to thaw or wearing the garment (with body heat) after freezer removal (outlined in Table B.2).

Table B.2 *Bacterial counts of Trial #2 with varying thawing conditions*

Treatment	Dilution	1	2	3	Average	CFU/mL
R1-NF	10^{-1}	1	1	0	0.67	4.44E+02
R1-NF	10^{-2}	1	0	0	0.33	2.22E+03
R1-NF	10^{-3}	0	0	0	0.00	0.00E+00
R2-NF	10^{-1}	1	1	0	0.67	4.44E+02
R2-NF	10^{-2}	1	0	0	0.33	2.22E+03
R2-NF	10^{-3}	1	0	0	0.33	2.22E+04
M1-NF	10^{-1}	1	2	0	1.00	6.67E+02
M1-NF	10^{-2}	0	0	0	0.00	0.00E+00
M1-NF	10^{-3}	0	0	0	0.00	0.00E+00
M2-NF	10^{-1}	8	10	8	8.67	5.78E+03
M2-NF	10^{-2}	2	3	1	2.00	1.33E+04
M2-NF	10^{-3}	1	0	0	0.33	2.22E+04
R1-NT	10^{-1}	4	6	3	4.33	2.89E+03
R1-NT	10^{-2}	2	1	0	1.00	6.67E+03
R1-NT	10^{-3}	1	0	0	0.33	2.22E+04
R2-NT	10^{-1}	1	0	1	0.67	4.44E+02
R2-NT	10^{-2}	0	1	0	0.33	2.22E+03
R2-NT	10^{-3}	1	0	0	0.33	2.22E+04
M1-NT	10^{-1}	2	1	0	1.00	6.67E+02
M1-NT	10^{-2}	0	0	0	0.00	0.00E+00
M1-NT	10^{-3}	0	0	0	0.00	0.00E+00
M2-NT	10^{-1}	2	2	3	2.33	1.56E+03
M2-NT	10^{-2}	1	0	1	0.67	4.44E+03
M2-NT	10^{-3}	0	0	0	0.00	0.00E+00
R1-R5	10^{-1}	5	2	4	3.67	2.44E+03
R1-R5	10^{-2}	3	1	3	2.33	1.56E+04
R1-R5	10^{-3}	1	0	0	0.33	2.22E+04
R2-R5	10^{-1}	1	1	1	1.00	6.67E+02
R2-R5	10^{-2}	1	0	0	0.33	2.22E+03
R2-R5	10^{-3}	0	0	0	0.00	0.00E+00
M1-R5	10^{-1}	2	1	0	1.00	6.67E+02
M1-R5	10^{-2}	0	0	0	0.00	0.00E+00
M1-R5	10^{-3}	0	0	0	0.00	0.00E+00
M2-R5	10^{-1}	1	0	0	0.33	2.22E+02
M2-R5	10^{-2}	0	0	1	0.33	2.22E+03
M2-R5	10^{-3}	0	0	0	0.00	0.00E+00
R1-I5	10^{-1}	0	0	0	0.00	0.00E+00
R1-I5	10^{-2}	0	0	0	0.00	0.00E+00
R1-I5	10^{-3}	0	0	0	0.00	0.00E+00
R2-I5	10^{-1}	0	0	0	0.00	0.00E+00

R2-I5	10^{-2}	1	0	0	0.33	2.22E+03
R2-I5	10^{-3}	0	0	0	0.00	0.00E+00
M1-I5	10^{-1}	2	0	0	0.67	4.44E+02
M1-I5	10^{-2}	0	0	0	0.00	0.00E+00
M1-I5	10^{-3}	0	0	0	0.00	0.00E+00
M2-I5	10^{-1}	3	1	2	2.00	1.33E+03
M2-I5	10^{-2}	1	0	1	0.67	4.44E+03
M2-I5	10^{-3}	1	0	0	0.33	2.22E+04
R1-R24	10^{-1}	0	1	0	0.33	2.22E+02
R1-R24	10^{-2}	0	0	0	0.00	0.00E+00
R1-R24	10^{-3}	0	0	0	0.00	0.00E+00
R2-R24	10^{-1}	0	0	0	0.00	0.00E+00
R2-R24	10^{-2}	0	0	0	0.00	0.00E+00
R2-R24	10^{-3}	0	0	0	0.00	0.00E+00
M1-R24	10^{-1}	0	0	0	0.00	0.00E+00
M1-R24	10^{-2}	0	0	0	0.00	0.00E+00
M1-R24	10^{-3}	0	0	0	0.00	0.00E+00
M2-R24	10^{-1}	1	0	0	0.33	2.22E+02
M2-R24	10^{-2}	0	1	0	0.33	2.22E+03
M2-R24	10^{-3}	0	0	0	0.00	0.00E+00
R1-I24	10^{-1}	0	0	0	0.00	0.00E+00
R1-I24	10^{-2}	0	0	0	0.00	0.00E+00
R1-I24	10^{-3}	0	0	0	0.00	0.00E+00
R2-I24	10^{-1}	0	0	0	0.00	0.00E+00
R2-I24	10^{-2}	0	0	0	0.00	0.00E+00
R2-I24	10^{-3}	0	0	0	0.00	0.00E+00
M1-I24	10^{-1}	0	0	0	0.00	0.00E+00
M1-I24	10^{-2}	0	0	0	0.00	0.00E+00
M1-I24	10^{-3}	0	0	0	0.00	0.00E+00
M2-I24	10^{-1}	0	0	0	0.00	0.00E+00
M2-I24	10^{-2}	0	0	0	0.00	0.00E+00
M2-I24	10^{-3}	0	0	0	0.00	0.00E+00

