The Use of Alternative (Non-Microbiological) Methods for Process Hygiene Monitoring and HACCP Verification in Red Meat Abattoirs

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Final Report

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Summary

The performance of rapid methods, alternative to the traditional microbial detection methods for monitoring hygiene in red meat abattoirs, was assessed both under experimental (laboratory and abattoir) and commercial conditions. The methods assessed were based on the detection of protein residues (Pro-tect and Flash sticks), total ATP (Hygiena Snapshot swabs) and porphoryn (a chlorophyll derivative present in the faecal material of animals fed on a diet rich in plant material; VerifEYE Solo). Microbiological testing (total aerobic viable count; TVC and *Enterobacteriaceae*) was also carried out on the carcasses and surfaces to provide a general background of hygiene against which the performance of the test methods could be assessed

An initial lab-based investigation was undertaken to assess each of the alternative methods under controlled conditions, and to identify any potential problems with the practical use of these methods. The VerifEYE Solo was found to be easy to operate. Optimal performance was observed at a distance of 16mm from the target surface. A range of surfaces found commonly within the abattoir were assessed for the ability to fluoresce as fluorescence can interfere with the detection of faeces by the machine. Objects such as abattoir wall cladding were found to fluoresce, and the background colour of the surface was shown to have an effect on the reading obtained by the VerifEYE Solo. Diluted faeces were more readily detected than faecal smears, particularly when the faecal smears had dried. Faecal spots as small as 0.3mm could be detected using the system. However, pig faeces did not fluoresce under the VerifEYE Solo system

The performance of the same detection methods were assessed under experimental abattoir conditions. Chlorophyll solutions were used to assess the practical effectiveness of the VerifEYE system. Chlorophyll "contamination" was tracked from the hides/fleece/skin of the cattle, sheep and pigs, to the resulting carcasses. TVC and numbers of *Enterobacteriaceae* were also determined from the coats and carcasses of the animals. Surfaces within the abattoir were also monitored using all the methods, including the traditional microbiological tests, both before and after routine cleaning. A correlation between the total ATP and both protein detection methods was observed from the environmental surfaces associated with slaughter of all three animal species. A correlation was also observed between the total ATP, Flash protein method and the TVC counts with the surfaces related to cattle slaughter. Transfer of bacteria from the coat/skin of the animal to the carcass was similar in the animals as the transfer of chlorophyll. Transferance of bacteria from coat to carcass was lowest for sheep when compared with the other two species. These findings are strongly indicative that monitoring of the transfer of bacteria to the carcass gives a good indication of the cleanliness of the dressing procedure for red meat animals. Under controlled conditions in a laboratory or experimental red meat plant, the detection of porphoryn (faeces) on sheep and cattle carcasses using the VerifEYE Solo and the detection of protein and total ATP on environmental surfaces were assessed favourably as monitors of process hygiene in red meat abattoirs

The final assessments were undertaken to examine the performance of the test methods on carcasses (cattle and sheep only) and surfaces (cattle, sheep and pigs) within commercial abattoirs. In order to carry these out, three commercial abattoirs were visited and carcasses sampled on four sites immediately prior to chilling. The surfaces were assessed both before and after routine cleaning. Assessment of the sheep carcasses indicated a potential correlation between the *Enterobacteriaceae* count and the VerifEYE Solo readings as both methods indicated the brisket area on the carcass to be the most frequently contaminated. No such correlation was observed when the data from the cattle carcasses was examined. The VerifEYE Solo readings from the carcasses were found to give a good overall indication of process hygiene as a whole with both cattle and sheep carcasses, but did give an indication as a potential use for assessing faecal contamination at individual sites for sheep carcasses

Analysis of the abattoir surface data indicated that the VerifEYE Solo did not detect significant levels of faecal contamination and therefore was not well suited to assessing the hygiene of surfaces. However, the other methods tested did show some strong relationships between each other when used to determine cleanliness of the surfaces, particularly in the sheep and pig plants.

Both protein detection methods used were able to detect that effective cleaning had taken place within the abattoirs, but it was felt that the Flash protein method would be better suited to the abattoir environment. Although more expensive, the measurement of total ATP to determine surface cleanliness was also thought to be a useful method within the abattoir to assess surface cleanliness

Overall, the results of these studies have indicated that the use of the VerifEYE Solo is a good method to measure overall carcass contamination within red meat abattoirs for cattle and sheep but has limited use for assessing surface cleanliness. The assessment of surface cleanliness in red meat abattoirs for all red meat species, was most cost-effectively achieved using either of the protein detection methods assessed

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Introduction

During slaughter and dressing of red meat animals, faecal contamination can be transferred to carcasses from a number of sources. These include the skin/hide of the animal, the gastrointestinal tract of the animal, and cross-contamination from environmental surfaces including processing equipment. In the UK there is a one in three chance that livestock-derived faecal material contains one of the five zoonotic agents (1) which cause over 80% of bacterial gastro-enteritis in humans

To aid hygienic meat production in red meat slaughterhouses, the Meat (HACCP) Regulations, were implemented in the UK in 2002. These regulations require that all red meat plants have a separate HACCP plan for the slaughter of each animal species and for any further processing that is carried out. In order to validate this HACCP plan, a number of carcasses and surfaces that have come into contact with carcasses during dressing, have to be sampled on a regular basis, and those samples tested to determine the total aerobic viable count (TVC) and numbers of *Enterobacteriaceae*. The carcass testing results give an indication of the process hygiene within the abattoir for each particular species

Testing using conventional lab-based microbiological methods are currently exclusively used, however the results can take several days to finalise. If results were available in real time, a more rapid response to processing problems would be possible. Another disadvantage of traditional microbiology is that the results are not always applicable to the smaller red meat abattoirs which sample on a less frequent basis and during a calendar year will sample less carcasses than larger plants. Partly as a response to these points, new regulations (H1-3) will be coming into force in various food establishments, including abattoirs, in January 2006. These new regulations will allow the use of rapid, non-microbiological sampling methods to be used to verify HACCP and give an indication of process hygiene, as long as these methods are shown to be suitable for use. The purpose of this study was to therefore evaluate rapid, non-microbiological methods for their suitability in determining process hygiene and validating HACCP in red meat slaughterhouses

Several rapid methods are currently in use in food processing plants. Commonly, such methods detect the presence of micro-organisms and/or food debris on food contact surfaces by detection of protein residues or ATP bioluminescence (2). Another rapid method being used extensively in the US meat industry is to detect chlorophyll and its breakdown products on carcasses by measuring an emission fluorescence after illumination at an appropriate excitation wavelength using the VerifEYE system (hand-held devices or a cabinet system). This detects emissions by chlorophyll a and its breakdown products at 675nm when excited by 420nm (3). Chlorophyll is broken down by the gut to fluorescent products which are present in the faeces of animals that have been fed a diet containing chlorophyll. Therefore any fluorescence detected on the carcasses indicates faecal, and hence possible bacterial, contamination.

In the UK rapid methods are not used extensively in red meat processing environments and the performance of non-traditional methods for the assessment of bacterial contamination is not known. Therefore this report will assess the performance of Flash protein sticks (Biocontrol, UK), Pro-tect protein swabs (Biotrace Fred Baker, UK), Hygiena Snapshot ATP swabs (Hygiena International Ltd, UK), and the VerifEYE Solo machine (Attec, UK), under laboratory conditions, in an experimental abattoir and under commercial abattoir conditions. The performance of these methods within the abattoirs will be assessed alongside the microbiological testing for total viable count (TVC) and *Enterobacteriaceae* currently carried out for HACCP verification

Objective 1: Experimental Evaluation of Alternative Methods for Carcasses and Surfaces

Task 1.1 – Experimental, laboratory-based evaluation of the rapid methods

Materials and Methods

Operation of the VerifEYE Solo

Operator familiarity with the VerifEYE Solo was established using the control solution provided by the manufacturer and cattle faeces, collected from fields where animals were grazing. The machine was used according to manufacturer's instructions. The machine was used only under fluorescent strip lighting

Operation of the ProTect protein detection system

The ProTect protein residue detection system (Biotrace, Bridgend, UK) was used according to manufacture's instructions. The swab end was pushed to force it through the tube membrane and the swab was moistened in detection chemical before use. The swab was used to test environmental surfaces. Typically, an area of $20cm^2$ was tested. After swabbing the surface, the swab was returned to the ProTect tube. After 5 min incubation, the colour of the detection chemical was inspected. A 5 point scale was used to quantify the colour change. Clean surfaces were scored as 0 (green colour), mildly soiled surfaces as 2 (grey colour) and soiled surfaces (4) as purple

Operation of the Flash Stick protein detection system

Flash sticks (Biocontrol, UK) were also used according to manufacturer's instructions. Briefly, the sticks were moistened from a sponge soaked in detection reagent before being used to swab an environmental surface. The percentage of the stick's tip that changed colour from yellow to blue was used to estimate surface contamination with protein residue. An integer-only, 5 point scale from 0 (100% yellow) to 4 (100% blue) with a value of 1 being added for each 20% of the surface that turned blue was used to assess the extent of protein soiling

Operation of the Snapshot ATP bioluminescence system

The Snapshot ATP detection system (Hygiena International, Watford, UK) was also used according to manufacturer's instructions. The end of the Snapshot swabs were broken allowing the ATP detection buffer to moisten the cotton end of the swab. Swabs were used to sample a $20cm^2$ area of environmental surface. After swabbing a surface, the swab was repeatedly run across the inside of a Snapshot cuvette. The bioluminescence generated by the Snapshot cuvette was quantified using the Snapshot luminometer.. The output from the luminometer was a digital reading between 0 and 999 relative light units (RLU). Typically, 1 hour elapsed between swabbing an environmental surface and activating the cuvette with the swab

Light level determination

Light levels for each experiment were measured as Exposure Value (EV) using a photographic exposure meter (D3B; Jessop, Leicester, UK), and EV values were converted into lux using the following equation:

$$\text{Lux} = 2^{EV}$$

a. Determination of the fluorescent properties of a range of materials and objects

A range of locally-sourced materials and objects were examined for fluorescence using the VerifEYE Solo (Table 1). Descriptions are provided in Table 1, with additional descriptive data below

Prepared concrete slabs coated with two different shades of grey floor paint, were used to simulate abattoir flooring. Sponge rollers were used to apply three coats of Ground Work Floor Paint (grey) (Plascon International Ltd., Winchester, UK) or two coats of Krylon Heavy Duty Floor Paint (slate) (Ronseal Ltd., Sheffield, UK) onto concrete slabs purchased from a garden centre. After the paint dried, the grey surface appeared a lighter colour than the slate surface to the naked eye

Polyvinylchloride extruded polymer wall panelling pieces in white colour and satin finish (Altro Whiterock grade W103/W104; Rudge Bros and James Bristol, UK) were obtained from a commercial abattoir. This panelling is common in UK abattoirs, and it includes an integral zinc-based biocide, AltroSan

High-gloss black or blue tiles, suitable for domestic use, were purchased from a local tile warehouse retail outlet

b. Detection limit of pure chlorophyll

Chlorophyll a (Sigma, Gillingham, UK) and chlorophyll b were dissolved individually in chloroform (BDH, Poole, UK) to produce a 10 µg μl^{-1} solution of each. Volumes (100 µl) of suitable tenfold dilutions, prepared in chloroform, were spotted onto a matt white plastic surface and a matt black plastic surface. The detection limits were determined using the VerifEYE Solo under bright light and dim light conditions

c. Detection limit of livestock faeces

Tissue samples (muscle, fat, pig skin) were obtained from slaughterlines before carcasses entered the chiller. Livestock coats (hide or fleece) were obtained simultaneously, from after de-pelting or de-hiding. Livestock faeces were obtained either from abattoir lairage areas at the same time as tissue samples were collected, or were picked up from fields where animals were grazing. Tissue and faecal samples were transported to the laboratory at ambient temperature. Tissue samples were used immediately, while faeces samples were used immediately for most studies, or were stored for up to one week

Well-mixed livestock faeces were diluted tenfold in HPLC water (Fisher, Loughborough, UK). Volumes (100 µl) were spotted onto tissue, skin and coat samples and onto inanimate surfaces (painted concrete slabs, black and blue tiles). The well-mixed faeces was also smeared onto the same surfaces. The detection limits for the faeces on the tissues and surfaces were determined using the VerifEYE Solo (at different sensitivity settings). The ability to detect faeces on environmental surfaces was also assessed using the Pro-tect, Flash Stick and total ATP bioluminescent systems

d. Effect of drying faeces on detection ability of VerifEYE Solo

The effect of drying at temperatures appropriate to the surface on the detection ability of the machine was examined. Meat and skin samples, with faecal spots in place, were stored at 4ºC for up to 24 h, while coat samples and inanimate surfaces, with faecal spots in place, were stored at ambient temperature for up to 24 h. The results of viewing the faeces and dilutions both before and after drying were compared

A second sample of fresh (wet) cattle faeces and suitable dilutions, were spotted onto the painted concrete surfaces, viewed, left to dry for 24 h, and viewed again. In addition, the same faeces and dilutions, which had been stored overnight at 4ºC, were again spotted onto the surfaces enabling us to compare, at the same time, both wet and dry faeces derived from the same sample

e. Effect of varying the proximity of VerifEYE Solo to the target surface on detection of faeces

