final report

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Prepared by: Peter Aust & Ray White
Food Science Australia

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Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Sensing for Tissue Characterisation-Contamination Detection

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EXECUTIVE SUMMARY

This report documents the methods and outcomes of Milestones 3 of the project, PRTEC.039 – Sensing for Tissue Characterisation – Contamination Detection. The research undertaken in Milestone 1 & 2 was focused on identifying the major factors relating to slaughter-floor carcass contamination, for both beef and sheep plants. The issue of defects, e.g. bruising & ecchymosis, was also investigated. Research indicated that faeces and ingesta were the main contaminants of concern to the industry, with urine, milk, bile and hair/wool also rating highly. The related costs to industry incurred by the prevention and removal of contamination and defects were also looked at.

The focus of the work presented in this document was the investigation of the actual constituents and the physical and chemical properties of the major contaminants previously recognised. Examples of this are faeces and ingesta, which are chiefly composed of plant matter and hence chlorophyll, particularly where animals are grass fed.

Subsequent to gaining an understanding of these constituents, the focus was on sensing techniques that may be appropriate for their detection. Some of these are in the form of existing systems that are currently being used in industry, e.g. the VerifEYE™; others would be classified as emerging technologies, such as the use of T-Rays. In-house verification trials and investigation at Food Science Australia were performed using thermal imaging and spectrometry. The thermal imaging was based around recently slaughtered carcasses, while the spectrometer was used in conjunction with meat samples

Although there has been a certain amount of research undertaken in the field of contamination detection, there is very little that has filtered through to industry. There does exist, however, a number of technologies that, with further development, may be applicable to an automated system. Methods such as spectrometry, vision systems and “electronic noses” may have a place in an in-line sensing system. These methods have a number of limitations, meaning that a considerable amount of research and development would be required before their adaptation into a slaughter floor was effective.

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1. INTRODUCTION

Contamination issues are increasing in focus for the meat industry in general and in particular processing areas due to regulatory body and consumer expectation of zero tolerance. The risk to communal health with the possibility of litigation means that all meat plants have food safety high on their list of priorities. From Milestone 1 & 2, the main forms of contamination which were found to be of concern to the meat production industry are faeces, ingesta, urine, milk, hair/wool and bile

All slaughter floors have a significant potential for the proliferation of micro organisms introduced through contaminants. The six main bacterial pathogens of concern are: Campylobacter spp., Escherichia coli (E *coli*) 0157:H7, Listeria monocytogenes, Salmonella spp., Clostridium perfringens, and Staphylococcus aureus.

Techniques to minimise the amount of external contamination on the animals prior to entry to the slaughter floor, such as pre-washing and “dag” removal, are currently employed at a number of works. However, the risk of spillage of substance such as ingesta and faeces is difficult to eliminate. During the throat incision of sheep for example (figure 1), substances almost certainly rich in pathogens invariably come into contact with meat surfaces. If contamination occurs, the carcass requires sterilisation and/or trimming. Obviously, all trimming must be performed as carefully as possible, without the removal of excess product.

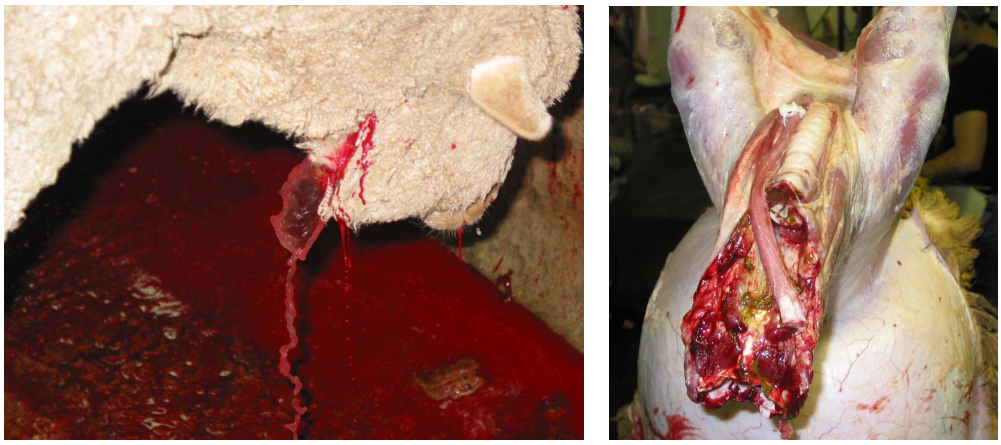

Figure 1a & b - Sheep throat incision, note mixture of blood & ingesta in both

Currently, the meat processing industry relies upon a variety of methods for the inspection of animal carcasses, typically involving human visual inspection, microbiological culture analysis, bioluminescent ATP-based assays, and antibody-based microbiological tests. Unfortunately, these procedures are labour intensive and time consuming, and have sensitivity that may not always be as high as desired. The techniques do not meet the requirements of a high speed, non-destructive system that is suitable for real-time in-line qualitative analysis.

