Identification of microbial contamination in body wax samples

Claire Bailey
Dr Alan Beswick

Health and Safety Laboratory
Harpur Hill
Buxton
Derbyshire
SK17 9JN

Currently, apart from non-UK case studies and anecdotal evidence, there are few published data available regarding infection prevention and control during the process of body waxing. The techniques available for waxing different parts of the body do vary, and have received some attention from the healthcare and enforcement community. However, the specific infection risks posed by used or unused waxes have not, as yet, been fully or scientifically characterised, with most publications taking the form of case studies of patient aftercare.

A focussed, but representative microbiological sampling study of wax pot residues in salons was therefore undertaken to inform HSE on this area of treatment. In particular, the study was required to provide accurate advice that could be offered by HSE in the area of body waxing, and to inform existing guidance provided by the standard setting body for the hair, beauty, nail and spa industries.

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EXECUTIVE SUMMARY

Objectives

Currently, apart from non-UK case studies and anecdotal evidence, there are few published data available regarding infection prevention and control during the process of body waxing. The techniques available for waxing different parts of the body do vary, and have received some attention from the healthcare and enforcement community. However, the specific infection risks posed by used or unused waxes have not, as yet, been fully or scientifically characterised, with most publications taking the form of case studies of patient aftercare.

A focussed, but representative microbiological sampling study of wax pot residues in salons was therefore undertaken to inform HSE on this area of treatment. In particular, the study was required to inform a planned Local Authority Circular on body waxing, and to allow more accurate information to be incorporated into that guidance.

The main aims of the study were to:

• Develop an evaluated method (or methods) that allowed bacteria (and potentially fungi) within a variety of submitted body wax types to be released and identified;

• Obtain wax samples from various UK locations – from both inner city areas as well as smaller towns, and typically from ‘open pot’ usage - to be tested with the developed approach, in order to identify any gross microbial contamination of used wax products; and

• Based on results obtained, determine whether body waxes routinely become contaminated with microorganisms during their use for hair removal in UK beauty salons and, if so, to what extent.

It was intended that the outcome of this assessment would then determine any associated risk from this contamination, and influence any recommendations to this industry sector.

Main Findings

A method was developed for the identification of microorganisms from solid and semi-solid wax samples, based on typical waxes used for hair removal within the UK beauty industry. Evaluation of seeded wax samples demonstrated that the method was capable of detecting microbial levels as low as 5-10 Colony Forming Units (CFU) per sample. In addition, microbial recovery was not inhibited by the presence of wax in the assay suspension or by the use of a mild solvent to allow wax solubilisation prior to microbial recovery.

Of the total number of samples submitted, most samples (78%) were found to be negative for any detectable microbial contamination (bacteria or fungi). Four used samples and one unused sample contained low to moderate numbers of bacteria but these results were not repeatable for reasons discussed in this report. The main bacteria identified were *Bacillus* and *Staphylococcus* species. Representatives from both groups are commonly found in the indoor environment and pose little risk to healthy individuals, though staphyloccal species are often associated with the skin. The study concluded that the findings were indicative of generally low level of contamination at the limit of available detection levels.
Recommendations

The levels of microorganisms detected in the supplied samples were generally low, and no evidence of gross contamination was noted from any salon sample received. However, this should not be seen as reason for complacency, since viewed in perspective this study represents only a modest cohort of ‘real’ samples, sourced from UK salons within a short time frame (Autumn 2008). Based on these findings, a useful insight in to the quality of used and unused wax products has been gained, and this allows some conclusions to be made.

It has been suggested within the industry that a change from open pot wax application to cartridge-based systems will assist infection prevention and control during body wax treatments. While cartridge system design may follow good infection control principles and offer choice within the sector, a change in wax application technique would not appear to be essential in order to achieve a safe, hygienic standard of body waxing. Our findings did, however, indicate that some contaminating bacteria were present within a minority of tested samples, and it is evident that the potential exists for waxes to become measurably contaminated with microorganisms, either during routine use or possibly prior to first use, e.g. during packaging, storage, transit etc. In view of this, and in order to minimise opportunities for wax contamination, industry good practice should always be adhered to by operators to ensure the highest level of hygiene is achieved for their treatments. Existing hygiene recommendations are available within the industry guidance cited within this report.
1 INTRODUCTION

During 2007 the relevant Government-approved standards setting body, the Hair and Beauty Industry Association (HABIA), published a new code of practice. That document, called Waxing Services, included information on infection control good practice for operators performing body-waxing procedures. It was apparent during HABIA consultation at that time that a lack of clarity existed regarding the extent of measures required by operators to prevent cross-contamination risks between their clients. Put simply, any contamination risks posed by re-using open wax pots – even when spatulas are regularly changed – was unknown in terms of the levels, and types of microbial contamination that might be expected. The HABIA document, though helpful in terms of recommending an appropriate technique, also had no firm data on which to base its recommendations. It chose to state the following in order to achieve some degree of hygiene consistency in this respect:

“Current accepted practice is that a new spatula is used for each client and the risk of cross-infection from re-dipping the spatula into the same pot used for more than one client is small. In these circumstances, i.e. when the wax pot is not single use and is reloaded with wax as necessary, in addition to general cleaning up of wax drips between clients, the wax pot must be regularly emptied, cleaned and dried, and the used wax discarded. How regularly depends on the number of clients, but should be at least once per week.”