The effect of VerifEYE Solo proximity to the faecal material being detected was examined using well-mixed cattle faeces, diluted in HPLC water, and smeared or spotted in 100 µl volumes, as described above, onto a light green work bench surface. The machine was held at distances ranging from 16 mm to 97 mm away from the bench surface, and the fluorescent properties of the faecal spots were viewed

f. Estimation of size limit of faecal spot detectable by VerifEYE Solo

Fresh cattle faeces was spotted in small quantities onto five substrates; glass microscope slide, a matt white plastic surface, beef cut muscle tissue, beef muscle + membrane and beef fat. The beef tissues originated from a retail sample of beef. The size of visibly fluorescing spots was estimated using an eyepiece graticule and stage micrometer in a microscope (Leitz Dialux 20, Leica, Milton Keynes, UK) with 4 x magnification objective and 6.3 x magnification eypiece. Standard microscope sub-stage illumination was used for transparent substrates, while non-transparent substrates were lit from above with 2 x 200 W halogen lamps

Results and Discussion

a. Determination of the fluorescent properties of a range of materials and objects

Under light conditions prevailing during the current study, enhanced processing and sensitivity setting 003 gave the most appropriate result with the control solution provided by the manufacturer, under both high (1000 + lux) and lower (<550 lux) light levels. When the machine was set on normal processing, the control solution was not able to be detected evenly and consistently via the view-screen. The machine settings were checked each time the VerifEYE Solo was used, and altered if necessary as recommended by the manufacturer. However, we frequently found enhanced processing with sensitivity setting of 003 were the most suitable settings under the lighting conditions used

The fluorescent properties of a range of materials and objects was determined using the VerifEYE Solo (Table 1). The abattoir wall cladding fluoresced strongly, while livestock faeces smeared on the surface of this material did not fluoresce, but appeared black in the viewer screen

Shiny materials and objects can reflect light into the instrument, and this reflectance can be viewed on-screen as fluorescence. Metal and glossy plastics regularly appear to fluoresce, and this can be difficult to differentiate from the fluorescence caused by cattle faeces. However, in some cases, turning the objects did aid with differentiation of background fluorescence. Although white wellington boots and hard hats did not in themselves fluoresce, they are shiny objects which also reflected light into the machine, and which were easily be confused with faecal contamination

Surfaces (laboratory benches, floors or walls) did not fluoresce, with the exception of portions of the laboratory safety signs

Green plant tissue, including grass and weeds, fluoresced strongly. However yellowed leaves either fluoresced strongly or not at all, while none of the red leaves examined fluoresced. Dark green leaves appeared to fluoresce less strongly than light green, visually brighter leaves

b. Detection limit of pure chlorophyll

Both pure forms of chlorophyll, a and b, could be detected with the VerifEYE Solo. The material was examined in two light levels (approximately 256 lux and 4096 lux). The light levels did not affect the ability of the VerifEYE Solo to detect either type of chlorophyll. On a white surface, dissolved chlorophyll (100 µl volumes) was detected at concentrations of 0.001 and 0.01 mg ml^{-1} for chlorophyll a and b respectively, but on the black surface detection limits were 10 x lower (Table 2). Therefore, the background surface colour itself can affect detection of chlorophyll by the VerifEYE Solo. In addition, chlorophyll a could be detected at ten times lower concentrations than chlorophyll b on both surfaces examined (Table 2). Once the solvent had evaporated, the remaining dry spots of pure chlorophyll could not be detected by the VerifEYE Solo

c. Detection limit of livestock faeces

The ability of the machine to detect solid smears of cattle faeces and diluted faeces on a range of beef and other surfaces is shown (Table 3). In this part of the study, three enhanced processing levels were used (decreasing sensitivities from 001, 003 to 005), although setting 003 was the most appropriate. On edible beef tissues, we detected faeces at concentrations ranging from 10 to 100 mg faeces ml^{-1} (Table 3). Clearly, decreasing the sensitivity to setting 005 resulted in reduced faecal detection rates (Table 3). Increasing the sensitivity to 001 was also inappropriate for the light levels used, as some parts of the beef fat tissue fluoresced even though no obvious faecal contamination was visible. At this setting, it was difficult to discriminate between the deliberate contamination with faecal spots and smears and background fluorescence from the fat tissue

During this part of the study, it became apparent that faeces smears were sometimes more difficult to discern than diluted faeces, even when both were clearly visible to the naked eye. The smears frequently exhibited a streaky luminous appearance on-screen, whereas diluted faeces, placed next to it on the same surface, was brightly and evenly luminous on-screen. This colour of the faeces smear or spot compared with the background colour also impacted on our ability to detect faeces smears and spots. We observed that cattle faeces were dark in appearance, and we had more difficulty discerning faecal smears with the VerifEYE Solo when the tissue or background surface was also dark-coloured. This occurred regularly, even when the machine sensitivity was optimal (003) for the lighting. We also observed that faeces diluted in HPLC water to contain 1 g or 0.1 g faeces ml^{-1} appeared brighter in colour to the naked eye than the solid faeces

In practice, during carcass dressing, faeces could arrive on carcasses by smearing (e.g. from animals' coats or gastrointestinal tracts), or via splashing from faecally-contaminated water. Direct spots of faecal contamination and smears from animals' coats may be more likely occurrences than water splashing during dressing of cattle and sheep carcasses

d. Effect of drying faeces on detection ability of VerifEYE Solo

After drying (4ºC for 24 h for beef samples, and ambient temperature for 24 h for other surfaces) the 100 µl spots of diluted faeces were dry, but the residues of spots containing 10 mg faeces ml^{-1} or more could still be seen with the naked eye. The drying regime used clearly affected the detection limits, and this was more noticeable when the machine was used on non-optimal sensitivity settings (001 and 005). Under optimal processing with sensitivity setting 003, drying at 4ºC for 24 h did not affect detection of the faecal spots on beef cut muscle or on beef fat ; detection limits before and after drying were 100 and 10 mg ml^{-1} respectively on these tissues (Table 3). In the case of beef membrane, the lowest concentration detected after chill drying was 10 mg ml^{-1}, a lower concentration that when faeces were not dried (Table 3). Under sensitivity setting 003, the faecal spots on inanimate surfaces were generally less visible than they had been before drying (Table 3)

When the machine sensitivity was set too high (001), the ability to visualise faecal material on fomite surfaces (concrete, tiles, hides) was not affected by 24 h drying. In contrast, the faecal material generally became easier to detect on meat and fat surfaces after 24 h drying

Both wet and dry faecal smears could be detected on clean and homogeneously painted concrete surfaces. However, dried faecal smears appeared quite "grainy" in the viewer, whereas the wet faeces smears were more luminously green (Table 4)

Both samples of cattle faeces used in this study were detectable at 1 mg faeces ml^{-1} on the grey-coloured surface (Tables 3 and 4), but the first sample of cattle faeces used was detectable only at 10 x higher concentration on the slate-coloured surface (Table 3)

e. Effect of varying the proximity of VerifEYE Solo to the target surface on detection of faeces

The distance of the VerifEYE Solo to the target surface greatly affected our ability to detect faeces smears and spots of diluted faeces on a workbench. We detected more of the faeces smears and spots when the machine was held 16mm distance from the bench than when it was held at any other distance (Table 5). Moving the machine closer than approximately 16 mm had a deleterious effect, and we had difficulty detecting faeces at these very proximal distances. On the other hand, faeces smears were consistently detected when the machine was at any distance between 16 to 81 mm away from the target surface, although at 64 to 81 mm distance, the images appeared grainy (Table 5)

The impact that distance from target surface has on visible detection limits could affect the way the machine is used in slaughterhouses. On the slaughterline, carcasses can vary in dimensions, so an immovable static machine may not be optimal for consistent faeces detection. However, as the VerifEYE Solo is moved away from the target surface, the on-screen image increases in size, even though the sensitivity of detection reduces. In practice, this image size increase may be beneficial under some circumstances on slaughterlines

f. Estimation of size limit of faecal spot detectable by VerifEYE Solo

Cattle faeces spots could not be produced any smaller than approximately 0.3 mm diameter, due to the viscous nature of the material. Spots of this size could be detected with the VerifEYE Solo. These faecal spots were also easily detectable to the naked eye on the smooth and uniformly-coloured surfaces used (glass microscope slide, white plastic beef muscle, beef fat, beef membrane). However, we believe that faecal contamination of this size on moving carcasses on the slaughterline would be difficult to see with the naked eye

g. Effects of liquid overlaying faeces smeared on surfaces on detection by VerifEYE Solo

Tap water, HPLC water and clear liquid solvents did not fluoresce, although liquid surfaces can also reflect light into the machine, and the resultant fluorescence may be confusing to operators. However, when droplets of moisture were turned and placed under the machine at different angles, it was possible to differentiate between clear liquids and cattle faeces

A variety of surfaces were used to determine the effects of overlaying fresh and dried cattle faeces with tap water. The surfaces examined were beef tissue covered with membrane, beef subcutaneous fat, grey-painted concrete and slate-painted concrete. Overlaying dried faecal smears or dried drops of diluted faeces with tap water did not affect the ability of the VerifEYE Solo to detect these faeces spots, as those which had been visible with the machine before being overlaid with water remained visible after water was deposited on top of them

Overlaying dried faecal smears with Meat Hygiene Service marking ink interfered with the ability of the VerifEYE Solo to detect these faeces spots. We could not detect any faeces under a flood of dark-coloured MHS ink

Cattle faeces were smeared onto stainless steel surfaces and submerged in 82ºC hot water for 30 seconds, 1 minute and 5 minutes. The hot water sterilisation treatment did not have any effect on the fluorescence of the faecal material, which remained as visible post-heat treatment as it had been prior to heat treatment

Conclusions

- VerifEYE Solo is easy to optimise and use according to the manufacturer's instructions
- Shiny materials, including liquids and solid objects can reflect light into the instrument, which can be mistaken as fluorescence
- Some common objects and materials fluoresce, including abattoir wall cladding, white paper, green plants and some livestock feeds
- The limit of detection of pure chlorophyll was much lower than cattle faeces
- The colour of the background surface can affect the detection ability of the machine
- Faecal smears were more difficult to detect than diluted faeces
- Dry faecal smears were sometimes more difficult to detect than fresh faecal smears, but this depended on the background substrate
- The proximity of the VerifEYE Solo greatly affected detection faeces on a workbench surface; 16 mm from this target surface appeared optimal
- Tiny spots of fresh faeces, ~ 0.3 mm diameter, could be detected
- Faeces covered in water could be detected
- Faeces covered in wet MHS ink could not be detected
- Faeces could be detected after hot water sterilisation treatment
- Pig faeces did not fluoresce – presumably the livestock had not been consuming a diet containing green plant tissue

Task 1.2 – Experimental, abattoir-based evaluation of the alternative methods

Aim

Three studies were carried out in a single low throughput abattoir in the South-West of England to evaluate the effectiveness of non-microbiological methods for the assessment of carcass and surface contamination during the dressing of cattle, sheep and pigs inoculated with chlorophyll, against the currently used microbiological methods

Materials and Methods

Sample collection

Three each of cattle, sheep and pigs were painted on the brisket or belly, with a 50µg/ml solution containing chlorophyll a and b (50% of each), using a paint-brush method. Immediately after bleeding out, but before removal of the hide/fleece/entry into the scald tank, the cattle, sheep and pigs were examined on the rump, flank, brisket; brisket, breast, neck and lateral thorax, and the neck, ham, back, belly and jowl, respectively, using a hand-held version of the VerifEYE solo machine (Attec, UK) to detect the presence of chlorophyll. The same four sites on each animal species were sampled for *Enterobacteriaceae* and total viable count (TVC) by excising an area of hide/fleece from the cattle and sheep, and a piece of skin from the pigs ($5cm^2$), using aseptic techniques, and placing into sterile stomacher bags. The animals were dressed using conventional techniques and the carcasses tested on the same sites as above using the VerifEYE Solo machine and the excision method

Six environmental surfaces were collected after the completion of dressing of each species, these being: the roll-out ramp, beef pram, flayer, knife, apron wash and the splitting saw, for cattle, and: the sheep dressing cradle (x2), knife, apron wash, splitting saw, and slaughtermans apron, for sheep, and the dehairing machine (inside and outside), the polishing table, knife, apron wash and splitting saw, for pigs. Surfaces were sampled using: a wet/dry swabbing method ($20cm^2$ area) to determine *Enterobacteriaceae* and TVC, two protein detection methods - Flash sticks (Biocontrol, UK), and Pro-tect swabs (Biotrace Fred Baker, UK), Hygiena snapshot total ATP swabs (Hygiena International Ltd, UK), and the VerifEYE Solo machine (Attec, UK)

After routine cleaning of the abattoir but before the start of slaughter the following day, the same six environmental surfaces for each species were sampled as outlined above

Flash protein stick method

A pair of latex gloves were put on and the outside disinfected with an alcohol wipe. An alcohol wipe was then used to disinfect a $20cm^2$ template, which was then placed on the area to be sampled. A Flash protein stick was removed from the container and the blue reactive end pressed firmly onto the hydrating pad, until the entire surface of the stick changed from blue to yellow. This end was then used to swab the templated surface in the horizontal, vertical and diagonal directions. The end of the stick was then examined immediately and the degree of colour change back to blue, scored from 0-4 (no change in colour to complete change)