R – Participant 1
M – Participant 2

NF – Not Frozen – fabrics were worn by participants and tested without freezing
NT – Not Thawed – bacteria was extracted from fabrics immediately after removal from freezer
I5 – Fabrics were incubated for 5 hours after removal from the freezer
R5 – Fabrics were put in a room temperature space for 5 hours after removal from the freezer
I24 – Fabrics were incubated for 24 hours after removal from the freezer
R24 – Fabrics were put in a room temperature space for 24 hours after removal from the freezer

B.2.4 Results

The highest bacterial count results for Participant R were from specimens that were not frozen, not thawed and thawing at room temperature for 5 hours (these results were similar and relatively low). Results for Participant M that had the highest bacterial counts were from specimens that were not frozen and incubated for 5 hours after freezing.

The specimens with the lowest bacterial counts for Participant R resulted from fabrics that were not frozen, incubated for 5 and 24 hours, and left at room temperature for 5 and 24 hour (with no observable bacterial colonies detected). For Participant M, the lowest bacterial counts resulted from specimens that were not frozen, not thawed, incubated for 5 hours, incubated for 24 hours, left at room temperature for 5 and 24 hours (with no observable bacterial colonies on the media).

B.2.5 Trial #3

Fabrics in Trial #3 were occluded to the skin on the top of each forearm for 24 hours. Denim fabrics were separated into equal pieces and were placed into the freezer for 95.5 hours (in a Petri dish) after removal from participants. Once fabrics were removed from the freezer, specimens were incubated (6 or 24 hours), left in a room temperature space (6 or 24 hours) before bacterial extraction or subjected to bacterial extraction without thawing.

Table B.3 *Bacterial counts of Trial #3 with varying thawing conditions*

Treatment	Dilution	1	2	3	Average	CFU/mL
R-NT	10^{0}	9	12	18	13	8.67E+02
R-NT	10^{-1}	2	1	1	1	8.89E+02
R-NT	10^{-2}	1	2	0	1	6.67E+03
R-NT	10^{-3}	0	0	0	0	0.00E+00
M-NT	10^{0}	15	10	21	15	1.02E+03
M-NT	10^{-1}	2	2	4	3	1.78E+03
M-NT	10^{-2}	0	0	0	0	0.00E+00
M-NT	10^{-3}	0	0	0	0	0.00E+00
R-I6	10^{0}	30	28	31	30	1.98E+03
R-I6	10^{-1}	16	12	10	13	8.44E+03
R-I6	10^{-2}	4	1	0	2	1.11E+04
R-I6	10^{-3}	0	0	0	0	0.00E+00
M-I6	10^{0}	17	28	29	25	1.64E+03
M-I6	10^{-1}	2	3	7	4	2.67E+03
M-I6	10^{-2}	0	0	0	0	0.00E+00
M-I6	10^{-3}	0	0	0	0	0.00E+00
R-R6	10^{0}	25	39	44	36	2.40E+03
R-R6	10^{-1}	16	23	25	21	1.42E+04
R-R6	10^{-2}	19	21	24	21	1.42E+05
R-R6	10^{-3}	6	8	8	7	4.89E+05
M-R6	10^{0}	15	10	26	17	1.13E+03
M-R6	10^{-1}	3	7	8	6	4.00E+03
M-R6	10^{-2}	5	4	5	5	3.11E+04
M-R6	10^{-3}	1	0	0	0	2.22E+04
R-I24	10^{0}	40	37	44	40	2.69E+03
R-I24	10^{-1}	20	16	15	17	1.13E+04
R-I24	10^{-2}	6	2	5	4	2.89E+04
R-I24	10^{-3}	1	0	1	1	4.44E+04
M-I24	10^{0}	25	30	31	29	1.91E+03
M-I24	10^{-1}	12	8	7	9	6.00E+03
M-I24	10^{-2}	2	1	0	1	6.67E+03
M-I24	10^{-3}	0	0	0	0	0.00E+00

