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Odour Evaluation on Antimicrobial Treated Fabrics:
An Assessment of Test Methods

by

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Abstract

Few studies have been carried out to determine the odour intensity retained on fabrics, particularly for antimicrobial treated textiles. The purpose of this study was to develop an *in vitro* method to collect human axillary odour on fabrics and compare this to the traditional *in vivo* method (i.e., wear trial); and also to investigate axillary odour intensity emanating from cotton and polyester fabrics without treatment and with antimicrobial treatments of polyhexamethylene biguanide (PHMB) and zinc pyrithione (ZP). Sensory measurement with line scale was used to determine the odour intensity retained on fabrics. Numbers of aerobic bacteria extracted from fabrics were counted to determine the effect of antimicrobial treatment. Findings suggest that odour can be generated and detected through the developed *in vitro* method by incubating 'fresh sweat' onto fabrics. However despite a reduction in bacteria due to the antimicrobial treatments they do not correspond to anti-odour as bacterial counts were not related to differences in odour intensity.

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LIST OF ABBREVIATIONS

AMS	AEGIS microbe shield
CFU	colony forming units
CGSB	Canadian General Standards Board
C.V.	coefficient of variation
cm	centimetre
C-N	cotton fabrics without treatment
C-PHMB	cotton fabrics treated polyhexamethylene biguanide
C-ZP	cotton fabrics treated zinc pyrithione
df	degree of freedom
E.	*Escherichia*
g	grams
GC-MS	gas chromatographic - mass spectrometry
g/m^2	grams per metre square
IVA	isovaleric acid
K.pneumoniae	*Klebsiella pneumoniae*
l	litre
M	mean
mg	milligram
ml	millilitre
ml/l	millilitre per litre
mm	millimetre
n (N)	number of subjects
p	significance value
PBS	phosphate buffer solution
PHMB	polyhexamethylene biguanide
P-N	polyester fabrics without treatment
P-PHMB	polyester fabrics treated polyhexamethylene biguanide
PPT	participant
PTR-MS	proton transfer reaction mass spectrometry
P-ZP	polyester fabrics treated zinc pyrithione
r	replicate
REB	Research Ethics Board
R.H.	relative humidity
R_H	reference sample represented high odour intensity
R_L	reference sample represented low odour intensity
S	sensory measurement
S.aureus	*Staphylococcus aureus*
SD	standard deviation
SIFT-MS	selected ion flow tube mass spectrometer
SEM	standard error mean
TRA	Thomson Research Associates

ZP	zinc pyrithione
°C	degrees Celcius
µl	microlitre
%	percent

CHAPTER 1 INTRODUCTION

Background

Strong body odour emanating from a person can be an embarrassing problem from the worldwide perspective, particularly in Western cultures where natural body odours are often viewed as unpleasant/unacceptable, and even considered unhygienic. People often try to mask, reduce or eliminate body odours by various methods, for example, through the use of perfumes and antiperspirants. Considerable amounts of money have been spent on these products annually, e.g. more than $2 billion in the United States alone in 2008 (Park, 2008).

Human body odour can be generated by many different regions on the body, among which the axillary region is a highly odorous area due to high density of apocrine and eccrine sweat glands and the types of resident bacteria (Kloos and Musselwhite, 1975; Sato, Kang, Saga & Sato, 1989). It has long been demonstrated that sterile axillary sweat is odourless and much of the malodour of the axillae resulted from microbial degradation, especially from the gram-positive aerobic bacteria, such as the *Corynebacterium* species (Leyden, McGinley, Holzle, Labows, & Kligman, 1981; Rennie, Gower, Holland, Mallet, & Watkins, 1990). Certain strains of corynebacteria defined as sub-group (A) or corynebacteria (A) by James, Casey, Hyliands, & Mycock (2004) are able to metabolize the long-chained fatty acids present in sebum and axillary secretions into volatile mid- and short-chained fatty acids, which have been identified as the main compounds responsible for axillary malodour (James et al., 2004; Zeng et al., 1991).

The rapid growth of bacteria in the axilla due to the nutrients present, and the warm, moist environment provided by eccrine sweat in this semi-occluded region, facilitate odour generation. Clothing can also play an important role in odour intensity as bodily secretions and skin bacteria are transferred from the body to the garment, and the absorbed sweat retained by textiles provides nutrients for bacterial growth (Teufel, Schuster, Merschak, & Bechtold, 2008). Even after garments have been removed from the body some clothing can still be perceived as odorous, even as long as 28 days

following removal (McQueen, Laing, Brooks, & Niven, 2007a). For some types of textiles, such as polyester, washing may not even be that effective in reducing odour as Munk, Johansen, Stahnke, & Adler-Nissen (2001) found that the amount of odorants remaining in polyester after 24 hours with or without washing was not significantly different.

As bacteria are known to be able to survive in many textiles (McQueen et al., 2007a; Neely, & Maley, 2000) a common method for controlling bacterial growth is to treat fibres/fabrics with antimicrobial agents. With more attention paid to healthy lifestyles, the use of antimicrobial textiles had extended from the potential use in hospital environments for reducing nosocomial infection, to everyday household and personal life. As well, consumers' demand for hygienic clothing and 'odour free' active wear has made the manufacturing of antimicrobial textiles increase rapidly in the last several years (Gao & Cranston, 2008).

Although there are several standard test methods for testing the efficacy of antimicrobial textiles in reducing bacterial populations (e.g., AATCC 100, AATCC 147, ISO 20743-Method 10.1), no standard methods for estimating the odour reduction efficacy on textiles have been established. The methods for assessing antimicrobial activity of textiles are not suitable for assessing odour control within anti-odour textiles, as test organisms used in standard methods tend not to be those which produce odour (McQueen, Keelan, & Kannayiram, 2010). Furthermore, evidence of antimicrobial activity *in vitro* does not always correspond to odour reduction *in vivo* (McQueen, Keelan, Xu, & Mah, 2012). Nonetheless, statements of odour reduction may still be made despite of the lack of such methods (Payne and Kudner, 1996). In fact, the extent to which the antimicrobial treatments actually reduce odour build-up in clothing is largely unknown since most testing involves monitoring bacterial growth *in vitro* rather than through sensory measurement. Instrumental methods are frequently used to evaluate odour, which are the physio-chemical approaches to analyze the chemical structure and concentration of an odorant (Neuner-Jehle and Etzweiler, 1991). Experimental instruments typically used are gas chromatographic-mass spectrometry (GC-MS), electronic nose, proton

transfer reaction mass spectrometer (PTR-MS) and selected ion flow tube mass spectrometer (SIFT-MS). Although these methods contribute a lot in the evaluation of odour, they are complicated and expensive, and are not capable of detecting odour intensity as the human sensor, which is a very important aspect in real life.

However, many problems may arise by relying solely on human participants as assessors or odour detectors, such as sensitivity to odorants can be quite variable among different assessors and even for the same assessor they may not be consistent across different times (Meilgaard, Civille, & Carr, 2007). Also, odour generated from the axillary region can be highly variable in intensity and quality over different times for the same individual and also variable from one individual to another (Leyden et at., 1981). However, replication of human odour in the laboratory can also be challenging due to the complicated processes involved in odour generation, which involves physiological sweat production, bacterial metabolism and transfer of odour onto fabrics. The use of human assessors in the assessment of odour is more practical as the unpleasant odours detected in daily life are perceived by the human sense of smell. Sensory measurement most often used in the area of textiles research generally has involved the tactile handfeel (drawing on texture perception) and visual assessment of colour and pilling (American Society for Testing and Materials, 2010; Winakor & Kim, 1980). Thus, using the human olfaction senses for detecting odour on textiles is applicable and suitable.

Statement of Problem and Purpose

Statement of Problem

Axillary odour can be retained on fabrics as sweat, bacteria and skin debris are transferred from the body to the clothing. Although instrumental methods have contributed to the process of odour study, the complicated test results are not easily understandable in some areas, such as consumer science. Further, the commonly used antimicrobial efficacy tests to investigate the effect of a treated fabric on controlling bacterial populations are not suitable for assessing odour control within anti-odour textiles. Sensory measurement as a scientific discipline is rarely used in the textile study

so sensory assessment of odour on fabrics is less common although it has begun to be addressed by some researchers (e.g., McQueen et al., 2007a; Munk et al., 2000). While in the pioneer studies, odour collected *in vivo* through the wear trial method is time consuming with only a limited number of samples being compared each time. So developing a new method to collect human axillary odour as well as to detect the intensity retained on fabrics is of practical significance.

Purpose

The purpose of the study was to investigate the odour intensity among fabrics that vary in fibre type, and fabrics which have been treated with antimicrobial treatments compared with those without treatments, following wear next to the skin. As well as develop an effective method to collect and measure odour on fabrics to widen the application of sensory evaluation technology in the textile area. Therefore it is hoped the effectiveness of this sensory measurement can be more generally known and used in the textile area and even provide the basis for a new test method.

Objectives

The objectives of this study were to:

1) develop a method to collect human axillary odour on fabrics that can be used for detecting odour intensity retained on fabrics and compare this method to the traditional method for axillary odour collection on fabrics (i.e., wear trial),

2) determine whether there is a difference in odour intensity following wear and incubation with axillary sweat between cotton and polyester untreated fabrics,

3) determine the effectiveness of two selected antimicrobial treatments on reducing odour intensity on polyester and cotton fabrics.

Hypotheses

To meet the second and third objectives, the following hypotheses are made:

Hypothesis 1: There are significant differences in odour intensity following wear between the cotton fabric and the polyester fabric.

Hypothesis 2: There are significant differences in odour intensity following wear between antimicrobial-treated fabrics and the non-treated control fabric.

Limitations and Delimitations

Limitations

The main limitation of this study was not being able to use all the different fabrics for collecting the human axillary odour. Although, fabrics used to make daily clothing can be classified into several large classes, a lot of different treatments, structures and fibre combinations can be applied to garments, it is impossible to include all the different fabrics. However, the fabrics used in the study represent the two most common fibre types available in the apparel market. As well, knit fabrics are commonly used for clothing that is worn in close proximity to the skin and therefore would be likely to retain axillary odours. The useful insights and practical information found in this study can still be useful and further the knowledge in textile research about odour retention in clothing fabrics. Another limitation was only three participants involved in the study as odour providers. Small number of participants may produce variable results, while it can make the experimental design less complicated in the sensory study and also acceptable for the new test method developed.

There were also challenges to using human beings as odour sources and as odour assessors. Sensory assessment can be inconsistent for different people and also inconsistent for a person at different times. Nevertheless, sensory measurement is a scientific discipline and the results presented can be easily understood by researchers in different disciplines.

Delimitations

In this study, the intensity of axillary odour that was retained on the fabrics were assessed and compared by the assessors, while the differences in quality of odour retained

on fabrics following wear next to the axillae by participants were not identified. Only three participants were selected and the odour intensity retained on fabrics and bacterial populations would be specific to these three participants.

The intensity of odour retained on the different fabrics were assessed and compared, but this study only evaluated a small selection of fabrics and the results cannot be applied to all the fabrics.

Definitions

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

- *Assessors* – assessors can be naive assessors, selected assessors and experts:

    1) "naive assessors" who do not have to meet any precise criterion, or "initiated assessors" who have already participated in sensory tests;

    2) "selected assessors" are assessors who have been selected and trained;

    3) "experts" can be "expert assessors" who have already demonstrated particular acuity in panel work and have developed a good long-term memory, or "specialized expert assessors" who draw on additional knowledge gained in particular fields (ISO 8586, 1993)

- *Intensity* – magnitude of the perceived sensation (ISO 5492, 2008)

- *Odour* – sensation perceived by means of the olfactory organ in sniffing certain volatile substances (ISO5492, 2008). The odour here refers to the undesirable smell emanated from the axillary region of the body and retained on the fabrics following wear next to the skin

- *Olfactory* – pertaining to the sense of smell (ISO 5492, 2008)

- *Sensory* – relating to the use of the senses, i.e. to the experience of a person (ISO 5492, 2008)

- *Sensory science* – a scientific method used to evoke, measure analyze and interpret those responses to characteristics of products as they are perceived by the senses of sight, smell, taste, touch, and hearing (Stone & Sidel, 2004)

- *Participants* – participants are those with strong body odour who wear the offered fabrics and generate odour that were collected in the study

CHAPTER 2 REVIEW OF LITERATURE

The literature review included four areas: 1) odour from the human body; 2) detecting axillary odour; 3) sensory measurement techniques; and 4) textiles properties.

Odour emitted from the human body

Secretions from the sweat glands, sebaceous glands, faeces, urine, expiration, salvia, skin, breasts and sex organs are all sources of human body odour. Especially the sweat glands and sebaceous glands located in the axillary region, anogenital area, scalp, mammary areolae, ear canals, hand and feet contribute a lot to body odour (Henkin, 1995). The axillary region is a highly odorous region with high density of sweat glands and bacteria (Kloos and Musselwhite, 1975; Sato et al., 1989). As a key source of human body odour, the axillary region can contribute to strong odours being retained in clothing, as sweat and odour can easily be transferred from the body to clothing worn next to this body region.

Compounds responsible for axillary odour

Axillary malodour is generated through the biotransformation of secretions from sweat and sebaceous glands in the axillae. Three groups of compounds among various compounds constituting axillary odour have been reported as contributing to overall axillary malodour: 1) 16-androstene steroids (Bird and Gower, 1982; Gower, Holland, Mallet, Rennie, & Watkins, 1994); 2) short-chained and medium-chained fatty acids (Zeng et al., 1991); and 3) volatile sulphur compounds with sulfanylalkanols identified as the most important components of total axillary odour (Natsch, Schmid, & Flachsmann, 2004).

The 16-androstene steroids have been considered as the compounds responsible for the typical odour of the axillary sweat in the 1970s-1990s, and the steroids 5α-androst-16-en-3-one and 5α-androst-16-en-3α-ol were the two mainly studied compounds. The 5α-androst-16-en-3-one steroid was found present in apocrine sweat in greater quantities than in skin extracts, whereas, the 5α-androst-16-en-3α-ol was absent in

apocrine sweat it was present in skin extracts (Gower et al., 1994). Results from an odour generation test showed that sterile apocrine sweat had no odour and therefore, it was suggested that 5α-androst-16-en-3α-ol was the predominant 16-androstene steroid causing axillary odour, as it was generated from the metabolism of gram-positive bacteria in the axilla (Gower et al., 1994). High levels of anosmia (odour blindness) to the 16-androstene steroids have been noted in a population while particularly high levels of sensitivity to these same compounds within the population could also be present. Interestingly, 5α-androst-16-en-3-one is one of the most common compounds for anosmia, with around 50% of individuals showing anosmia to it (Amoore, 1977; Lundström, Hummel, & Olsson, 2003; Wysocki and Beauchamp, 1984). Furthermore, there are only a very small number of bacterial species capable of biotransforming 16-androstenes to the odorous 16-androstene steroids (Austin and Ellis, 2003). Therefore, 16-androstene steroids may contribute only a very small part to axillary odour and not be as important as early studies stated (Austin and Ellis, 2003).

Zeng et al. (1991) found the C_6-C_{11} carboxylic acids represented typical axillary odour. Some of the early studies also showed that short-chained C_2-C_5 volatile fatty acids and mid-chained C_6 –C_8 were responsible for axillary odour (Nitta and Ikai, 1953). The short-chained fatty acids present in axillary odour have been long accepted, although generally they have been considered more important contributors in foot odour (Kanda et al., 1990). So later, focus has been mainly on the chemical analysis of mid-chained fatty acids. Through gas chromatography-olfactometry (GC-O) analysis of axillary sweat the characteristic axillary odour was shown to consist primarily of C_6 -$C_{11,}$ saturated, branched, and unsaturated acids, with the main component being (E)-3-methyl-2-hexenoic acid (Zeng et al. 1991; Zeng, Leyden, Spielman, & Preti, 1996). The E-isomer represented the typical axillary odour and had a very low olfactory threshold which is 20 times lower than that of Z-isomer. Nonetheless, 20% of people in one study still showed anosmia to (E)-3-methyl-2-hexenoic acid (Baydar, Petrzilka, & Schott, 1992). In another study, high levels of short- and mid-chained fatty acids were found as the results of the incubation of axillary sweat and sebum on polyester fabrics (Munk, Munch, Stahnke, Adler-Nissen, & Schieberle, 2000).

The volatile sulphur compounds, which are released by bacterial enzymes, have more recently been identified as important components of total axillary odour (Natsch et al., 2004; Troccaz, Starkenmann, Niclass, van de Waal, & Clark, 2004). Although these compounds are found in very small numbers, the olfactory thresholds are also very low.

Bacteria related to axillary odour

It has long been demonstrated that sterile axillary sweat is odourless and much of the odour emitted from the body results from microbial degradation (Shelley, Hurley, & Nichols, 1953). In the axillary region, gram-positive aerobic bacteria have been found to be the major types of bacteria to cause malodour (Leyden et at. 1981; Shehadeh and Kligman, 1963; Taylor et al., 2003). Bacteria play an important role in the formation of human axillary odour, as short-/mid-chained fatty acids as well as the volatile sulphur compounds are formed from the bacterial degradation and bacterial enzymes released (Leyden et at., 1981; Taylor et al., 2003; Troccaz et al., 2004).

A high density of microbial populations is present in the axillary region. This has been found to be much greater than those found on the legs, arms and head (Kloos and Musselwhite, 1975). Gram-positive cocci (staphylococci) and corynebacteria tend to be the most common types of microflora present in the axilla, followed by micrococci and propionibacteria which were also present in the axillary microflora (Kloos and Musselwhite, 1975; Leyden et al., 1981; Shelley et al., 1953; Taylor et al., 2003). *Micrococcus*, Klebsiella and Enterobacter were also found in the axilla for some participants, with the latter two only being found in adults (Kloos and Musselwhite, 1975). The number of micrococci are much lower than that of staphylococci and aerobic coryneforms (Kloos and Musselwhite, 1975; Taylor et al., 2003).

Some researchers indicate that high densities of bacteria are the prerequisite for the production of axillary odour (Guillet, Zampetti, & Aballain-Colloc, 2000; Rennie et al., 1991; Taylor et al., 2003). Rennie et al. (1991) found that axillary extracts were not perceived to be odorous until the density of coryneform isolates (e.g., *Corynebacterium xerosis*) incubated with the extracts reached 10^5 /cm^2 and 10^6 /cm^2. In another study, it

was found that patients' bromidrosis (strong malodour) was reportedly improved by washing with an antiseptic soap when there was a reduction of microorganisms such as micrococci and diphtheroids (corynebacteria), while no improvement was found for the patients' bromidrosis when the bacteria was not significantly reduced (Guillet et al., 2000). Several other studies found axillary odour intensity was related to bacteria numbers, as high-odour persons were typically found to have higher numbers of coryneforms; whereas lower numbers of coryneforms were found for low-odour persons (Leyden et al., 1981; Rennie et al., 1991; Taylor et al., 2003). Also, a positive relationship between odour intensity and micrococci numbers (although present in low amounts in the axilla) has also been shown (Taylor et al., 2003).

Although mid- and short-chained fatty acids are considered as the main chemical component responsible for axillary odour, they are not the initial product of the secretion from axillary sweat. It is evident that corynebacteria are responsible for the majority of axillary odour by metabolizing long-chained fatty acids initially present in the sebum and sweat secretions from the axillary region to the volatile mid- and short-chained fatty acids associated with axillary odour. However, not all the corynebacteria are responsible for causing axillary odour, and those capable of metabolizing fatty acids have been grouped as sub-group (A) or corynebacteria (A) by James et al. (2004).