Pro-tect protein swabs
The outside of the latex gloves were disinfected with an alcohol wipe. An alcohol wipe was then used to disinfect a 20cm^2 template, which was then placed on the area to be sampled. A Pro-tect swab was removed from the protective tube. The end of the swab was wetted by dipping into the detection chemical in the tube and then the templated area was swabbed horizontally, vertically and diagonally. The swab was pushed firmly into the protective tube until it reached the detection chemical in the bottom. The swab was shaken in the liquid for 10 seconds and then left for 10 minutes to react. After this time, the colour of the liquid was examined and compared to that noted on the side of the swab. The colour change was then given a score of 0-3. (green = 0, grey = 1, light purple = 2, dark purple = 3)

Hygiena Snapshot total ATP swabs
The outside of the latex gloves were disinfected with an alcohol wipe. An alcohol wipe was then used to disinfect a 20cm^2 template, which was then placed on the area to be sampled. An ATP swab (already pre-wetted) was removed from the protective tube and used to swab the templated area horizontally, vertically and diagonally. The swab was carefully replaced into the protective tube and placed into a pot covered with tin foil. The swabs were then transported back to the laboratory for further processing

VerifEYE Solo machine
The hand held device was used to determine chlorophyll on the coat of the animals and on the carcass. The device was used according to manufacturers instructions under good lighting conditions, on setting 003. Care was taken to hold the device at the correct distance from the surfaces being examined. The degree and amount of fluorescence was examined visually by the operator for each sample site and a score of 0-4 recorded (0 = absence, 4 = bright fluorescence in almost all the area tested)

Wet/dry swabbing
The outside of the latex gloves were disinfected with an alcohol wipe. An alcohol wipe was then used to disinfect a 20cm^2 template, which was then placed on the area to be sampled (abattoir surface samples). A jumbo cotton swab (Sterilab Services, UK) was moistened in maximum recovery diluent (MRD, Oxoid, UK) and used to swab the templated area horizontally, vertically and diagonally. The swab was rotated between the thumb and index finger during swabbing. After sampling the area, the template was kept in the same area and the swab was broken off into a pot containing 10ml MRD. A second, dry jumbo swab was then rubbed over the same area as above, using the same technique. This was then broken off into the same pot of MRD as the previous swab. Swabs were transported to the laboratory and stored under chilled conditions until processed

Laboratory analysis
To the excised pieces of hide/fleece and carcass, 25ml of Maximum Recovery Diluent (MRD; Oxoid, UK) was added and the sample stomached for 2 min. The universals containing the wet/dry swabs and 10ml MRD were vortexed for 1 min. All samples were further serially diluted in MRD and plated onto VRBG agar (Oxoid, UK) for enumeration of *Enterobacteriaceae*, and PCA (Oxoid, UK) for enumeration of TVC, using the standard ISO pour plate methods (ISO 4833:1991 and ISO 5552:1997)

The Hygiena snapshot total ATP swabs were further processed by turning the swab upside down, snapping the valve, then carefully turning the swab back the other way. The swab was removed from the tube and put into a cuvette (supplied with the swabs). The bulb on the swab was squeezed to expel the liquid and the swab rotated in the liquid for 10 seconds. The swab was then removed from the cuvette and the cuvette capped. The capped cuvette was placed into a luminometer and the light output read as Relative Light Units (RLU) from the display

Analysis of results

The counts of *Enterobacteriaceae* and TVC were calculated for the hide/fleece/skin, carcass, and environmental swab samples, to determine Log_{10} CFU/cm^2. For the two protein detection methods a score of between 0-4 was assigned to each sample depending on the degree of colour change, with 4 being the strongest. A score of 0-4 was also assigned to each sample examined using the VerifEYE solo machine, depending on the degree and extent of fluorescence found on the area (with 4 being the highest degree of fluorescence over the maximum area; subjective). The reading obtained on the luminometer from the total ATP swabs taken was displayed as Relative Light Units (RLU)

Results and Discussion

Hide/fleece/skin and carcasses

The data obtained from the carcasses of cattle, sheep and pigs was calculated to show the difference in both microbial counts and chlorophyll levels between the hide/fleece/skin and the carcass (Figure 1). The average TVC count (Log_{10} CFU/cm^2, from four sampled areas of each species) obtained from cattle hide, sheep fleece and pig skin was similar (Log_{10} 5.2, 5.8 and 5.3, respectively). However, the same count detected on the finished carcasses was varied, with that obtained from the pig carcasses being the highest and that obtained from the sheep carcasses being the lowest i.e. 3.5 Log_{10} for pigs, 1.8 for sheep and 2.8 for cattle. When the difference between the carcass TVC count and the hide/fleece/skin TVC count was calculated for each species, the difference was higher with the sheep followed by the cattle (Figure 1). This indicates that the transfer of bacteria from the fleece to the carcass of sheep was lower than with either cattle or pigs. When the difference between the carcass and the hide/fleece/skin *Enterobacteriaceae* count was calculated, the levels for all animal species were found to be similar, indicating a similar transfer of this bacterial group to the resulting carcasses (cattle: 1.41 Log_{10}, sheep: 1.43 Log_{10}, pigs: 1.23 Log_{10}). The average level of chlorophyll detected on the hide/fleece/skin and carcasses of cattle and pigs varied greatly (cattle: 2.9 and 0.9, sheep: 3.0 and 2.1, pigs:1.1 and 0.2). The transfer of chlorophyll from the hide/fleece/skin to the carcass in the three species showed that the transfer of chlorophyll in pigs and sheep was the same (0.9), but was found to be greater in cattle (2.0) (Figure 1). The difference in the level of chlorophyll detected on the hide/skin and carcasses from cattle showed a similar level as that obtained with the TVC count, and the corresponding difference in pigs, showed a similar level as obtained with the *Enterobacteriaceae* count. The corresponding difference in sheep was not similar to either the TVC or *Enterobacteriaceae* count

The results obtained indicate that the transfer of contamination from the hide/fleece/skin to the carcass was less with sheep and cattle dressing than with pig dressing, potentially indicating better process hygiene during the dressing of sheep and cattle in this abattoir. The results obtained also indicate that the detection of chlorophyll on carcasses may be a good indication of process hygiene, particularly in terms of the total bacterial load on cattle carcasses and bacteria from faecal origin on pig carcasses

Surfaces

The environmental surfaces were examined pre- and post-cleaning to determine the effect of cleaning at reducing the levels of bacteria and visible contamination (Tables 13, 14 and 15). With the cattle surfaces on average a 2 fold decrease in TVC count was observed which was also detected with the total ATP method and the Flash protein method (Table 1). Higher fold reductions were observed with the *Enterobacteriaceae* count (15 fold) and the Pro-tect protein method (8 fold). However, the chlorophyll levels detected using the VerifEYE solo machine showed no reduction between pre-and post-cleaning (Table13)

The reductions observed for the total ATP, Pro-tect protein swabs and the Flash protein method for the sheep environmental surfaces were similar (4-fold, 2-fold and 3-fold, respectively) (Table 14). The chlorophyll levels detected post-cleaning were very slightly higher than those detected pre-clean, showing an increase in chlorophyll detected rather than a reduction (Table 14). The reduction in TVC count observed post-clean was the highest observed with all three animal species (20-fold). There was a slight reduction in the count of *Enterobacteriaceae* observed (0.2 to 0), but due to the nature of the calculation method, was shown as zero (Table 14)

The reductions observed with the *Enterobacteriaceae* count from the surfaces sampled after pig slaughter were similar to those observed with the total ATP method (4-fold, Table 15). Both of the protein detection methods used showed a 2-fold reduction in contamination after the surfaces had been cleaned (Table 15). The chlorophyll levels detected post-cleaning were higher than those detected pre-cleaning, and hence a reduction level was not obtained using this method (Table 15). The 15-fold reduction in TVC count observed post-cleaning was greater than the reduction detected with any of the other methods examined

The results obtained for the environmental surfaces indicate a potential correlation between the measured total ATP method and at least one of the protein detection methods, for the surfaces from all three species. A potential correlation was also indicated between the measured total ATP, the Flash protein method, and TVC count with surfaces related to cattle slaughter. A similar correlation between the detection of microbial ATP and TVC from the surfaces of beef carcasses has already been found (4). Therefore, the detection of total ATP and protein may have a potential use to assess the effectiveness of surfaces cleaning in abattoirs

Conclusions

- The transfer of bacteria (TVC) from coat to carcass was lowest with sheep
- The transfer of *Enterobacteriaceae* from coat to carcass was similar with all three animal species
- The transfer of chlorophyll from coat to carcass was similar in pigs and sheep but higher in cattle
- Transfer of chlorophyll from hide to carcass in cattle was similar to the transfer of bacteria (TVC)
- Transfer of chlorophyll from skin to carcass in pigs was similar to the transfer of *Enterobacteriaceae*
- Transfer of contamination from coat to carcass of animals gave a good indication of process hygiene
- A potential correlation between total ATP and the protein detection methods was found for the surfaces tested when all three animal species were slaughtered
- A potential correlation between the total ATP method, Flash protein method and TVC was observed for surfaces related to cattle slaughter

- Overall the results indicate that the detection of chlorophyll on carcasses from some red meat species, and methods to detect protein and total ATP on surfaces may have a potential for use in the assessment of abattoir process hygiene

Objective 2: Validation of the Alternative Methods under Commercial Conditions

Aim

The alternative methods already examined under both laboratory and experimental abattoir conditions, will be assessed under commercial abattoir conditions to determine their ability to assess process hygiene. Commercial validation of carcasses will be carried out in a cattle and sheep abattoir only, and validation of surfaces will be carried out in cattle, sheep and pig abattoirs

Materials and Methods

Carcass validation

Sixty each of cattle and sheep carcasses were sampled from one abattoir processing cattle and one abattoir processing sheep in the South-West of England. Once bled, but before skinning each animal was examined and allocated a cleanliness score (1-5), based on the MHS scoring system. The animals were then dressed using conventional methods. Each carcass was sampled at four different sites after inspection and before entering the chiller using the excision method detailed in **Objective 1, Task 1.2.** Sites sampled on the carcasses for cattle were rump, flank, brisket, and neck and those sampled from the sheep were flank, breast, brisket, and lateral thorax. Each piece of excised tissue was placed into a sterile, labelled stomacher bag and stored in a cool box for transportation back to the laboratory

Each carcass sampled above was also examined at the same four sites, adjacent to the excised area, using the VerifEYE Solo device. The VerifEYE Solo was used under good lighting conditions and operated as setting 003. The amount and degree of fluorescence was scored between 0-4 (0 = absent, 4 = very high), subjectively, by the operator

Surface validation

Three commercial plants; which processed cattle, sheep and pig respectively were visited at the end of the day's processing but before the start of cleaning. The same plants were visited the next day after cleaning had occurred but before the start of the day's processing. Environmental surfaces (15) such as those which would be expected to come into contact with food as well as large plant objects such as chiller doors were sampled during both visits. All of the surfaces sampled were appropriate targets for sampling and testing for plants to ensure that their HACCP scheme pre-requisites were in place. Surfaces were sampled using the Snapshot system to determine surface levels of ATP as described above. Protein residues on surfaces were assessed using the Pro-tect and Flash stick systems. For comparison, total aerobic bacterial and *Enterobacteriaceae* numbers on surfaces were also determined using a wet-dry swabbing method as described for **Objective 1, Task 1.2**. The VerifEYE solo was used to determine if faecal contamination was present on surfaces in the sheep and cattle plants only

Laboratory analysis

Total aerobic viable counts (TVC) and *Enterobacteriaceae* counts were determined for both carcass and surface samples by standard plate count methods according to the criteria specified by ISO 4833:1991 (10) and ISO 5552:1997 (11), respectively. Briefly, microbiological analyses involved the addition of 25ml of peptone saline [10g protease peptone, 5g NaCl, 9g $Na_2HPO_4.12H_2O$, 1.5g KH_2PO_4, to 1000ml] to each stomacher bag containing excised slivers of tissue, followed by homogenisation for 1 minute using a stomacher (Model number BA 6021, Seward, UK). The containers containing the swabs were vigorously vortexed (Model SGP 202 O1OJ Fisons, Ipswich,

UK) for 1 min. Each sample homogenate (excision or swab) was then diluted decimally in peptone saline and 1 ml aliquots added to appropriately labelled Petri dishes. For TVC, 15 ml of tempered (46°C) PCA [Oxoid CM325] was added to each Petri dish, mixed and allowed to harden. For *Enterobacteriaceae*, 15 ml of tempered (46°C) VRBGA [Oxoid CM485] was added, mixed and allowed to harden as before. TVC were incubated at 30^{o}C for 72h before colonies were counted. *Enterobacteriaceae* were incubated at 37^{o}C for 24h. Confirmation of *Enterobacteriaceae* was by oxidase testing and the ability to metabolise glucose. Bacterial numbers on decimally-diluted plates were converted into CFU cm^{-1} according to the criteria described by ISO 6887-1:1999 (12)