What remained unclear was whether operators following this industry guidance could expect to wholly prevent, or at least mitigate, any risk of cross infection from client to client. Subsequent discussions undertaken for the current study - between HSL researchers and beauty sector training staff - have confirmed that heated, open wax pots may be retained and used for several clients, and typically this at least equates to the course of a working day. The issue of cross-contamination potential between those treated therefore remains one of some importance, and the current study was undertaken to fundamentally determine if microorganisms were detectable in used wax samples, and if so, whether their levels and types present could be regarded as hazardous to health.

Following the 2007 publication of the report ‘Identification and prioritisation of emerging health and safety issues in the beauty industry’1, the Local Authority Co-ordinators of Regulatory Services (LACORS) asked that HSE draft a Local Authority Circular on Body Waxing. However, because it was not possible confirm the above statement with scientific data, LACORS sought support from the funding made available by the Health and Safety Commission, and asked HSL to undertake this study. This funding is provided for Local Authorities to allow them to make increased use of science.

1.1 EVIDENCE FROM THE LITERATURE

A literature review performed by HSL staff during Spring 2008 uncovered a number of relevant publications on the subject of body waxing and reported post-waxing complications that could be reliably linked with the procedure. The identified reports were limited, and those found were all overseas publications. This further underlines the fact that only limited research has been conducted on this subject, despite much anecdotal evidence in the UK suggesting that post-waxing skin problems are common. Twelve peer-reviewed publications were identified, all of which strongly indicated that body waxing was linked to cases of folliculitis and other skin

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1 Identification and prioritisation of emerging health and safety issues in the beauty industry. Available via the LACORS web site via login only at: http://www.lacors.gov.uk/lacors/Home.aspx
complications. The predominant bacterium implicated in these case reports was \textit{Pseudomonas aeruginosa}, a common environmental bacterium that can reside harmlessly on the body but which may cause human infection when present on broken skin\textsuperscript{5,7}. The majority of these reports were sourced from Spain, including an additional case report for \textit{Mycobacterium chelonae} infection\textsuperscript{8}, and also an Australian report of \textit{Streptococcus pyogenes} and \textit{Herpes simplex} infection in a diabetic individual\textsuperscript{9}. Other case studies identified from the literature search mainly related to physical skin damage after waxing, including burns and scarring. A repeat literature search, conducted by HSL staff in January 2009, failed to reveal any recently published UK or overseas papers on this topic.

\section*{1.2 THE PROCESS OF HAIR REMOVAL USING A TRADITIONAL, HEATED ‘OPEN POT’ WAXING METHOD}

To perform traditional body waxing the wax is heated up in a large pot to a high temperature, usually 60-70°C, to melt the wax. The required temperature may vary from one wax product to another, and in our own experience some waxes may be soft and sticky at room temperature, with others perfectly solid. Following initial heating, the pot is then allowed to cool slightly and left to stand for the purposes of treatment at approximately 50°C. Retention at this heat ensures that wax stays molten, but is not hot enough to burn the client when the product is applied to the skin. Following detailed discussions with the University of Derby on this subject, it appears that accurate measurement of wax temperature is rare; melting blocks are typically supplied with a numbered dial and scale (e.g. 1 to 10), but do not display actual temperature. The operator typically learns from fellow operators – or from trial and error – the optimal numerical level at which the heater dial needs to be set in order to initially melt the wax, and then to maintain its molten state. This means that a good degree of uncertainty exists over the actual temperatures used for this process, though these temperatures are undoubtedly relevant for infection control purposes. This is because the process of initial heating is likely to reduce the levels of any viable, contaminating microorganisms in the wax, and this effect could continue even at lower (maintenance) temperatures, particularly if held at 50°C or higher.

Following on from the HABIA statement (above), we assume that most operators who adhere to good practice guidelines would use a fresh spatula to scoop out the molten wax for each new client commencing treatment. This is then spread thinly over the client’s skin and a material strip is firmed over the wax and leg and then pulled off to remove the hair. The same spatula is then typically used to remove more wax from the pot as the process continues, and this is repeated until the area of concern is hair free. Although spatulas are cheap to purchase in bulk (pennies each), the informed feedback from those teaching within this sector suggests that so many are used per treatment and per day, that using a new spatula for every dip of the wax pot would be excessively wasteful and ultimately costly. However, the waxing process can cause blood spots and in-growing hairs, and results in some surface skin loss. A concern therefore exists that skin bacteria and fungi might be transferred back to the wax, and subsequently be transferred back to the next treated client. There is also a theoretical risk from blood borne virus transmission for the reasons stated, though detection of any virus is outside the scope of this study, and no reported evidence of their transmission via waxing could be found.