Evaluation of axillary odour

Evaluation and description of odour is not simple, and the most commonly used methods for detecting odour involve instrumental methods and sensory measurement. Instrumental methods are physio-chemical approaches to analyze the chemical structure and concentration of an odorant (Neuner-Jehle & Etzweiler, 1991); sensory measurement can be used to measure intensity and quality of odour (Stone & Sidel, 2004). As the current research involved using sensory methods for detecting odour, only sensory methods for detecting odour were reviewed.

Sensory measurement in odour detection

Sensory measurement is a scientific discipline and widely used in the evaluation and product development of foods, beverages and cosmetics. Sensory science is defined as "a scientific method used to evoke, measure, analyze and interpret those responses to characteristics of products as they are perceived by the senses of sight, smell, taste, touch, and hearing" (Stone & Sidel, 2004). In textile research sensory measurement that is generally used involves tactile hand-feel and visual assessment of colour change and surface change such as pilling or creasing (International Organization for Standardization, 2005; Winakor & Kim, 1980). Sensory assessment of odour on fabrics is less common although it has begun to be addressed by some researchers (McQueen, Laing, Wilson, Niven & Delahunty, 2007b; Munk et al., 2000).

The relationship between the given physical stimulus and the subject's response has at least three steps in the process: 1) the stimulus hits the sense organ, followed by a converted nerve signal travelling to the brain; 2) the brain then interprets the incoming sensations into perception with previous memories; and 3) based on the perception, a response is formulated (Schiffman, 1996).

The odour sensation is perceived by the olfactory system when the volatiles of the odour enter the nasal passage, which must be transmitted by gas that can be in the form of atmosphere, water vapour or an industrial gas. The amount of volatiles transmitted is affected by the temperature and the compound's nature, and the intensity of the odour perceived is affected by the proportion of the odour gas in contact with the olfactory receptors (Laing, 1983). The airborne odour is sensed by millions of tiny, hair-like cilia of the olfactory epithelium which is in the ceiling of the nasal cavity, as shown in Figure 2.1 (Meilgaard et al., 2007).

Once odorants enter the nose, they will stimulate the olfactory receptors which are located within the olfactory neuroepithelium; when activated, the receptor cells send electrical signals which are then relayed in glomeruli, followed by transmission to the brain. The process is described in Figure 2.2 (SensaSlim, 2011).

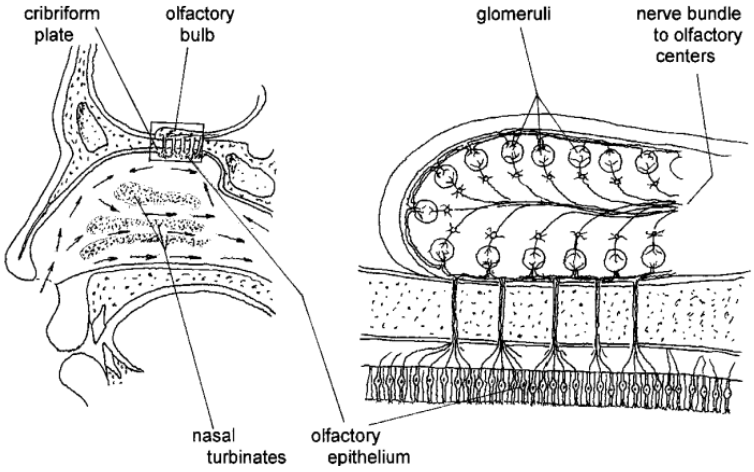


Figure 2.1 Anatomy of the olfactory system (Meilgaard et al., 2007)

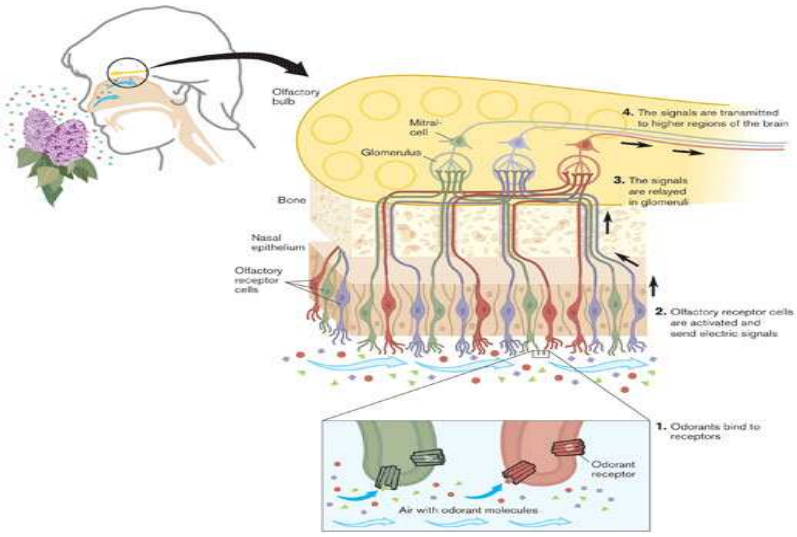


Figure 2.2 Odour sensation process (SensaSlim, 2011)

Methods used in sensory tests

Methods selected in sensory studies may depend on the objective of the study. For example, in the food industry the research department may be interested to know whether a change in an ingredient can be perceptible to consumers, or if there is any

difference for some specific attribute after improving the recipe or production technology, this may also include the degree of the difference. In such sensory studies, one of the following methods or a combination of these methods would be commonly used.

Discrimination tests

Discrimination tests are used to measure whether there is a difference between samples. Discriminations tests are efficient and frequently used to detect small differences that may exist between samples, as they are easy and simple to use, while size of difference among samples is not measured. Commonly used tests include the paired comparison test, triangle test, duo-trio test, two-out-of-five test, same/different test, A-not-A test and different-from-control test. The details and comparisons of these tests are listed in Table 2.1 (Meilgaard et al., 2007).

In some discrimination tests a 'forced-choice' is required, which means that even if there is no perceived difference, assessors still need to choose one sample over another/others in a particular property. In some cases the guessing chance would be as high as 50%, therefore, fairly large numbers of test samples are required (Meilgaard et al., 2007).

Among the discrimination test methods, paired comparison and variations of this, such as quad analysis, have been used as they have an advantage of being simple and quick without the need for highly trained sensory panels. However, a problem with the paired comparison method is when multiple samples need to be evaluated, for example, when sample size increased from 4 to 6, the pairs would increase from 6 to 15 respectively, and when sample size is large the number of pairs that need to be evaluated would be huge. An alternative method to the traditional paired comparison method is the quad design (Miller, 2002). In a quad design, four samples will be randomly selected from all samples to form a 'quad' with only five pairs compared instead of six pairs as shown in Figure 2.3 (Miller, 2002) and all quads presented in a study will include all the samples with each sample having equal chance for evaluation, so this can reduce the number of test samples compared with paired comparison. For example, the quad

analysis was well described and used to assess the appearance retention of carpet (Miller, 2002), and the softness, drape, lustre and elasticity of silk (Kim, Yoo & Kim, 2005). McQueen, Laing, Wilson, Niven & Delahunty (2007b) applied this method to the evaluation of underarm odour intensity among different fabrics.

Table 2.1 Discrimination tests

Test	Details	Sample example	Guessing chance	Number of assessors	Advantages	Limits
Paired comparison	Pick one sample from 2 samples according to specific attributes	AB	1/2	>20	Easy and quick	Less statistically efficient
Triangle	3 samples presented, 2 are the same and the goal is to find the odd one	ABB	1/3	20-40	Statistically efficient	Sensory fatigue
Duo-trio	3 samples presented in which one is a reference (R) sample, find the one that matches R from the other 2 samples	A(R)AB	1/2	15-30	Simple	Less statistically efficient
Two-out-of-five	Present 5 samples in which 2 are the same, find the 2 samples different from the other 3	ABAAB	1/10	10-20	Even small differences can be detected	Affected by sensory fatigue and memory
Same/ different	Present 2 samples, ask whether they are the same or different (same/different=50/50)	AB or AA	1/2	20-50	Used in complex stimuli and mentally confused situations	Time consuming
A-not-A	Present samples of product A or not A, judge it is A or not A	A or not A	1/2	10-50	Used when one product has importance as a standard or reference	Need large sample numbers
Different-from-control	One sample set as a control, measure how different other samples are from the control with a scale	-	None	20-50	Can detect the size of difference	Time consuming

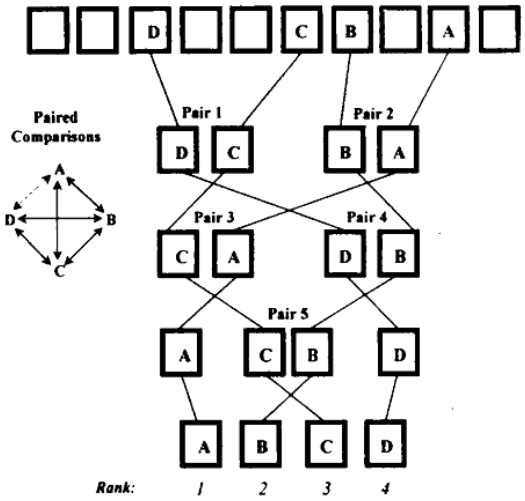


Figure 2.3 Process of quad analysis (Miller, 2002)

Ranking tests

Ranking tests involve arranging three or more samples in order of the degree of some specified attributes (e.g., order the fabrics in stiffness with the stiffest one ranked first). Ranking tests are widely applied and tend to be rapidly carried out with little training required. However, the size of difference among samples is not measured in a ranking test. Furthermore, as direct comparisons are made among samples, results from ranking tests are hard to compare from one session to another (Pangborn, 1984). An example of a ranking test is shown in Figure 2.4, and the ranked order from the smallest to largest should be B, C, D, A.

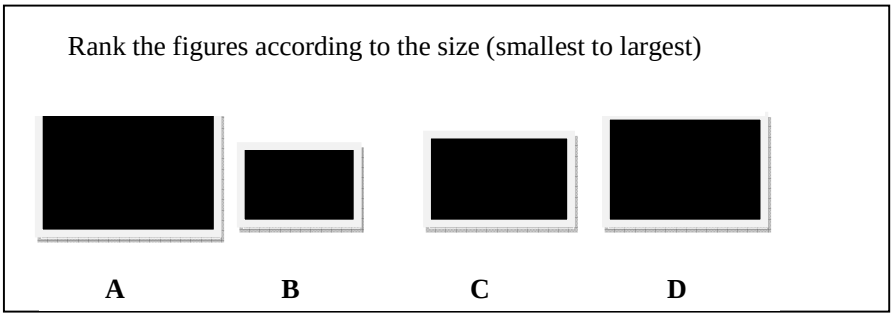


Figure 2.4 Ranking example

An example of ranking test used in the textile area was to apply rank ordering method for aiding fabric wrinkle evaluation, and the test results showed that the method was reliable and could even overcome the problems connected with the conventional standard method (i.e. grading), such as the limited grading range (Memarian, Amni-Tehran & Latifi, 2011).

Scaling tests

In scaling tests, numbers or words are used to measure the intensity of a perceived attribute. Category scales, line scales and magnitude estimation are commonly used scaling tests. For *category scales*, there is a limited number or word categories reflecting different degrees of intensity and assessors rate certain attributes by choosing a number or descriptor. With a *line scale*, assessors can mark on a line that reflects the level of their response to certain stimulus. By *magnitude estimation*, assessors can give a free number to the test samples usually according to the number given to the first sample which is like a reference (Meilgaard et al., 2007). Examples of the three scales are given in Figures 2.5 a) category scale, b) line scale, and c) magnitude estimation scale.

Sensory measurement in textiles

Regardless of which method will be used in the sensory study, people's sensory response to test samples would be converted to data for statistical analysis, which can be categorized at least to one of four ways (Gravetter and Wallnau, 2009; Meilgaard et al., 2007). They are nominal data, ordinal data, interval data and ratio data.

1. *Nominal data* – items are assessed and classified into different groups that differ in names, without any quantitative observation or order (e.g., gender, colour).

2. *Ordinal data* – assessors place the items examined in an ordered sequence in terms of size or magnitude, while equal difference between items does not indicate equal magnitude of difference being measured.

3. *Interval data* – items are assessed in ordered categories that all intervals of exactly the same size, although there is no true zero (e.g., Celsius or Fahrenheit temperature scale).

4. *Ratio data* – it is an interval data with an additional feature of an absolute zero point (e.g., distance, volume).

Please sniff the sample and indicate odour intensity on the scale below (circle the number):

SAMPLE_____

0	1	2	3	4	5	6	7	8	9	10
No odour		Low			Med			High		Extreme odour

a) category scales

Touch the fabric sample and indicate the hand feel (soft/stiff) by marking the following line

Sample_____

soft ⊢——————————————————⊣ stiff

b) line scale

The reference yogurt that you taste has the intensity of vanilla flavor at 10, rate other samples for the vanilla flavor intensity based on the reference one, e.g. if the vanilla flavor intensity of any sample is twice of the reference one, assign the sample value of 20.

Reference sample 10
Sample 265 _____
Sample 798 _____

c) magnitude estimation

Figure 2.5 Examples of scaling tests, a) category scale; b) line scale; and c)magnitude estimation

Although the discrimination and ranking tests can be easier for assessors to carry out, most of these tests can not measure the magnitude of difference among different

samples. Also, there could be a high chance of guessing (e.g., 50% in a paired comparison test) which therefore a large number of correct assessments is required to acquire statistically valid results. Thus, scaling test methods have been used in many studies (e.g., Cardello and Winterhalter, 2003; McQueen et al., 2007a; 2007b) because they produce more information. In several studies for assessing body odour intensity, category scales were used, such as a 4-point category scale from no odour to foul odour (Bowler, Davies & Jones, 1999) or from none to severe odour (Karlsmark et al., 2003). Both scales were used to evaluate wound odour in clinical settings (Bowler et al., 1999; Karlsmark et al., 2003). A 0-5 scale from absence of odour to very strong odour was used for the efficacy assessment of antiperspirants and deodorants (Piérard et al., 2003). In another study, a scale which ranged from 0-10 for assessing odours of T-shirts was used to test the hypothesis relating to a particular group of genes influencing mate choice (Wedekind & Füri, 1997). An extremely high number of categories (99-points) was used in a tactile fabric hand study (Winakor and Kim, 1980).

The category scale can overcome some problems existing with discrimination and ranking tests, however there are still some limitations for such a method. For example, the limited numbers on a category scale cannot express all the sensory responses; or when the assessor has a response located between two consecutive numbers there is no choice for the situation rather than choosing either of the close-by numbers which may lead to bias in the final analysis (Lawless & Heymann, 1998). Also, if there are only low number of categories on a scale this could result in it being harder to detect differences among samples; whereas, a high number of categories may result in small differences becoming significant (Lawless and Heymann, 1998).

For these reasons, some researchers choose a line scale method which is ‘free’ to use. For example, Munk et al. (2001) used a 15 cm line scale with ‘nothing’ labeled at the beginning and ‘very strong’ at the end to evaluate odour intensity of soiled then washed cotton and polyester fabrics. In comparing a line scale method with the quad analysis method, McQueen et al. (2007b) also used a 15 cm (150 mm) line scale, with ‘low intensity’ on the left and ‘high intensity’ on the right, to detect axillary odour on fabrics.

They found there was a positive correlation in rank order for fabric odour intensity between the quad analysis test and the line scale test, and therefore indicated that the line scale method could be an efficient method for assessing odour intensity where sensory fatigue is possible and test samples are limited (McQueen et al., 2007b). Kalinski et al. (2005) used a 10 cm scale with labelled words along the line (0 - no wound odour, 1 to 4 cm - mildly offensive, 5 to 8 cm - moderately offensive and 9 to 10 cm - extremely offensive) to detect wound odour. The labels on the line scale may ease the measurement by extra information but too many labels tend to make the line scale into a category scale, therefore, care should be taken when using labels on line scales.

Magnitude estimation is another 'freely assigned' scaling test. Magnitude estimation has been used to evaluate fabric handle and level of comfort of different fabrics (Cardello and Winterhalter, 2003; Cho, Kim, & Casali, 2002). Both groups compared the subjective sensation with Kawabata data for mechanical properties in order to predict the relationship between the two (Cardello & Winterhalter, 2003; Cho et al., 2002).

Other researchers have found that test results were similar no matter which scaling test method was used. Hein and colleagues compared a 9-point hedonic scale (category scale), an unstructured line scale (line scale) and labelled affective magnitude scale (magnitude estimation) for consumer preference of commercial breakfast bars, and obtained similar conclusions using the three methods (Hein, Jaeger, Carr & Delahunty, 2008). Pearce, Korth, & Warren (1986) also reached similar results when comparing the preference of the feel of different fabrics, regardless of whether a category scale or magnitude estimation was used. Although selection of test methods may not result in many differences of the overall ranking of test samples, considerations such as sample type and predicted type of data still need to be taken into account (Hein et al., 2008).

Test controls, sample preparation and assessor selection

Test controls

To minimize the extraneous variables that may influence the detection of true differences, many variables should be controlled. First, the temperature and humidity of sensory testing rooms should be comfortable for the assessors (room temperature of 22-24°C with 45-55% relative humidity (R.H.) are suggested) and the air in the room should be filtered/recirculated to remove all detectable odours (Meilgaard et al., 2007). Second, the colour and lighting in the room should permit sufficient viewing of samples without shadow illumination in certain distances, and walls should be off-white to prevent any unwanted differences in appearance. Third, materials used for furnishing or construction should be non-odorous, easy to clean, smooth and non-absorbent, and a natural colour is preferred. Finally, the test room should be quiet and easy to access, with separate booths and a preparation place (International Organization for Standardization, 1998; Meilgaard et al., 2007).

Sample preparation

All the materials for a sample should come from one source and the amount should be precise. The containers for preparation, storage and serving should reduce the transfer of volatiles, such as glass, stainless steel or china. Plastic can be used when the test is held less than 10 minutes. Three-digit random numbers for coding should be used instead of letters to reduce biases. The sample presentation should be random with all the samples having equal chance to be selected (Meilgaard et al., 2007).

Assessors selection and training

The validity of sensory measurement mostly depends on the test results made by assessors. Thus emphasis should be given to the importance of selection and training of assessors. The former provides a way of selecting better assessors among the available candidates, and the latter ensures that the selected assessors are sufficiently trained in the activities they are being asked to perform.

Selection Recruitment and screening of assessors is the first process before selection. There are many resources that can be used for recruitment, such as websites, bulletin boards, seminars, questionnaires, colleges/schools (American Society for Testing and Materials, 1999; Meilgaard et al., 2007). During recruitment, some information about candidates can be collected which provides the base for screening candidates, such information is listed in Table 2.2 (American Society for Testing and Materials, 1999; International Organization for Standardization, 1993). The number of persons recruited needs to be 2-3 times the number of persons to constitute the final panel (International Organization for Standardization, 1993).

Table 2.2 Background information about candidates

Information collected	**Requirement for assessors**
Interest	Should be interested in sensory analysis and the products
Availability	About 80% attendance insured
Health	Generally in good health; no allergies to test materials
Attitude to products	Be willing to use the test products
Communication	Good communication skills
Others	Certain age groups, sex etc. may be required for some tests

Although assessors' selection is important, especially in some specific sensory studies such as the smelling (odour) test where some people may be anosmic to some odorous compounds, the selection process is seldom reported in the literature with a few exceptions. One example was where Rennie, Gower, Holland, Mallet, & Watkins (1990) asked candidates to smell steroids and rank the halving dilutions of butanoic acid for selection of their olfactory sensitivity. Another example was to screen assessors using isovaleric acid, androstenone and androstenol via 3-Alternative forced choice threshold tests (McQueen et al., 2007b). The final selection is based on the candidates' performance/acuity, with other information (i.e. availability, health, interest etc.) considered as well (International Organization for Standardization, 1993; Meilgaard et al., 2007).