Results and Discussion

Carcass validation

Two different plants were visited (one each for cattle and sheep), three times each, to collect samples from 60 each of cattle and sheep (4 sites per animal, processed separately). The average *Enterobacteriaceae*, TVC and VerifEYE results for each site and both animal species is shown in Table 16

For the sheep carcasses the average *Enterobacteriaceae* count ranged from Log 1.35-2.15, the average TVC count ranged from Log 4.84-7.22, and the average VerifEYE reading ranged from 0.6-3.4 (Table 16). The highest average *Enterobacteriaceae* count was found on the brisket and the highest average TVC count was found on the flank. The highest VerifEYE Solo reading was found on the brisket of the carcass, which correlated with the highest *Enterobacteriaceae* count. When the data collected was examined, it was found that the site which showed the most contamination (TVC, *Enterobacteriaceae* and VerifEYE) most frequently, was also the brisket site. The brisket area of these sheep is where the first cut is made to remove the fleece and it is well documented that the cut line areas are the most likely areas on the carcass to become contaminated (5). The correlation between the *Enterobacteriaceae* counts and the VerifEYE readings observed may not be a true correlation as the VerifEYE readings are subjective and dependant on the operator, which varied between two trained personnel during the study. However, it is more likely that the VerifEYE readings would correlate with the *Enterobacteriaceae* rather than the TVC count as the *Enterobacteriaceae* counts give an indication of the faecal contamination on the carcass, which the VerifEYE system is designed to measure

For the cattle carcasses the average *Enterobacteriaceae* count ranged from 0-Log 2.88, the average TVC count ranged from Log 3.24-4.19, and the average VerifEYE reading ranged from 0.02-0.7 (Table 16). The highest average *Enterobacteriaceae* count and TVC count was found on the neck area of the carcasses. This may be due to bacteria present running down the carcass and collecting at the neck one the carcass is washed due to the carcass being hung by the back leg. The highest average VerifEYE results were seen at the rump site of the carcass, which did not correlate with the counts from either of the bacterial species examined for. When the data was examined, it was found that the sites which showed the most contamination (TVC, *Enterobacteriaceae* and VerifEYE) most frequently, was the brisket site for TVC and *Enterobacteriaceae*, and the rump site for the VerifEYE Solo. The rump area of the animal is one of the areas where most faecal material will adhere as it is very close to the anus. Therefore it may not be surprising that the VerifEYE machine detected more faecal material at this site.

The results obtained for the cattle and sheep carcasses also showed that the *Enterobacteriaceae* counts were similar for both species, but the TVC counts and the VerifEYE results were higher for the sheep carcasses. The TVC counts obtained indicated that the dressing of cattle at this particular plant results in less bacteria on the carcass than the dressing of sheep at the plant we visited, and hence cleaner cattle carcasses are produced. This was also highlighted by the much lower VerifEYE readings obtained from the cattle carcasses. This indicates that the VerifEYE

Solo machine may be a good indicator of overall process hygiene of carcasses when the carcass is examined as a whole, instead of examining individual sites

Conclusions

- The highest average TVC count was found on the flank of the sheep carcasses
- The brisket site of the sheep carcasses showed the highest average *Enterobacteriaceae* count and the highest average VerifEYE readings
- A potential correlation was observed between the *Enterobacteriaceae* counts and the VerifEYE readings
- The brisket area of the sheep carcasses had the highest level of contamination most frequently
- With the cattle carcasses the highest average *Enterobacteriaceae* and TVC counts were found on the neck area, but the highest average VerifEYE readings were observed on the rump area
- The VerifEYE Solo device appeared to be effective under commercial conditions as a good indicator of the overall process hygiene for the dressing of cattle and sheep, and may give an indication of *Enterobacteriaceae* levels on sheep carcasses

Surface validation

Three different cattle, sheep and pig plants were each visited. On consecutive days, environmental surfaces were sampled after production had finished but before the cleaning crew had begun to sanitise the plant. Prior to the commencement of production the following day, the cleaned surfaces were re-sampled

The results of the surface testing in the cattle plant are shown in Table 17. The VerifEYE machine did not detect significant amounts of faecal contamination of environmental surfaces in plants. Before cleaning, the fat conveyor was strongly fluorescent when viewed with the machine. However, the conveyor was heavily contaminated with fat and it is likely that reflection from condensate on the surface of the fat is what caused the strong signal. The outer surface of the offal chiller door was constructed of polyvinyl chloride, a substance which we previously showed was reflective and thus could give false positive readings

A comparison between the two protein detection systems showed that the Pro-tect swab was less sensitive than the Flash stick. The entire detection end of the Flash stick did not always turn from yellow to blue and thus it was straightforward to assign a number between 0 and 4 to protein contamination when using the Flash stick. The Pro-tect swabs however had a tendency to undergo an "all or nothing" change from no reaction to fully contaminated. Consequently, it was rare that we recorded a middle of the range value for the Pro-tect swabs

Microbiological testing of the surfaces showed that although detectable numbers of total aerobes were present, the surfaces were not contaminated with faecal indicator organisms. There was a marked, but not statistically significant (t-Test $P>0.05$), reduction in bacterial numbers on surfaces after cleaning

There was a single anomalous result within the cattle surfaces that we cannot satisfactorily explain. The carcass hook test sample measured higher numbers of *Enterobacteriaceae* than total aerobes. Although the media and incubation conditions are different for the two tests, it is unlikely that the result is a true reflection of the microbiology of the carcass hook

Relationships between each of the testing methods were investigated using standard Pearson Correlations. A pre-calculated statistical lookup table was used to determine an appropriate threshold for the significance of presumptive relationships between each of the test method results.

For cattle, our combined datasets had 24 degrees of freedom and the lookup table provided a value of 0.388 as an appropriate test for significance. There were no significant relationships identified

The surface sampling results for the sheep processing plant are shown in Table 18. In general, the results are not significantly different to those we observed for the cattle plant. As before, viewing surfaces with the VerifEYE revealed that there was no gross contamination of surfaces with faeces. The notable exception was the hide puller which could reasonably be expected to be contaminated with manure that had transferred from the fleece. The material that the fleece conveyor belt was made from, although not reflective, was strongly fluorescent when viewed with the VerifEYE machine. For this reason the fleece conveyor results were not used for statistical analysis of the test results

Detection of protein residues on surfaces in the plants also mirrored our findings in the cattle plant. The Pro-tect system again appeared to be less sensitive than the Flash sticks, with the majority of results being either "all or nothing". As with the sheep plant, protein residues were reduced as a consequence of cleaning. The reduction was significant for both protein detection systems (t-Test $P<0.05$)

Although the datasets were quite small, Pearson correlation coefficients were undertaken to determine if there were relationships between the test results for each of the assessment methods used. The pre and post cleaning data were analysed as a single dataset. This combined dataset had 28 degrees of freedom and so we used a value of 0.361 as he threshold for significance. There were significant relationships identified between Pro-tect and VerifEYE, Pro-tect and Flash, and Flash and ATP. However, critical assessment of the data revealed that the relationships are likely to be artefacts caused by interaction between the majority of VerifEYE numbers being zero, a significant number of the Pro-tect scores being either 4 (before clean) or 0 (after clean) and the majority of the Flash readings being 0 post clean. It is likely the presumptive relationships are a consequence of the lack of sensitivity of the Pro-tect system and the lack of faecal contamination of the environmental surfaces

The results obtained from sampling environmental surfaces in a pig plant are shown in Table 19. There was a significant reduction in total bacterial numbers and *Enterobactericeae* (t-Test $P<0.05$) after cleaning. Pearson correlations (22 degrees of freedom and significance threshold of 0.404) revealed that there were a number of relationships between the different test methods. Most strong was a relationship between the two protein detection systems (0.698) and a correlation between Flash and ATP results (0.623). The surfaces in the pig plant were particularly dirty and thus the relationship stems from the fact that most of the protein detections were at the top end of the scale before cleaning and the bottom end of the scale after cleaning. The correlation between ATP and total counts is also worthy of mention (0.505) although scrutiny of the microbiological counts revealed that before cleaning there was an unusually small range in the numbers of bacteria that were measured. It is likely that the correlations are the result of the small relative range of the ATP test systems and the uncharacteristically narrow range of the counts before cleaning

The presence of any relationships from the entire test results from all three plants was also investigated. Since there was not gross faecal contamination of surfaces observed in plants, and because the VerifEYE machine was not used in the pig plant, we excluded these results from the correlations. These comparisons have 75 degrees of freedom and a significance threshold of 0.202 was applied. Taken overall, the best relationships were between the two microbiological methods (0.671) and as was identified at the sheep and pig plants, the two protein residue detection systems had a significant correlation (0.50)

With the possible exception of the relationship between the two protein systems, it is likely that the relationships identified from this study apply only to specific conditions. Over a range of processing conditions, and levels of surface cleanliness, the relationships are probably too week to make meaningful conversions between surface microbiology and the rapid methods that were trialled

It is perhaps not surprising that significant differences between any of the testing systems were not detected. Surfaces are not uniformly contaminated (either with visible detritus or

microorganisms) and thus even sampling sites that are close together on the surface may mean there are large differences between the samples collected. One commonly-encountered method of solving this problem is to sample large numbers of surfaces. Such an approach was beyond the scope of this pilot study

In terms of evaluating the individual rapid methods for their ability to verify that proper cleaning had been undertaken, both protein systems were able to detect that effective cleaning had occurred. The Pro-tect system was more sensitive to protein residues than the Flash Stick system. The two protein detection systems evaluated have different strengths however. For checking the status of surfaces during processing the Pro-tect swabs less suited because they are too sensitive and have a tendency to turn purple when only a small amount of protein is present. The range of protein concentrations covered by the Flash sticks is wider and thus these provide more meaningful assessments when surfaces are in use. The ATP Snapshot machine covers a similarly wide range of soiling and has an added advantage of being able to read swabs up to two hours after samples have been taken. From a practical point of view the ATP machine performed well in these trials. Financially, it is the second most expensive system that was trialled

The VerifEYE machine is not well suited for the assessment of environmental surfaces in meat plants. It does not detect non-ruminant faeces and during processing, environmental surfaces are not contaminated with faeces. Although the machine detects faeces on carcases, it has limited usefulness for environmental surfaces

Conclusions

- Within the cattle and sheep plants, the VerifEYE Solo was not able to detect significant levels of faecal contamination on abattoir surfaces
- No significant relationships between the methods tested were found with the surfaces within the cattle plant, but from the surfaces found within the sheep plant, strong relationships were found between; Pro-tect and VerifEYE, Pro-tect and Flash, and Flash and total ATP
- Strong relationships were also found between Pro-tect and Flash, and Flash and total ATP, with the surfaces tested within the pig plant
- The two protein detection methods used in the trial were able to detect that effective cleaning had occurred
- For monitoring hygiene on surfaces during working, the Flash protein detection method will provide a more meaningful and accurate result (the Pro-tect method is too sensitive for this type of monitoring)
- The total ATP system used to detect hygiene of plant surfaces performed well during the trial
- The VerifEYE Solo device was not thought to be well suited to assessing the hygiene of plant surfaces

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Table 1. Detection of fluorescence of locally-available items, read with VerifEYE Solo set on enhanced mode, sensitivity setting 003, under strip fluorescent lighting of 2050 lux intensity

Material that fluoresced	Materials that did not fluoresce
• Wall cladding from abattoir (Altro Whiterock W103/W104)	• White wellington boots
• Shiny metal surfaces, including rivets, screws, stainless steel cutlery, baking tray, jewellery, drawing pins	• White hard hat
• Shiny plastic surfaces, including clingfilm, meat wrap,	• Tyvek overalls (DuPont (UK) Ltd., Stevenage, UK)
• Autoclave indicator tape	• Meat Hygiene Service marking ink
• Laboratory safety signs	• Mop head
• White copier paper, 80 gsm (Whitegrove W1202), envelopes, other white paper	• Broom bristles
• Fresh plants including grass, dandelion, other weeds	• Kitchen sponges
• Green leaves	• Laboratory benches, floor, walls
• Some yellow leaves	• Twigs; tree bark
• Moss	• Dry rosehip
• Polystyrene boxes	• Dry pine cone or needles
• Livestock feed – cracked maize	• Dry brown tree leaves
• Livestock feed – extruded protein soya	• Red holly berries
• Livestock feed – high protein soya	• Some yellow leaves
• Livestock feed – grass pellets	• Red leaves
• Livestock feed –compound pig feed (n = 2)	• Livestock feed – barley
	• Livestock feed – sunflower

Table 2. Detection limits of pure chlorophyll a and chlorophyll b on two coloured matt surfaces observed with VerifEYE Solo set on enhanced mode, sensitivity setting 003, under strip fluorescent lighting of 4096 lux intensity

	Detection limits (mg ml^{-1})	
	White	Black
Chlorophyll a	0.001	0.01
Chlorophyll b	0.01	0.1

Table 3. Detection limits of cattle faeces on a range of beef tissues and surfaces at three sensitivity settings, enhanced processing, 1024 lux

	Highest concentration of cattle faeces (mg ml^{-1}) detected in 100 µl spots before/after drying for 24 h at three sensitivity settings:		
	001	003	005
Beef muscle + membrane	100/10	100/10	100/10
Beef cut muscle	100/10	100/100	100/Smear[§]
Beef fat	100/10	10/10	10/10
Grey concrete	1/0.1	1/0.1	10/0.1
Slate concrete	1/1	10/0.1	ND/0.1
Blue tile	10/10	100/ND	ND/ND
Black tile	100/100	100/ND	ND/ND
Cattle hide (brown)	100/10	100/ND	ND/ND
Cattle hide (black)	100/10	Smear/ND	ND/ND

* ND; not detected under these conditions
[§]; Smear of cattle faeces (not quantifiable, but clearly visible to naked eye).