At HSL there is often a requirement to develop new methods to satisfactorily analyse challenging samples; in the case of body wax this constituted a sample matrix that was impervious to aqueous treatments, and which varied considerably across of wide range of available brands. At the commencement of the study a published method for this kind of assay was not available, and there was a need to develop a method that would allow HSL to reliably retrieve any contaminating bacteria from within test samples. The required approach would
facilitate detection and identification of any microorganisms present. Once optimised, the intention was then to apply the assay to samples obtained from beauty salons.
2 METHODS

2.1 SAMPLE COHORT

HSL used existing contacts to approach Environmental Health Officers (EHOs) throughout the country for help in contacting beauty salons that offer waxing services. Seven EHOs offered their help, each with access to multiple businesses. In some cases the EHO supplied business addresses, which were contacted directly following an explanatory visit from the EHO, e.g. Derbyshire and Dagenham businesses (Table 1). In other cases the EHO obtained the samples themselves following liaison with their local businesses, e.g. Birmingham and Sheffield. Once confirmed, HSL’s contacts were sent pots, disposable gloves, pre-paid packaging and an instruction/response sheet (see Appendix); salons then provided used and unused wax samples either directly, or via the assisting EHO. In total thirteen beauty salons around the country agreed to participate in the study, providing samples for analysis at HSL. All participating businesses were asked to fill in a short questionnaire, detailing the wax they used and had supplied. As part of that information gathering exercise, respondents were also asked to indicate how many customers the product had been used on. An opportunity was also provided for them to include any additional information they thought relevant. Twelve salons responded to the requests for samples, and each sent two samples; a used sample and an unused sample (control). All used samples were obtained from an open heated pot - not a cassette system - as earlier discussions indicated that contamination was most likely to occur with open systems, and that resources should be focussed on these.

2.2 IMPACT ASSESSMENT

From the outset it was anticipated that the outcome of this study might influence the types of body waxing products and systems recommended by regulators for use in the UK. The Health and Safety Executive and local authority Environmental Health Officers recognise that any recommendations for a change in waxing systems could have a financial impact on the beauty salons, and this effect therefore had to be considered as part of the study. In view of this, HABIA, (the government body appointed to set standards for the hair, beauty, nails and other aspects of the beauty industry), was approached directly and asked to provide HSL with cost-impact comparisons for various waxing approaches, including cartridge and open systems. In order to standardise the comparison, HSL asked that the cost assessment be based on a single type of treatment - a leg and bikini wax. Wholesale prices were obtained for different systems, including the routine approach to open pot waxing with molten wax and HABIA-recommended spatula use, compared with a cartridge-based approach, e.g. as sold by the PhD system, a well known brand used for cartridge-based waxing.

In order to provide a more accurate comparison of prices for different target markets and locations, the data sourced by HABIA from some of its members included a range of geographical locations to account for price variation across the UK (Table 1). Further background information on the sources of data used is provided in the Results section.
2.3 DEVELOPMENT OF A WAX ANALYSIS METHOD

2.3.1 Initial wax dispersal tests and evaluation of detergent
For the purposes of this study, the process of disrupting or dispersing wax samples in order to release microbial cells contained within them proved more challenging than expected. The chosen method was required to preserve the integrity and viability of any contaminating microbes wherever possible, so harsh treatments such as extreme heat, concentrated industrial detergent or pure organic solvents were avoided. During lengthy method development a range of milder solvents and detergents were evaluated for their ability to disperse samples of body wax available on the UK market.

Initial efforts focussed on the use of 2% and 3% solutions of warm Neutracon™ (www.decon.co.uk), a powerful neutral detergent capable of dispersion of petrochemical-based products, but of a neutral pH that retains microbial viability. Consultation with Decon staff indicated that, with regards to organic chemical wax dispersal, Decon Neutracon is a near neutral (pH7+/_) product, which does not possess bacterial activity and would, therefore, be suitable. A detailed assessment of Neutracon™ use with and without physical mixing was therefore conducted. Where used, a mixing action was applied using either sterile plastic rods (using a grinding action) or by the addition of two to three 5 mm diameter (sterile) glass beads followed by vigorous mixing. In all cases wax was added to a warmed detergent mix, but repeated attempts to disperse or break up the body wax either failed or had insufficient effect. Raising the temperature of the wax suspension in order to promote solubility conferred some benefits to the process, but it became evident that maintenance temperatures in excess of 65°C would be required for this. Such temperatures would quickly kill most of the microorganisms sought from the test samples, and consequently the use of powerful, neutral detergent was discounted as a method of choice and is not further reported on here.