R – Participant 1
M – Participant 2

NT – Not Thawed – bacteria was extracted from fabrics immediately after removal from freezer
I6 – Fabrics were incubated for 6 hours after removal from the freezer
R6 – Fabrics were put in a room temperature space for 6 hours after removal from the freezer
I24 – Fabrics were incubated for 24 hours after removal from the freezer
R24 – Fabrics were put in a room temperature space for 24 hours after removal from the freezer

B.2.6 Results

The fabrics from Trial #3 that had the highest bacterial counts for Participant R were from fabric specimens that were incubated for 24 hours as well as fabrics that were left at room temperature for 6 hours. For Participant M, the highest bacterial count results were from specimens that were incubated for 24 hours as well as fabrics that were left at room temperature for 6 hours.

The lowest bacterial counts for Participant R were from specimens that were not thawed as well as specimens that were incubated for 6 hours. For Participant M, the lowest bacterial counts were from specimens that were not thawed as well as specimens that were incubated for 6 hours.

B.2.7 Trial #4

Fabric specimens in Trial #4 were occluded to the top of the forearm of both participants for 24 hours; results are displayed in Table B.4. Fabrics were not frozen in this trial. Instead, worn fabrics were removed from participants and separated into two pieces, where one piece was inoculated with a diluted broth (a 9:1 ratio of nutrient broth to phosphate buffering solution) to explore potential methods to encourage bacterial growth while the other piece was left untreated. The addition of nutrient broth was intended to increase bacterial growth with the inclusion of an additional nutrient source. Next, specimens from one arm were incubated for six hours and specimens from the other arm were placed in a room temperature space for six hours before bacterial extraction.

Table B.4 *Bacterial counts of Trial #4 with varying growth conditions*

Treatment	Dilution	1	2	3	Average	CFU/mL
R-I-B	10^{-1}	TNTC	TNTC	TNTC	N/A	N/A
R-I-B	10^{-2}	TNTC	TNTC	TNTC	N/A	N/A
R-I-B	10^{-3}	TNTC	TNTC	TNTC	N/A	N/A
R-I-0	10^{-1}	TNTC	TNTC	TNTC	N/A	N/A
R-I-0	10^{-2}	TNTC	TNTC	TNTC	N/A	N/A
R-I-0	10^{-3}	TNTC	TNTC	TNTC	N/A	N/A
R-RT-B	10^{-1}	TNTC	TNTC	TNTC	N/A	N/A
R-RT-B	10^{-2}	TNTC	TNTC	TNTC	N/A	N/A
R-RT-B	10^{-3}	TNTC	TNTC	TNTC	N/A	N/A
R-RT-0	10^{-1}	TNTC	TNTC	TNTC	N/A	N/A
R-RT-0	10^{-2}	59	TNTC	TNTC	59.00	3.93E+05
R-RT-0	10^{-3}	21	22	39	27.33	1.82E+06
M-I-B	10^{-1}	7	8	1	5.33	3.56E+03
M-I-B	10^{-2}	3	2	1	2.00	1.33E+04
M-I-B	10^{-3}	2	0	0	0.67	4.44E+04
M-I-0	10^{-1}	0	0	0	0.00	0.00E+00
M-I-0	10^{-2}	0	0	0	0.00	0.00E+00
M-I-0	10^{-3}	0	0	0	0.00	0.00E+00
M-RT-B	10^{-1}	3	2	1	2.00	1.33E+03
M-RT-B	10^{-2}	1	0	0	0.33	2.22E+03
M-RT-B	10^{-3}	0	0	0	0.00	0.00E+00
M-RT-0	10^{-1}	1	0	0	0.33	2.22E+02
M-RT-0	10^{-2}	0	0	0	0.00	0.00E+00
M-RT-0	10^{-3}	0	0	0	0.00	0.00E+00

R – Participant 1
M – Participant 2

I-0 - Fabric was incubated but not placed in nutrient broth
I-B - Fabric was incubated and placed in nutrient broth
RT-0 - Fabric was placed in a room temperature space and not placed in nutrient broth
RT-B - Fabric was placed in a room temperature and placed in nutrient broth

B.2.8 Results

The highest bacterial counts for Participant R were observed for all of the treatments, colonies were too numerous to count (with the exception of the room temperature without broth treatment at the higher dilutions). For Participant M, the highest bacterial counts resulted from fabrics incubated with nutrient broth.