Table 4. Detection of cattle faeces on painted concrete surfaces, enhanced processing, sensitivity 003, under strip fluorescent lighting of 2048 lux intensity

Surface, faeces condition; conditions of storage	Detection of cattle faeces deposited as smear or 100 µl spots before/after drying for 24 h and after storage at 4ºC				
	Smear	100 mg ml^{-1}	10 mg ml^{-1}	1 mg ml^{-1}	0.1 mg ml^{-1}
Grey concrete					
Wet faeces; 0 h	+	+	+	+	-
Dried faeces; 24 h at ambient temperature on concrete	±	±	+	+	-
Wet faeces; 24 h storage at 4ºC, but freshly-spotted	+	±	+	-	-
Slate concrete					
Wet faeces; 0 h	+	+	+	+	-
Dried faeces; 24 h at ambient temperature on concrete	±	±	+	±	-
Wet faeces; 24 h storage at 4ºC, but freshly-spotted	+	±	+	-	-

+; detected as bright, homogeneous fluorescence
±; detected as streaky fluorescence
ND; not detected under these conditions

Table 5. Effect of proximity of VerifEYE Solo to faeces on workbench surface, enhanced processing, under strip fluorescent lighting of 2048 lux intensity

Sensitivity and distance (mm) of machine from faeces	Concentration of cattle faeces, deposited as smear or as 100 µl spots				
	Smear	100 mg ml^{-1}	10 mg ml^{-1}	1 mg ml^{-1}	0.1 mg ml^{-1}
001					
16	+	+	+	ND	ND
32	+	+	±	ND	ND
49	+	+	±	ND	ND
64	+	+	ND	ND	ND
81	+	+	ND	ND	ND
97	±	±	ND	ND	ND
003					
16	+	+	+	+	ND
32	+	+	±	ND	ND
49	+	±	ND	ND	ND
64	±	±	ND	ND	ND
81	±	ND	ND	ND	ND
97	ND	ND	ND	ND	ND
005					
16	ND	ND	ND	ND	ND
32	ND	ND	ND	ND	ND
49	ND	ND	ND	ND	ND
64	ND	ND	ND	ND	ND
81	ND	ND	ND	ND	ND
97	ND	ND	ND	ND	ND

+; detected as bright, homogeneous fluorescence
±; detected as streaky fluorescence
ND; not detected under these conditions

Table 6. Detection limits for cattle faeces on a range of beef tissues and surfaces at three sensitivity settings, enhanced processing, 512 lux, before and after drying for 24 h at appropriate temperature

Processing setting and surface examined	Faecal smear		10^{-1} 100 mg ml^{-1}		10^{-2} 10 mg ml^{-1}		10^{-3} 1 mg ml^{-1}		10^{-4} 0.1 mg ml^{-1}	
001	0	24	0	24	0	24	0	24	0	24
Beef muscle + membrane	+	+	+	+	-	+	-	-	-	-
Beef cut muscle	+	+	+	+	-	+	-	-	-	-
Beef fat	+	+	+	+	-	+	-	-	-	-
Grey concrete	+	±	+	+	+	+	±	+	-	±
Slate concrete	+	±	+	+	-	+	±	+	-	-
Blue tile	+	±	+	+	±	±	-	-	-	-
Black tile	+	±	+	+	-	-	-	-	-	-
Beef hide (brown)	+	+	+	+	-	+	-	-	-	-
Beef hide (black)	+	±	+	+	-	+	-	-	-	-
003										
Beef muscle + membrane	±	±	+	+	-	+	-	-	-	-
Beef cut muscle	+	+	+	+	-	-	-	-	-	-
Beef fat	+	±	+	+	+	±	-	-	-	-
Grey concrete	+	±	+	±	+	+	±	+	-	+
Slate concrete	±	±	±	-	+	+	-	+	-	±
Blue tile	±	-	±	-	-	-	-	-	-	-
Black tile	±	-	±	-	-	-	-	-	-	-
Beef hide (brown)	±	-	±	-	-	-	-	-	-	-
Beef hide (black)	±	-	-	-	-	-	-	-	-	-

Table 6 continued. Detection limits for cattle faeces on a range of beef tissues and surfaces at three sensitivity settings, enhanced processing, 512 lux, before and after drying for 24 h at appropriate temperature

005	Faecal smear		10^{-1} 100 mg ml^{-1}		10^{-2} 10 mg ml^{-1}		10^{-3} 1 mg ml^{-1}		10^{-4} 0.1 mg ml^{-1}	
	0	24	0	24	0	24	0	24	0	24
Beef muscle + membrane	±	±	±	+	-	+	-	-	-	-
Beef cut muscle	+	+	±	-	-	-	-	-	-	-
Beef fat	+	+	+	+	+	+	-	-	-	-
Grey concrete	±	±	-	±	+	+	-	+	-	±
Slate concrete	-	-	-	-	-	±	-	±	-	±
Blue tile	-	-	-	-	-	-	-	-	-	-
Black tile	-	-	-	-	-	-	-	-	-	-
Beef hide (brown)	-	-	-	-	-	-	-	-	-	-
Beef hide (black)	-	-	-	-	-	-	-	-	-	-

-: no green patch visible on VerifEYE Solo viewer.
±; green patch on VerifEYE Solo viewer appears grainy

Table 7. Detection limits for sheep faeces on a range of sheep tissues and surfaces at three sensitivity settings, enhanced processing, 1024 lux

Processing setting and surface examined	Faecal smear		10^{-1} 100 mg ml^{-1}		10^{-2} 10 mg ml^{-1}		10^{-3} 1 mg ml^{-1}		10^{-4} 0.1 mg ml^{-1}	
001	0	24	0	24	0	24	0	24	0	24
Sheep muscle + membrane	+		+		+		-	-	-	-
Sheep cut muscle	+		+		±		-	-	-	-
Sheep fat	+		+		+		-	-	-	-
Grey concrete	+	±	+	±	+	+	±	±	-	-
Slate concrete	+	±	+	±	+	±	±	±	-	-
Blue tile	+	+	+	+	±	-	-	-	-	-
Black tile	+	+	+	+	-	-	-	-	-	-
Sheep fleece (white)										-
Sheep fleece (brown)										-
003										
Sheep muscle + membrane	+		+		-		-	-	-	-
Sheep cut muscle	+		+		±		-	-	-	-
Sheep fat	+		+		±		-	-	-	-
Grey concrete	±	±	±	±	+	+	-	±	-	-
Slate concrete	±	±	±	±	-	±	-	-	-	-
Blue tile	±	±	±	±	-	-	-	-	-	-
Black tile	±	±	±	±	-	-	-	-	-	-
Sheep fleece (white)										
Sheep fleece (brown)										

Table 7 cont. Detection limits for sheep faeces on a range of sheep tissues and surfaces at three sensitivity settings, enhanced processing, 1024 lux

005	Faecal smear		10^{-1} 100 mg ml^{-1}		10^{-2} 10 mg ml^{-1}		10^{-3} 1 mg ml^{-1}		10^{-4} 0.1 mg ml^{-1}	
	0	24	0	24	0	24	0	24	0	24
Sheep muscle + membrane	+		+		+		-	-	-	-
Sheep cut muscle	+		+		-		-	-	-	-
Sheep fat	+		+		-		-	-	-	-
Grey concrete	±	-	±	-	±	±	-	-	-	-
Slate concrete	-	-	-	-	±	-	-	-	-	-
Blue tile	-	-	-	-	-	-	-	-	-	-
Black tile	-	-	-	-	-	-	-	-	-	-
Sheep fleece (white)										
Sheep fleece (brown)										

-: no green patch visible on VerifEYE Solo viewer
±; green patch on VerifEYE Solo viewer appears grainy

Table 8. The detection of cattle faeces by Flash protein detection system

Surface, faeces condition; conditions of storage	Detection of cattle faeces deposited as 100 µl over a 20cm^2 area before/after drying for 24 h				
	100 mg ml^{-1}	10 mg ml^{-1}	1 mg ml^{-1}	0.1 mg ml^{-1}	0.01 mg ml^{-1}
Grey concrete					
Wet faeces; 0 h	+	+	+	+	ND
Dried faeces; 24 h at ambient temperature on concrete	ND	ND	ND	ND	ND
Blue gloss tile					
Wet faeces; 0 h	+	+	+	ND	ND
Dried faeces; 24 h at ambient temperature on tile	ND	ND	ND	ND	ND

+; detected, colour change from yellow to blue
ND; not detected, no colour change under these conditions

Table 9. The detection of cattle faeces by Pro-tect protein detection system

Surface, faeces condition; conditions of storage	Detection of cattle faeces deposited as 100 µl over a 20cm^2 area before/after drying for 24 h *				
	100 mg ml^{-1}	10 mg ml^{-1}	1 mg ml^{-1}	0.1 mg ml^{-1}	0.01 mg ml^{-1}
Grey concrete					
Wet faeces; 0 h	XX	?	ND	ND	ND
Dried faeces; 24 h at ambient temperature on concrete	X	ND	ND	ND	ND
Blue gloss tile					
Wet faeces; 0 h	XX	?	ND	ND	ND
Dried faeces; 24 h at ambient temperature on tile	XX	?	ND	ND	ND

* values shown in the above table are as described in the manufacturers manual
XX / X; fail result detected, colour change from green to purple
?; caution result detected, colour change from green to grey
ND; not detected, no colour change (stays green) under these conditions

Table 10. The detection of sheep faeces by Flash protein detection system

Surface, faeces condition; conditions of storage	Detection of sheep faeces deposited as 100 µl over a 20cm^2 area before/after drying for 24 h				
	100 mg ml^{-1}	10 mg ml^{-1}	1 mg ml^{-1}	0.1 mg ml^{-1}	0.01 mg ml^{-1}
Grey concrete					
Wet faeces; 0 h	+	+	+	+	ND
Dried faeces; 24 h at ambient temperature on concrete	ND	ND	ND	ND	ND
Blue gloss tile					
Wet faeces; 0 h	+	+	+	ND	ND
Dried faeces; 24 h at ambient temperature on tile	ND	ND	ND	ND	ND

+; detected, colour change from yellow to blue
ND; not detected, no colour change under these conditions

Table 11. The detection of sheep faeces by Pro-tect protein detection system

Surface, faeces condition; conditions of storage	Detection of sheep faeces deposited as 100 µl over a 20cm^2 area before/after drying for 24 h *				
	100 mg ml^{-1}	10 mg ml^{-1}	1 mg ml^{-1}	0.1 mg ml^{-1}	0.01 mg ml^{-1}
Grey concrete					
Wet faeces; 0 h	XX	?	ND	ND	ND
Dried faeces; 24 h at ambient temperature on concrete	XX	?	ND	ND	ND
Blue gloss tile					
Wet faeces; 0 h	XX	?	ND	ND	ND
Dried faeces; 24 h at ambient temperature on tile	XX	?	ND	ND	ND

* values shown in the above table are as described in the manufacturers manual
XX / X; fail result detected, colour change from green to purple
?; caution result detected, colour change from green to grey
ND; not detected, no colour change (stays green) under these conditions

Table 12. The detection of ATP aliquots by Hygiena snapshot and Lightning MVP detection systems

Concentration of ATP used (mg $ml^{-1)}$	Detection of ATP aliquot (10 µl directly onto swab tip)	
	Lightning (zones/ml)	Snapshot (RLU/ml)
Background reading	/	PASS
Control reading	1.8	5
10	6.3	2918202
1	6.3	2591824
0.1	5.1	1634478
0.01	4.4	96405
0.001	4.6	9536
0.0001	4.3	1329
0.00001	3.3	191
0.000001	2.5	36
0.0000001	1.9	5
0.00000001	1.6	0
0.000000001	1.5	0
0.0000000001	1.4	0

Figure 1. Difference between average detected *Enterobacteriaceae*, TVC and chlorophyll on hide/fleece/skin and carcass for cattle, sheep and pigs