2.3.2 Evaluation of purified organic oils to liquidise wax
Discussions with organic chemists at HSL provided a possible method for rendering solid wax to the molten state: Filter sterilized mineral oil and virgin olive oil was applied (separately) to a range of test waxes in the presence of heat and agitation in a shaking water bath. Several different brands of wax product were heated up to 60°C in this way, and although partial melting was observed for some waxes, no total melt was achieved. It was also recognised that even a temperature of 60°C was likely to kill some types of microorganism, particularly Gram-negative bacteria that are unable to form protective spores. Temperatures below 60°C showed no observable melt effect with this approach, and for this reason the use of added oils was also discounted as a method of choice for wax assay and is not further reported on here.

2.3.3 Evaluation of solvent to dissolve wax

2.3.3.1 Acetone
Some organic solvents are capable of solubilising solid and semi-solid waxes, and are incorporated in to beauty salon products to assist surface cleaning following waxing procedures. Ethanol was avoided because of its known anti-microbial action but, following discussions with HSL chemists, acetone was assessed at varying concentrations (in de-ionised water) to determine its capability in this respect. Despite assessments that saw acetone use spanning concentrations of between 20% and 100%, no wax solubility was noted. Additional physical disruption using a BIO-101 bead beater failed to improve results sufficiently to consider using this approach for test samples.
2.3.3.2 Babyliss Spring Clean™

This is a commercially available wax surface removal product used widely in the beauty sector. This product is miscible with water, and although it is not designed for use on skin, its action is dependent on isopropyl alcohol (isopropanol), an alcohol that is used for degreasing delicate electricals and other surfaces, and which has been used extensively in the past for tissue fixation. As such, the physical damage this active agent causes to cells is limited, and it was decided to test this solvent at various concentrations in order to assess its ability to dissolve body wax, while retaining microbial viability.

To determine the optimal level of Spring Clean™ solvent in which to dissolve the wax, a dilution series was set up using filter-sterilized solvent product in sterile, de-ionised water. Solvent concentrations of between 5% and 50% were prepared in 1 ml tubes and then inoculated with 20µl of a seeded skin bacterium in order to assess the ability of this microorganism to survive exposure to the solvent at various concentrations. The chosen bacterium was a strain of *Staphylococcus aureus*, previously used at HSL for other HSE and external project work. *Staphylococcus aureus* is a common skin coloniser, does not form resistant spores, and as such it was anticipated that, should it survive exposure to Spring Clean™ solvent, then the method would prove suitable for other skin microbes implicated in wax contamination. Initially, this recovery evaluation was done without the presence of wax products, simply to determine whether the solvent water mix was lethal to the seeded *S. aureus* cells, or not. The samples were exposed to the solvent series for 10 minutes – a period calculated as necessary for the assay of actual test samples in this way – then serially diluted and plated out onto Tryptone Soy agar (TSA). As a process control during this work, the *S. aureus* stock used to inoculate the sample was also serially diluted and plated (without solvent) on to TSA, in order to determine the quantity of bacteria that might ideally be recovered from the Spring Clean™ seeding if no solvent lethality was observed. From this test it was possible to determine the maximum Spring Clean™ concentration at which *S. aureus* cell death remained minimal, but wax was dissolved. Results are shown in Figure 1.

Following the above assessment, and having already gained information on the concentration of solvent that could be used with seeded bacteria, the assay sensitivity was investigated. This further test was to establish the recovery of the same bacterium from a mixture of Spring Clean™ and combined wax product. *S. aureus* was again prepared as above and the resulting stock was serially diluted to generate different starting concentrations of bacteria in the extraction suspension. This series of inocula was then used to seed tubes with an established 10% solvent solution (Spring Clean™ and de-ionised water), containing sterile grated wax, or else to seed tubes containing 10% solvent alone (Spring Clean™ and water).

- The process of grating the wax before addition to the Spring Clean™ solvent was found to improve the speed of solubilisation.
- The seeding of solutions with and without wax was undertaken to allow comparison, i.e. to determine if the presence of wax in the solvent had a detrimental effect on bacterial recovery when compared to solvent alone.
- The samples were again left exposed for 10 minutes, to allow wax solubilisation, then serially diluted and then plated out on to TSA to establish the degree of bacterial recovery.

Results are shown in Figure 2.

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2.3.4 Sample analysis
For solid waxes test samples received, wax was grated from bulk samples using a sterile, fine metal grater, as available from most kitchen shops. From the well-mixed, bulk gratings generated, 0.01 g of wax was then removed and mixed with 1 ml of 10% sterile, filtered, Spring Clean™ solvent and water. Although the amount of wax used per sample represented only a small percentage of the total product received, it was found to be the optimal amount that would dissolve in the 10% solvent solution. In addition, the fact that the grated bulk wax was well mixed, and the assay volume small (within 1 ml total volume), meant that sensitivity for the assay remained high. This was previously confirmed from testing of seeded samples, which used suspensions of similar composition (section 2.3.3.2).