Training The training procedure usually involves helping the assessors to understand certain terminologies; teaching them how to precondition, how to correctly conduct the test procedure and how to use sensory measurement scales. The assessors are instructed to avoid contact with tobacco, strong odours/tastes for at least 60 min prior to a test, and not to use any perfume/cosmetics prior to or during the test (International Organization for Standardization, 1993). Also, in the training process the assessors should also be informed of some of the techniques required in the test, such as the sniffing techniques. The assessors should take short sniffs with their mouths closed and avoid long, deep inhalation; usually three short sniffs are recommended for measuring one sample and 30 seconds are required between sample evaluations (American Society of Testing and Materials, 1999).

In the literature on evaluating axillary odour, the training sessions have been even less frequently reported than that on selection. For example, in one study on odour retention on a variety of knit fabrics McQueen et al., (2007b) reported that six female assessors were selected for their odour acuity and then trained; the process of selection was given while not for the training.

Odour collection and measurement

Many different methods have been used to collect human body odour from the axillary region, although the research objectives for collecting odour may differ. Axillary odour/secretions can be collected directly or with the assistance of other materials such as textiles. Direct methods involve collecting sweat from the underarm in a plastic goblet placed against the axilla during a sauna or while cycling (Troccaz et al., 2004); scrubbing the axilla surface using a solution placed against the axilla (Rennie et al., 1990; Taylor et al., 2003); and even letting secretions remain in the axillae for 6 hours after washing and have assessors smell the axillary region directly (Rennie et al., 1991). Other collection methods were conducted with the assistance of textile materials, such as wiping the armpit area with gauze pad or textile swatches after exercise (Curran, Rabin, Prada, & Furton, 2005; Munk et al., 2000); wearing a cotton pad in the axilla three times a week (Preti et al., 1987; Zeng et al., 1996); wearing an 100% cotton T-shirt during two

consecutive nights (Wedekind and Füri, 1997); or attaching/stitching the test fabric to the armpits of cotton T-shirts for wearing several times during exercise (McQueen et al., 2007b; Munk et al., 2000).

For detecting and comparing human axillary odour retained on different fabrics, the wear trial method for collecting odour is the most "natural" way that replicates how odour is retained on fabrics in daily life. However, this method of collection is quite time-consuming when there are several different fabrics to test (one wear trial could be 1-2 days) (e.g., McQueen et al., 2007a). Furthermore, a difference may exist between the two arms for one person which could account for a difference in odour intensity between two different fabrics. Directly rubbing the underarm with test fabrics as a means to collect odour is an easy and quick method which was employed by Munk et al., (2000). However, a major problem is that the amount of sweat which may be absorbed onto different fabric specimens cannot be controlled. Thus, the ideal method requires controlling the amount of sweat being inoculated onto fabrics that are the same-size among different fabrics.

Odour measurement can be conducted via instrumental techniques or sensory measurement. Although the sensory method depends highly on human assessors who can be quite variable, instrumental methods are not capable to detect the odour intensity as human sensors, and furthermore the replication of human odour in laboratory is almost impossible. Sensory measurement of odour evaluation on fabrics is practical and applicable as the malodour problem in daily life results from the human olfactory assessment, and it has been also successfully used in some textile areas, such as tactile hand-feel (drawing on texture perception) and visual assessment of colour and pilling (American Society for Testing and Materials, 2010; International Organization for Standardization, 2005).

Textiles properties

Although the generation of axillary odour is predominantly due to the bacterial metabolism of secretions and the high density of sweat glands in the underarm region, clothing also plays an important role in the odour intensity. Clothing may even

potentially increase odour intensity over that which was originally produced in the axillae itself, as the bodily secretions and skin bacteria can continue to produce odour in the clothing even after removal from the human body (Dravnieks, Krotoszynski, Lieb, & Jungermann, 1968; McQueen et al., 2007a).

Odour on textiles without treatment

Difference in odour intensity retained on fabrics is strongly associated with the fibre type from which a fabric is made. A common belief was that fabrics made from natural fibres tend to generate less odour than fabrics made from synthetic fibres. This was confirmed by the work conducted by McQueen and colleagues recently (McQueen et al., 2007a; McQueen et al., 2007b; McQueen et al., 2008). In their studies, intensity of axillary odour collected, via the wear trial method, on fabrics made of 100% wool, 100% cotton and 100% polyester were compared. Results showed that polyester fabrics were rated much higher in odour intensity than either wool or cotton fabrics, with wool fabrics rated the lowest. They also found there was a fabric structure effect on the difference of odour intensity, with the heavier and thicker interlock fabrics having more intense odour than the light weight and thinner single jersey fabrics, although this was only apparent for the high-odour polyester fabrics (McQueen et al., 2007a; McQueen et al., 2007b).

Despite the scientific confirmation that fabrics made from natural fibres generate lower odour than those from synthetic fibres, the reason why this may differ has not been studied. Some manufactures and suppliers claimed that the low odour intensity of wool was due to a 'natural antimicrobial' property in the wool fibre, while evidence proved this was not the case (McQueen et al., 2007a). Bacterial counts were similar at 1 day following wear for wool, cotton and polyester fabrics, but changes were significant for fabric types over time with a significant decline in bacterial numbers on polyester fabrics whereas numbers were relatively unchanged on the wool fabrics up to 28 days of storage (McQueen et al., 2007a).

It is likely that the difference in odour intensity on the fabrics relates to the chemical structure and physical morphology of the fibre, and proposed explanations were

given in another study by McQueen and coworkers (McQueen et al., 2008). In summary, they proposed that differences in odour intensity on fabrics varying by fibre type resulted from differences in absorption of odorous compounds as odour intensity was inversely related to moisture regain of the fabrics. The polyester fabric can easily attract oily soils, such as the odorous metabolites of long-chained fatty acids due to its hydrophobic nature, while the hygroscopic cotton and wool fabrics may be able to absorb fatty acids and may retain fewer odour precursors at least on the fibre surface (McQueen et al., 2008). Oily soils can penetrate into the cotton fibre, but not into the polyester fibre (Obendorf, Namasté & Durnam, 1983), with soils remaining on the polyester surface making the oily metabolites more readily available for bacteria to metabolise them into odorous compounds. Also, the microclimate between the underarm and clothing could be different due to the different fibre types worn next to the axillary region. It may be warmer and more humid when polyester is worn than when cotton and wool are worn, which may favour a higher level of potential bacterial metabolites and bacterial metabolism leading to increased odour (McQueen et al., 2008).

Odour control with antimicrobial treated fabrics

Several different ways to treat textiles for odour control can be used, for example, adding antimicrobial agents into textiles to reduce bacterial populations and subsequently odour; scented textiles by incorporating fragrances into textiles to mask odour; odour-absorbing textiles by adding odour absorbent materials, such as activated charcoal and cyclodextrins to absorb odours; and refurbishing textiles by rigorous laundering to remove microorganisms or kill bacteria that may cause odour (McQueen, 2011). Among these treatments, a common method for controlling odour on textiles is to treat textiles with antimicrobial agents as odour is generated through bacterial metabolism of sweat. Also, with more attention paid to healthy lifestyles in recent years, the use of antimicrobial textiles is becoming increasingly popular (Gao & Cranston, 2008).

PHMB treated textiles

Polyhexamethylene biguanide (PHMB) is a biocidal antimicrobial agent for killing microorganisms rather than inhibiting their growth. It has been successfully and widely used in the food industry, mouthwashes, wound dressings and sanitization of swimming pools for its broad-spectrum bactericidal properties with low toxicity (Gao & Cranston, 2008). The chemical structure of polyhexamethylene biguanide is shown in Figure 2.6.

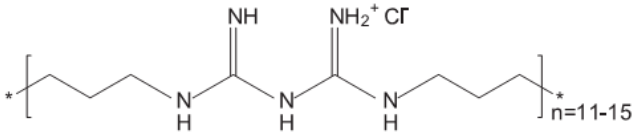


Figure 2.6 Chemical structure of PHMB (Gao & Cranston, 2008)

As an antimicrobial agent applied in textiles, PHMB has proved to be effective at killing bacteria and is durable against repeated laundering, as well it can be economically and easily applied to textiles (Gao & Cranston, 2008). Chen-Yu and co-workers compared the antimicrobial properties of cotton/polyester fabrics treated with PHMB and AEGIS Microbe Shield (AMS) (Chen-Yu, Eberhardt & Kincade, 2007). They evaluated the treated fabrics up to 25 laundering cycles, and found that fabrics treated with PHMB exhibited better antibacterial properties against *Staphylococcus aureus* and *Klebsiella pneumoniae* than that treated with AMS either with 0, 5, 10 or 25 laundering cycles (Chen-Yu et al., 2007). Even after 25 laundering cycles PHMB treated fabrics still had over 90% bacterial reduction of the two bacterial species, while fabrics treated with AMS did not exhibit any antibacterial property at 25 laundering cycles (Chen-Yu et al., 2007). The explanation for why PHMB treated fabrics had better antibacterial properties related to the chemical structure of the agent. In the PHMB molecules a larger number of biguanide groups (12 per molecule) provided multiple sites for bacteria killing activities, as well as providing multiple sites for binding onto the fabric surface, compared with only one silanol group per molecule in the AMS (Chen-Yu et al., 2007).

Zinc Pyrithione treated textiles

Zinc pyrithione (ZP) is known as an antibacterial and antifungal agent (chemical structure shown in Figure 2.7). The wide use of zinc pyrithione is as an antidandruff agent applied in hair care products (Arch, 2008). It also has been used in building products, antifouling paints, some personal care products, as well as biocide in textiles (Arch, 2008).

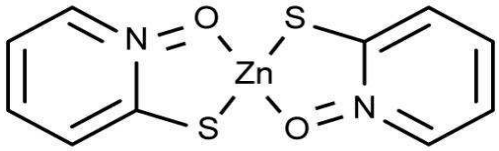


Figure 2.7 Chemical structure of ZP (About.com.Chemistry,2012)

Studies evaluating textiles which have been treated with a ZP antimicrobial agent are much less common than textiles treated with PHMB antimicrobial agents. Morris and Welch (1983) invented a new method to incorporate zinc pyrithione in textiles by adding urea into the ZP solution prepared for the antimicrobial treatment. Their test results showed fabrics treated with the method had better antibacterial durability, which could effectively inhibit the growth of *Staphylococcus aureus* even after fifty launderings, although its antifungal activity had poor durability against laundering. However, after five launderings the presence of ZP was no longer present on the fabric suggesting that ZP is not very durable to laundering.

Walter, McQueen & Keelan (2012) carried out a study recently to compare the antimicrobial effectiveness of fabrics treated with different antimicrobial agents, and they found that fabrics treated with ZP possessed better inhibitory activity against *S. aureus* and *K. pneumoniae* as well as the skin bacterial populations *in vivo* compared with fabrics treated with triclosan or silver chloride-titanium dioxide (Walter et al., 2012).

CHAPTER 3 METHODS

The purpose of this study was to develop a protocol to collect axillary odour on fabrics and assess the odour on fabrics using sensory measurements. This protocol includes: the screening and selection of body odour providers and assessors, methods for collecting body odour on fabrics generated from the axillary region, and specific test procedures for measuring odour intensity emanating from fabrics. Fabrics which differ in fibre content (polyester and cotton) and two types of antimicrobial treatments (zinc pyrithione and polyhexamethylene biguanide) were compared with fabrics without treatments.

Experimental fabrics

Fabric types and treatment

Fabrics used in this study were sourced from Testfabrics Inc, (West Pittston, PA, USA) and antimicrobial treatments were provided by Thomson Research Associates (TRA) (Toronto, Ontario, Canada). Preparation of antimicrobial treatments to fabrics was carried out by TRA. Fabrics used in this study were cotton (Style #460 bleached cotton interlock knit, 187 g/m^2) and polyester (Style #720 texturized Dacron 56T double knit jersey, 200 g/m^2) knit fabrics. The antimicrobial products were Ultra-Fresh GH-20 (20% polyhexamethylene biguanide [PHMB]) and Ultra-Fresh KW-48 (48% zinc pyrithione [ZP]). Each antimicrobial treatment was applied to each fabric and each untreated fabric acted as the controls, resulting in six fabrics in total. A description and codes of the experimental fabrics are listed in Table 3.1.

Each treated fabric was evaluated for antimicrobial activity against *Staphylococcus aureus* (*S. aureus*) using the ISO 20743 (International Organization for Standardization, 2007). The antimicrobial activity of each fabric was shown in Table 3.1.

Each fabric sample was conditioned before testing all physical properties (i.e., fabric mass and thickness) according to CAN/CGSB-4.2 No.2-M88 (Canadian General

Standards Board, 2001) at a temperature of 20°C±2°C and a relative humidity of 65% ±2% for at least 24 hours. No fabric specimens contained the same courses or wales.

Table 3.1 Fabric types and treatment

Fabric code	Fibre content	Antimicrobial treatment	Fabric structure	*S. aureus* Reduction (%)	Mass per unit area (g/m^2)	Thickness (mm)
C-N	Cotton	None	Interlock knit	-	203.7	0.38
C-PHMB	Cotton	PHMB	Interlock knit	>99.9	218.2	0.49
C-ZP	Cotton	ZP	Interlock knit	>99.9	217.0	0.48
P-N	Polyester	None	Double knit jersey	-	174.3	0.38
P-PHMB	Polyester	PHMB	Double knit jersey	>99.9	166.7	0.38
P-ZP	Polyester	ZP	Double knit jersey	>99.9	168.3	0.37

Measurement of physical properties

Standard test methods were used to characterize the fabric properties, such as mass per unit area and thickness. Tests of unit mass of fabrics was taken under CAN/CGSB- 4.2 No.5.1-M90 (Canadian General Standards Board [CGSB], 1990), and thickness under CAN/ CGSB 4.2 NO. 37-M 87 (CGSB, 1987).

Ethical requirement

Two parts of the study involved humans as participants: 1) axillary odour providers who wore T-shirts with test fabric specimens during normal daily routine and exercise, and had their underarm scrubbed; 2) assessors for evaluating odour intensity. Ethical approval at the University of Alberta's Research Ethics Board 3 (REB 3) was sought and obtained.

Collection of axillary odour on textile fabrics

Two methods for collecting axillary odour onto fabrics used in this study were carried out. Three participants and 11 assessors were recruited in the study and they were the same for both methods.

Wear trial collection of odour in vivo

The first collection method was through a wear trial which involved participants wearing a T-shirt with fabric swatches sewn into the underarm region. In the wear trial, each participant was given a 100% cotton T-shirt with test fabric swatches and a matched control swatch stitched into either the left or right underarm region. The placement of test or control fabric swatches were assigned first to the left or right side of the T-shirt and for the duplicate study the fabrics were then swapped for left and right sides (see Table 3.2). The participants wore the T-shirt in the morning and followed their daily routine during the day, as well as carrying out at least 30 min exercise session (e.g. brisk walk on the treadmill) in the evening before returning the T-shirts. The participants wore the test T-shirts for about 8 h in total each test day. The test fabrics were removed from the T-shirts and cut into small fabric specimens (30 mm x 30 mm) as per the sampling plan (see Figure 3.1). This process of collecting odour on fabrics and preparing the fabrics was similar to that carried out by McQueen and colleagues (McQueen et al., 2007a).

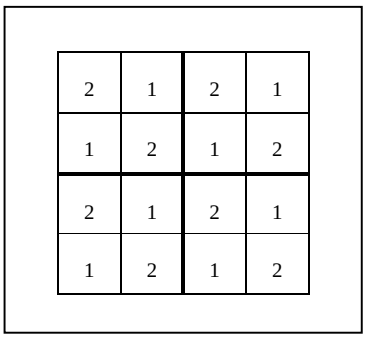


Figure 3.1 Sampling plan

Specimens were grouped by the number on the grid of the sampling plan and specimens labelled the same number were placed into the same glass bottles (60 ml) before being evaluated by the sensory panel.

Incubation method collection of odor in vitro

The second collection method involved incubating test fabric samples with fresh axillary sweat solution. The method for collecting sweat and bacteria from the axilla was similar to that for collecting axillary microflora (Jackman & Noble, 1983; Taylor et al., 2003). Before scrubbing of the underarm took place, participants carried out at least 30 min of physical activity to facilitate sweating in the axillary region. Participants then lay down on a table with their hands behind their heads, a Teflon cylinder (4.9 cm^2) was held firmly against the center area of the axilla, and 2 ml of phosphate buffer solution (PBS) with 0.05% Tween80 was added to the cylinder. The skin was then scrubbed with a skin cell scraper for 1 min and the solution was removed into a 40 ml sterile glass beaker. The cylinder was shifted to cover a region of the underarm which had not previously been scrubbed so that the procedure was repeated twice for each arm. The sweat/PBS solution for the two underarms was pooled (to make 8 ml solution in total). Following collection 1.2 ml of solution was pipetted onto the prepared fabrics (4 layers of 30 mm x 30 mm specimens). The inoculated fabric specimens were then incubated for three days at 37°C before sensory measurement and microbiological analysis was carried out.

In preliminary testing, other solutions were tried for inoculation onto fabrics, i.e., the artificial sweat used to test colour fastness to perspiration (International Organization for Standardization, 2008), but no odour was generated even following five days of incubation as well as no bacterial growth was found on untreated fabrics.

Selection and screening of participants

It was important that participants who were involved in providing odour had sufficiently high axillary odour so that it could be detectable on fabrics following wear. Therefore it was necessary to screen participants for axillary odour prior to final selection. The screening procedure for participants was as follows: each candidate was asked to

wear a 100% cotton T-shirt which had polyester fabric swatches (20 cm x 20 cm) stitched into the underarm area for a one hour period during exercise. The candidate participants were asked to ensure that the exercise they did involved moderate to strenuous exercise to make sure they 'sweated' in the T-shirts during this one-hour period (e.g., running, lifting weights). Following the hour of exercise, the worn T-shirts were line dried before the polyester fabric swatches were removed from the T-shirts and each swatch was placed into a separate glass jar (i.e., fabrics worn in the right and left axilla were in separate glass jars). Polyester was chosen for the fabric swatch because polyester has been found to have higher odour intensity following wear than fabrics made from other fibre types such as cotton (McQueen et al., 2007a; Munk et al., 2000).

Selection of participants was determined by three expert assessors who evaluated odour intensity of each fabric and right-left odour imbalance. The three experts assessed the odour intensity of the fabric swatches worn in the right and left axillae of each candidate on a 0- (non odorous) to10-point (extremely odorous) category scale, in which a score above 7.0 was considered high odour and that below 3.0 considered low odour. A difference over 20% (i.e., 2.0 points on the scale) between the scores obtained from the right and left axilla meant a large right-left axillae odour imbalance (American Society for Testing and Materials, 2009). Thus candidates whose odour scores were around 5.0, with their right-left axillae odour imbalance within 20% were selected in the study.

Assessor selection, screening and training

The process of assessor selection is to screen for their olfactory sensitivity and acuity, which is an important part of sensory evaluation of axillary odour. This is because a portion of the population can be anosmic to some odorous compounds found in axillary odour. For example, as high as 50% of individuals could be anosmic to 5α-androst-16-en-3-one, which is one of the compounds responsible for the human axillary malodour (Amoore, 1977; Lundström et al., 2003).