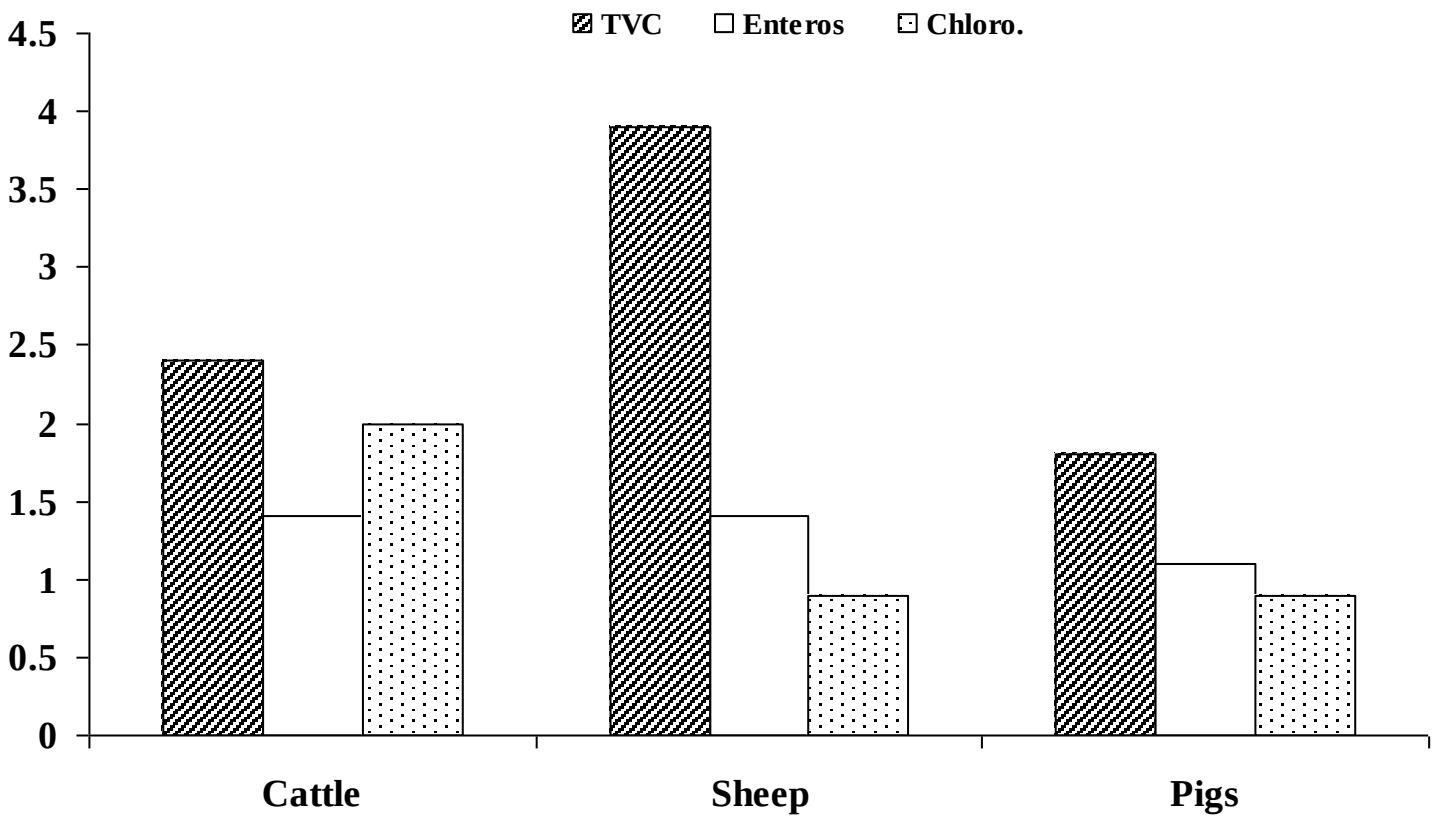

Table 13. Reduction in contamination parameters observed pre- and post-cleaning of surfaces in cattle slaughter

	TVC		Enteros.		VerifEYE		Total ATP		Pro-tect		Flash	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Roll-out ramp	4.8	3.1	2.7	0	3	3	928	1558	3	0	1	0
Beef pram	4.8	3.6	3.5	0	2	2	1860	2044	5	1	3	2
Knife	0	3.7	0	0	0	0	195	337	0	0	1	1
Flayer	3.8	1.5	0.2	0	1	1	16361	8305	3	0	1	1
Apron wash	1.5	1.9	0	0	1	0	1011	2	1	0	2	1
Saw	4.4	3.1	1.8	1.1	0	1	4566	504	0	0	3	1
Mean	3.2	2.8	1.4	0.2	1.2	1.2	4153	2125	1.7	0.2	1.8	1.0
Reduction	**2 fold**		**15 fold**		**0**		**2 fold**		**8 fold**		**2 fold**	

Table 14. Reduction in contamination parameters observed pre- and post-cleaning of surfaces in sheep slaughter

	TVC		Enteros.		VerifEYE		Total ATP		Pro-tect		Flash	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Sheep cradle 1	3.0	1.8	0	0	1	1	24635	17171	3	1	1	1
Sheep cradle 2	2.5	2.1	0	0	3	3	357594	28223	3	2	2	1
Knife	3.8	1.0	0	0	2	2	59992	424	3	2	2	0
Apron wash	1.5	0.7	0	0	0	2	4203	533	0	0	2	2
Saw	1.6	1.9	0	0	1	1	88979	105405	3	3	1	0
Apron	3.9	0.7	1.2	0	3	2	46287	2290	3	1	3	0
Mean	2.7	1.4	0.2	0	1.7	1.8	96948	25674	2.5	1.5	1.8	0.7
Reduction	**20 fold**		**0**		**Increase**		**4 fold**		**2 fold**		**3 fold**	

Table 15. Reduction in contamination observed pre- and post-cleaning of surfaces in pig slaughter

	TVC		Enteros.		VerifEYE		Total ATP		Pro-tect		Flash	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
Dehairer (inside)	5.0	2.6	2.2	0	0	0	2760	903	3	3	3	1
Dehairer (outside)	1.1	1.2	0	0	0	1	120	542	0	0	1	1
Polishing table	4.6	0.2	2.2	0	0	0	1159	5696	1	0	4	3
Knife	2.9	4.2	0.2	0.7	0	0	48578	5724	3	3	3	0
Apron wash	0.8	1.0	0	0	0	0	2855	1547	0	0	0.5	2
Saw	1.9	0.5	0	0	0	1	8491	3791	2	0	2	1
Mean	2.7	1.6	0.8	0.1	0	0.3	10660	3034	1.5	1.0	2.2	1.3
Reduction	**14 fold**		**4 fold**		**Increase**		**4 fold**		**2 fold**		**2 fold**	

Table 16. Average counts of TVC and *Enterobacteriaceae* (Log_{10} CFU/cm^2) and average VeifEYE readings obtained from each of four sites from cattle and sheep carcasses in commercial plants

		Enterobacteriacea	TVC	VerifEYE
Sheep:	**Lateral Thorax**	1.52	6.19	0.6
	Breast	1.85	6.37	1.3
	Brisket	**2.15**	4.84	**3.4**
	Flank	1.35	**7.22**	0.8
Cattle:	**Rump**	0	3.28	**0.7**
	Flank	1.41	3.24	0.5
	Brisket	1.64	3.77	0.5
	Neck	**2.88**	**4.19**	0.02

Table 17. Hygienic status of surface samples collected in a beef plant after a day's processing but before cleaning and after cleaning but before the start of processing

After the day's processing:

Surface	VerifEYE	Flash stick	Pro-Tect swab	ATP swab	TVC (CFU/cm^2)	Ents (CFU/cm^2)
Hand rail	1	3	4	49	8	0
Sprayer	0	2	1	166	78	0
Derinder	0	0	4	49	29	0
Fat conveyor	10	2	4	46	75	2
Band saw	0	3	4	0	11	0
Wall #1	0	2	4	308	0	0
Sink	0	2	4	169	400	0
Chainmail gauntlet	0	2	1	89	0	0
Blue crate	0	4	1	155	520	0
Carcase hooks	0	3	0	224	6	0
Offal chiller door	4	0	0	152	300	0
Wall #2	0	3	2	50	94	6

Before the day's processing:

Surface	VerifEYE	Flash stick	Pro-Tect swab	ATP swab	TVC CFU/cm2)	Ents CFU/cm2)
Hand rail	0	1	0	13	0	0
Sprayer	2	1	0	110	0	0
Derinder	0	1	0	8	0	0
Fat conveyor	0	2	0	5	0	0
Band saw	1	1	0	106	0	0
Wall #1	0	0	0	5	4	0
Sink	0	1	0	18	0	0
Chainmail gauntlet	0	0	0	26	0	0
Blue crate	0	0	0	101	300	0
Carcase hooks	0	1	4	219	1900	3600
Offal chiller door	4	0	0	23	0	0
Wall #2	4	2	0	6	0	0

TVC = total numbers of aerobic bacteria; Ents = numbers of *Enterobacteriaceae*

Table 18. Hygienic status of surface samples collected in a sheep plant after a day's processing but before cleaning and after cleaning but before the start of processing

After the day's processing:

Surface	VerifEYE	Flash stick	Pro-Tect swab	ATP swab	TVC (CFU/cm^2)	Ents (CFU/cm^2)
Hock hook	1	2	4	1326	735	3
Fleece puller	4	3	4	0	950	19
Knife steriliser #1	0	2	2	213	1	0
Fleece conveyor	4*	0	2	794	745	1
Sink	0	0	0	1534	4	3
Washer	0	0	0	31	250	0
Knife steriliser #2	0	0	4	144	6	0
Carcase hook	0	0	0	106	5	0
Hock cutter	2	1	0	1171	54	0
Metal table	2	4	4	13887*	58500	17000
Organ pan	3	3	0	766	31500	16300
MHS saw	0	0	2	1791	81500	75000
Offal chiller door	0	0	0	0	4	0
Cutting block	2	4	1	117	35500	25500
Grading platform	0	2	2	225	1	1

Before the day's processing:

Surface	VerifEYE	Flash stick	Pro-Tect swab	ATP swab	TVC CFU/cm2)	Ents CFU/cm2)
Hock hook	0	0	0	117	0	0
Fleece puller	0	1	0	2	0	0
Knife steriliser #1	0	0	0	143	5	0
Fleece conveyor	4	1	0	36	14	2
Sink	0	1	0	4	0	0
Washer	0	0	0	51	1	1
Knife steriliser #2	0	0	0	1	37	15
Carcase hook	0	0	0	658	1	0
Hock cutter	0	0	0	497	101000	36
Metal table	0	1	0	86	25	0
Organ pan	0	1	0	0	255	1
MHS saw	0	2	0	171	5	1
Offal chiller door	0	1	0	1	1	0
Cutting block	0	2	0	115	13	0
Grading platform	0	1	0	8	6	0

TVC = total numbers of aerobic bacteria; Ents = numbers of *Enterobacteriaceae*

Table 19. Hygienic status of surface samples collected in a pig plant after a day's processing but before cleaning and after cleaning but before the start of processing

After the day's processing:

Surface	Flash stick	Pro-Tect swab	ATP swab	TVC (CFU/cm^2)	Ents (CFU/cm^2)
Polisher	4	4	44	2100	7
MHS platform rail	2	1	2	3300	18
Rinse head	1	1	76	4000	21
Flame surround	1	1	2	590	23
Band saw blade	3	4	233	3800	39
Organ pan	4	4	266	3100	6
Wall	3	4	85	1500	6
Trotter snipper	4	4	213	6100	34
Gambrell table	4	1	214	2200	30
Chiller door	3	0	194	3900	37
Carcass hook	2	4	271	2100	7

Before the day's processing:

Surface	Flash stick	Pro-Tect swab	ATP swab	TVC CFU/cm2)	Ents CFU/cm2)
Polisher	2	4	42	7	0
MHS platform rail	1	0	4	119	0
Rinse head	1	0	1	8	0
Flame surround	0	1	3	12	0
Band saw blade	0	0	60	66	0
Organ pan	0	0	25	14	0
Wall	1	1	159	41	0
Trotter snipper	2	1	229	33	0
Gambrell table	1	1	120	3	0
Chiller door	0	0	53	22	0
Carcass hook	0	0	4	17	0

TVC = total numbers of aerobic bacteria; Ents = numbers of *Enterobacteriaceae*

Appendix 1

Objective 1, task 2 – Experimental, abattoir-based evaluation of the alternative methods

Cattle hides and carcasses

	Bacterial counts ($Log_{10}CFU/cm^2$)		
	Enterobacteriaceae	**TVC**	**Chlorophyll**
HR1	2.30	5.00	4
HB1	<0.7	4.36	3
HF1	3.98	6.00	3
HN1	<0.7	4.77	2
HR2	3.86	6.07	4
HB2	1.00	4.85	3
HF2	1.30	5.19	2
HN2	1.78	4.65	3
HR3	2.84	5.92	4
HB3	2.57	4.89	4
HF3	2.36	5.74	2
HN3	1.00	4.83	1
CR1	<0.7	2.23	1
CB1	<0.7	2.78	2
CF1	<0.7	3.33	0
CN1	2.48	5.12	0
CR2	<0.7	2.59	1
CB2	<0.7	2.78	3
CF2	<0.7	1.00	0
CN2	2.74	4.30	0
CR3	0.7	2.68	2 (cut line)
CB3	<0.7	3.00	1
CF3	<0.7	1.70	0
CN3	<0.7	1.81	1

H = hide, C = carcass; R = rump, B = brisket, F = flank, N = neck

Appendix 2
Objective 1, task 2 – Experimental, abattoir-based evaluation of the alternative methods