For softer wax products that could not be grated, preparation was more challenging: One ml tubes of 10% solvent were weighed before use. Semi-solid waxes were then scooped out using a sterile spatula and dissolved in 10% sterile, filtered, spring clean solvent and water. The tubes were then post-weighed and the mass of wax determined using a simple mathematical formula (weight after – weight before = wax weight tested).

In all cases, the suspensions of wax and solvent were agitated for 10 minutes at 37°C before serial dilution and were plated out onto TSA plates. The plates were incubated overnight at 37°C before counting. If no colonies were present the samples were re-incubated for (up to) a further 5 days.

2.4 FORMAL IDENTIFICATION OF CULTURED COLONIES
In order to obtain some insight into the nature of the colonies grown from wax, representative colonies were taken from sample W16 and analysed using DNA based techniques. This sample was chosen because it provided the highest level of microorganisms across initial and repeat analysis, and was seen to generate at least 2 different colony types on the related culture plates. A well-established HSL method, using the polymerase chain reaction (PCR) and gene sequencing approaches, was used to amplify up genetic material from the colonies in order to formally identify them. DNA sequences, once obtained, were analysed using the NCBI Blast database.

Colony identification was made on colonies grown from samples W11 and W12, based on observations of colony morphology. Certain microorganisms grow characteristically on agar plates and can be identified to the genus level based on their appearance.

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3 BLAST DNA alignment tool. Accessed via:
3 RESULTS

3.1 IMPACT ASSESSMENT RESULTS

In its response to HSL’s request, HABIA was able to supply data relating to the cost of various wax treatments, and emphasised that the length of treatment and cost vary according to target market – i.e. luxury/prestige vs mass market, quality of product, single dip or multiple dip use of spatulas, client size and age, hair type and skill of the therapist. We received five replies – three from salons and two from suppliers. Not all offered each type of system. Some provided upper and lower limits according to quality of product and skill of therapist. In other words the data available are only indicative and serve as a reflection of typical sector values. The results shown below might therefore be best regarded as pilot data, and although representative, a more thorough costing exercise would be required to achieve a comprehensive assessment of treatment cost variations.

HABIA made a request of its members for an estimated cost per client of the services offered in salons (or by them as a supplier). Results are given in Table 1 below for the standardised treatment of a full leg wax and bikini line.

Times varied considerably and agreed maximum service times for the assessment are 60 minutes for bikini line and full leg. Most salons would book between 45 and 60 minutes depending on the client.

All HABIA-sourced prices below are supplied inclusive of VAT. (N.B. suppliers’ catalogue prices are typically exclusive of VAT). All prices are exclusive of “Other” items i.e. they are just for wax, spatulas and strips (if applicable) because the items were (or should have been) fairly consistent between systems. Items under “Other” included pre- and post-wax cleansers/treatments, gloves, aprons, tissues, couch roll, cotton wool, aftercare advice sheet, clinical waste disposal costs.

In order to place these costs in to context with typical earnings, HABIA also inform us that average gross weekly pay in the 2006 National Earnings Survey for “Beauticians and related occupations” was £269.30 per week for full time workers, or £7.05 per hour.

Table 1. Cost of full leg wax undertaken using various waxing treatments

<table>
<thead>
<tr>
<th></th>
<th>Hot wax (n=2)</th>
<th>Warm wax (n=6)</th>
<th>Cassette Phd (n=2)</th>
<th>Roller wax (n=5)</th>
<th>Sugaring* (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time (mins)</strong></td>
<td>60+</td>
<td>45</td>
<td>30-60</td>
<td>30+</td>
<td>90</td>
</tr>
<tr>
<td><strong>Consumables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Wax</td>
<td></td>
<td></td>
<td>1.5 tubes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b) Spatulas</td>
<td>50</td>
<td>24-40</td>
<td>NA</td>
<td>NA</td>
<td>40</td>
</tr>
<tr>
<td>c) Strips</td>
<td>NA</td>
<td>12-17</td>
<td>10-17</td>
<td>10-24</td>
<td>24</td>
</tr>
<tr>
<td>d) Other (please state)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Mean total cost of consumables</strong></td>
<td>£2.65</td>
<td>£2.55</td>
<td>£2.67</td>
<td>£3.28</td>
<td>£2.98</td>
</tr>
</tbody>
</table>

*For this technique, a paste made primarily of sugar is applied to the surface of the skin. The sugaring paste is then removed taking the hair along with it.

**The Industry-sourced data supplied confirm that use of a roller wax system would be £0.30p per treatment more expensive than sugaring, and £0.73p per treatment more expensive than the cheapest (warm wax) treatment listed in Table 1.
3.2 METHOD VALIDATION

The resulting counts for samples mixed with differing solvent concentrations are shown below (Fig 1). The results, although not a true standard curve, do show a loss of viability as the solvent concentration is increased. Data generated without the presence of wax are more consistent than the results with wax, but both show the same downward trend in bacterial viability as solvent concentration increases.