Assessor recruitment

Assessors were recruited from the University of Alberta campus. Candidates answered a brief questionnaire (see Appendix A) concerning demographic questions, general health status and smoking habits. Other information to confirm their availability and willingness to participate in the sensory panel were also included in the questionnaire.

The purpose of the process was to attract as many candidates as possible for the initial screening. Potential assessors were selected based on their feedback of the questionnaire, such as interest, availability etc., as well as their olfactory sensitivity. According to the ISO standard (International Organization for Standardization, 1993), the number of persons recruited should be at least two to three times the number required in the final test, which should be no less than 10 selected assessors. Thus, about 20-30 persons were expected in the recruitment. In total 28 persons participated in the screening process and 11 were selected and trained to make up the final sensory panel.

Screening

Candidate assessors were screened for their olfactory sensitivity and acuity by using two odorous compounds (i.e., isovaleric acid [IVA] and androstenone) (American Society for Testing and Materials, 2009). The screening test in this study was conducted in two phases. The phase I test involved a two alternative forced choice threshold test for IVA and the phase II involved a triangle test for anosmia to androstenone. Detailed information about the screening protocol is given in Appendix B.

Training

Assessors selected as being suitable candidates for the final sensory panel (e.g., having good odour acuity) were then trained. The training session included three parts: i) assessor orientation, ii) introduction of odour evaluation and iii) how to scale odour intensity. The training notice is given in Appendix C.

Orientation. A brief orientation was held for the assessors to help them understand the purpose and importance of the study as well as to allow them to introduce themselves to each other.

Introduction of odour evaluation. In the introduction part, the same types of samples which would be used in the final test were presented and a demonstration was given step by step informing the selected assessors how to carry out the test. The precautions in which to follow on the test day (i.e., no smoking two hours before the test, not use perfume etc.) were also given.

Scaling of odour intensity. The assessors were trained how on to use a line scale which would be used to record odour intensity in the final test (Figure 3.2). Assessors were asked to rate odour intensity by marking on the line with a vertical line. The selected assessors were presented with two reference samples at the beginning of the session. The reference samples comprised of fabrics which had been inoculated with varying concentrations of IVA solution. One reference sample represented low odour intensity (R_L) with IVA concentration of 0.22 ml/l; and the second reference sample represented high odour intensity (R_H) with IVA concentration at 0.89 ml/l. When assessors measured the samples with a lower intensity than R_L, they were asked to mark the line in the "Low" area, and if they assessed samples with a higher intensity than R_H, they were asked to mark located in the "High" area of the scale. If they assessed samples which were higher than R_L and lower than R_H they were to mark the line scale in the "Middle" area.

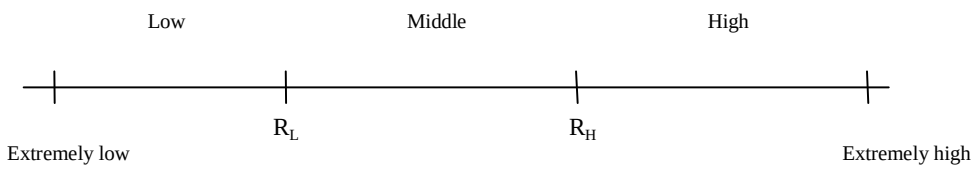


Figure 3.2 Line scale using reference samples to train assessors

The training sessions were carried out over two different days with 30-40 min each time. On the first day, orientation, introduction of the study and practice using the line scale with figures and reference samples was involved, and on the second day the training session involved assessors being presented with 'real' fabric samples which had been worn by a selected participant. Results provided at both times were recorded for testing their consistency over time.

Sensory measurement of odour intensity

Test samples either collected via the wear trial (*in vivo*) or incubation method (*in vitro*) followed the same procedure of sensory measurement. Specimens of the same fabric type obtained from each participant were placed in sterile Petri dishes separately and left overnight in the conditioning room at 20°C and 65% R.H. for at least 8 h before each test day. In the morning of the test day, fabrics in each Petri dish were transferred to sterile 60 ml amber glass bottles with screw lids. Disposable and odour-free gloves were used as well as the sterile tweezers (flame sterilized with alcohol) between each sample to decrease the chance of cross contamination. Control test samples (i.e., non-worn cotton and non-worn polyester for the *in vivo* method and cotton and polyester inoculated with PBS solution only for *in vitro* method) were also placed into the test bottles.

The sensory tests were carried out in the standard sensory test room (International Organization for Standardization, 1998) (Figure 3.3) by the selected panel of 11 assessors. All the test bottles were placed in a water bath at 37±2°C to simulate the human body temperature. All the tested fabrics were randomly assigned a three digit number and 7 samples presented (control sample included and always presented the first) as a group to each assessor with a different order following the 6-treatment designs described by Macfie and Bratchell (1989) to avoid order effects.

Figure 3.3 Sensory test set-up in the standard room

Each assessor was asked to take 2-3 short sniffs over samples in the given order with their mouth closed, and then mark on the 150 mm line scale (Figure 3.4) with a vertical line indicating the intensity of the odour. Thirty seconds between each sample was taken and the assessors were to refresh their noses by sniffing the glass of clean water. Re-sniffing samples was not allowed.

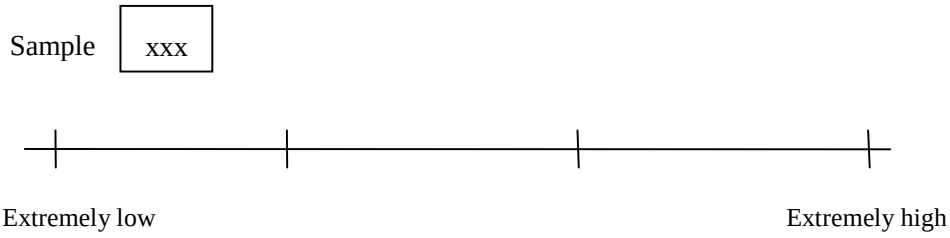


Figure 3.4 Line scale used in training and for final sensory assessment

Test data from one of the 11 assessors was removed from the final data, as this individual's results were found to be inconsistent with the panel mean. So, the panel mean and statistical analysis of the data was carried out based on the results of ten assessors (n=10).

Microbiological measurement for bacterial growth

Bacterial populations were extracted from the test fabrics and colony forming units (CFU) were counted. The procedure for extracting from the test fabrics was similar to that described by McQueen et al. (2007a). After each sensory test, each sample was placed in 50 ml Corning tubes with sterile glass beads, after which 20 ml of PBS solution amended with 0.05% Tween 80 was added. The tubes were vortexed for one minute. Ten-fold dilutions were made in PBS-Tween 80 solution and 20 µl of each dilution were placed in triplicate onto the culture media of a non-selective blood agar media (19.75g blood agar base No.2, 1.5g yeast extract, 1g glucose, 2.5ml Tween 80 and 25ml defibrinated horse blood for making 500ml agar). The agar plates were incubated at 37°C for 48 h. After incubation the bacteria were counted for each plate and CFU/ml were calculated based on the following formula:

CFU/ml = mean (bacteria number) x 1/dilution x 1000/20

Research design

Two fibre types (cotton, polyester), two treatments for the fabric (PHMB, zinc pyrithione) and two different odour collection methods (T-shirt wear trial, fabric incubation) were variables under investigation, which resulted in eight variables in total.

The research design of the wear trial

Six different fabrics were evaluated in this study with five comparisons made (i.e., C-N vs. P-N, C-N vs. C-PHMB, C-N vs. C-ZP, P-N vs. P-PHMB and P-N vs. P-ZP). Fabrics for each comparison were worn by each participant in each underarm (i.e., for the C-N vs. P-N fabric pair C-N was worn in right underarm once and P-N in the left; then for the duplicate test C-N was worn in the left underarm and P-N in the right). Thus each fabric pair was compared twice for each participant. The fabric assignment to each underarm for each participant is shown in Table 3.2.

Table 3.2 Test fabric assignment design for wear trial

	Participant	Trial No.				
		1	2	3	4	5
Replicate 1	1(A)	C-N/P-N	C-N/C-PHMB	C-N/C-ZP	P-N/P-PHMB	P-N/P-ZP
	2(B)	C-N/P-N	C-N/C-PHMB	C-N/C-ZP	P-N/P-PHMB	P-N/P-ZP
	3(C)	C-N/P-N	C-N/C-PHMB	C-N/C-ZP	P-N/P-PHMB	P-N/P-ZP
Replicate 2	1(A)	P-N/C-N	P-ZP/P-N	P-PHMB/P-N	C-PHMB/C-N	C-ZP/C-N
	2(B)	P-N/C-N	P-ZP/P-N	P-PHMB/P-N	C-PHMB/C-N	C-ZP/C-N
	3(C)	P-N/C-N	P-ZP/P-N	P-PHMB/P-N	C-PHMB/C-N	C-ZP/C-N

Note: samples posted on the left or right site of the bar (/) indicate which side of the T-shirt the test fabric were sewn (e.g., C-N/P-N means C-N on the left side and P-N on the right)

Considering the potential existence of imbalance of two arms for the same person, fabrics were reversed under the arms for each participant for each replicate. To reduce the impact of antimicrobial treatments influencing skin microflora, at least one day was taken off between the wear trials following exposure to an antimicrobial treated fabric (see Table 3.3). Since there was no need to have any "rest time" following the untreated C-N and P-N fabrics, the C-N/P-N combination was always the first fabric pair in each replicate, and an antimicrobial treated fabric was worn the next day.

Schedule for collecting and assessing in vitro and in vivo methods of collection

The total experimental procedures were carried out over a six-week period. The schedule for when the wear trial and scrub methods of odour collection were evaluated is indicated in Table 3.3. For the wear trial, all three participants wore the same fabric pairs (e.g. C-N/P-N) at the same time. This resulted in six test samples (and one control sample) for sensory measurement. For the incubation method, one or two participants would be scrubbed on the same test day, which resulted in six or 12 test samples (and one or two control samples) on an assessment day. For the incubation method an incubation time of three days was required, so the test samples were assessed three days following the scrubbing procedure.

Table 3.3 General schedule of test procedure

	Monday	Tuesday	Wednesday	Thursday	Friday
Week 1	C-N/P-N	C-N/C-PHMB S(6) [C-N/P-N]	S(6) [C-N/ C-PHMB]	C-N/C-ZP	S(6) [C-N/C-ZP]
Week 2	Scrub 'P1'	P-N/P-PHMB	S (6) [P-N/ P-PHMB]	P-N/P-ZP	S (6+6) ['P1']+[P-N/P-ZP]
Week 3	P-N/C-N	P-ZP/P-N S (6) [P-N/C-N]	S(6) [P-ZP/P-N]	P-PHMB/ P-N	S(6) [P-PHMB/P-N]
Week 4	Scrub 'P2'	C-PHMB/C-N	S(6)[C-PHMB/ C-N]	C-ZP/C-N	S(6+6) ['P2']+[C-ZP/C-N]
Week 5	Scrub 'P1+P3'				Scrub 'P2+P3' S(12)['P1']+['P3']
Week 6		S(12)['P2']+['P3']			

Note: S stands for sensory measurement and the number means number of test samples (e.g., S (6) = six test samples for sensory measurement, control fabrics was prepared elsewhere); P1, P2 and P3= Participant1, Participant 2 and Participant 3.

Statistical analysis

Descriptive statistics were calculated (i.e. mean, standard deviation etc.), and CFU/ml were $\log_{10}$ transformed before data analysis. Panel mean scores were calculated for statistical analysis. For the wear trial method paired t-tests were carried out for the data of each matched pair collected. For the incubation method a multiple analysis of variance (ANOVA) was performed on the data with factors of fibre, treatment, and participant. Tukey's HSD tests were used to identify the specific differences using IBM SPSS Statistics version 19 (SPSS Inc, Chicago, 2010).

CHAPTER 4 RESULTS

In vivo wear trial method

Odour intensity on fabrics

Descriptive data of odour intensity collected using the wear trial method for fabrics worn by three participants are shown in Table D.1 (Appendix D). Mean (±SEM) odour intensity values for the matched pairs for each fabric and treatment type, for all three participants are shown in Figure 4.1. Of the worn fabrics odour intensity ranged from 28.5±3.6 for cotton fabric treated with PHMB worn by Participant 3 to 118.4±5.2 for non-treated cotton fabric worn by Participant 1 (Figure 4.2, Table D.1). Although odour perceived from control fabrics (i.e., unworn cotton or polyester fabrics) were consistently rated as lower than the lowest worn fabric (i.e., <23.3) (Table D.1).

Effect of fibre content and fabric treatment on odour intensity

Figure 4.1a shows the matched pairs for each participant and each replicate for untreated cotton and untreated polyester fabrics. Paired t-tests were carried out to determine significance of fibre type (untreated cotton versus untreated polyester) and effectiveness of antimicrobial treatment (i.e., an untreated fabric versus the matched antimicrobial treated fabric). Overall means are shown in Table 4.1 and t-test statistics are shown in Table 4.2. The overall mean results for untreated cotton fabrics compared to untreated polyester fabrics worn in the opposite underarm are 64.97±16.91 and 69.94 ±15.03 respectively. This was not significant (t_5=-0.660, p=0.539). However, the odour intensity between cotton and polyester for each individual participant varied and it appears that for Participant 3 cotton fabrics were perceived to be lower in odour intensity than polyester fabrics as for both replicates the polyester fabric was rated higher (Figure 4.1a).

For the PHMB antimicrobial treated fabrics differences in odour intensity were overall rated to be non-significant. For cotton fabrics treated with PHMB (M=45.78±7.01) compared with the matched untreated cotton fabrics (M=50.93±8.03) means were not significantly different (t_5=0.839, p=0.440). A similar overall result was apparent for

polyester fabrics treated with PHMB, as the odour intensity of the treated fabric (M=63.30±17.04) was lower (but not significant) than the matched untreated polyester fabric (M=67.85±14.02) (t_5=0.783, p=0.469). For Participant 3, however, the PHMB did noticeably reduce odour intensity in the polyester fabrics only, whereas for Participant 1 the PHMB did reduce odour intensity in the cotton fabrics (Figure 4.1b and 4.1d).

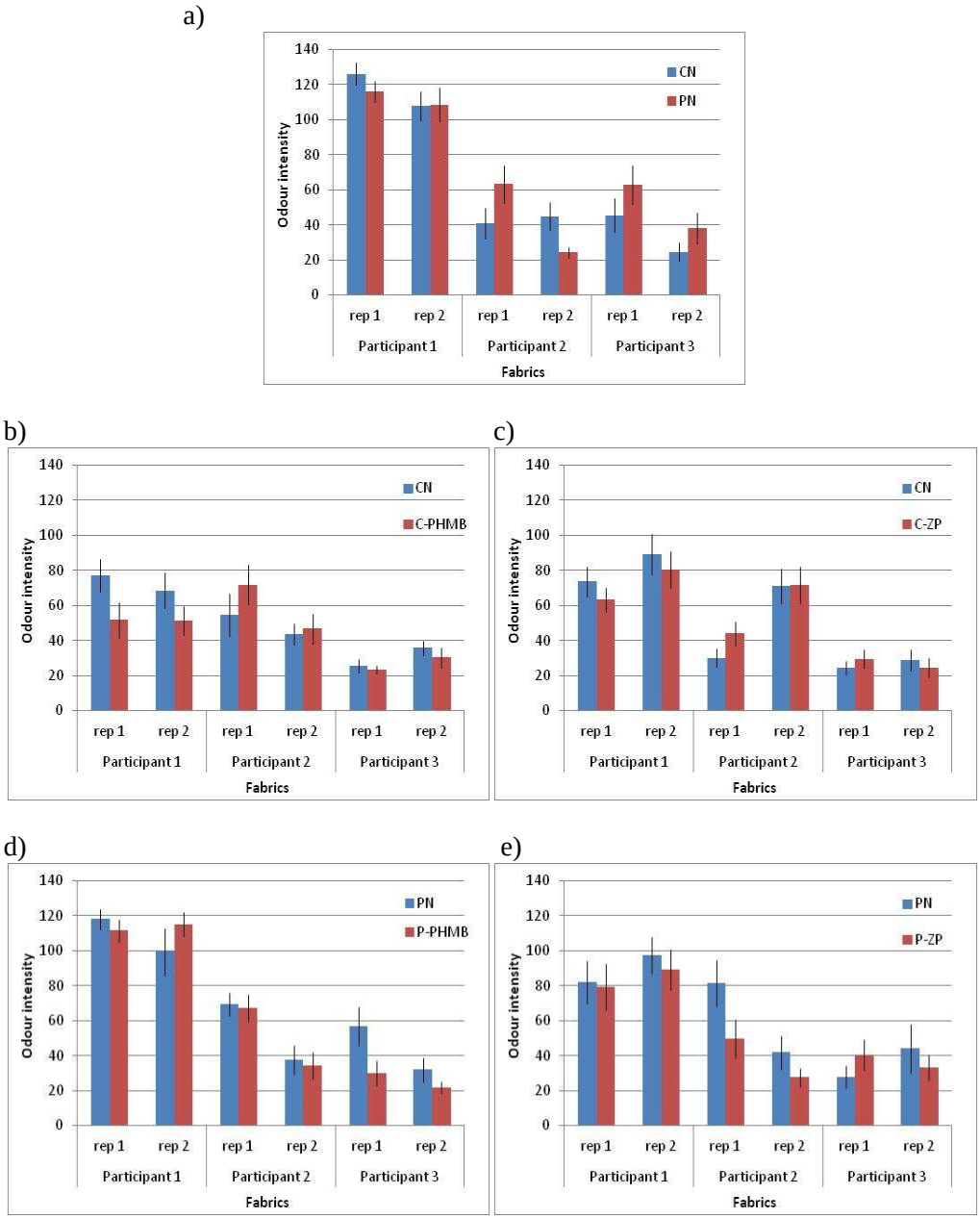


Figure 4.1 Mean (± SEM) ratings of odour intensity of different wear trial fabrics a) CN/PN; b) CN/C-PHMB; c) CN/C-ZP; d) PN/P-PHMB; and e) PN/P-ZP

The ZP antimicrobial treatment did not have a significant effect on odour intensity either as odour intensity for cotton treated with ZP (M=52.20±9.41) was not significantly lower than the matched untreated cotton (M=52.88±11.53) (t_5=0.181, p=0.863). For polyester fabrics treated with ZP (M=53.16±10.34) compared with the matched untreated polyester fabrics (M=62.45±11.44) mean odour ratings were not significantly different (t_5=1.566, p=0.178).