An issue with the implementation of a contamination detection system is that it would need to be robust enough to withstand the abattoir environment. The main issues here are water and steam/moisture, as well as the presence of washdown sanitation products, such as soda-ash. Also, most abattoirs are constructed such that there is a minimum of surplus floor space available, particularly older plants. If a cabinet is required for the mounting of the sensing device and ancillary equipment, finding room for its installation could be a significant problem.

2. ATTRIBUTES/CONSTITUENTS OF CONTAMINANTS

Most of the contaminants of concern on the slaughter floor are organic in nature; the main exceptions being grease, oil and metal fragments from for example, the conveying system, which are mineral based. Contaminants from the hide could be inorganic soil; however it is inevitable that the significant amount is derived from faecal matter. Organic material always has the potential to harbour micro-organisms, which can be the source of food spoilage and/or food borne illnesses. Given this, most industry focus is on organic contaminants.

2.1 FAECAL, INGESTA PLANT MATTER

The main constituent of plant leaf is chlorophyll, along with digestive metabolites of this pigment like the chlorophyll metabolite, pheophytin, and other compounds such as protoporphyrin IX. Chlorophyll can be subdivided into either type '*a'* or type '*b'*. They differ only slightly, in the molecular composition of a side-chain (in '*a'* it is $-CH_3$, in '*b'* it is CHO). Ingesta and faecal matter from beef and sheep will generally contain these substances, with chlorophyll being of particular interest from a sensing aspect. Chlorophyll, it has been determined, has an important sensing characteristic. When activated by a light source with a specific wavelength range, chlorophyll will "fluoresce". Fluorescence, essentially, is electro-magnetic radiation of a certain wavelength, which is emitted when a substance or surface is illuminated by a particular form of light. This property can provide a useful tool for detecting the presence or absence of chlorophyll, and thus possible contaminants.

The amount of chlorophyll present in animal's ingesta/faeces is dependant on the specific diet of the animal. Grass has a high percentage of chlorophyll; whilst grains and other feed components such as fruit peels, have lower concentration levels.

It should be noted that animal diets can vary substantially. According to the (Australian) National Registration Authority for Agricultural and Veterinary Chemicals, the following percentages of feed can be used to make up the long-term diet of cattle and sheep:

Cattle:	**Sheep:**
pasture 100	pasture 100
grain 100	grain 100
pulses/legumes 100	pulses/legumes 100
fodder and forage 100	fodder and forage 100
processed grain fractions 40	molasses 40
molasses 40	fruit by-products 20
fruit by-products 20	oilseeds 30
oilseeds 30	plant protein meals 20
plant protein meals 30	other 5
other 5	

As can be seen from this table, the amount of grass (and therefore chlorophyll) in the diet can vary to a substantial degree. With some diets, the levels of chlorophyll could be virtually non-existent. A number of the plants that lot feed their cattle have indicated that these animals have their diets supplemented with a certain amount of grass/hay before slaughter. This is promising from a sensing point of view due to the aforementioned characteristics of chlorophyll.

2.2 SEEDS

The detection of seeds is an issue mainly with sheep processors. The animals pick up the seeds from grass stalks in the pastures and they can penetrate under the skin and into the flesh. Seeds are found much less in cattle due to the thicker, tougher hide and the fact that their torso is higher above the ground. When detected on the slaughter floor seeds are treated as a contaminant, and are removed by trimming. Visual inspection is used. The main seeds of concern in Australia are Silver Grass, Broome Grass, Barley Grass, Wire Grass, Geranium (Erodium), Chilean Needle Grass and Spear Grass.

Seeds consist of a high proportion of cellulose and have a very low level of chlorophyll; therefore fluorescence would not appear to be a suitable technique. No biological detection methods for seeds were uncovered through this project. Ultrasonic detection is feasible, but would require surface contact with a probe. The use of such a probe would be highly labour intensive or alternatively requiring a complex robot control system. Thermal imaging would prove difficult for assessing for seeds due to temperature equilibration of the seed and the surrounding tissue. The lack of strong contrast between colour of most seeds and carcass fat colour would mean that detection would be difficult with most colour vision systems. This, however, would appear to be the most promising technique to pursue for external contamination.

2.3 HAIR, WOOL

Hair and wool consist 90% of a biological polymer, α-keratin, and about 10% water. They both come in a wide variety of colours, although sheep's wool in Australia is predominately a light cream colour. The resultant contrast of this material against a backdrop of meat or fat is a major issue when using colour as a criterion for detection.

These particles generally have moderate to good visibility to the human eye; however it is much more difficult for detection with a machine vision system, particularly single fibres. A computing system with a set of potentially complex software algorithms would be required to "isolate" the fibres from other features/colours of the surface in question.

Loose surface hair/wool may be visible by thermal imaging due to the inherent lower temperature of the fibres. However, any contaminant of this type trapped in surface fat or selvedge by moisture etc, would have a temperature signature that is insignificant.

2.4 DIRT, SOIL

"Dirt" is a very broad title and it could be any one or a combination of a number of different substances. Possibilities include grease or oil from the conveyor system, fragments of faecal matter, mud or the like transferred from the hide/pelt, and debris, possibly airborne, from various areas of the plant. Due to its diverse nature, there would appear to be few specific features of dirt through which it could be classified by a sensing system.