Figure 1. Recovery of seeded *Staphylococcus aureus* cells from the Babyliss Spring Clean™ solvent extraction system, with and without the presence of wax. NB. The Figure shows the log reduction (i.e. cell loss) from known numbers of seeded cells following a typical extraction procedure.

Based on the above data, a level of 10% solvent was chosen for subsequent test samples as it dissolved most types of wax and did not appear to affect the cell viability/count too markedly. Below this level of solvent the solubility of the waxes was incomplete.

The results of method sensitivity tests are given below in Figure 2:
Figure 2. Sensitivity of *Staphylococcus aureus* recovery from seeded extraction suspensions containing 10% Babyliss Spring Clean™. NB. 1 x 10⁷ seeded cells (neat) were diluted out and assessments made with and without the presence of grated sterile wax.

The assay was confirmed as sensitive enough to detect less than 10 colony forming units (CFU); evident from cell recovery even when the dilution factor of 10⁻⁷ was used. For example when ~3 x 10⁷ cells were used, 1 x 10⁷ cells were recovered from both the sample with wax + Babyliss Spring Clean solvent and the sample with the solvent but no wax. When the number of cells seeded in to the mix was reduced to a level in the order of 300, the cell recovery was between 170 – 150 CFU. It was also possible to detect 5-10 CFU from the sample when only ~30 CFU were seeded into the sample initially. These results show that if viable bacteria are freely released within the sample – as should be the case with a largely solubilised wax – then the proposed method is able to detect them.
### 3.3 TABLE 2. TEST SAMPLE RESULTS – PAIRED OR GROUPED COLOURS DENOTE RELATED SAMPLES RECEIVED IN THE SAME BATCH

<table>
<thead>
<tr>
<th>Lab number</th>
<th>HSL sample Number</th>
<th>Wax product description</th>
<th>Geographical source area</th>
<th>Detected CFU/g of wax</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>07414/08</td>
<td>Unused</td>
<td>Dagenham, Essex</td>
<td>Negative</td>
</tr>
<tr>
<td>W2</td>
<td>07413/08</td>
<td>Used</td>
<td>Dagenham, Essex</td>
<td>Negative</td>
</tr>
<tr>
<td>W3</td>
<td>07529/08</td>
<td>Unused</td>
<td>Sheffield, S. Yorks</td>
<td>Negative</td>
</tr>
<tr>
<td>W4</td>
<td>07528/08</td>
<td>Used</td>
<td>Sheffield, S. Yorks</td>
<td>Negative</td>
</tr>
<tr>
<td>W5</td>
<td>07493/08</td>
<td>Unused</td>
<td>Sheffield, S. Yorks</td>
<td>1.25 x 10^4 - not detectable on repeat**</td>
</tr>
<tr>
<td>W6</td>
<td>07492/08</td>
<td>Used</td>
<td>Sheffield, S. Yorks</td>
<td>Negative</td>
</tr>
<tr>
<td>W7</td>
<td>07763/08</td>
<td>Unused</td>
<td>Birmingham, West Mids</td>
<td>Negative</td>
</tr>
<tr>
<td>W8</td>
<td>07764/08</td>
<td>Used</td>
<td>Birmingham, West Mids</td>
<td>Negative</td>
</tr>
<tr>
<td>W9</td>
<td>07526/08</td>
<td>Used</td>
<td>Sheffield, S. Yorks</td>
<td>Negative</td>
</tr>
<tr>
<td>W10</td>
<td>07527/08</td>
<td>Unused</td>
<td>Sheffield, S. Yorks</td>
<td>Negative</td>
</tr>
<tr>
<td>W11</td>
<td>07817/08</td>
<td>Used</td>
<td>Beverley, Lincs</td>
<td>500 - not detectable on repeat**</td>
</tr>
<tr>
<td>W12</td>
<td>07818/08</td>
<td>Unused</td>
<td>Beverley, Lincs</td>
<td>500 - not detectable on repeat**</td>
</tr>
<tr>
<td>W13</td>
<td>07844/08</td>
<td>Unused</td>
<td>Buxton, Derbyshire</td>
<td>Negative</td>
</tr>
<tr>
<td>W14</td>
<td>07845/08</td>
<td>Used</td>
<td>Buxton, Derbyshire</td>
<td>Negative</td>
</tr>
<tr>
<td>W15</td>
<td>07846/08</td>
<td>Unused</td>
<td>Leeds, North Yorks</td>
<td>Negative</td>
</tr>
<tr>
<td>W16</td>
<td>07847/08</td>
<td>Used</td>
<td>Leeds, North Yorks</td>
<td>14,000 – this is an average value; repeat levels were lower**</td>
</tr>
<tr>
<td>W17</td>
<td>07838/08</td>
<td>Unused</td>
<td>Sowerby Bridge, West Yorks</td>
<td>Negative</td>
</tr>
<tr>
<td>W18</td>
<td>07839/08</td>
<td>Used</td>
<td>Sowerby Bridge, West Yorks</td>
<td>Negative</td>
</tr>
<tr>
<td>W19</td>
<td>07848/08</td>
<td>Unused</td>
<td>Birmingham, West Mids</td>
<td>Negative</td>
</tr>
<tr>
<td>W20</td>
<td>07849/08</td>
<td>Used</td>
<td>Birmingham, West Mids</td>
<td>500 - not detectable on repeat**</td>
</tr>
<tr>
<td>W21</td>
<td>07850/08</td>
<td>Unused</td>
<td>Birmingham, West Mids</td>
<td>Negative</td>
</tr>
<tr>
<td>W22</td>
<td>07851/08</td>
<td>Used</td>
<td>Birmingham, West Mids</td>
<td>Negative</td>
</tr>
<tr>
<td>W23</td>
<td>07852/08</td>
<td>Used</td>
<td>Birmingham, West Mids</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Secondary testing of these samples was undertaken to double check the initial test result. In each case the secondary analysis failed to repeat the first result, despite the fact that the initial result was clear and well supported by controls in place at the time. Possible reasons for this are discussed in section 4.**
3.4 FORMAL IDENTIFICATION OF CULTURED COLONIES