Table 4.1 Overall mean of odour intensity for matched pairs of wear trial fabrics

Matched pairs		Mean	N	SD	SEM
Pair 1	CN	64.97	6	41.41	16.91
	PN	69.94	6	36.81	15.03
Pair 2	CN	50.93	6	19.68	8.03
	C-PHMB	45.78	6	17.30	7.06
Pair 3	CN	52.88	6	28.24	11.53
	C-ZP	52.20	6	23.05	9.41
Pair 4	PN	67.85	6	34.34	14.02
	P-PHMB	63.30	6	41.75	17.04
Pair 5	PN	62.45	6	28.02	11.44
	P-ZP	53.16	6	25.32	10.34

Table 4.2 t-test statistics of odour intensity for the paired samples of wear trial fabrics

Matched pairs		Paired differences			t	df	p
		Mean	SD	SEM			
Pair 1	CN- PN	-4.963	18.429	7.523	-0.660	5	0.539
Pair 2	CN - C-PHMB	5.152	15.046	6.143	0.839	5	0.440
Pair 3	CN- C-ZP	0.679	9.168	3.743	0.181	5	0.863
Pair 4	PN - P-PHMB	4.544	14.208	5.801	0.783	5	0.469
Pair 5	PN - P-ZP	9.289	14.534	5.933	1.566	5	0.178

Effect of participant on odour intensity

The odour intensity rating for both replicates for fabric types for each individual participant is shown in Figure 4.1a-e. The participant who wore the fabric had the greatest effect on overall odour intensity ($F_{2,59}$=54.844, p≤0.001). Test results were highly

variable for each individual participant with respect to fibre type and/or fabric treatment, i.e., the ZP antimicrobial treatment reduced odour intensity in both cotton and polyester fabrics for Participant 1 for both replicates, while for Participant 2 and 3 the results were variable for fibre types and replicates. Fabrics worn in the underarm region of Participant 1 were overall more odorous than those worn by either Participant 2 or Participant 3, who was the only female in the study.

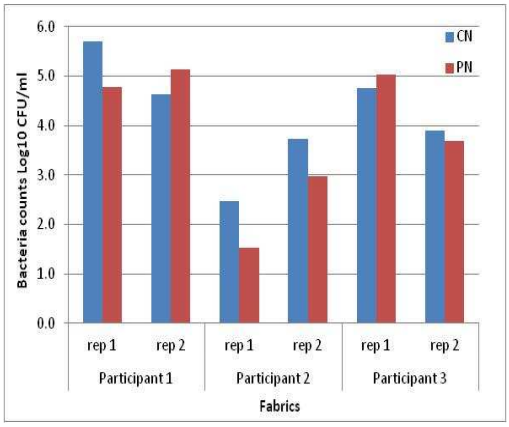
Bacterial counts on fabrics worn in the wear trial

Bacterial counts obtained from the fabrics worn by three participants are shown in Appendix D, Table D.2. Overall bacterial counts for matched pairs for each fabric and treatment are shown in Figure 4.2a-e. Bacterial counts were log transformed and the overall means are shown in Table 4.3 for each of the matched pairs. Fabrics with antimicrobial treatments had lower bacterial counts than fabrics without any treatment for both cotton and polyester. Of the two antimicrobial treatments, fabrics treated with PHMB tended to have lower bacterial counts than those treated with ZP. The highest bacterial count was from a non-treated cotton fabric worn by Participant 3 ($9.70x10^5$ CFU/ml), while the PHMB treated cotton and polyester fabrics had the lowest bacterial counts as bacterial growth was below the minimum detectable level of <16.7 CFU/ml.

Effect of fibre content and fabric treatment on bacterial counts

Paired t-tests were carried out to determine significance of fibre type (Table 4.4) and effectiveness of antimicrobial treatment for bacterial counts as carried out for odour intensity. The overall mean results for untreated cotton fabrics (M=4.20 Log CFU/ml) and untreated polyester fabrics (M=3.86 Log CFU/ml) worn in the opposite underarm were not significantly different (t_5=1.317, p=0.245). That meant the fibre type did not have a significant effect on the bacterial counts. Overall, the fabric that had the highest bacterial counts was from the first replicate of the untreated cotton fabric worn by Participant 3 (9.70×10^5 CFU/ml), followed by the same fabric worn by Participant 1 (5.07×10^5 CFU/ml) (rep 1). Fabrics from Participant 2 had the lowest bacterial populations compared with other two participants.

a)



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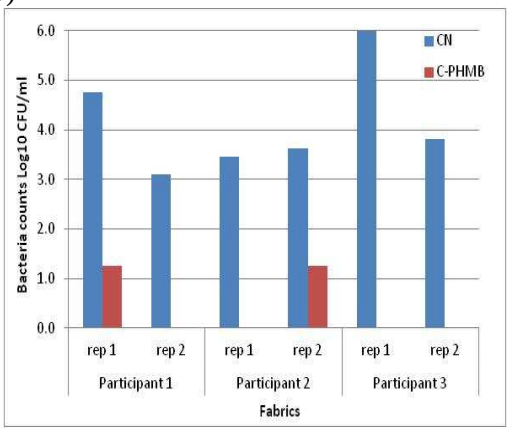

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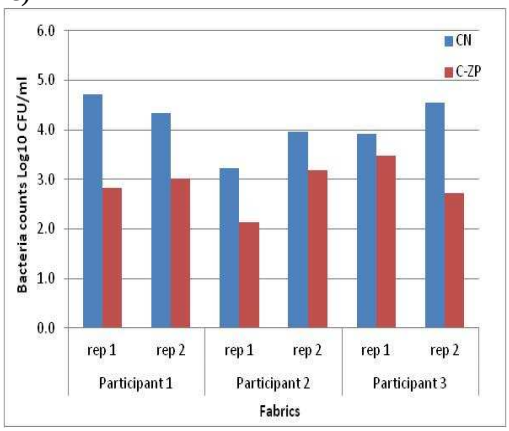

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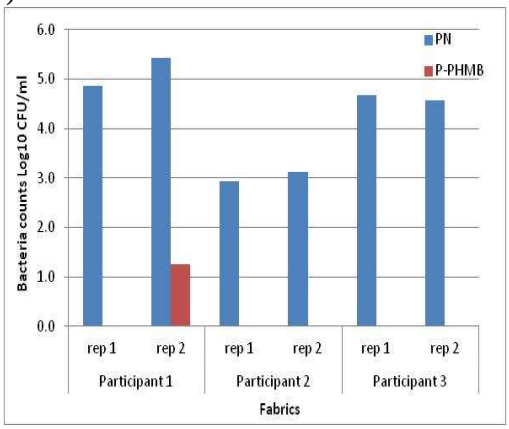


e)

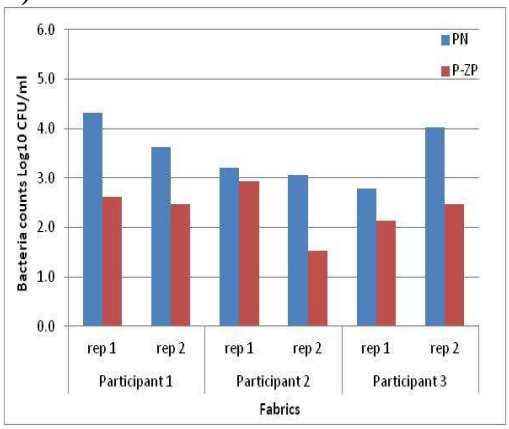


Figure 4.2 Overall bacterial counts for matched pairs of wear trial fabrics (Log10 CFU/ml) a) CN/PN; b) CN/C-PHMB; c) CN/C-ZP; d) PN/P-PHMB; and e) PN/P-ZP

Antimicrobial treatment had a significant effect on reducing bacterial counts as fabrics treated either with PHMB or ZP regardless of fibre types all had significantly lower bacterial counts than the matched untreated fabrics (Table 4.4). Meanwhile, fabrics treated with PHMB even had lower bacterial populations compared with the ZP treated fabrics.

Table 4.3 Summary of bacterial counts (Log_{10} CFU/ml) for matched pairs of wear trial fabrics

Matched pairs		Mean	N	SD	SEM
Pair 1	CN	4.20	6	1.42	0.58
	PN	3.86	6	1.01	0.58
Pair 2	CN	4.12	6	0.54	0.22
	C-ZP	2.89	6	0.46	0.19
Pair 3	CN	4.13	6	1.10	0.45
	C-PHMB	0.42	6	1.07	0.44
Pair 4	PN	4.27	6	0.65	0.26
	P-PHMB	0.21	6	0.51	0.21
Pair 5	PN	3.51	6	0.59	0.24
	P-ZP	2.36	6	0.48	0.20

Table 4.4 t-test statistics of bacterial counts for the paired samples of wear trial fabrics

Matched pairs		Paired differences			t	df	p
		Mean	SD	SEM			
Pair 1	CN- PN	0.340	0.632	0.258	1.317	5	0.245
Pair 2	CN - C-PHMB	3.708	1.221	0.498	7.441	5	0.001
Pair 3	CN- C-ZP	1.230	0.565	0.231	5.328	5	0.003
Pair 4	PN - P-PHMB	4.060	0.831	0.339	11.967	5	0.000
Pair 5	PN - P-ZP	1.142	0.564	0.230	4.962	5	0.004

Effect of participant on bacterial counts

The bacterial counts obtained from fabrics of both replicates for each individual participant are shown in Figure 4.2 a-e. The effect of participants who wore the fabric was significant on overall bacterial counts ($F_{2,59}$=2.047, $p \leq 0.05$), while it was not as

dominant as the treatment effect. Bacterial counts on fabrics worn by Participant 2 were significantly lower than counts on fabrics worn by Participant 1 or Participant 3, who were not significantly different from each other.

In vitro incubation method

Odour intensity on incubated fabrics

Descriptive statistics for odour intensity ratings for fabrics incubated with fresh sweat collected from three participants are given in Table D.3 (Appendix D). Mean (±SEM) odour intensity values for each fabric and treatment type for all three participants combined are shown in Figure 4.3. Excluding the non-worn control fabrics (which were rated the lowest in odour intensity), odour intensity ranged from 30.9±3.6 for cotton fabric treated with PHMB incubated with fresh sweat from Participant 1 to 83.8±6.9 for polyester fabric treated with ZP incubated with sweat from Participant 2 (Figure 4.4). The overall results showed that fabrics made of cotton, regardless of whether they had an antimicrobial treatment or not, had lower odour intensity than those made of polyester. Also, the same trend among treatments, for each fibre type, was perceived with fabrics treated with ZP having the highest odour intensity, fabrics treated with PHMB the lowest and the non-treated fabrics in the middle.

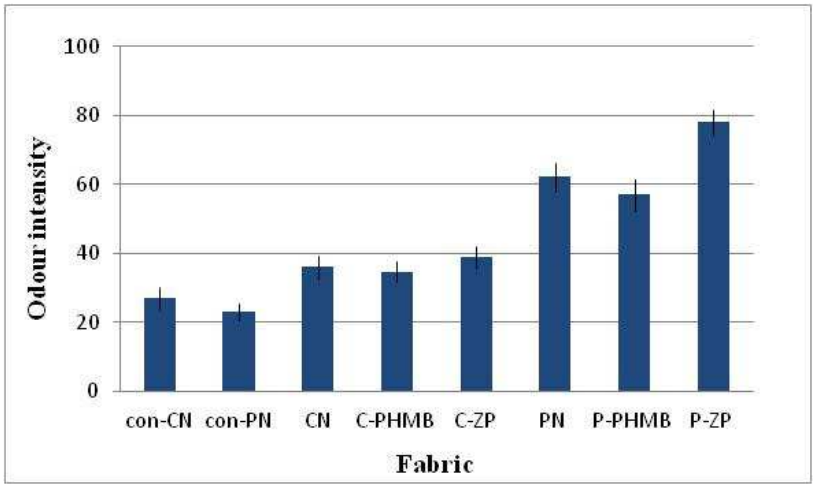


Figure 4.3 Mean (± SEM) ratings of overall odour intensity for incubated fabrics

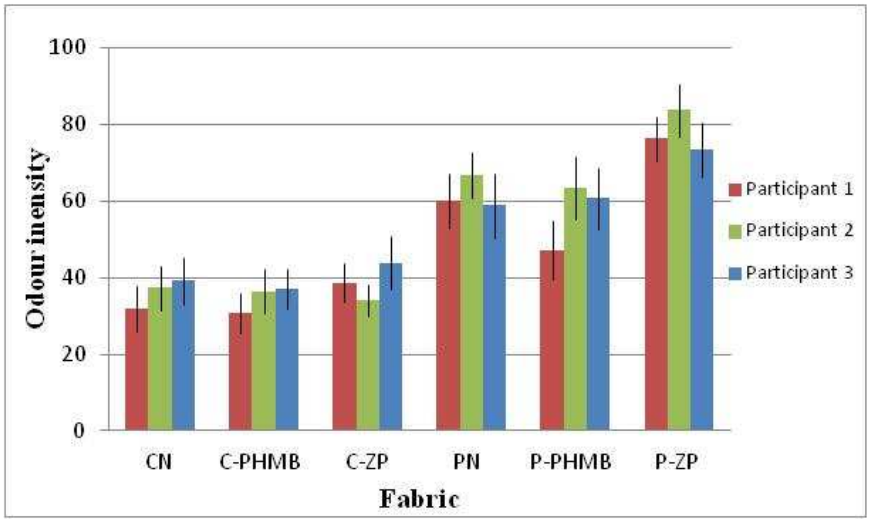


Figure 4.4 Mean (±SEM) ratings of odour intensity for different incubated fabrics by each participant

Effect of fibre content and fabric treatment on odour intensity

Significance of variables and interactions for odour intensity collected on fabrics by the incubation method is shown in Table 4.5. Fibre content from which the fabric was made had a significant effect on perceived odour intensity ($F_{1,18}$=132.609, p≤0.001) as cotton fabrics were rated much lower in odour intensity (M=31.63) compared to polyester (M=60.80). Overall, the fabric perceived the most intense in odour was polyester fabric treated with ZP (73.67±3.76), this was closely followed by the non-treated polyester fabric (57.70 ±4.06). The fabric which was perceived overall as the lowest in odour intensity was cotton fabric treated with PHMB (28.97±3.06). Differences were also apparent in odour intensity due to the type of treatment ($F_{2,18}$=11.907, p≤0.001), as fabrics treated with PHMB and the untreated fabrics were perceived to be significantly lower than fabrics treated with ZP (Table 4.6).

Table 4.5 Significance of variables affecting odour intensity on incubated fabrics ANOVA

Source	df	SS	MS	F	Sig.	p≤
Fibre	1	7661.112	7661.112	132.609	0.000	0.001
Treatment	2	1375.872	687.914	11.907	0.001	0.001
Participant	2	448.069	224.305	3.878	0.040	0.05
Fibre/Treatment	2	395.621	197.810	3.424	0.055	NS
Fibre/Participant	2	173.590	86.795	1.502	0.249	NS
Treatment /Participant	4	195.119	48.780	0.844	0.515	NS
Fibre/Treatment/Participant	4	118.276	29.569	0.512	0.728	NS
Error	18	1039.902	57.772			

Table 4.6 Differences in odour intensity for participant and treatment Tukey's test for significant differences

Source	Mean	n	Tukey's groupings
Participant			
1	41.23	12	⌈
3	48.61	12	|
2	48.80	12	⌊
Fibre			
Cotton	31.63	18	⌈
Polyester	60.80	18	⌊
Treatment			
PHMB	40.01	12	⌈
No treatment	43.99	12	|
ZP	54.65	12	⌊
Fibre/treatment			
Cotton-PHMB	28.97	6	⌈
Cotton-no treatment	30.27	6	|
Cotton-ZP	35.64	6	⌊
Polyester-PHMB	51.05	6	⌈
Polyester-no treatment	57.70	6	|
Polyester-ZP	73.67	6	⌊

Mean Square (Error) = 57.772
Mean grouped by lines are not significantly different at p≤ 0.05

Difference in odour intensity due to participant

The odour intensity rating for fabric types for each individual participant is shown in Figure 4.4. There are also significant differences in odour intensity depending on the participant ($F_{2,18}$=3.878, p≤0.05). Fabrics worn by Participant 1 had a slightly lower overall odour intensity (M=41.23) than either Participants 2 or 3 (M=48.80 & M=48.61 respectively). However, the post-hoc Tukey's HSD tests were not sensitive enough to differentiate among the three participants. For each individual participant, the same general trend with respect to fibre type and/or fabric treatment was apparent as polyester was most often perceived to be higher in odour intensity than cotton fabrics. Fabrics worn by Participant 2 were often rated higher in odour intensity for the polyester fabrics compared with other two participants, while for the cotton fabrics Participant 3 were rated higher than others.

Bacterial counts on fabrics in the incubation method

Bacterial counts obtained from fabrics inoculated with sweat solution and incubated are shown in Table D.4 (Appendix D) for all three participants. Overall bacterial counts for each fabric and treatment type are shown in Figure 4.5 (bacterial growth was typically below the limit of detection on the cotton control fabrics and polyester fabrics treated with PHMB and ZP). Fabrics which had an antimicrobial treatment applied to them had much lower bacterial counts than those without the treatment for both cotton and polyester. The antimicrobial treated polyester fabrics had no bacterial growth on them. The highest bacterial counts were observed on the non-treated polyester fabrics incubated with the fresh sweat collected from Participant 3 ($3.95x10^4$ CFU/ml) in replicate 1 (Table D.4).

Effect of fibre content and fabric treatment on bacterial counts

Significance of variables and interactions for bacterial counts on incubated fabrics is shown in Table 4.7. There was a significant effect due to the fibre content from which the fabrics were made for bacterial counts ($F_{1,18}$=7.370, p≤0.05). Overall results showed that cotton fabrics retained significantly higher bacterial counts (1.61 Log

CFU/ml) compared to the polyester fabrics (1.10 Log CFU/ml). The antimicrobial treatment had an even greater effect on bacterial counts than fibre type ($F_{2,18}$=146.058, p≤0.001), as the non-treated fabrics retained significantly higher bacterial counts than fabrics treated with either PHMB and ZP, which were not significantly different from each other (Table 4.8).