Fabrics for Participant M with the lowest bacterial counts were from fabrics that were incubated without nutrient broth, put in nutrient broth at room temperature and without nutrient broth at room temperature.

B.2.9 Trial #5

Denim specimens in Trial #5 were occluded to the top of the forearm of participants, results displayed in Table B.5. In this trial, fabrics for one of the arms for each participant were placed in ambient conditions for 24 hours before attachment. The fabrics for the other arms were place in a room temperature space for 24 hours before attachment. Fabrics were worn for 22 hours before removal. Upon removal of fabrics from participants, they were each separated into two equal pieces using sterile scissors. Fabrics were not frozen in this trial. Instead, one piece from each arm was placed in 900 μL of Milli-Q water in a conical tube, whereas the opposing piece was subjected to bacterial extraction without any additional treatment. The addition of Milli-Q water was included in this trial to determine if increased bacterial growth could be achieved with the addition of a nutrient source.

Table B.5 *Bacterial counts of Trial #5 with varying thawing and growth conditions*

Treatment	Dilution	1	2	3	Average	CFU/mL
R-Hum-0	10^{-1}	26	29	32	29	1.93E+05
R-Hum-0	10^{-2}	4	4	6	5	3.11E+05
R-Hum-0	10^{-3}	2	1	1	1	8.89E+05
R-Hum-H20	10^{-1}	TNTC	TNTC	TNTC	N/A	N/A
R-Hum-H20	10^{-2}	TNTC	TNTC	TNTC	N/A	N/A
R-Hum-H20	10^{-3}	33	23	34	30	2.00E+07
R-RT-0	10^{-1}	7	3	6	5	3.56E+04
R-RT-0	10^{-2}	1	4	0	2	1.11E+05
R-RT-0	10^{-3}	1	1	0	1	4.44E+05
R-RT-H20	10^{-1}	TNTC	TNTC	TNTC	N/A	N/A
R-RT-H20	10^{-2}	TNTC	TNTC	TNTC	N/A	N/A
R-RT-H20	10^{-3}	8	16	11	12	7.78E+06
M-Hum-0	10^{-1}	45	34	42	40	2.69E+04
M-Hum-0	10^{-2}	5	15	7	9	6.00E+04
M-Hum-0	10^{-3}	2	0	1	1	6.67E+04
M-Hum-H20	10^{-1}	TNTC	TNTC	TNTC	N/A	N/A
M-Hum-H20	10^{-2}	TNTC	TNTC	TNTC	N/A	N/A
M-Hum-H20	10^{-3}	TNTC	TNTC	TNTC	N/A	N/A
M-RT-0	10^{-1}	1	1	0	1	4.44E+02
M-RT-0	10^{-2}	1	0	1	1	4.44E+03
M-RT-0	10^{-3}	0	0	0	0	0.00E+00
M-RT-H20	10^{-1}	TNTC	TNTC	TNTC	N/A	N/A
M-RT-H20	10^{-2}	TNTC	TNTC	TNTC	N/A	N/A
M-RT-H20	10^{-3}	52	TNTC	55	54	3.57E+06

R – Participant 1
M – Participant 2

Hum-0 – Fabrics were placed into the conditioning room for 24 hours, but not placed in water
Hum-H20 - Fabrics were placed into the conditioning room for 24 hours, and placed in water
RT-0 – Fabrics were placed into a room temperature space for 24 hours, but not placed in water
RT-H20 - Fabrics were placed into a room temperature space for 24 hours, and placed in water

B.2.10 Results

The highest bacterial counts for Participant R in this trial were from fabrics that were in a conditioned space, placed in water as placed in a room temperature space (placed in water)

where the results were too numerous to count. For Participant M, the highest bacterial counts were from fabrics that were placed in a conditioned space (placed in water) as well as placed in a room temperature space in water (where the bacterial counts were too numerous to count).

The lowest results for Participant R were from fabrics that were not placed in water in a room temperature space. For Participant M, the lowest results were from fabrics that were not placed in water in a room temperature space.