One main exception would be if the dirt had a high concentration of organic matter as it could contain chlorophyll, possibly from faeces, thus opening up the option of sensing that substance. Dirt being generally dark in colour means that larger particles or smears may be detectable by a vision system. This type of contaminant is generally non-specific as to where it can be found; therefore a whole carcass detection system would probably be required, with an associated increase in set-up cost.

2.5 MILK

Cow's milk is chiefly composed of (with approximate percentages): water (87%), fat (4%), protein (3.4%) lactose (4.8%) and minerals (0.8%). Sheep milk is: water (82%), fat (6.2%), protein (6.2%), lactose (4.8%) and minerals (0.8%). Lactose is a disaccharide consisting of two subunits, a galactose and a glucose linked together. Its empirical formula is $C_{12}H_{22}O_{11}$ and its molecular weight is 342.3.

Milk itself can be tested for contamination in isolation; this can be performed for index organisms in the milk to predict the presence of pathogens, while levels of indicator organisms serve to assess process control. However, this type of testing is not applicable for milk as a contaminant.

After spillage, the white/cream colour of milk is quickly dissipated into the animal surface, which would make it very difficult to detect by vision systems. There is a certain amount of odour emanating from milk that may make a form of technology known as an "electronic nose" feasible. These devices have a sensing receptor that is linked to a computer system with specific software that can identify odours that match the built-in data-base.

2.6 BILE

Spillage of bile can occur during the later stages of evisceration, when the paunch is separated from the red offal. It is produced in the gall bladder on the liver. It has a distinctive yellow/green colour and a relatively strong odour. It is a complex fluid containing water, electrolytes and a range of organic molecules including bile acids, cholesterol, phospholipids and bilirubin that flows through the biliary tract into the small intestine.

The colour and odour both potentially lend themselves to sensing by vision systems and "electronic nose" technology, respectively. However, in the case of the former, the colour of the surface fat can have a bearing on the visibility of any bile present.

3. SUBSTANCES APPLICABLE TO ADVANCED SENSING

Any contamination sensing apparatus that is introduced on a slaughter floor would actually be detecting key physical or chemical properties of particular substances. Physical properties include colour and shape, and chemical properties include features that allow discreet differentiation of constituents.

3.1 CHLOROPHYLL

The natural constituents of plants include chlorophyll *a*, chlorophyll *b* and protoporphyrin IX. Chlorophyll *a,* which is mainly found in the leaves, possesses some useful properties. One of these is that it absorbs electromagnetic radiation (i.e. a light source) at a wavelength (λ) of 400-475 nm (the violet region), and emits electromagnetic radiation (i.e. fluoresces) at λ630-700 nm (red). In comparison, meats fluoresce at λ420-520 nm (blue), when excited by a light source at λ360 nm (ultra-violet). Fluorescence is suppressed if an excitation wavelength of around 420 nm is used.

3.2 TOTAL ATP

The Adenosine Triphoshate (ATP) test is currently used for testing contact surfaces in areas such as the slaughter floor. Protein test methods are unsuitable for carcasses as meat proteins will give a false positive result.

ATP is found in all living cells. Total ATP Bioluminescence kits are available on the market at this time. A swab is taken and Lysol is added to it. The latter causes the release of cytoplasmic and mitochondrial ATP. A sample taken by swab is mixed with reagents and then placed in measuring instrument (luminometer). This test detects the presence of living cells; providing a result in a few minutes. This test does not distinguish between eukaryotic and prokaryotic cells (i.e. animal cells versus bacteria) and can be affected by some cleaning chemicals, e.g. alkaline cleaner may decrease the sensitivity.

Due to the fact that this is not a real time test, it is not conceivable that this method could be readily adapted to an effective automated system. It perhaps could have an application as an overall system checking tool.

3.3 MICROBIAL ATP AND PHOSPHATASE

ATP systems are becoming more sophisticated and can now give results within 5 minutes. They allow detection of 2-3 log cfu/cm^2 (colony-forming units per square centimetre) total viable bacteria count (TVC) on carcasses. The test involves sampling a carcase with a sponge from the templated area. Research is currently in progress to assess the potential in a commercial environment. There is also research being undertaken on specific ATP tests for individual micro-organisms.

Phosphatase (an enzyme occurring naturally in most raw foods and micro-organisms) testing is commonly used in the dairy industry. The time required is about 20 minutes and at present further research is required to determine the potential of this test in a commercial situation.

Again, due to these tests not being “real time”, these methods could not be readily adapted to an effective automated system for individual carcass contaminant control.

4. SENSING OPTIONS

In general, the sensing techniques discussed here would be applicable for external sensing only, i.e. contamination on the outer surfaces of the carcase. Most internal sensing methods such as X-Ray are essentially unsuitable for sensing external contamination and were also deemed to be unsuitable due to the inherent problems involved, such as the complexity of the infrastructure required. This type of apparatus requires full personal exclusion for OH&S reasons. Any introduced system would obviously need to be safe for the plant staff involved. Also, as well as having a primarily objective of improving food safety for the product, it would need to be proved that the system did not have any possible ill-effect on the safety qualities of the product. There has been, for example, substantial resistance to irradiating of meat, due to a number of factors such as residual toxins.