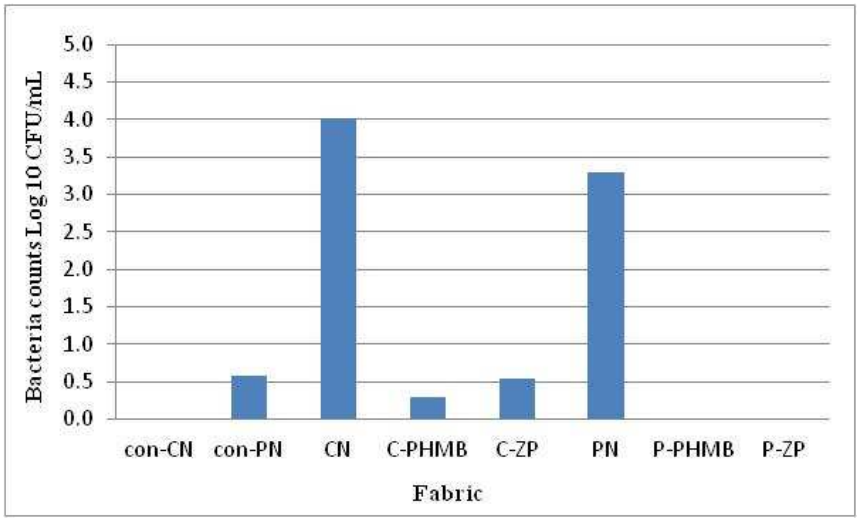


Figure 4.5. Overall bacterial counts for different incubated fabrics

Table 4.7 Significance of variables affecting bacterial counts on incubated fabrics ANOVA

Source	df	SS	MS	F	Sig.	p≤
Fibre	1	2.402	2.402	7.370	0.014	0.05
Treatment	2	95.227	47.613	146.058	0.000	0.001
Participant	2	0.146	0.073	0.224	0.802	NS
Fibre/Treatment	2	0.291	0.146	0.446	0.647	NS
Fibre/Participant	2	0.116	0.058	0.178	0.178	NS
Treatment/Participant	4	1.163	0.291	0.892	0.489	NS
Fibre/Treatment/Participant	4	1.177	0.294	0.902	0.483	NS
Error	18	5.868	0.326			

Table 4.8 Differences in bacterial counts for participant and treatment Tukey's test for significant differences

Source	Mean	n	Tukey's groupings
Participant			
2	1.29	12	]
1	1.33	12	]
3	1.44	12	]
Fibre			
Polyester	1.10	18	]
Cotton	1.61	18	]
Treatment			
PHMB	0.14	12	]
ZP	0.27	12	]
No treatment	3.65	12	]
Fibre/treatment			
Polyester-PHMB	0.00	6	]
Polyester-ZP	0.00	6	]
Cotton-PHMB	0.29	6	]
Cotton-ZP	0.54	6	]
Polyester-no treatment	3.29	6	]
Cotton-no treatment	4.02	6	]

Mean Square (Error) = 0.326
Mean grouped by lines are not significantly different at $p \leq 0.05$

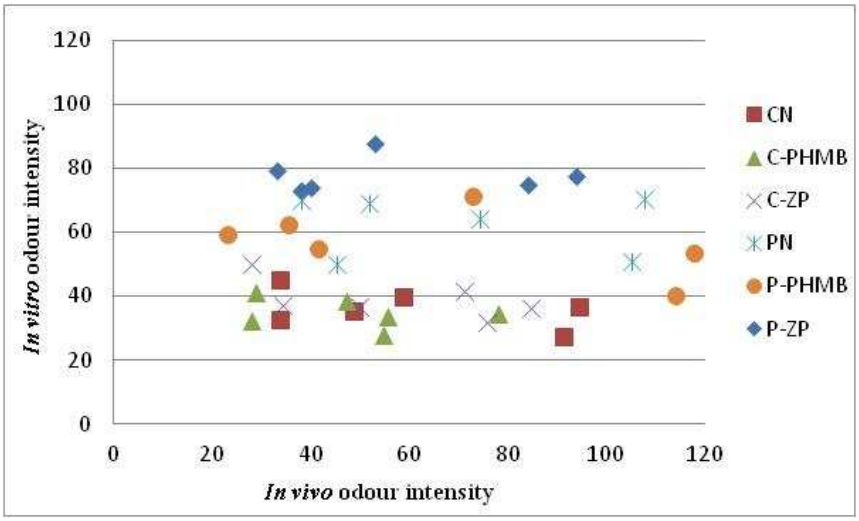
Effect of participant on bacterial counts

For the incubation method there was no effect on bacterial counts due to the participant from whom the fresh sweat came from ($F_{2,18}$=0.224, p=0.802), as the overall mean bacterial counts ranged from 1.29 CFU/ml for Participant 2 to 1.44 CFU/ml for Participant 3.

Relationship of odour intensity between the *in vivo* and *in vitro* collection methods

Figure 4.6 shows the mean odour intensity ratings from the wear trial fabrics (*in vivo*) plotted against the mean odour intensity from the incubated fabrics (*in vitro*) for all participants, depicted by fabric types (Figure 4.6a) and participant (Figure 4.6b). Comparatively cotton fabrics were rated at the lower end of the scale, and polyester fabrics were at the upper end. This is particularly noticeable along the y-axis for the *in vitro* collection method where the cotton fabrics ranged from 27.36 to 50.00. However, the trend for the *in vivo* collection method was not so obvious as the odour intensity for all the cotton fabrics are spread over the x-axis direction ranging from 28.00 to 94.57. Far less variability in odour intensity for fibre content was observed overall for the odour intensity results using the *in vitro* incubation method, compared with the *in vivo* method. This can be explained by the effect of participants (Figure 4.6b), as the participant who wore the fabric had the greatest effect on the overall odour intensity in the *in vivo* wear trial method. Along the y-axis, odour intensity for each participant is consistent with data scattered comparatively even in the *in vitro* method. Whereas, in the *in vivo* method odour intensity is really variable depending on participant as Participant 1 has the highest odour with data clustered in the right and Participant 3 has the lowest odour with data clustered in the left along the x-axis direction.

a)



b)

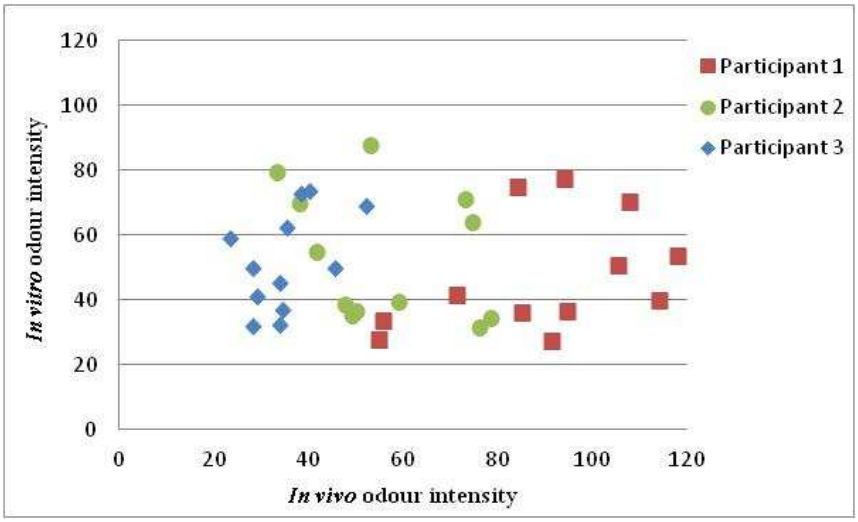


Figure 4.6. Relationship for odour intensity between in vivo and in vitro collection methods for fabrics (a); and participant (b)

CHAPTER 5 DISCUSSION

Axillary odour collected from three participants via two different collection methods (*in vivo* and *in vitro*) were assessed for odour intensity by a sensory panel and bacterial counts obtained from the fabrics were also measured. Findings from the current study and factors that may influence the odour intensity and bacterial counts obtained as a result of fibre type and antimicrobial treatment are discussed in this chapter.

Odour intensity

Difference in odour intensity due to fibre type

In the *in vivo* method, cotton fabrics were slightly lower in odour intensity than polyester fabrics they were matched with, however, the difference was not statistically significant (M=65 for cotton, M=70 for polyester). Therefore, intensity of axillary odour emitted from fabrics following the *in vivo* collection method was not influenced by the fibre type from which the fabrics were made, with odour intensity found to be much higher on cotton fabrics than has been previously reported in other studies involving wear trials. However, significant differences in odour intensity due to fibre type were found following the *in vitro* incubation method, with the mean ratings being different from the wear trial method (M=32 for cotton, M=61 for polyester).

That there were no significant differences found between cotton and polyester fabrics following wear against the underarm in the *in vivo* method was unexpected as previous studies, using wear trial methods for odour collection, found odour intensity on fabrics made from cotton to be significantly lower than that from polyester (McQueen et al., 2007a). For example, McQueen and colleagues (2007a) examined odour retention on a range of cotton, wool and polyester fabrics after being worn next to the underarm for two consecutive days. They found that polyester fabrics had the highest odour intensity (ratings ranged from 60 to 80), wool the lowest and cotton fabrics in the middle (ratings ranged from 30 to 40) (McQueen et al., 2007a). In another study, the difference in odour intensity on cotton and polyester fabrics following multiple wear (worn for a minimum of one hour of exercise) and wash cycles was assessed (McQueen et al., 2012a). Cotton

fabrics were found to retain significantly lower axillary odour intensity than polyester fabrics, both before and following washing (M=31.1 for cotton, M=60.2 for polyester for unwashed fabrics; M=20.6 and M=33.5 for washed fabrics) (McQueen et al., 2012a). Interestingly, the results of the *in vitro* incubation collection method were more consistent with findings of previous studies.

Effect of antimicrobial treatment on odour intensity

Neither antimicrobial treatment (i.e., PHMB or ZP) had any significant effect on reducing odour intensity in the wear trial method regardless of the fibre content. Although very few studies have been done to link antimicrobial treated fabrics to odour intensity, the finding that neither treatment influenced odour intensity *in vivo* was still unexpected. In other studies, researchers have found that some antimicrobial treated fabrics can improve odour emanating from fabrics (Mao & Murphy, 2001; Payne & Kudner, 1996). In Mao & Murphy's (2001) study, 20 participants (12 males, 8 females) wore fabrics (fibre content not reported) with/without Tinosan AM 100 a triclosan based antimicrobial under each armpit. Results from a participant-evaluated paired-comparison odour assessment indicated that 90% of the evaluations of treated fabrics were perceived to be 'fresher', and thus were preferred, compared with the untreated fabrics (Mao & Murphy, 2001). However, an actual rating of odour intensity was not performed so the degree of difference between the treated and untreated fabrics could not be determined. In another study, it was reported that no odour was detected on cotton towelling treated with 0.2% PHMB (Payne & Kudner, 1996). Conversely, McQueen and colleagues (2012b) carried out a study to determine whether polyester textiles treated with a silver chloride antimicrobial agent were effective in reducing axillary odour as well as reducing axillary bacterial populations via a wear trial method (with treated and matched-control fabrics worn by each participant similar to the current study). Their results showed that the treated fabrics did not lower odour intensity compared with the untreated fabrics with comparatively high bacterial populations extracted from the treated fabrics despite evidence of the antimicrobial treatment being effective in *in vitro* testing (McQueen, Keelan, Xu & Mah, 2012b). In all these studies, fabrics used were different, as were the

antimicrobial treatments, so results obtained from each study cannot be compared directly to the results obtained in the current study.

A possible reason for the non-significant effect of antimicrobial treatment may be due to the low number of participants. In fact, the participants had the greatest effect on overall odour intensity in the wear trial method. The antimicrobial treatment on fabrics worn by Participant 2 was generally not effective as odour intensity of the treated fabrics were close to or even higher than that of the non-treated matched fabrics, with ZP treated polyester fabrics being an exception. Results for Participant 1 and Participant 3 were very variable as in some replicates the treated fabrics tended to have lower odour intensity than the untreated ones, while for others the results were opposite. So, it is possible that the low number of participants and the great effect of participant on overall odour intensity influenced or even restrained the evidence of any treatment effect.

For the *in vitro* incubation method, a significant difference was found in odour intensity with respect to the antimicrobial treatment applied to the fabric. A similar trend was apparent for both cotton and polyester fabrics with ZP having the highest odour intensity, fabrics treated with PHMB the lowest and non-treated fabrics in the middle. Studies evaluating textiles which have been treated with the PHMB antimicrobial agent are more common than textiles treated with the ZP antimicrobial agent. Chen-Yu and co-workers found that 65% polyester/35% cotton fabrics treated with PHMB had better antimicrobial properties and were more durable compared with fabrics treated with AEGIS Microbe Shield (Chen-Yu et al, 2007). In their research paper, Chen-Yu et al. also pointed out that in the PHMB molecules there were a large number of biguanide groups to provide multiple sites for bacteria inhibition activities as well as providing multiple bindings on the fabric surface for antimicrobial finishes. This could be one reason to explain the lower odour retention on the PHMB treated fabrics. Nonetheless, it was still surprising to find that the ZP treatment had higher odour intensity than the non-treated polyester and cotton fabrics.

The effect of the antimicrobial treatment on overall odour intensity showed differences for the *in vivo* method compared to the *in vitro* method. This difference may

be ascribed to the differences in the odour collection procedures between the two methods. In the *in vitro* method the 'fresh sweat' applied to the fabric was not necessarily odorous at the beginning and three days of incubation was required to allow sufficient odour to build up in the fabrics, so there was more time for the antimicrobials to have an effect. Whereas, in the *in vivo* method odour intensity was assessed less than one day after removal from the body so less time was given for odour to accumulate within the fabric, as well as the antimicrobial treatment to take effect. These are two very different conditions and therefore the effect that the antimicrobial could have had on preventing odour in the first place may be quite different. Another possible reason could be due to the difference of participant effect created by the two methods. It seemed that with small participant effect in the incubation method, the treatment effect was more easily evident.

Effect of participant on odour intensity ratings

The participant who wore the fabric had the greatest effect on overall odour intensity in the wear trial method. This was not completely unexpected, as interpersonal differences in axillary odour intensity can be influenced by lifestyle, gender, genetics, health etc. (Pastor & Harper, 2012). As well as interpersonal differences there appears to also be a difference due to time-variation (i.e., odour was different on different days for the same person) which may be due to the many uncontrolled factors on different days, such as, work load, metabolism level, weather, diet, environment, mood, etc. The interpersonal variations that occur on different days mean that in the wear trial method only fabric matched pairs could be reasonably compared. Differences due to participant were also found to be significant in other studies. McQueen et al., (2007a) found that differences in odour intensity were significant depending on participant although in their study the fibre content of the fabric was also an influence, as for each participant the same general trend among fibre types occurred (i.e., that polyester was more odorous than cotton which was more odorous than wool).

In the current study, only three participants (2 males, 1 female) were involved in the trial. This number was lower than that reported by other research groups. In other studies assessing odour intensity usually five or more participants were used. For

example, two rugby teams (18-23 males in total) participated in one study (McQueen et al., 2007b), six males took part in Munk and colleagues study (Munk et al., 2000), and eight participants (4 males, 4 females) participated in McQueen and colleagues study (McQueen et al., 2012a). Another, the difference in gender in this small group may increase the effect of participant, as women were reported to have lighter axillary odour than men (Labows, McGinley & Kligman, 1982). This was the case in the current study, as the only female in the study (Participant 3) had lower odour intensity than the two males. However, having only the small number of three participants made the experimental design less complicated and also made the whole study easier to control. Only three participants also provided a sufficient number to use when the new *in vitro* method was tried.

Interestingly, participant did not have a major effect on odour intensity in the incubation method. The fairly even results of odour intensity for each participant in this method were not expected as Participant 1 was much more odorous than Participant 2 and Participant 3 in the wear trial method. An apparent difference between the two methods is that all the test fabrics can be inoculated with the same amount of 'fresh sweat' from one participant at a time in the incubation method, while in the wear trial method the amount of sweat transferred from participant to the fabrics could be highly variable due the metabolic and other differences that may occur on a day-to-day basis. Another difference is that in the wear trial method sweat was more or less continuously transferred from the underarm to fabric while in the incubation method it was transferred in a single process. Also, the left/right arm odour imbalance could be removed in the incubation method by pooling the sweat collected from the two arms.

Bacterial counts

Effect of fibre on bacterial counts

In the wear trial method it was found that the bacterial numbers were not influenced by the fibre type from which the fabrics were made. The results corresponded to findings of the study carried out by McQueen and colleagues (McQueen, et al., 2007a).

They found that bacterial numbers did not differ on fabrics made from wool, cotton or polyester one day after fabrics had been removed from the body. However, they did find significant differences as the days increased with bacterial counts declining more rapidly on polyester fabrics than on cotton or wool fabrics (McQueen et al., 2007a). In another study, McQueen et al. (2012a) found that bacterial counts were not significantly different on cotton and polyester fabrics either before or after laundering.

A significant difference was found in bacterial counts due to fibre type in the *in vitro* incubation method, with polyester fabrics having significantly lower bacterial counts than cotton fabrics. This was in agreement with McQueen and co worker's study where they found there were significant differences in bacterial counts between polyester, cotton and wool after 7 days incubation with bacterial counts declining more rapidly on polyester fabrics than on cotton or wool fabrics compared with that on the first day (McQueen et al., 2007a). Teufel and colleagues found differences in bacterial counts on different textiles in their *in vivo* study while not in their *in vitro* study (Teufel, Pipal, Schuster, Staudinger, & Redl, 2010; Teufel et al., 2008), although in both their studies they found that the type of material had a strong impact on the bacterial colonization of the textile. In their *in vivo* study, five participants were asked to wear T-shirts made of hydrophobic fabrics (polyester or polypropylene) and hydrophilic fabrics (70% lyocell/30% cotton) during a fast walk over 5 h before fabrics were sampled from the back, front and axilla (Teufel et al., 2008). Test results showed that bacterial growth on the hydrophilic material was significantly lower than on the hydrophobic materials (Teufel et al., 2008). For the *in vitro* study, five different fabrics were used (i.e., lyocell, cotton, polyamide, polyester and polypropylene) and 100 µL of subaxillary sweat collected from participants (five women and five men) were inoculated onto the textiles and incubated for 24 h at 37°C (Teufel et al., 2010). Test results showed that, for men, polypropylene fabrics displayed significantly lower bacterial growth than the other materials, with cotton, polyamide and polyester fabrics all having the highest growth and lyocell in the middle. While for women, polyamide and polyester fabrics had significantly higher colonization than cotton, polypropylene and lyocell (Teufel et al., 2010). The two studies carried out by Teufel were through a new test method of DNA quantification, which is said to be

more reliable compared with the impression plating method and can quantify all bacteria not just those which can be cultured in the laboratory (Teufel et al., 2008).

It is important to note that the results obtained in the current study were based on all the test fabrics including the antimicrobial treated fabrics, while for other studies cited, only untreated fabrics were compared without any antimicrobial treated fabrics included. Probably, the significant difference here was due to the influence of the antimicrobial treated fabrics on bacterial counts. Although untreated polyester was lower than untreated cotton these differences were not found to be significant in post-hoc Tukey's tests.

Bacterial counts on the same fabrics were higher in the wear trial method than they were found to be in the incubation method (especially the untreated fabrics). The reason for this would likely be due to greater quantity of nutrients and higher humidity provided in the wear trial method.

Effect of treatment on bacterial populations

There was a significant effect due to antimicrobial treatment on bacterial populations found in both methods with the antimicrobial treated fabrics effectively reducing the bacterial counts in both the *in vivo* and *in vitro* test methods. The results were expected as the two antimicrobial agents (ZP and PHMB) were reported to be effective in reducing *Staphylococcus aureus* as tested by the ISO 20743 quantitative antibacterial assessment method. In other studies, PHMB applied to cotton towels was found to effectively reduce *Staphylococcus aureus* (Payne and Kudner, 1996). In another study 65% polyester/35% cotton fabrics treated with PHMB had better antimicrobial properties against *Staphylococcus aureus* and *Klebsiella pneumoniae* and were more durable compared with that treated with AEGIS Microbe Shield (Chen-Yu et al., 2007). In a recent study, Gao & Cranston (2010) developed a new and effective method to treat wool fabric with PHMB and also found the treated wool fabrics could reduce more than 90% of *Staphylococcus aureus* and *Escherichia coli*.

The reports of ZP treated textiles are not so common as PHMB treated ones. Morris and Welch (1983) invented a new method to incorporate zinc pyrithione into

cotton textiles and test results showed the treated fabrics had good antibacterial activity against growth of *Staphylococcus aureus* up to fifty launderings, although the presence of ZP itself in the fabric had disappeared after five laundering cycles, indicating that ZP was not very durable to laundering. Walter and colleagues carried out a study recently to compare the impact of antimicrobials on normal skin microflora treated with different antimicrobial agents (Walter et al., 2012). They found that fabrics treated with ZP possessed better inhibitory activity against *S. aureus* and *K. pneumonia* as well as the skin bacterial populations *in vivo* compared with fabrics treated with triclosan or silver chloride-titanium dioxide (Walter et al., 2012).

Differences in bacterial populations among participants

A significant difference in bacterial counts due to participant was found in the wear trial method. The difference was not unexpected due to high variability in microbial population, density and composition of bacteria in the axillary region among individuals (Leyden et al., 1981; Rennie et al., 1991). Also, the secretion transferred from the underarm to the adjacent fabrics may differ among persons as some participants may sweat more than others and therefore this could influence the transfer of bacteria to the fabric.