DNA samples from two distinct colony types were obtained from sample W16. Reliable, genus level identification was achieved for both colonies; one was found to be a *Bacillus* species (data suggested it was most likely *Bacillus simplex*), the other was confirmed as a *Staphylococcus* species; species from this group are commonly found on the skin and are related to the seeding organism used for method development. Colony morphology observations made for samples W11 and W12 confirmed that these samples also contained *Bacillus* species, which were detected during the initial analysis. These bacterial groups are common environmental contaminants and are likely to be present wherever human activity takes place. *Bacillus* species are capable of forming resting endospores, which can survive drying and starvation stresses and are associated with organic dusts, soil etc. Staphylococcal species are commonly associated with skin squames, and as such are often detectable in dusts from areas used for human activity, or on the skin surface itself.
4 DISCUSSION

A method was developed within the laboratory that can quantify and identify typical skin bacteria within a wax sample. Evaluation of the method on a variety of UK-sourced body waxes purchased by HSL demonstrated that the method could be effective for solid and softer waxes alike. Several types of sample were subsequently submitted for testing at HSL by salon businesses, either directly or via EHOs, and the majority of waxes were of the softer variety, with some more viscous than others. The nature of these samples was challenging, but the developed assay - which used a commercially available 10% solvent – allowed analysis of all samples to be performed. For a minority of the (hardest) wax samples tested, the method did not completely homogenise some solids, but the bulk of these samples were still successfully dissolved by the process. It was felt that this was sufficient to release bacteria and allow detection by plate count. We showed that it was possible to detect bacteria if present and that the assay was of high sensitivity. Once successfully released from the wax matrix it was shown possible to detect these contaminants down to less than <10 CFU per sample.

The samples obtained from salons did not routinely show high levels of contaminating microorganisms, with the majority of test (used) and control (unused) samples showing no evidence of microbial contamination. Two used samples and one unused (W11, W12 and W20) initially showed evidence of low numbers of detectable colonies from the least diluted (neat) plated samples. When counted colonies were used to provide a value for cfu per gram of wax, they indicated ~500 cfu/g for each of the three samples. This is low when put in to context, i.e. compared to the possible levels of microorganisms already present on healthy skin. These are normally present in at least the hundreds to thousands per sq. cm on dryer areas of the skin, but can colonise at much higher levels in the axilla (armpit), between the toes and in the groin. The types of bacteria detected here are consistent with environmental contamination, either from skin surfaces or from organic dusts present in areas where the waxes were previously stored and/or used.

It should also be noted that when samples generating these low, but positive microbial counts were re-tested, no detectable microorganisms were detected. This may have occurred for a number of reasons:

- The original count was at the limit of detection, since colony counts on the plate were low, and were then extrapolated to calculate a CFU value per gram of wax. Subsequent analysis may have failed to detect contaminants due to these generally low levels;
- In addition, it remains possible that some degree of clumping of contaminating organisms remained, despite thorough mixing of samples during the assay, and this may have caused sporadic detection trends. This would have increased the chances of ‘missing’ contaminants if their levels were already low; and
- A final possibility relates to the age of the samples: received samples were promptly analysed within 1 to 2 days of receipt, so any recently contaminating microbes were likely to still be viable. Repeat testing was, by necessity, performed some days later, once initial data had been assessed. This time delay is likely to have resulted in a decline in the viability of some contaminants and so contributed to a failure to detect them on repeat analysis. It should be noted that any viable microorganisms resident in wax would be unlikely to multiply, due to an absence of water availability or suitable nutrients.

Two additional samples initially showed high levels of microorganisms (in the thousands per gram) on first analysis, but on retesting some days later one of the samples showed no evidence of contamination (W5), and the other demonstrated a lower level of contamination (W16). It is
felt that these results may again have been attributable to one or more of the points discussed above.