Sheep fleece and carcasses

	Bacterial counts ($Log_{10}CFU/cm^2$)		
	Enterobacteriaceae	**TVC**	**Chlorophyll**
FB1	2.30	6.19	3
FBr1	<0.7	5.42	3
FF1	3.98	4.65	3
FT1	<0.7	6.18	3
FB2	3.86	5.70	3
FBr2	1.00	5.23	3
FF2	1.30	7.87	3
FT2	1.78	5.11	3
FB3	2.84	5.84	3
FBr3	2.57	6.47	3
FF3	2.36	5.54	3
FT3	1.00	5.84	3
CB1	<0.7	2.06	3
CBr1	<0.7	1.48	2
CF1	<0.7	1.54	3
CT1	2.48	3.20	1
CB2	<0.7	<0.7	2
CBr2	<0.7	1.54	3
CF2	<0.7	1.00	2
CT2	2.74	1.00	0
CB3	0.70	2.31	3
CBr3	<0.7	1.70	3
CF3	<0.7	2.39	3
CT3	<0.7	3.02	0

F = fleece, C = carcass; B = breast, Br = brisket, F = flank, T = lateral thorax

Appendix 3

Objective 1, task 2 – Experimental, abattoir-based evaluation of the alternative methods

Pig skin and carcasses

	Bacterial counts ($Log_{10}CFU/cm^2$)		
	Enterobacteriaceae	**TVC**	**Chlorophyll**
SH1	<0.7	5.04	1
SB1	<0.7	4.90	1
SBr1	3.72	6.15	1
SJ1	0.70	5.35	1
SH2	1.93	5.18	1
SB2	1.30	4.96	1
SBr2	1.98	5.09	1
SJ2	0.70	5.30	1
SH3	0.70	5.56	1
SB3	<0.7	4.30	1
SBr3	4.20	6.46	1
SJ3	3.06	5.60	1
CH1	<0.7	3.74	0
CB1	<0.7	3.40	0
CBr1	0.70	3.98	0
CJ1	<0.7	3.00	0
CH2	1.65	2.93	1
CB2	<0.7	3.63	0
CBr2	<0.7	3.58	1
CJ2	<0.7	3.33	0
CH3	1.18	4.41	0
CB3	<0.7	3.41	0
CBr3	<0.7	2.94	0
CJ3	<0.7	3.16	0

S = skin, C = carcass; H = ham, B = back, Br = belly, J = jowl

Appendix 4

Objective 1, task 2 – Experimental, abattoir-based evaluation of the alternative methods

Cattle surfaces – before cleaning

	Bacterial counts (CFU/cm^2)			Protein detection		
	Enterobacteriaceae	**TVC**	**Chlorophyll**	**Flash**	**Pro-tect**	**Total ATP (RLU)**
Roll-out ramp	4.60 x 10^2	7.00 x 10^4	3	1	2	928
Beef pram	3.00 x 10^3	6.60 x 10^4	2	3	2	1860
Knife	<0.5	0.5	0	1	0	195
Flayer	1.5	5.80 x 10^3	1	1	2	16361
Apron wash	<0.5	29	1	2	0/1	1011
Saw	58	2.76 x 10^4	0	3	0	4566

Cattle surfaces – after cleaning

	Bacterial counts (CFU/cm^2)			Protein detection		
	Enterobacteriaceae	**TVC**	**Chlorophyll**	**Flash**	**Pro-tect**	**Total ATP (RLU)**
Roll-out ramp	1	1.35 x 10^3	3	0	0	1558
Beef pram	<0.5	3.80 x 10^3	2	2	0/1	2044
Knife	0.5	5.70 x 10^3	0	1	0	337
Flayer	<0.5	36	1	1	0	8305
Apron wash	<0.5	89	0	1	0	2
Saw	13	1.13 x 10^3	1	1	0	504

Appendix 5

Objective 1, task 2 – Experimental, abattoir-based evaluation of the alternative methods

Sheep surfaces – before cleaning

	Bacterial counts (CFU/cm^2)			Protein detection		
	Enterobacteriaceae	**TVC**	**Chlorophyll**	**Flash**	**Pro-tect**	**Total ATP (RLU)**
Sheep cradle 1	<0.5	1.05 x 10^3	1	1	3	24635
Sheep cradle 2	0.5	3.50 x 10^2	3	2	3	357594
Knife	<0.5	5.70 x 10^3	2	2	3	59992
Apron wash	<0.5	35.5	0	2	0	4203
Saw	<0.5	44	1	1	3	88979
Apron	16.5	9.00 x 10^3	3	3	3	46287

Sheep surfaces – after cleaning

	Bacterial counts (CFU/cm^2)			Protein detection		
	Enterobacteriaceae	**TVC**	**Chlorophyll**	**Flash**	**Pro-tect**	**Total ATP (RLU)**
Sheep cradle 1	<0.5	56	1	1	1	17171
Sheep cradle 2	<0.5	1.30 x 10^2	3	1	2	28223
Knife	<0.5	10	2	0	2	424
Apron wash	<0.5	5	2	2	0	533
Saw	<0.5	90.5	1	0	3	105405
Apron	<0.5	5	2	0	1	2290

Appendix 6

Objective 1, task 2 – Experimental, abattoir-based evaluation of the alternative methods

Pig surfaces – before cleaning

	Bacterial counts (CFU/cm^2)			Protein detection		
	Enterobacteriaceae	**TVC**	**Chlorophyll**	**Flash**	**Pro-tect**	**Total ATP (RLU)**
Dehairer (in)	1.80×10^2	1.14×10^5	0	3	2	2760
Dehairer (out)	<0.5	13.5	0	1	0	120
Pig table	1.40×10^2	4.40×10^4	0	4	0/1	1159
Knife	1.5	8.60×10^2	0	3	2	48578
Apron wash	<0.5	6	0	0/1	0	2855
Saw	0.5	76	0	2	1	8491

Pig surfaces – after cleaning

	Bacterial counts (CFU/cm^2)			Protein detection		
	Enterobacteriaceae	**TVC**	**Chlorophyll**	**Flash**	**Pro-tect**	**Total ATP (RLU)**
Dehairer (in)	<0.5	3.75×10^2	0	1	2	903
Dehairer (out)	<0.5	14	1	1	0	542
Pig table	<0.5	1.5	0	3	0	5696
Knife	5.5	1.48×10^4	0	0	2	5724
Apron wash	<0.5	1	0	2	0	1547
Saw	<0.5	0.5	1	1	0	3791

Appendix 7

Objective 2 – Validation of the alternative methods under commercial conditions (carcass data)

	Log_{10} CFU/cm^2				Log_{10} CFU/cm^2		
	TVC	**Ents**	**Verifeye**		**TVC**	**Ents**	**Verifeye**
C1N	3.31	<0.7	0	**S1T**	1.60	<0.7	0
C1R	3.84	<0.7	0	**S1B**	3.49	<0.7	3
C1F	3.57	<0.7	0	**S1F**	2.40	0.7	0
C1Br	4.17	<0.7	0	**S1Br**	1.95	<0.7	2
C2N	3.75	<0.7	0	**S2T**	4.08	2.04	0
C2R	3.44	<0.7	2	**S2B**	4.23	1.30	1
C2F	3.42	<0.7	0	**S2F**	2.67	1.00	0
C2Br	3.92	2.57	0	**S2Br**	3.09	<0.7	4
C3N	3.18	<0.7	0	**S3T**	4.31	1.48	0
C3R	2.89	<0.7	1	**S3B**	3.17	0.7	1
C3F	2.84	<0.7	0	**S3F**	2.29	<0.7	0
C3Br	3.39	2.39	0	**S3Br**	2.77	<0.7	3
C4N	3.02	<0.7	0	**S4T**	1.00	<0.7	1
C4R	3.45	<0.7	2	**S4B**	1.54	1.40	0
C4F	3.59	<0.7	0	**S4F**	3.99	<0.7	3
C4Br	3.48	<0.7	0	**S4Br**	5.46	2.24	3
C5N	2.56	<0.7	0	**S5T**	7.97	0.70	0
C5R	1.60	<0.7	1	**S5B**	8.14	2.29	1
C5F	2.04	0.70	0	**S5F**	8.00	<0.7	0
C5Br	3.28	2.11	0	**S5Br**	6.33	1.40	4
C6N	2.27	<0.7	0	**S6T**	2.68	<0.7	0
C6R	2.66	<0.7	0	**S6B**	4.12	1.60	1
C6F	2.16	<0.7	0	**S6F**	2.95	1.00	0
C6Br	2.58	0.70	0	**S6Br**	3.44	1.00	4
C7N	2.72	<0.7	0	**S7T**	2.34	<0.7	0
C7R	3.19	<0.7	1	**S7B**	4.04	1.00	1
C7F	2.99	<0.7	1	**S7F**	2.52	1.60	0
C7Br	5.00	2.60	1	**S7Br**	3.20	1.00	4
C8N	2.73	<0.7	0	**S8T**	3.28	0.70	0
C8R	2.91	<0.7	0	**S8B**	2.10	<0.7	1
C8F	2.95	<0.7	1	**S8F**	1.93	0.70	0
C8Br	3.24	0.70	0	**S8Br**	3.28	1.00	4
C9N	2.40	<0.7	0	**S9T**	1.88	<0.7	1
C9R	2.89	<0.7	1	**S9B**	2.81	<0.7	1
C9F	3.15	<0.7	0	**S9F**	2.82	<0.7	1
C9Br	2.57	<0.7	3	**S9Br**	4.25	1.40	3
C10N	3.10	0.70	0	**S10T**	2.72	1.84	1
C10R	2.50	<0.7	0	**S10B**	3.27	<0.7	0
C10F	3.41	<0.7	0	**S10F**	3.26	1.00	0
C10Br	2.83	<0.7	3	**S10Br**	4.02	<0.7	4

	Log_{10} CFU/cm^2				Log_{10} CFU/cm^2		
	TVC	**Ents**	**Verifeye**		**TVC**	**Ents**	**Verifeye**
C11N	2.66	0.7	0	**S11T**	2.95	1.48	0
C11R	2.63	<0.7	0	**S11B**	3.29	1.40	0
C11F	2.04	<0.7	0	**S11F**	4.00	1.40	0
C11Br	3.51	<0.7	0	**S11Br**	4.15	1.74	4
C12N	5.95	4.65	1	**S12T**	3.16	1.30	0
C12R	2.50	<0.7	1	**S12B**	2.11	<0.7	0
C12F	1.78	<0.7	0	**S12F**	2.30	<0.7	0
C12Br	2.89	<0.7	0	**S12Br**	3.61	0.70	4
C13N	3.00	<0.7	0	**S13T**	2.56	1.00	0
C13R	3.16	<0.7	4	**S13B**	3.88	2.68	0
C13F	3.13	<0.7	3	**S13F**	2.66	1.40	1
C13Br	3.28	<0.7	0	**S13Br**	2.20	<0.7	4
C14N	3.01	0.70	0	**S14T**	2.61	<0.7	0
C14R	3.17	<0.7	4	**S14B**	2.90	1.40	0
C14F	3.18	<0.7	2	**S14F**	2.82	1.60	0
C14Br	3.45	1.40	3	**S14Br**	2.66	<0.7	4
C15N	3.45	2.00	0	**S15T**	2.84	2.02	0
C15R	2.15	<0.7	1	**S15B**	2.94	1.84	1
C15F	2.74	<0.7	0	**S15F**	2.99	2.16	0
C15Br	2.70	<0.7	2	**S15Br**	2.87	2.13	2
C16N	2.84	<0.7	0	**S16T**	2.82	0.70	0
C16R	3.12	<0.7	1	**S16B**	2.57	1.00	0
C16F	3.36	2.23	4	**S16F**	2.77	1.84	0
C16Br	3.35	<0.7	4	**S16Br**	2.52	<0.7	3
C17N	3.02	1.84	0	**S17T**	3.32	1.00	0
C17R	2.90	<0.7	2	**S17B**	3.88	1.30	0
C17F	3.22	<0.7	0	**S17F**	2.85	<0.7	2
C17Br	3.22	<0.7	0	**S17Br**	4.01	0.70	4
C18N	3.56	2.25	0	**S18T**	2.94	1.00	0
C18R	3.09	<0.7	2	**S18B**	2.75	0.70	0
C18F	2.89	<0.7	0	**S18F**	3.83	2.66	3
C18Br	3.15	<0.7	0	**S18Br**	5.33	2.76	3
C19N	3.23	1.90	0	**S19T**	2.04	0.70	0
C19R	2.29	<0.7	2	**S19B**	4.13	1.74	2
C19F	3.38	<0.7	4	**S19F**	3.20	1.74	0
C19Br	4.20	1.30	2	**S19Br**	4.38	2.22	2
C20N	3.04	2.56	0	**S20T**	2.00	<0.7	0
C20R	2.73	<0.7	2	**S20B**	4.30	0.70	0
C20F	2.88	<0.7	3	**S20F**	3.26	1.54	0
C20Br	3.20	2.90	2	**S20Br**	3.65	1.30	4