However, it was surprising that the participant difference in bacterial populations was not found in the incubation method. This may be due to the collection method itself, as in the incubation method the bacterial counts were evaluated after three days incubation which allows the number of bacteria to reach a steady state, while in the wear trial method the bacterial counts were measured just one day after the fabric removal where bacterial growth at the very beginning could highly depend on participant.

Interaction between odour intensity and bacterial counts

There was no direct relationship between odour intensity and bacterial counts in the current study either in the wear trial method or incubation method. This was in agreement with the study carried out by McQueen and colleagues, in which they found odour intensity to be significantly different on fabrics made from wool, cotton and

polyester yet bacterial counts on fabrics were similar at one day following removal from the body. Differences in odour intensity still existed up to 28 days after removal from the body, but bacterial counts had changed with a significant decline in numbers on polyester fabrics (high odour intensity) and no change on wool fabrics (low odour intensity) (McQueen et al., 2007a).

However, some interesting results were still found. In the wear trial method, the overall results showed that the treated fabrics had lower bacterial counts although odour intensity was not significantly different between treated and untreated fabrics. For the antimicrobial treatment, the agent of PHMB showed a better effect on reducing bacterial numbers than that of ZP for both cotton and polyester fabrics, while this was not the case for odour intensity. For cotton fabrics, similarly the PHMB treated fabrics were found to have lower odour intensity than the ZP treated fabrics, while in contrast, the PHMB treated polyester fabrics were perceived to have higher odour intensity than the ZP treated ones. The varied results may be due to the low number and great effect of participants.

Another, Participant 1 had high odour on cotton as well as polyester in the wear trial method, which could mean that he had particular compounds that strongly adhere to cotton (and to polyester). In one sensory study, Munk et al. (2001) found cotton was more odorous than polyester after washing and with slow drying. They also reported that a compound called skatole was detected to have a very high odour impact through gas chromatography-olfactometry analysis on cotton only. Unfortunately, in the current study, with sensory measurement only, if some particular odorant was adhering to cotton after being worn by Participant 1 there was no chemical analysis used in conjunction with the sensory evaluation to confirm the odorants. While the significantly higher odour intensity from Participant 1 in the *in vivo* results did not occur in the *in vitro* method, as odour intensity of Participant 1 was much lower in the incubation method and even a bit lower than the other two participants. It is possible that high odour intensity is a cumulative 'build-up' process of certain smelling chemical compounds as the process of sweat being transferred from the body to fabrics was a repeated process in the wear trial method, while in the incubation method it was a single process. McQueen et al. (2012a) found that

C4-C8 chained carboxylic acids were more prevalent on unwashed polyester than unwashed cotton using comprehensive two-dimensional gas chromatography. This suggested that odour build-up in polyester may be cumulative as the carboxylic acid odorants were not effectively removed from polyester compared to cotton. Again, without chemical analysis the proposed reason can not be confirmed.

In the incubation method, the strong effect of antimicrobial treatment of PHMB and ZP on bacterial populations was also found, however antimicrobial treatment did not necessarily have an effect on lowering odour intensity. Odour intensity was rated highest for the ZP treated fabrics, the untreated fabrics in the middle and the PHMB treated fabrics the lowest for both fibre types. It was surprising that the ZP treated fabrics were rated the highest in odour intensity (significantly higher for polyester fabrics) since none to very low numbers of bacteria were obtained from the fabrics following incubation. It is not possible to determine why ZP was more odorous than the untreated fabric in the incubation method. However, one possible explanation could be that some chemical reaction could be occurring between specific odorants and the ZP treatment, but further work would be required to check this.

For the incubation test method, lower odour intensity on cotton fabrics with/without treatment compared to the polyester fabrics was found. However, bacterial counts were higher on cotton compared to the polyester. This may be due to the selective growth of bacteria (possibly the odour-causing bacteria) underrepresented in sweat on the synthetic fabrics (Teufel et al., 2010).

Sensory panel

Screening and selection

In sensory studies test results depend greatly on the assessors' response to certain stimuli. Thus, the screening and selection of assessors is important especially in sensory studies such as the current study where people may be anosmic to some odorous compounds responsible for axillary odour (i.e., about 50% of individuals may be anosmic

to 5α-androst-16-en-3-one). Unfortunately in many published articles the screening process is seldom reported.

In the current study, the screening tests involved a two alternative forced choice threshold test for isovaleric acid and a triangle test for androstenone. All the candidate assessors were asked to carry out the two tests in order to test their olfactory sensitivity and acuity. Based on their test results 11 out of 28 assessors were selected to participate in the final study. Although the compounds selected for screening were recommended in the ASTM E1207-09 standard for sensory evaluation of axillary deodorancy, isovaleric acid and androstenone are only two possible odorants in a large array of odorants which make up overall axillary odour. Other compounds such as 3-methyl-2-hexenoic acid and 3-methyl-3-sulfanylhexan-1-ol would also be important to include in screening tests for assessors as these odorants have been identified to be strong contributors to odour intensity (Troccaz et al., 2004; Zeng et al., 1991). Unfortunately, in the current study these compounds were not sourced as neither could be purchased and needed to be synthesized in a laboratory.

Training

In the literature, training of sensory panels has been reported even less than that of screening and selection of assessors. However, training of sensory panels is highly important as it can help the assessors to become familiar with the stimuli and better understand the sensory scales. In the current study, two training sessions were carried out after the assessors had been selected, which included information on how to use a line scale with figures and also axillary odour on fabric samples collected from selected participants.

In one training session, reference samples with low and high odour intensity were given with example scales (Figure 3.2) in order to inform the assessors on how to use the line scale in a 'uniform' way. However, in reality assessors still used the line scale differently with some specific assessors tending to use the left or right part of the line scale more consistently than others. Nonetheless, the majority of assessors' results

correlated well with the panel mean and the results of only one assessor were removed from the final data due to poor correlation with the panel mean.

The initial training of assessors occurred before another research study (i.e., McQueen et al., 2012a) and therefore there was a time delay of a few months between the first training sessions and research carried out in the current study. Therefore, one possible reason for the differences in how assessors used the scale in the current study may be due to the time delay between the training sessions and the current study which may have resulted in assessors being out of practice. For future studies, a retraining session closer to the time of each study is recommended.

Comparison of the *in vivo* and *in vitro* methods

Axillary odour emitted from fabrics collected by the two different methods (i.e., *in vivo* wear trial method and *in vitro* incubation method) was assessed. Microbiological tests were also carried out which involved extracting bacteria from the fabrics after sensory evaluation had been conducted. The wear trial method is a traditional method for collecting human body odour on fabrics and has been used in previous studies (McQueen et al., 2007a; McQueen et al., 2007b; Munk et al., 2000). The greatest advantage of the wear trial method is that it mimics the real-life situation of odour formation on fabrics during wear. However, a disadvantage of the method is that it is time-consuming to compare multiple fabrics and ideally requires a relatively large number of participants to partake in the wear trial. Furthermore, biases can easily occur due to the interpersonal variability related to different participants, which may be more noticeable with a smaller number of participants as was used in the current study.

The new incubation method developed in the current study, had test results that were similar to those found in other studies, for example, the relationship between cotton and polyester fabrics for odour intensity perceived in the current *in vitro* method was similar to findings from other wear trials (e.g., McQueen et al., 2007a; McQueen et al., 2007b; McQueen et al., 2012a). Some other advantages of using the incubation method

were found, such as less time needed to finish the experiment, more samples collected each time etc. A comparison of the two methods is shown in Table 5.1.

Table 5.1 Comparison of the in vivo and in vitro methods

	In the current study		*Summary*	
	Wear trial in vivo	*Incubation in vitro*	*Wear trial in vivo*	*Incubation in vitro*
Time efficiency	20 days in total	12 days in total	Time-consuming	Time efficient
Exhibit in-use situation	Replicates real-life situation	Does not replicate what happens in real-life	Replicates real-life situation	Does not replicate what happens in real-life
Number of samples obtained each day of testing	6 fabrics at most	6-12 fabrics	Limited	More
Influence that individual participant has on odour intensity	Greatest effect compared to others	Small effect compared to others or no effect	Large	Small
Number of participants required	3 used in the study with results varying widely	3 used in the study with relatively consistent results	Large	Small
Right/left arm imbalance	Exist with one fabric only worn under one arm each time	Not exist by pooling the sweat from the right and left arm	Inevitable	Removed

Therefore, the new *in vitro* incubation method tried in the study has shown to be a practical method for collecting axillary odour on fabrics, which also overcame some problems associated with the wear trial method (see Table 5.1), especially the participant effect and requirement. In the current study the effect of participant in the overall odour intensity was not significant so the *in vitro* method has potential to result in less variable data. Sweat samples collected via the incubation method from different participants could also be pooled together which would further reduce any potential variability due to participants and may even intensify the odour.

As the *in vitro* method allows for a greater number of fabrics, rather than just two per person, to be compared at the same time this method is more time efficient. So, the incubation method is particularly recommended when the participant number is small for the study, or at least it can be a pre-test for its time efficient property and ease of use. However, one limitation is that three days were required in the incubation method to allow sufficient odour to build-up on the fabrics. This does not replicate the real-life situation where odour may be detected on the clothing during and/or shortly after wear. Furthermore, results obtained did not correspond to that found in the *in vivo* test in the current study. No clear reasons can be given to explain why there were differences in the results between the two different methods as what was occurring with respect to odour build-up and bacterial growth during the three days' incubation was not studied in the current investigation. Therefore, more work to improve the incubation method to make it better represent real-life circumstances are recommended for future work.

CHAPTER 6 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The first purpose of the study was to develop a method to collect human axillary odour on fabrics that can be used for detecting odour intensity retained on fabrics and compare this method to the traditional method for axillary odour collection on fabrics (i.e., wear trial). The second purpose of the study was to determine whether there were differences perceived in odour intensity following wear and incubation with axillary sweat between fibre type (cotton and polyester), as well as determining whether two types of antimicrobial treatment (zinc pyrithione [ZP], polyhexamethylene biguanide [PHMB]) had any effect on reducing odour intensity.

An *in vitro* method was developed to collect axillary odour and bacteria on apparel fabrics. A mechanical 'scrubbing' technique was used to collect underarm sweat and bacteria which were then inoculated onto the test fabrics for odour generation, and odour was assessed after three days of incubation. The traditional *in vivo* wear trial method to collect axillary odour was also used. A sensory panel (n=10) was selected to assess the intensity of odour on fabrics that had been provided by three participants (two males and one female). Aerobic bacteria were also extracted from the worn and incubated fabrics and colony forming units were counted.

In the incubation method, cotton was found to have significantly lower odour intensity than polyester fabrics, however, these differences were not apparent in the wear trial method. In the wear trial method fabric treatment had no effect on reducing odour intensity compared with the untreated matched control fabrics. Fabric treatment did have an influence on odour intensity in the incubation method. PHMB treated fabrics were not different from the non-treated fabrics, while ZP treated polyester fabrics were significantly higher in odour intensity than untreated fabrics and PHMB treated fabrics. Both ZP and PHMB were found to significantly reduce bacterial populations regardless of fibre type in both the incubation and wear trial method.

Conclusions

Odour can be generated and detected through the incubation of 'fresh sweat' onto fabrics. Therefore the *in vitro* method developed in the current study appears to be a practical method for collecting axillary odour onto fabrics. Compared with the traditional wear trial method, this incubation method has demonstrated it is more time efficient and easier to use, with it being possible to compare a greater number of fabrics each time.

Following three days of incubation, odour intensity on fabrics was measured in the *in vitro* method, with the intensity of odour being strongly influenced by fibre type and to a lesser extent by antimicrobial treatment. Polyester fabrics were perceived to have significantly higher odour than the cotton fabrics. Fabrics treated with PHMB or without treatment emanated odour of much lower intensity compared with fabrics treated with ZP. The individual participant who provided the axillary sweat had a far lesser effect on overall odour intensity in the *in vitro* method. However, in the wear trial method, fibre type and fabric treatment did not show any significant effects on odour intensity, and the participant who wore the fabric had the greatest impact on odour intensity.

Both antimicrobial treatments (PHMB and ZP) significantly reduced aerobic bacteria extracted from fabrics following the *in vivo* wear trial and the *in vitro* incubation method. However, despite a reduction in bacteria due to the antimicrobial treatments they do not correspond to anti-odour as bacterial counts were not related to differences in odour intensity.

Recommendations

For future work, the following recommendations are suggested based on the findings and limitations in the current study:

1) More participants than three are recommended to participate in the wear trial in any future work to reduce the overall impact that individual participant's can have on overall odour intensity.

2) Carrying out chemical analysis in conjunction with sensory measurement to find whether there are some particular odorants that adhere to certain fabrics generating overall higher odour intensity.

3) Investigate the odour build up and the antimicrobial effect over time of other antimicrobial treated fabrics not examined in the current study.

4) Refine certain parameters of the incubation method to intensify odour intensity and potentially better replicate the *in vivo* wear (e.g., an alternative solution than PBS to collect sweat, alternative methods to collected undiluted 'fresh sweat', inclusion of friction in contamination process).

5) Investigate an *in vitro* method that does not rely on human participants, such as a synthetic sweat and odour-causing bacteria mixture onto fabrics for better repeatability in the laboratory experiments.

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APPENDIX A. QUESTIONNAIRE FOR SCREENING OF ASSESSORS

Questionnaire

Demographics

Please circle the letter that applies to you.

1. Your age:
   a) 18-29 years
   b) 30-39 years
   c) 40-49 years
   d) 50-59 years
   e) 60-69 years
   f) Over 70 years

2. Gender:
   a) Male
   b) Female

3. How would you rate your sense of smell?
   a) Less than average
   b) Average
   c) Better than average
   d) I cannot smell at all

Interest

1. Have you ever noticed that body odour (from the underarm) can remain on clothing and may differ depending on different types of clothing worn?
   a) Yes
   b) No

2. Are you interested in being an assessor on an olfactory measurement panel?
   a) Yes
   b) No
3. Do you have any sensory measurement experience before?
   a) Yes
   b) No
   If Yes, can you specify: _____

4. For detecting the odour intensity, members should follow some precautions, such as not using perfumes, eating spicy food etc on each test day (prior to conducting the test). Would you be willing to do these?
   a) Yes
   b) No

Time availability

1. Are you generally available to be on a sensory panel in September, October and November?
   a) Yes
   b) No

2. Are there any weekdays (Monday-Friday) that you will not be available on a regular basis? Which day?
   _____

Health

1. Are you a smoker?
   a) Yes
   b) No

2. Have you previously been diagnosed with any smell disruptions?
   a) Yes
   b) No

3. Do you have any allergies to some smell?
   a) Yes
   b) No

4. Do you have frequent colds or sinus conditions?
   a) Yes
   b) No

5. Do you take medication which may affect your sense of smell?
   a) Yes
   b) No

THANK YOU FOR YOUR INTERRST!

APPENDIX B. PROTOCOL FOR SCREENING ASSESSORS

Two phases of screening tests

Phase I Threshold test

Threshold test is the test for the limits of sensory capacities, which can be used to compare the sensitivity levels of different individuals and also as a reference to examine their anosmia. A standard Isovaleric Acid (IVA) solution was used in the test, and six modified IVA concentrations were prepared in distilled water (American Society for Testing and Materials, 2009), as shown in Table B.1.

Table B.1 The concentration of IVA solution

Solution No.	IVA concentration (ml/L)
6	3.56
5	1.78
4	0.89
3	0.22
2	0.055
1	0.014

The threshold test procedure was conducted in the following steps:

1. Label six 50 ml cups 1A, 2A, 3A, 4A, 5A and 6A and label another set as 1B, 2B, 3B, 4B, 5B and 6B. For each set A or B, randomly assign the IVA solution or water to the A's and B's. The researcher shall record the identity of what is served in each of A and B (see Table B.2).

2. Pour 40 ml of the appropriate solutions into each labelled cups and cover the cup with lid after pouring. Arrange the cups onto a tray in ascending order (bottom is the lowest concentration and top the highest)

3. Ask the candidates to smell the prepared solutions in the cups from the bottom to the top (that is from the lowest concentration to the highest) and record the results on the answer sheet.

4. Repeat the test for each candidate with the previous steps but using the different serving order as shown in Table B.2b to check the candidates' sensory consistency.

Table B.2 Threshold test serving order

(a)

Sample	Identity	Sample	Identity
6A	IVA	6B	W
5 A	W	5B	IVA
4 A	IVA	4 B	W
3 A	W	3 B	IVA
2 A	IVA	2 B	W
1 A	IVA	1 B	W

(b)

Sample	Identity	Sample	Identity
6A	IVA	6B	W
5A	W	5B	IVA
4A	W	4B	IVA
3A	IVA	3B	W
2A	IVA	2B	W
1A	W	1B	IVA

IVA (isovaleric acid); W (distilled water)

The main principle for selecting assessors is to choose individuals who perform best on the screening tests and who are most available and interested in the odour evaluation study. For this threshold test, the detection threshold for IVA is the concentration level at which the first correct identification of three successive sets of solutions. The candidates' who have a threshold level at lower concentration would be the priority selection.

Sensory Panel Analysis of Odour Intensity on Fabrics
(Answering sheet for Phase I test)

Participant Number: _____

Date: _____

You have been given six sets of samples to evaluate. Smell each set starting with the sample set on the left (1A and 1B). Unscrew the lid of the bottle and smell the headspace air in the bottle with your mouth closed. Immediately replace the lid on the bottle and allow the headspace to build up for the next participant.

Record "**YES**" if you detect the presence of something in the bottle other than water, or "**NO**" if you do not detect anything. You **must** make a choice in each set. Do not re-smell the bottles.

Allow **30 seconds** between sample pairs. You can refresh your nose between sample pairs by sniffing the glass of water. Smell all the samples in the order presented.

Sample	Detection (Yes or No)	Sample	Detection (Yes or No)
1A	Yes	1B	No
2A	No	2B	Yes
3A	Yes	3B	No
4A	No	4B	Yes
5A	Yes	5B	No
6A	Yes	6B	No

Do not write here, for researcher use only

Isovaleric Acid recognition threshold: _____

Phase II test Triangle test

To further screen the candidate assessors' olfactory acuity, the Phase II test which is a triangle test was also carried out. In this test, the odorous compound androstenone was used and the test procedures are listed as the following:

1. An androstenone solution for screening concentration was made based on the procedure described by Amoore (1979) with some minor changes.

    a. An intermediate solution was prepared by weighing 1 mg 5α-androst-16-en-3-one in a dry 10 ml flask and dissolved in 4.2 ml glacial acetic acid. The intermediate solution can be stored in a freezer for one month.

    b. A stock dilution was prepared one day before the test, using the following procedure: in a 125 ml flask, 100 ml 0.05 M Borate buffer (pH 7.0) and 0.05 ml of the intermediate solution was added. It was stoppered and immediately shaken for 1 minute. The 0.05 M borate buffer (pH 7.0) was prepared in a 4000 ml beaker with a magnetic stirrer, 2000 ml of water and 38.2 g sodium borate decahydrate, $Na_2B_4O_7.10\ H_2O$ was added. It was stirred to dissolve. While continuing to stir, concentrated sulphuric acid (96%) H_2SO_4 was dropped in to adjust the pH to 7.0 (measured by a pH meter).

        Concentration for the stock: (1/ 4.2) * 0.05/100=0.12 mg/L

    c. Screening dilutions were prepared just before beginning the test: in a 30 ml glass bottle with screw lid, add 20 ml 0.05 M borate buffer and 3 ml stock dilution as mentioned in **b**.