B.3 Summary of trials

Preliminary trials were designed to explore various methods and treatments for the final trial. In Trial #2, fabrics were occluded to the legs, which would be similar to wearing a pair of jeans, however, the participants noted that this location was very uncomfortable and caused blistering as well. As a result, placing fabrics on the arm was deemed to be the most comfortable placement for fabrics for the final trial. Additionally, fabrics that were placed in ambient or conditioned spaces displayed an increase in bacterial counts for one of the participants when compared to results from fabrics that are not in ambient conditions. As a result, denim fabrics will be conditioned for 24 hours before participant occlusion. Additionally, while results from fabrics placed in nutrient broth, Milli-Q water or incubated after freezing did result in increased bacterial counts in the preliminary trials, they were notably time consuming and may not be feasible as time after treatments is part of the research hypothesis.

Appendix C

Bacterial Counts

Table C.1 *Bacterial Counts (CFU/mL) on fabrics immediately after wear*

Treatment	Participant	Rep	Baseline	0 h	24 h
Freeze	1	1	8.59×10^3	5.93×10^2	6.59×10^3
		2	5.81×10^3	7.41×10^2	3.33×10^3
	2	1	5.93×10^3	3.70×10^2	7.63×10^3
		2	9.74×10^3	1.33×10^3	3.70×10^3
	3	1	6.59×10^3	1.11×10^3	4.81×10^3
		2	8.93×10^3	8.89×10^2	7.70×10^3
	4	1	1.00×10^4	5.93×10^2	7.63×10^3
		2	1.09×10^4	8.89×10^2	8.15×10^3
	5	1	9.22×10^3	1.48×10^2	6.22×10^3
		2	9.67×10^3	5.56×10^2	9.63×10^3
	6	1	1.44×10^4	8.15×10^2	1.10×10^4
		2	1.35×10^4	6.67×10^2	9.63×10^3
Iron	1	1	5.67×10^3	2.74×10^3	8.44×10^3
		2	9.26×10^3	1.56×10^3	6.44×10^3
	2	1	4.59×10^3	2.30×10^3	6.81×10^3
		2	8.04×10^3	1.85×10^3	4.81×10^3
	3	1	3.52×10^3	6.67×10^2	5.70×10^3
		2	6.70×10^3	8.89×10^2	5.44×10^3
	4	1	5.11×10^3	1.63×10^3	8.22×10^3
		2	8.59×10^3	1.19×10^3	6.30×10^3
	5	1	4.70×10^3	2.37×10^3	9.41×10^3
		2	8.67×10^3	2.15×10^3	5.78×10^3
	6	1	6.78×10^3	1.70×10^3	1.02×10^4
		2	1.22×10^4	1.56×10^3	4.59×10^3

Table C.1 *Continued*

	Participant	Rep	Baseline	0 h	24 h
UV	1	1	8.15×10^3	3.11×10^3	5.85×10^3
		2	8.00×10^3	3.04×10^3	6.30×10^3
	2	1	8.00×10^3	2.59×10^3	2.00×10^3
		2	6.07×10^3	1.56×10^3	4.59×10^3
	3	1	5.78×10^3	1.33×10^3	2.07×10^3
		2	7.11×10^3	2.15×10^3	7.48×10^3
	4	1	7.85×10^3	6.00×10^3	8.15×10^3
		2	9.67×10^3	2.00×10^3	6.89×10^3
	5	1	1.69×10^4	6.96×10^3	1.36×10^4
		2	1.00×10^4	2.74×10^3	8.44×10^3
	6	1	1.61×10^4	8.00×10^3	1.59×10^4
		2	1.46×10^4	5.11×10^3	8.22×10^3
Wash	1	1	5.93×10^3	$<7.41 \times 10^1$	$<7.41 \times 10^1$
		2	7.19×10^3	$<7.41 \times 10^1$	$<7.41 \times 10^1$
	2	1	6.37×10^3	$<7.41 \times 10^1$	$<7.41 \times 10^1$
		2	6.78×10^3	$<7.41 \times 10^1$	$<7.41 \times 10^1$
	3	1	2.67×10^3	$<7.41 \times 10^1$	$<7.41 \times 10^1$
		2	3.59×10^3	$<7.41 \times 10^1$	$<7.41 \times 10^1$
	4	1	1.21×10^4	$<7.41 \times 10^1$	$<7.41 \times 10^1$
		2	1.19×10^4	$<7.41 \times 10^1$	$<7.41 \times 10^1$
	5	1	9.44×10^3	$<7.41 \times 10^1$	$<7.41 \times 10^1$
		2	1.04×10^4	$<7.41 \times 10^1$	$<7.41 \times 10^1$
	6	1	1.40×10^4	$<7.41 \times 10^1$	$<7.41 \times 10^1$
		2	1.27×10^4	$<7.41 \times 10^1$	$<7.41 \times 10^1$