The options in available sensing systems include the technologies discussed below. Some of these are well developed, others are emerging (e.g. T-rays). Some, e.g. vision systems are currently being investigated for use in the specific application of contamination detection.

4.1 FLUORESCENCE

The principle behind fluorescence is that certain substances, when excited by electromagnetic radiation (generally from around the visible spectrum range) will emit radiation that is of a measurably different wavelength. If the expected wavelength (or range) is known, this property can be used as a positive marker for that substance. Detection would be via a spectroscope or camera hardware with a wavelength range sensitive grid or filter treatment. For example, chlorophyll, as found in plant matter, exhibits a particular behaviour when illuminated with radiation around the 450 nm wavelength region.

One commercial system developed for the detection of chlorophyll is the VerifEYE™. These are manufactured in the USA, where they are in common use in meatworks, with a few being taken up in Australia. The process is such that results from the target surface are available immediately on the display screen. Any chlorophyll detected is portrayed as a change in colour in the image, i.e. from purple to red. It has been claimed the VerifEYE™ instrument can detect rumen and faecal contamination up to 100 times (2 log) more diluted than that detectible by human vision. With re-tuning, this procedure can also be used to detect nerve tissue, a factor where BSE is concerned.

The mode of operation of this apparatus involves the shining of light at specific wavelengths (~440 nm) onto the surface of a carcass and subsequently monitoring, through cameras, the wavelength of the fluoresced light emanating from the surface. Chlorophyll fluoresces at around 650 nm.

A limitation is that this system is dependant upon the animal having a certain amount of chlorophyll in their diet just prior to slaughter. Also, detection results can be affected by ambient lighting levels and the presence of other substances such as vegetable-based marking inks, bone and some connective tissues. The presence of excessive surface water, i.e. droplets, can also give spurious results.

Currently the VerifEYE™ is available in two configurations, a cabinet unit for testing of whole sides and a hand-held unit for spot checking. The cabinet set-up is suitable for production up to ~450 head per hour, and its purchase cost is very high. The hand held unit with an on-board video screen display, is significantly cheaper. The cabinet unit has the potential for automated side tracking of positive-reading carcasses

A wide literature and World-Wide-Web searches failed to uncover any other commercial units based on this type of technology, no doubt influenced somewhat by the patent for the VerifEYE™ system.

4.1.1 SPECTROMETER VERIFICATION TESTS

Tests were performed at FSA using an Ocean Optics® HR2000 Spectrometer. This has been set up to be sensitive in the 300 – 700 nm wavelength region, with a 200 m aperture slit to enable fluorescence measurement Basically this is a device that connects to a computer by a USB cable, with a hand-held probe connected to both a light source and the spectrometer via fibre-optic cables. As part of the set-up, three Ocean Optics® light sources were evaluated: LS1, PX2 and 2000 BAL. The 2000 BAL was found to have the strongest light output in the desired UV region; therefore it was used for all the tests. This unit has two light sources: a halogen bulb and a deuterium bulb, which provide a wide output spectrum. The system has provision for the insertion of filters to reduce or eliminate the intensity of specific wavelengths of light. The filters can be inserted in the light source output stream (light going to the probe) and/or the spectrometer input stream (light coming back from the probe). Various combinations of in-line filters were inserted at stages of the trials, to provide the most appropriate signal from the samples.

The samples were pieces of bovine brisket meat, with some subcutaneous fat still intact if required for comparative measurements. The portions were smeared over half their area with the appropriate contaminants for the tests. Substances used were: ingesta, faeces, urine, milk and bile. Tests were carried out under controlled conditions, in restricted ambient lighting to maximise sensitivity of results.

The following five graphs (figures 2 – 6) show results from the sample with and without the above–mentioned contaminants. It can be seen that the signal from each of the five different pieces of meat bare close resemblance in profile, as one would expect. These results were taken from exposed meat, because fat and selvedge coverings gave significantly different profiles, dependant on the actual colour of the surface. In reality, the intensity varied significantly over different parts of the meat surface, with the profile maintaining a consistent shape. It would appear that variations were due to factors such as specific moisture levels (i.e. minute droplets) and changes in the surface texture and contour. The intensity level of the signal as indicated by the results varied substantially with small changes in probe – surface distance. The recorded data were obtained with the probe located around 3 mm from the test piece surface.

As mentioned previously, chlorophyll (found in most ingesta and faeces) will fluoresce (give off light radiation) at a wavelength of 630 – 700 nm when illuminated by a source of wavelength 450 – 500 nm. For the tests with faeces and ingesta, the light sources were set up such that they were supplying most intensity in the required frequency. For the other materials, a broad spectrum light output was used to facilitate the highest likelihood of determining if a signal different from that with the corresponding meat tissue could be obtained.

A spike in response at around the 700 nm wavelength region can be detected for the ingesta and faeces samples (figures 4 & 5), with ingesta giving a stronger response on this occasion. There is also a reduced response noticeable at around 400 nm wavelength in both these two results. This corresponds with the radiation absorbed by the chlorophyll. No discernable specific responses (with relation to meat only readings) could be found with the other three samples.