        Concentration for the screening dilution: 0.12 * 3/20 = 0.018 mg/L

2. Label six sets of 30 ml bottles (three bottles in each set) with the random 3-digit number. In each set, pour 20 ml screening dilution as mentioned in 1(c) in some of the numbered bottles, and others with 20 ml 0.05 M borate buffer, while pouring the same solution into the three glass bottles is not allowed (that is there should be screening dilution and 0.05 M borate buffer in each set), the solutions can be assigned to the six sets of bottles as that:

Table B.3 Example for the assignment of screening and buffer solutions

Set No.	Identity
1	BBS
2	BSB
3	BSS
4	SSB
5	SBS
6	SBB

S-screening dilution; B- borate buffer

3. Arrange the six sets of bottles in a tray with a random order for each candidate.

4. Ask the candidates to smell the solutions set by set, compare the three solutions in each set and find out the odd one. Record their results on the answer sheet shown below.

Decision for the acceptance of candidates based on triangle test is by counting the number of correct responses, and then consulting to the table of *Critical Number of Correct Response in a Triangle Test* (Meilgaard et al., 2007).

Sensory Panel Analysis of Odour Intensity on Fabrics
(Answering sheet for Phase II test)

You have been presented with three samples. Two of the three on your tray are identical and one is different. Smell each of the coded samples from left to right as they are presented on the tray and **identify the odd or different sample**.

Wait **30 seconds** between each sample set. You can refresh your nose between each sample set by sniffing the glass of water. Smell all the samples in the order presented.

Sets of three samples (sample code)	***Which is the odd or different sample***	***Comments***
656 734 316	_____	_____
998 624 495	_____	_____
415 217 829	_____	_____

Sets of three samples (sample code)	***Which is the odd or different sample***	***Comments***
587 126 491	_____	_____
763 256 178	_____	_____
306 591 716	_____	_____

APPENDIX C. TRAINING DOCUMENTS

Training Notice

Introduction

Body odour can be an embarrassing problem, and clothing can play an important role in odour intensity as bodily secretions and skin bacteria are transferred from the body to the garment. Body odour in clothing can even become higher and more intense than the odour which originates on the body. The demand for odour control in clothing is increasing, however, the extent to which the clothing actually manages odour is still largely unknown.

The purpose of this study is to help us understand how odour builds up in clothing over time and how this may be influenced by different fabric properties. As a part of the sensory panel for assessing odour intensity you will evaluate odour on different fabrics (polyester, untreated cotton and antimicrobial treated cotton). You will assess fabrics which have been worn by different people before and after laundering.

Sniff techniques

As part of this training session and for the experimental work you will be sniffing samples. Please use the following sniff technique:

When sniffing, you are asked to take short sniffs and avoid long, deep inhalations; three short sniffs are recommended with your mouth closed. To avoid adaptation, smell clean water as a blank and wait about 30 seconds between samples.

Ranking odour intensity

There is a set of solution with different concentrations, please rank them from the lowest intensity to the highest intensity.

(Lowest) _____ _____ _____ (Highest)

Measurement using a line scale

In this study, you will be asked to smell the odour on fabrics and record the intensity on a line scale. A line scale is commonly used in sensory science. Assessors may mark the intensity of something on a line that reflects their response to a certain stimulus, for example marking the proportion of the shaded area in the following figures.

None ——/—— All

For the following diagrams, please indicate how much of the area is shaded:

None —————— All

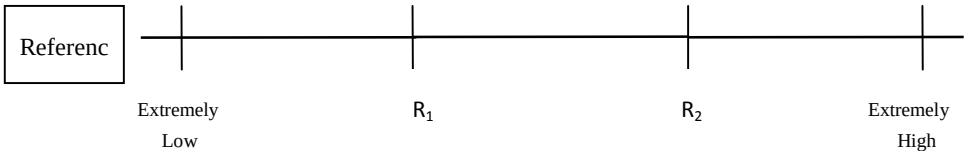
None —————— All

None —————— All

None —————— All

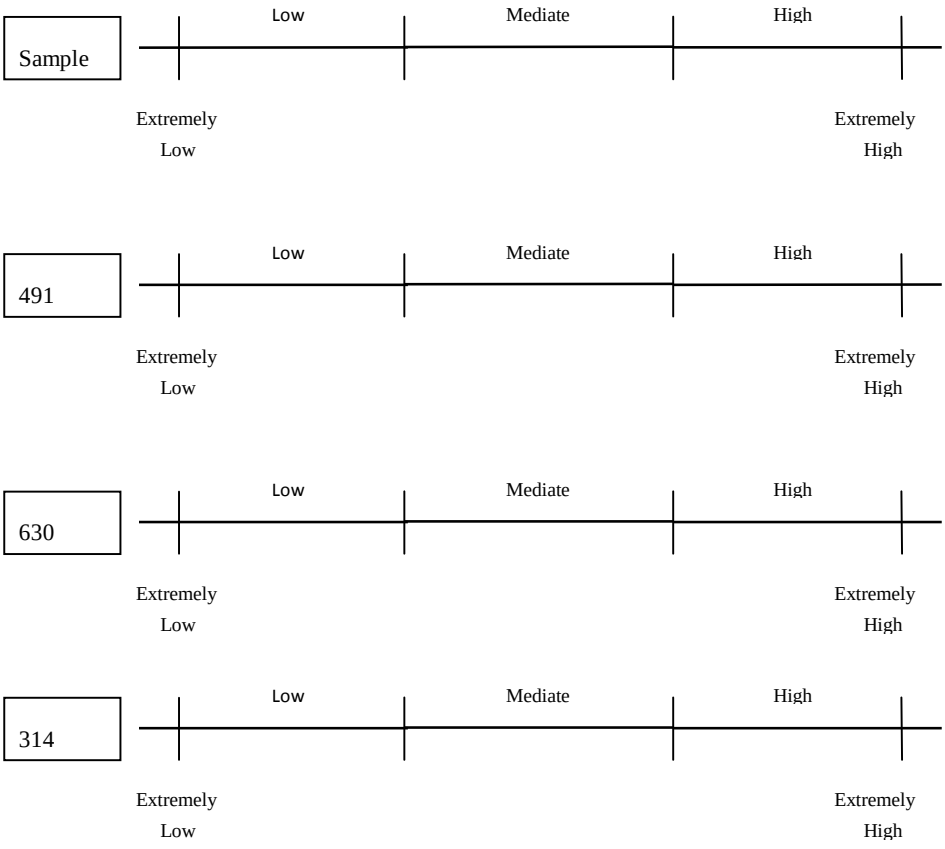
“Standardize your smell sensitivity”

Unlike the previous figures where there are correct answers, there are no universal answers for odour intensity because sensitivity is quite variable among different people. Also, how the same person may perceive an odour could differ on different time. Therefore, the purpose of this training session is to attempt to “standardize” your smell sensitivity by using a reference sample.



Practice

According to the training so far, please practice using the line scale with real fabric samples. Shake the bottle before smelling.



PRECAUTIONS FOR ASSESSORS BEFORE ODOUR DETECTION

There are some items every assessor should pay attention to before each test, which are important because it can reduce the discrepancy of each individual and thus ensure the validity of the test results to some extent:

1. No perfumed substances such as perfumes, hair spray, strong body lotions and soaps can be used on the testing day;
2. No physical exertions such as tennis, swimming, running etc. just before each test;
3. Must not consume alcohol at least 3 h prior to odour measurement;
4. No strong taste food (including spicy food) prior to odour measurement (i.e. on the test day);
5. Avoid the use of the toothpaste, chewing gum, mouth rinse and sprays, breath mints and avoid drinking coffee or tea at least 1h prior to each test;
6. Tie you hair up when you have long hair
7. Must be free of colds or other physical conditions (e.g. fatigue, tiredness)

Thank you very much for your cooperation!

APPENDIX D. RAW DATA OF TEST RESULTS

Table D.1 Intensity of odour collected via wear trial

		Combined results			Replicate 1				Replicate 2			
PPT	Fabric	Mean	S.D.	SME	Arm	Mean	S.D.	SME	Arm	Mean	S.D.	SME
0	CON	22.9	9.4	2.2	0	25.0	7.9	2.6	0	20.9	10.9	3.6
1	CN	118.4	22.0	5.2	L	126.2	18.4	6.1	R	110.7	23.4	7.8
1	PN	113.7	22.4	5.3	R	116.1	18.0	6.0	L	111.3	27.1	9.0
2	CN	47.3	27.4	6.1	L	45.0	29.8	9.0	R	50.0	25.8	8.6
2	PN	49.2	35.5	7.9	R	68.7	37.4	11.3	L	25.3	8.8	2.9
3	CN	36.2	24.2	5.7	L	45.3	28.7	9.6	R	27.0	15.4	5.1
3	PN	55.5	35.3	8.3	R	63.0	33.3	11.1	L	48.0	37.5	12.5
0	CON	20.0	7.0	1.5	0	20.0	7.3	2.2	0	20.0	7.1	2.2
1	CN	75.8	29.9	6.5	L	78.8	29.2	8.8	R	72.5	31.9	10.1
1	C-PHMB	55.3	29.0	6.3	R	54.9	31.7	9.6	L	55.7	27.3	8.6
2	CN	55.1	32.6	7.3	L	61.8	40.7	12.9	R	48.3	22.1	7.0
2	C-PHMB	62.9	34.7	7.8	R	78.3	37.9	12.0	L	47.5	24.2	7.6
3	CN	32.7	14.7	3.2	L	27.5	13.5	4.1	R	38.4	14.4	4.5
3	C-PHMB	28.5	16.4	3.6	R	28.0	17.1	5.1	L	29.0	16.6	5.3
0	CON	26.3	14.8	3.4	0	28.3	18.7	6.6	0	24.8	11.9	3.6
1	CN	87.5	33.4	7.7	L	80.6	28.6	10.1	R	92.5	36.9	11.1
1	C-ZP	79.1	32.2	7.4	R	71.1	27.7	9.8	L	84.9	35.3	10.6
2	CN	59.8	35.1	8.1	L	38.3	26.4	9.3	R	75.6	33.0	10.0
2	C-ZP	65.0	32.4	7.4	R	49.9	23.6	8.3	L	76.0	34.4	10.4
3	CN	33.2	24.4	5.6	L	30.0	18.4	6.5	R	35.5	28.7	8.7
3	C-ZP	30.7	19.3	4.4	R	34.4	19.0	6.7	L	28.0	19.9	6.0
0	CON	23.1	18.0	3.9	0	23.1	16.1	4.8	0	23.2	20.9	6.6
1	PN	112.1	30.7	6.7	L	120.4	18.6	5.6	R	103.0	39.2	12.4
1	P-PHMB	115.9	20.6	4.5	R	114.1	21.1	6.4	L	117.8	20.9	6.6
2	PN	57.3	28.1	6.1	L	71.8	21.5	6.5	R	41.3	26.5	8.4
2	P-PHMB	58.0	34.3	7.5	R	73.0	30.4	9.2	L	41.5	31.9	10.1
3	PN	51.8	35.5	7.7	L	62.8	38.8	11.7	R	38.3	27.1	8.6
3	P-PHMB	29.7	22.0	4.8	R	35.5	28.1	8.5	L	23.3	10.7	3.4
0	CON	17.7	7.6	1.7	0	19.0	8.7	2.8	0	16.3	6.4	2.1
1	PN	93.8	34.1	7.8	L	86.6	36.7	11.6	R	101.9	31.0	10.3
1	P-ZP	88.8	36.6	8.4	R	84.2	40.0	12.6	L	94.0	34.1	11.4
2	PN	66.5	38.2	8.8	L	83.8	37.4	11.8	R	47.3	30.4	10.1
2	P-ZP	43.7	29.7	6.8	R	53.0	33.3	10.5	L	33.3	22.6	7.5
3	PN	39.6	32.2	7.4	L	30.4	19.3	6.1	R	49.8	41.2	13.7
3	P-ZP	39.2	26.5	6.1	R	38.2	26.5	8.4	L	40.2	28.1	9.4

Table D.2 Summary of bacterial counts obtained from fabrics worn during the wear trial (×10^3 CFU/ml)

	Combined results		Replicate 1			Replicate 2		
PPT	Fabric	Log	Arm	CFU/ml	Log	Arm	CFU/ml	Log
0	CON	0.97	0	0.02	1.25	0	0.00	0.00
1	CN	5.44	L	506.67	5.71	R	42.17	4.63
1	PN	5.00	R	60.50	4.78	L	138.83	5.14
2	CN	3.45	L	0.30	2.48	R	5.32	3.73
2	PN	2.69	R	0.03	1.54	L	0.95	2.98
3	CN	4.51	L	56.00	4.75	R	7.98	3.90
3	PN	4.75	R	106.67	5.03	L	4.93	3.69
0	CON	0.00	0	0.00	0.00	0	0.00	0.00
1	CN	4.46	L	57.00	4.76	R	1.27	3.10
1	C-PHMB	0.97	R	0.02	1.25	L	0.00	0.00
2	CN	3.55	L	2.87	3.46	R	4.28	3.63
2	C-PHMB	0.97	R	0.00	0.00	L	0.02	1.25
3	CN	5.69	L	970.00	5.99	R	6.45	3.81
3	C-PHMB	0.00	R	0.00	0.00	L	0.00	0.00
0	CON	0.00	0	0.00	0.00	0	0.00	0.00
1	CN	4.57	L	53.00	4.72	R	22.00	4.34
1	C-ZP	2.93	R	0.68	2.84	L	1.03	3.01
2	CN	3.74	L	1.67	3.22	R	9.32	3.97
2	C-ZP	2.92	R	0.13	2.13	L	1.53	3.18
3	CN	4.34	L	8.43	3.93	R	35.00	4.54
3	C-ZP	3.24	R	2.98	3.47	L	0.52	2.71
0	CON	0.00	0	0.00	0.00	0	0.00	0.00
1	PN	5.23	L	73.67	4.87	R	266.67	5.43
1	P-PHMB	0.97	R	0.00	0.00	L	0.02	1.25
2	PN	3.04	L	0.87	2.94	R	1.33	3.12
2	P-PHMB	0.00	R	0.00	0.00	L	0.00	0.00
3	PN	4.63	L	47.50	4.68	R	37.17	4.57
3	P-PHMB	0.00	R	0.00	0.00	L	0.00	0.00
0	CON	0.00	0	0.00	0.00	0	0.00	0.00
1	PN	4.10	L	20.83	4.32	R	4.30	3.63
1	P-ZP	2.56	R	0.42	2.62	L	0.30	2.48
2	PN	3.14	L	1.62	3.21	R	1.17	3.07
2	P-ZP	2.65	R	0.85	2.93	L	0.03	1.54
3	PN	3.74	L	0.62	2.79	R	10.33	4.01
3	P-ZP	2.34	R	0.13	2.13	L	0.30	2.48

Log=Log_{10} CFU/ml; minimum detection limit<16.7 CFU/ml

Table D.3 Odour intensity on incubated fabrics

		Combined results				Replicate 1				Replicate 2			
PPT	Fabric	Mean	S.D.	C.V. %	SME	Mean	S.D.	C.V. %	SME	Mean	S.D.	C.V. %	SME
0	Con-PN	27.2	19.3	70.9	4.2	21.6	8.7	40.3	2.7	32.3	24.8	76.9	7.5
1	CN	31.9	27.5	86.4	6.0	36.8	34.1	92.8	10.8	27.4	20.4	74.7	6.2
1	C-PHMB	30.9	23.7	76.8	5.2	27.7	24.2	87.2	7.6	33.7	24.1	71.3	7.3
1	C-ZP	38.7	23.6	60.9	5.1	41.5	21.1	50.9	6.7	36.2	26.4	72.9	8.0
1	PN	60.2	32.0	53.2	7.0	70.3	33.9	48.2	10.7	51.0	28.6	56.2	8.6
1	P-PHMB	47.1	34.8	73.7	7.6	40.1	35.3	88.1	11.2	53.5	34.6	64.6	10.4
1	P-ZP	76.3	26.1	34.2	5.7	75.0	25.3	33.8	8.0	77.5	27.9	36.1	8.4
0	Con-CN	22.4	13.8	61.8	3.0	21.3	5.5	25.8	1.7	23.6	19.7	83.5	6.2
2	CN	37.4	26.3	70.3	5.7	35.4	24.1	68.2	7.3	39.6	29.6	74.8	9.4
2	C-PHMB	36.5	26.1	71.3	5.7	34.6	26.1	75.3	7.9	38.6	27.3	70.7	8.6
2	C-ZP	34.2	18.7	54.8	4.1	36.5	19.7	53.9	5.9	31.6	18.3	57.8	5.8
2	PN	66.9	27.3	40.9	6.0	64.1	21.2	33.1	6.4	69.9	33.8	48.4	10.7
2	P-PHMB	63.4	37.7	59.4	8.2	71.1	37.8	53.2	11.4	55.0	37.7	68.5	11.9
2	P-ZP	83.8	31.5	37.5	6.9	87.8	30.6	34.8	9.2	79.4	33.5	42.2	10.6
0	Con-CN	25.5	15.0	58.7	3.4	27.0	19.3	71.4	6.4	24.1	10.6	44.1	3.4
3	CN	39.2	26.4	67.4	6.1	32.6	17.1	52.5	5.7	45.2	32.5	71.8	10.3
3	C-PHMB	36.9	22.5	60.8	5.2	32.2	17.9	55.7	6.0	41.2	26.1	63.3	8.3
3	C-ZP	43.8	30.1	68.6	6.9	37.0	18.2	49.2	6.1	50.0	37.7	75.5	11.9
3	PN	58.9	36.6	62.1	8.4	69.0	36.5	53.0	12.2	49.8	36.0	72.2	11.4
3	P-PHMB	60.7	34.4	56.7	7.9	62.3	34.2	54.9	11.4	59.2	36.4	61.5	11.5
3	P-ZP	73.4	30.8	42.0	7.1	72.9	24.4	33.5	8.1	73.8	36.9	50.1	11.7

Table D.4 Summary of bacterial counts obtained from incubated fabrics trial (×10^3 CFU/ml)

PPT	Fabric	Combined Log	Replicate 1 CFU/ml	Replicate 1 Log	Replicate 2 CFU/ml	Replicate 2 Log
0	CON	0.00	0.00	0.00	0.00	0.00
1	CN	4.19	24.00	4.38	6.98	3.84
1	C-PHMB	0.00	0.00	0.00	0.00	0.00
1	C-ZP	1.41	0.05	1.71	0.00	0.00
1	PN	3.03	0.88	2.95	1.25	3.10
1	P-PHMB	0.00	0.00	0.00	0.00	0.00
1	P-ZP	0.00	0.00	0.00	0.00	0.00
0	CON	0.00	0.00	0.00	0.00	0.00
2	CN	3.92	10.00	4.00	6.68	3.82
2	C-PHMB	0.00	0.00	0.00	0.00	0.00
2	C-ZP	1.25	0.03	1.54	0.00	0.00
2	PN	3.12	1.93	3.29	0.73	2.87
2	P-PHMB	0.00	0.00	0.00	0.00	0.00
2	P-ZP	0.00	0.00	0.00	0.00	0.00
0	CON	1.41	0.00	0.00	0.05	1.71
3	CN	4.10	19.33	4.29	5.90	3.77
3	C-PHMB	1.41	0.05	1.71	0.00	0.00
3	C-ZP	0.00	0.00	0.00	0.00	0.00
3	PN	4.31	39.50	4.60	0.88	2.95
3	P-PHMB	0.00	0.00	0.00	0.00	0.00
3	P-ZP	0.00	0.00	0.00	0.00	0.00

Log=Log_{10} CFU/ml; minimum detection limit<16.7 CFU/ml