The ability to detect such low numbers of microorganisms might also be achieved using a DNA method such as the polymerase chain reaction (PCR). However, standard PCR detects both live and dead cells, and use of this technique also adds considerably to analysis costs. The benefits of the PCR method may therefore be questionable under these circumstances.

The HABIA cost impact assessment provided background information for the front line cost and effort involved for common waxing procedures, and included data for sugaring, traditional waxing and cartridge based methods. The latter approach was seen as the most expensive, and this would be particularly relevant if the results from wax testing had shown large-scale bacterial contamination of used wax samples; this may have led to recommendations for businesses to change their way of working. From the data obtained here from wax testing we believe that this will not be the case, as no consistently high levels of microorganisms were detected in the ‘open pot’ waxes. Of the higher contamination levels detected, these were moderate at the point of first analysis, and did suggest significant environmental contamination of the wax. However, repeat testing of these samples also indicated that bacterial decline was rapid for these, and across the range of samples tested. Good practice techniques can act to minimise such contamination events, and would logically include correct use of spatulas, gloves, wax pot hygiene and other recommendations currently made by HABIA. These routine measures, along with use of high quality wax products and their sensible storage, should ensure that contamination opportunities are minimal, and risk to the client effectively controlled.
5  APPENDIX

Instructions for sending body wax samples to the Health and Safety Laboratory as part of the research study

Thank you for previously offering to send body wax samples to assist HSL’s study of waxing methods and good infection control. To learn more about this topic we are asking volunteers to provide us with small amounts of used and unused samples of their regular body wax product for analysis here at HSL. The findings will be confidential.

This study is for research purposes only and will not involve any action if your samples are found to contain microorganisms. What we will do, however, is inform you of the results if you provide contact details, and we will offer you supportive advice if we feel it is necessary.

The types of wax samples we need for the study are as follows:

- **Used body wax** (but NOT wax that has been on the client’s skin) – Please send us samples of used wax left in the pot after treatment, once you have finished with it. Please do not treat it in any special way. Just a small amount of wax (10 ml) is needed and this will be tested for any microorganisms that might have been transferred to it during routine waxing treatment;
- **Unused body wax** - we also need a small sample of unused wax as a control sample – this is for comparison with the used wax.

Please read the following information before you send any wax samples to us:

- Within this pack you will have received
  - 3 sterile pots (1 for used wax, 1 for unused wax and 1 spare);
  - A sealable bag; and
  - An SAE ‘Jiffy’ envelope for sending the samples back to us

PTO
How to obtain the samples:

• When you take the sample of used wax please do the following:
  o Open one of the sterile pots provided;
  o Wearing disposable gloves if you have them, scrape or pour at least 10 ml of warm, used wax in to the sample pot;
  o Screw the lid back firmly on the sample pot, and
  o Label the bottle ‘used wax’ with a biro or marker pen

• When you take the sample of unused wax please do the following:
  o Open one of the sterile sample pots provided;
  o Wearing plastic gloves if you have them, take a small sample of the unused wax either: (i) straight from the sachet if powdered or in pellet form; or (ii) if solid you could either scrape it or pour it warm (but unused) in to the sample pot;
  o Screw the lid back firmly on the sample pot, and
  o Label the bottle ‘unused wax’ with a biro or marker pen

A third pot is provided as a spare if, for example, you drop one of the other pots and need to repeat the sampling procedure.

Once the samples are in the pots please place the pots in the sealable bag provided and post them back to HSL in the pre-paid Jiffy bag. Please also be sure to complete and return the form below.

Please use the box below to enter your contact details if you wish to receive the results obtained for your samples. If you prefer to keep your business anonymous then we would still be grateful if you could enter (i) your geographical area, (ii) the name of the product you have supplied and (iii) for used samples, the number of client treatments the wax has been used on before sending to us. THEN PLEASE RETURN THIS COMPLETED FORM WITH THE SAMPLES.

Contact name or company name:........................................................................................................
Address of premises and/or email address:...........................................................................................
...............................................................................................................................................................
Product name:.....................................................................................................................................
If known, please indicate how many clients the supplied (used) wax was used on before sending to HSL:
.............................................................................................................................................................
For any further comment, e.g. on the way your wax is used: please use margin below..........................

THANK YOU FOR YOUR ASSISTANCE
6 REFERENCES


Identification of microbial contamination in body wax samples

Currently, apart from non-UK case studies and anecdotal evidence, there are few published data available regarding infection prevention and control during the process of body waxing. The techniques available for waxing different parts of the body do vary, and have received some attention from the healthcare and enforcement community. However, the specific infection risks posed by used or unused waxes have not, as yet, been fully or scientifically characterised, with most publications taking the form of case studies of patient aftercare.

A focussed, but representative microbiological sampling study of wax pot residues in salons was therefore undertaken to inform HSE on this area of treatment. In particular, the study was required to provide accurate advice that could be offered by HSE in the area of body waxing, and to inform existing guidance provided by the standard setting body for the hair, beauty, nail and spa industries.

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