The tests performed here give only a brief snapshot of what could be undertaken in a full scale series of trials. Further investigation could look at more intense light sources, a wider range of filters as well as some of the more advance features of the spectrometer, including transmittance and absorption. Different surface characteristics such as fat and selvedge covering could also be another variable.

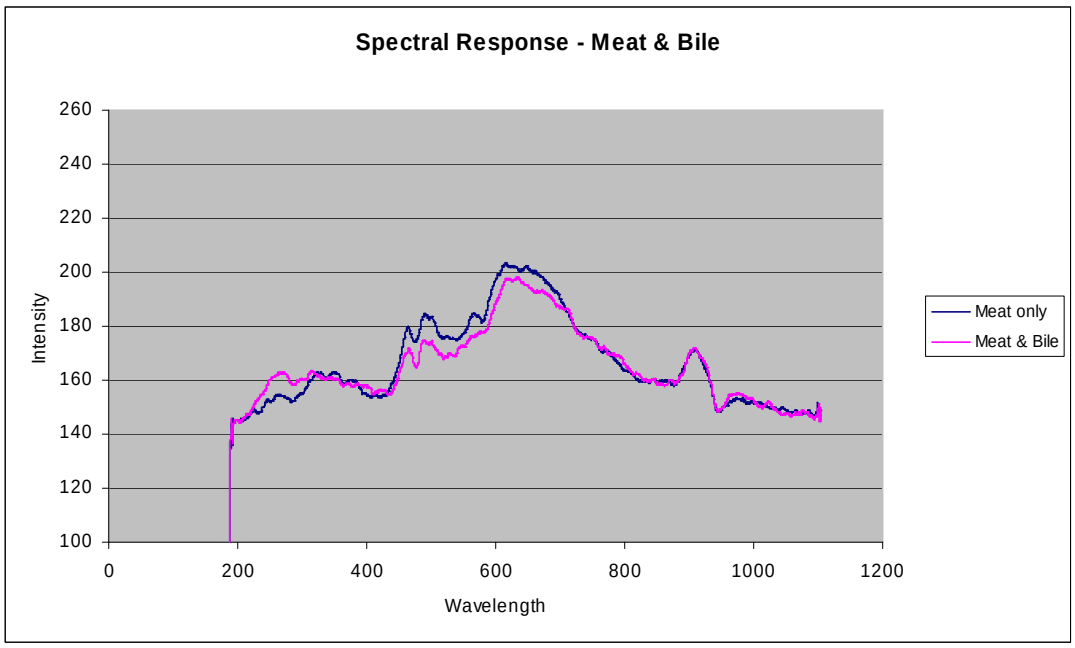

Figure 2 - Meat & Bile

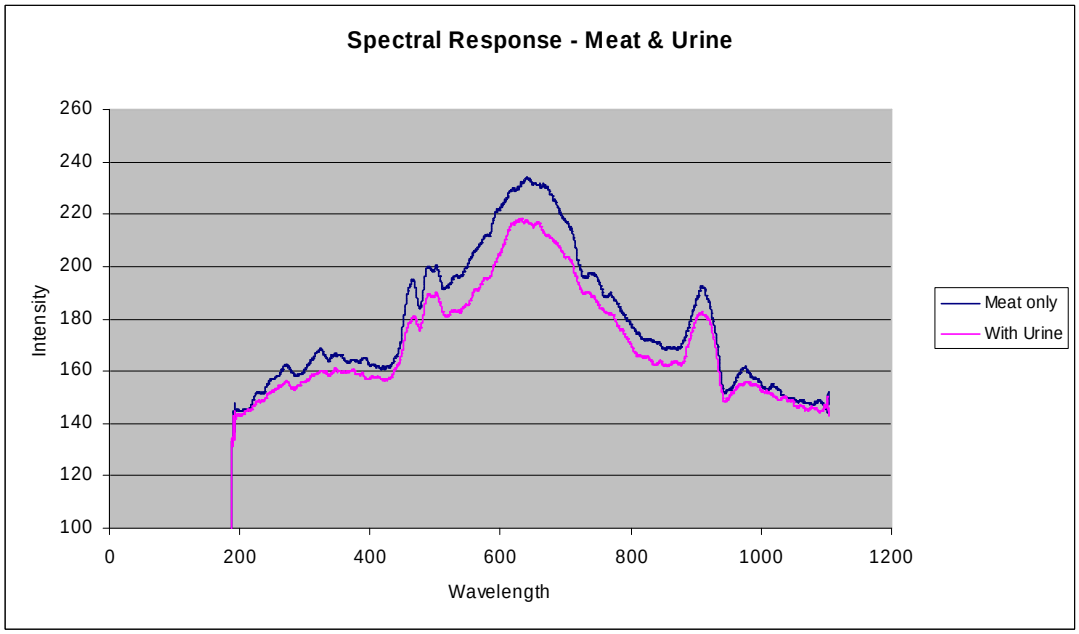

Figure 3 - Meat & Urine

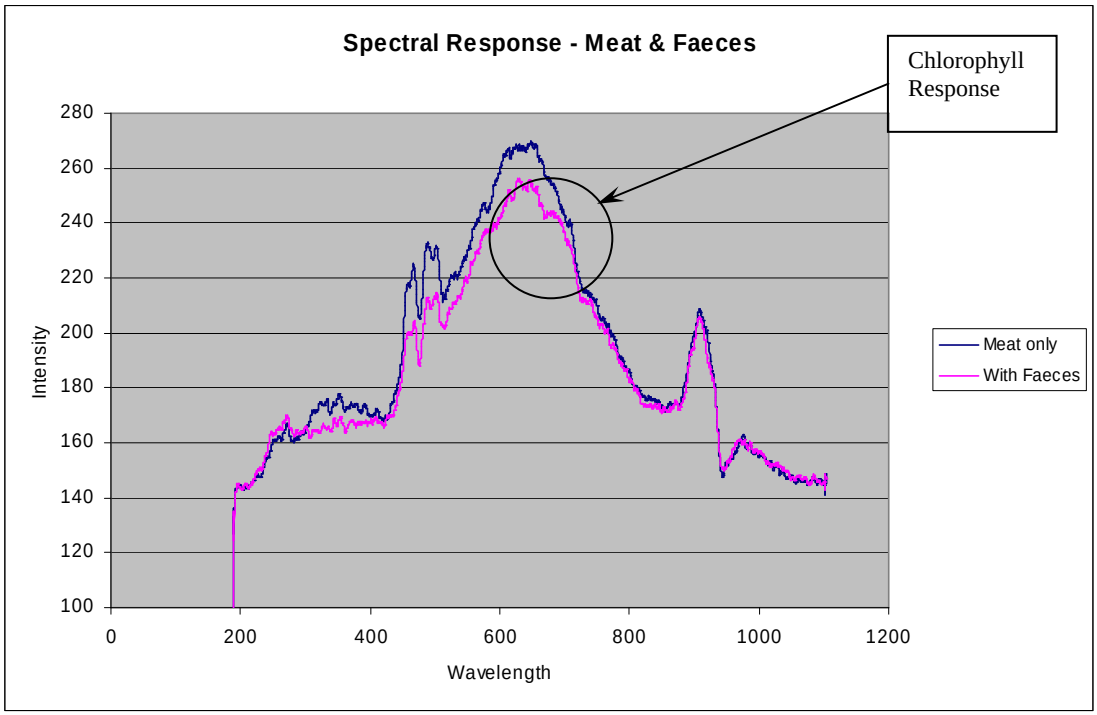

Figure 4 - Meat & Faeces

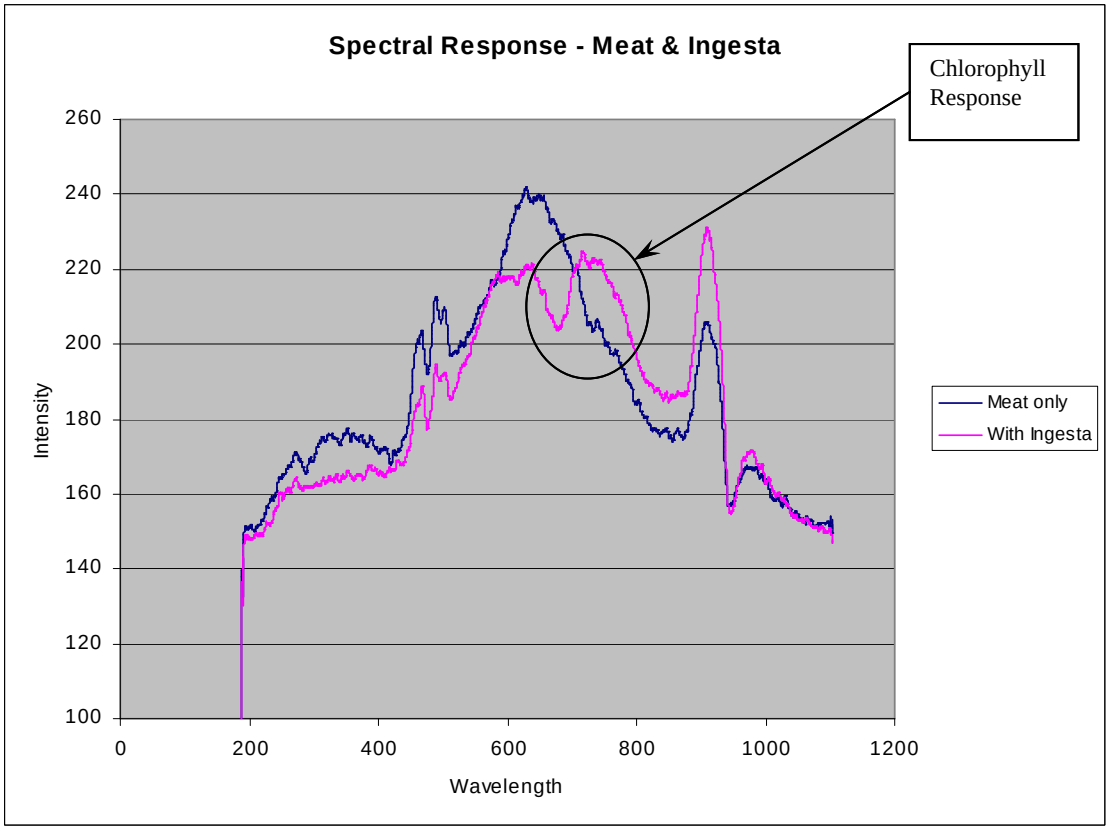

Figure 5 - Meat & Ingesta

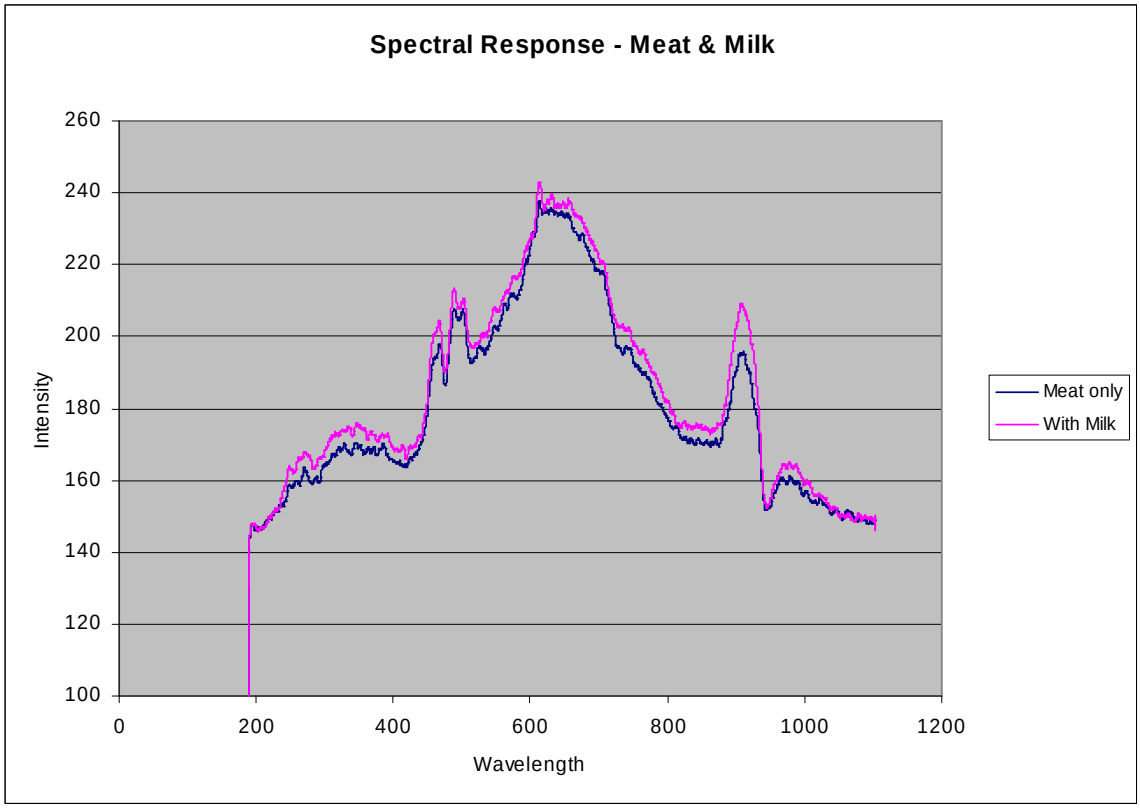

Figure 6 - Meat & Milk

4.2 T-RAYS

T-rays are from a part of the electromagnetic radiation spectrum that includes microwaves, infrared, visible light, ultraviolet and X-rays; with a frequency of around one trillion ("tera") cycles per second, which puts them in the far-infrared zone. Terahertz radiation, known as T-rays, is emitted when electrons traveling at nearly the speed of