	Log_{10} CFU/cm^2				Log_{10} CFU/cm^2		
	TVC	**Ents**	**Verifeye**		**TVC**	**Ents**	**Verifeye**
C21N	2.35	<0.7	0	**S21T**	3.57	1.60	0
C21R	2.90	<0.7	0	**S21B**	3.97	1.30	0
C21F	2.10	<0.7	0	**S21F**	2.94	<0.7	0
C21Br	3.20	<0.7	0	**S21Br**	2.84	<0.7	3
C22N	2.02	<0.7	0	**S22T**	3.62	<0.7	0
C22R	4.86	<0.7	0	**S22B**	3.95	1.78	3
C22F	2.57	<0.7	0	**S22F**	2.28	<0.7	0
C22Br	3.35	<0.7	0	**S22Br**	4.20	<0.7	3
C23N	3.00	<0.7	0	**S23T**	3.23	1.93	4
C23R	3.19	<0.7	0	**S23B**	4.62	2.53	4
C23F	4.58	<0.7	0	**S23F**	2.96	1.81	3
C23Br	<0.7	<0.7	0	**S23Br**	5.29	3.10	3
C24N	1.00	<0.7	0	**S24T**	4.77	2.11	4
C24R	4.08	<0.7	0	**S24B**	4.23	1.54	2
C24F	4.12	<0.7	0	**S24F**	3.46	<0.7	3
C24Br	0.70	<0.7	0	**S24Br**	3.54	<0.7	3
C25N	<0.7	<0.7	0	**S25T**	3.78	<0.7	1
C25R	1.18	<0.7	0	**S25B**	3.82	1.70	0
C25F	<0.7	<0.7	0	**S25F**	3.84	2.16	0
C25Br	<0.7	<0.7	0	**S25Br**	3.41	0.70	3
C26N	1.40	<0.7	0	**S26T**	5.20	2.84	0
C26R	2.24	<0.7	0	**S26B**	1.90	<0.7	0
C26F	1.95	<0.7	0	**S26F**	2.38	0.70	2
C26Br	1.65	<0.7	0	**S26Br**	4.20	0.70	3
C27N	1.40	<0.7	0	**S27T**	2.52	<0.7	0
C27R	1.18	<0.7	1	**S27B**	4.22	3.00	0
C27F	0.70	<0.7	2	**S27F**	2.83	0.70	0
C27Br	<0.7	<0.7	2	**S27Br**	3.30	1.18	4
C28N	<0.7	<0.7	0	**S28T**	2.97	0.70	1
C28R	1.00	<0.7	0	**S28B**	3.28	1.00	0
C28F	1.74	<0.7	0	**S28F**	1.30	<0.7	0
C28Br	1.54	<0.7	0	**S28Br**	4.26	1.40	3
C29N	0.70	<0.7	0	**S29T**	3.36	1.40	0
C29R	1.00	<0.7	1	**S29B**	2.40	1.40	0
C29F	0.70	<0.7	4	**S29F**	2.64	<0.7	1
C29Br	1.30	<0.7	1	**S29Br**	3.18	<0.7	3
C30N	1.40	<0.7	0	**S30T**	2.84	<0.7	2
C30R	2.59	<0.7	3	**S30B**	4.43	2.13	1
C30F	1.84	<0.7	0	**S30F**	3.05	0.70	1
C30Br	2.45	<0.7	1	**S30Br**	5.33	2.70	3

	Log_{10} CFU/cm^2				Log_{10} CFU/cm^2		
	TVC	**Ents**	**Verifeye**		**TVC**	**Ents**	**Verifeye**
C31N	<0.7	<0.7	0	**S31T**	3.41	1.18	0
C31R	<0.7	<0.7	3	**S31B**	4.12	2.24	0
C31F	0.70	<0.7	2	**S31F**	2.16	0.70	2
C31Br	3.52	2.50	1	**S31Br**	3.86	1.70	3
C32N	2.34	<0.7	0	**S32T**	3.43	1.78	0
C32R	0.70	<0.7	0	**S32B**	4.41	1.74	0
C32F	1.18	<0.7	0	**S32F**	2.04	<0.7	0
C32Br	5.27	2.34	1	**S32Br**	3.02	<0.7	4
C33N	2.23	<0.7	0	**S33T**	2.75	1.00	0
C33R	<0.7	<0.7	4	**S33B**	3.45	1.18	0
C33F	<0.7	<0.7	2	**S33F**	2.95	<0.7	0
C33Br	2.75	<0.7	1	**S33Br**	5.83	3.46	4
C34N	1.74	<0.7	0	**S34T**	3.40	1.48	1
C34R	<0.7	<0.7	0	**S34B**	4.83	2.98	2
C34F	0.70	<0.7	3	**S34F**	2.02	<0.7	1
C34Br	2.58	<0.7	1	**S34Br**	4.95	3.06	3
C35N	1.18	<0.7	0	**S35T**	2.79	1.40	0
C35R	2.76	<0.7	0	**S35B**	4.28	2.02	1
C35F	1.81	<0.7	0	**S35F**	3.05	<0.7	0
C35Br	1.87	<0.7	0	**S35Br**	3.68	<0.7	4
C36N	1.48	<0.7	0	**S36T**	3.88	1.40	3
C36R	1.60	<0.7	0	**S36B**	4.58	1.93	0
C36F	1.18	<0.7	0	**S36F**	2.45	<0.7	4
C36Br	3.06	<0.7	0	**S36Br**	4.68	2.54	4
C37N	2.48	1.48	0	**S37T**	3.27	1.30	0
C37R	1.78	<0.7	0	**S37B**	3.41	1.70	0
C37F	1.40	<0.7	0	**S37F**	4.02	1.00	1
C37Br	1.60	<0.7	0	**S37Br**	2.48	<0.7	4
C38N	2.30	<0.7	0	**S38T**	3.50	2.53	1
C38R	2.76	<0.7	0	**S38B**	4.21	1.81	0
C38F	2.50	1.98	0	**S38F**	1.84	<0.7	3
C38Br	<0.7	<0.7	0	**S38Br**	4.12	1.18	4
C39N	0.70	<0.7	0	**S39T**	2.79	1.00	1
C39R	<0.7	<0.7	0	**S39B**	2.94	<0.7	0
C39F	1.00	<0.7	0	**S39F**	4.29	1.78	0
C39Br	1.60	<0.7	0	**S39Br**	3.80	1.30	4
C40N	2.75	1.54	0	**S40T**	3.81	1.84	2
C40R	<0.7	<0.7	0	**S40B**	3.81	1.74	0
C40F	1.81	<0.7	0	**S40F**	3.22	1.98	1
C40Br	2.74	<0.7	0	**S40Br**	4.91	3.00	3

	Log$_{10}$ CFU/cm^2				Log$_{10}$ CFU/cm^2		
	TVC	**Ents**	**Verifeye**		**TVC**	**Ents**	**Verifeye**
C41N	1.88	1.00	0	**S41T**	2.13	<0.7	1
C41R	1.00	<0.7	0	**S41B**	3.62	<0.7	4
C41F	1.00	<0.7	0	**S41F**	2.46	0.70	3
C41Br	2.34	<0.7	0	**S41Br**	2.72	<0.7	4
C42N	1.30	0.7	0	**S42T**	3.30	<0.7	2
C42R	2.36	<0.7	1	**S42B**	2.36	1.18	3
C42F	<0.7	<0.7	0	**S42F**	2.82	0.70	0
C42Br	3.22	1.65	0	**S42Br**	2.45	<0.7	4
C43N	0.70	<0.7	0	**S43T**	3.25	<0.7	2
C43R	<0.7	<0.7	0	**S43B**	2.49	<0.7	3
C43F	1.00	<0.7	0	**S43F**	2.11	0.70	0
C43Br	1.88	0.70	0	**S43Br**	1.81	<0.7	4
C44N	2.60	1.74	0	**S44T**	2.78	<0.7	0
C44R	<0.7	<0.7	0	**S44B**	2.31	<0.7	0
C44F	1.90	<0.7	0	**S44F**	0.70	<0.7	0
C44Br	2.56	<0.7	0	**S44Br**	3.00	<0.7	4
C45N	2.10	<0.7	0	**S45T**	2.78	<0.7	0
C45R	<0.7	<0.7	0	**S45B**	2.93	<0.7	2
C45F	2.37	<0.7	0	**S45F**	2.45	<0.7	1
C45Br	2.46	0.70	1	**S45Br**	2.80	<0.7	4
C46N	<0.7	<0.7	0	**S46T**	1.18	<0.7	0
C46R	<0.7	<0.7	0	**S46B**	1.74	<0.7	1
C46F	2.75	2.84	0	**S46F**	1.93	<0.7	0
C46Br	1.48	<0.7	0	**S46Br**	2.99	<0.7	2
C47N	2.62	0.70	0	**S47T**	1.48	<0.7	0
C47R	<0.7	<0.7	0	**S47B**	2.11	<0.7	3
C47F	<0.7	<0.7	0	**S47F**	1.60	<0.7	1
C47Br	2.46	<0.7	1	**S47Br**	1.30	<0.7	4
C48N	2.10	1.40	0	**S48T**	1.90	<0.7	0
C48R	0.70	<0.7	0	**S48B**	2.93	<0.7	3
C48F	1.18	<0.7	0	**S48F**	2.11	<0.7	1
C48Br	1.48	<0.7	0	**S48Br**	3.61	0.70	1
C49N	1.18	1.00	0	**S49T**	3.62	<0.7	0
C49R	<0.7	<0.7	0	**S49B**	2.45	<0.7	4
C49F	2.25	<0.7	0	**S49F**	3.20	<0.7	0
C49Br	2.19	<0.7	0	**S49Br**	1.93	<0.7	4
C50N	3.11	1.95	0	**S50T**	2.15	<0.7	0
C50R	2.00	<0.7	0	**S50B**	3.04	<0.7	3
C50F	3.00	<0.7	0	**S50F**	2.00	<0.7	2
C50Br	1.18	<0.7	0	**S50Br**	2.04	<0.7	4

	Log_{10} CFU/cm^2				Log_{10} CFU/cm^2		
	TVC	**Ents**	**Verifeye**		**TVC**	**Ents**	**Verifeye**
C51N	0.70	<0.7	0	**S51T**	2.52	<0.7	0
C51R	0.70	<0.7	0	**S51B**	2.69	<0.7	1
C51F	<0.7	<0.7	0	**S51F**	2.19	<0.7	1
C51Br	2.15	<0.7	0	**S51Br**	1.65	<0.7	3
C52N	1.95	<0.7	0	**S52T**	2.11	<0.7	1
C52R	1.90	1.18	0	**S52B**	2.22	<0.7	0
C52F	<0.7	<0.7	0	**S52F**	2.54	<0.7	2
C52Br	1.00	<0.7	0	**S52Br**	2.79	<0.7	3
C53N	1.18	<0.7	0	**S53T**	2.44	<0.7	1
C53R	<0.7	<0.7	0	**S53B**	2.42	<0.7	1
C53F	2.63	<0.7	0	**S53F**	1.70	<0.7	0
C53Br	1.40	<0.7	0	**S53Br**	2.93	<0.7	4
C54N	1.65	<0.7	0	**S54T**	1.74	<0.7	0
C54R	<0.7	<0.7	0	**S54B**	1.70	<0.7	4
C54F	<0.7	<0.7	0	**S54F**	2.27	<0.7	0
C54Br	2.93	0.70	0	**S54Br**	2.74	<0.7	3
C55N	3.38	2.19	0	**S55T**	1.30	<0.7	0
C55R	2.08	<0.7	0	**S55B**	3.10	<0.7	3
C55F	2.78	<0.7	0	**S55F**	2.57	<0.7	4
C55Br	3.04	1.54	0	**S55Br**	2.40	<0.7	4
C56N	3.35	<0.7	0	**S56T**	1.00	<0.7	0
C56R	1.81	<0.7	0	**S56B**	2.68	<0.7	1
C56F	1.60	1.00	0	**S56F**	1.40	<0.7	0
C56Br	<0.7	<0.7	0	**S56Br**	3.28	<0.7	3
C57N	1.70	<0.7	0	**S57T**	2.28	<0.7	0
C57R	<0.7	<0.7	0	**S57B**	2.41	<0.7	3
C57F	2.28	1.30	0	**S57F**	2.81	0.70	0
C57Br	2.08	0.70	0	**S57Br**	3.20	<0.7	2
C58N	3.27	0.70	0	**S58T**	2.57	<0.7	0
C58R	1.30	<0.7	0	**S58B**	2.77	<0.7	3
C58F	<0.7	<0.7	0	**S58F**	2.38	<0.7	4
C58Br	2.81	<0.7	0	**S58Br**	3.22	1.18	4
C59N	1.40	<0.7	0	**S59T**	3.54	<0.7	0
C59R	<0.7	<0.7	0	**S59B**	2.06	<0.7	3
C59F	3.48	1.81	0	**S59F**	1.30	<0.7	1
C59Br	2.06	<0.7	0	**S59Br**	2.42	<0.7	3
C60N	2.79	1.30	0	**S60T**	0.70	<0.7	1
C60R	<0.7	<0.7	0	**S60B**	1.00	<0.7	2
C60F	4.07	2.68	0	**S60F**	1.90	<0.7	1
C60Br	2.83	1.30	0	**S60Br**	3.02	<0.7	3

C = cattle; N = neck, R = rump, F = flank, Br = brisket
S = sheep; T = lateral thorax, B = belly, F = flank, Br = brisket