light are deflected by a magnetic field. Objects at room temperature emit thermal energy in the THz range. T-rays have a potential for medical imaging, communications and even quality control. T-rays can pass through many substances with the main exception being metal and water. Due to this ability, the result of their short wavelengths makes T-rays a candidate for many applications.

Among the potential uses and benefits proposed for this technology are: safer alternative to X-rays, scanning baggage at airports, and identification of large biological molecules such as proteins, viruses and bacteria. Unlike X-rays, they do not require shielding, with photon energies that are 1 million times weaker than X-ray photons. T-ray systems can offer more than just images: they can provide valuable spectroscopic information about the composition of a material, especially in chemical and biological species. Many packaging materials are transparent to T-rays, making them ideal for use in non-destructive mail screening. Using *Bacillus thurengiensis* (Anthrax) bacteria, researchers in Germany were able to use T-rays to image bacterial spores inside an envelope.

It appears that although there are developments occurring in this field, particularly in the area of bacteria, at this stage there are no recommended systems applicable to sensing of contaminants in the meat industry.

4.3 VISION SYSTEM

A computerised vision system generally consists of five basic components: illumination (light source), camera, an image capture board (frame grabber or digitiser), and computer hardware and software. As with the human eye, vision systems are affected by the level and quality of illumination. The source can either be in the UV (200-400 nm), VIS (400-700 nm), or NIR (700-2500 nm) band. There are also applications in thermal imaging (above 2500 nm) for agricultural products. Various aspects of illumination including location, lamp type and colour quality will have a marked bearing on the results. Front lighting would be required for a carcass surface application. Back lighting can be used for analysis of a silhouette shape, but this would not be suitable for surface contamination identification.

The image sensors used in machine vision are usually based on solid state, charged couple device (CCD) camera technology. CCD cameras are either array or line scan types. Array or area type cameras consist of a matrix of minute photosensitive elements (known as photosites) from which the complete image of the object is obtained based on output proportional to the amount of incident light. Alternatively line scan cameras use a single line of photosites which repeatedly scan up to 2000 times per minute to provide an accurate image of the object as it moves under the sensor. Cameras can be either colour or monochrome depending on the requirements. (Chen et al, 2003))

Machine vision can be performed using X-ray imaging and nuclear magnetic resonant imaging (MRI). These imaging technologies are already widely used in medical applications. Even though they have potential for detecting diseases and defects in agricultural products and food, their applications in the agricultural sector were previously limited because of the high cost of equipment investment and low operational speed.

With a downward trend in hardware and software costs, computer vision systems are now being used increasingly in the food industry for quality assurance purposes. The possibility exists for a conventional vision system to be utilised for detection of surface particles such as seeds or faecal clumps. To achieve detection, the contaminant would require a physical attribute such as a well defined outline or a three dimensional aspect/texture to make it distinguishable from the surrounding tissue. These systems are not generally as effective for detection of contamination which may have indistinct boundaries such as smeared faeces or spilt milk, bile or urine. In basic terms, if the item/material can be clearly seen by the naked eye, there is some chance that a vision system could be utilised.

Food Science Australia has already undertaken a substantial amount of research into the use of vision systems on the slaughter floor. Carcass parts such as hocks and briskets are being assessed as possible subjects of vision sensing for production challenges. The detection of carcass contamination through such a system would be far more challenging; particularly due to the need to simultaneously sense and identify many contaminant types.

4.3.1 MULTISPECTRAL

Multispectral imaging consists of a set of several images, each acquired at a narrow band of wavelengths. The simplest method to obtain images at a discrete spectral region is by positioning a bandpass filter (or interference filter) in front of a monochrome camera lens. A more advanced approach in multispectral imaging is the use of a common-aperture multi-channel imaging camera. A three channel common-aperture Multispectral imaging camera is similar to the three chip colour camera. The ranges of spectral regions are accomplished by proper selections of dichroic coatings and bandpass filters. In conjunction with an appropriate image processing algorithms, the multispectral imaging system has been used as an effective technique for the real-time identification of fecal and ingesta contaminants on poultry carcasses (Park et al, 2004). This system has been demonstrated at a processing speed of 140 chickens per minute.

In relation to carcass sensing, further research is required into image data from different diet samples, such as milo and wheat. This is because the type of feed ingredients used in animal finishing diets is an important factor in the selection of the optimum wavelength to differentiate faeces and ingesta contamination. Further research in the development of classification algorithms of individual faeces and ingesta would enable the implementation of a multispectral imaging system for hazard analysis critical control point (HACCP) application.

4.3.2 HYPERSPECTRAL

In recent years, hyperspectral imaging has emerged as a powerful technique in areas such as earth remote sensing and medical diagnosis. This technique combines the features of imaging and spectroscopy to acquire both spatial and spectral information from an object. The technique yields much more useful information than other imaging techniques, because each pixel on the image surface possesses a spectral signature of the object at that pixel.

Spectroscopic data analysis techniques can be used to extract chemical composition from each or an aggregate of pixels. Because of these combined features, hyperspectral imaging can greatly enhance the capability to identify materials and detect subtle and/or minor features in an object. Applications range from precision agriculture applications, such as detection of plant stress or crop infestation, to medical applications, as well as agricultural product quality and safety sensing. (CSIRO - MIS, 2005)

Two general approaches have been used in the development of hyperspectral imaging techniques. One of the approaches is to sequentially capture a series of narrow-band spectral images to accomplish a three-dimensional image cube. Another approach is a “pushbroom” method where a line of spatial information with a full spectral range per spatial pixel is captured sequentially to complete a volume of spatial-spectral data. The fact that CCD detectors have two-dimensional arrays and a spectrograph allows simultaneous recording of a line of spatial and a multiple of spectral information. The advantage of this type of system is that sample sizes in one of the spatial directions are not limited by the size of CCD as compared to the first approach that sequentially captures a series of narrow-band spectral images.

4.4 THERMAL IMAGING

This technique is based on detecting the amount of heat energy being emitted by the subject in question. Specific devices are available which generally consist of a camera (using what is known as a micro bolometer array), an on-board processor and a video screen. A number of settings and adjustments are also available; to select temperature and distance ranges, etc. The cameras are hand held and most can be connected to a video or computer for image downloading.

A thermal camera was used during the MLA project PRTEC.040 - Interface Detection. Images applicable to the Tissue Characterisation project were obtained at this time. Promising results came from the use of this apparatus, particularly in the detection of hair on beef carcases, see figure 7, taken of a carcass lying down in a cradle. The centre of this image depicts the brisket area with the hide opened up. Ticks also were found to have an identifiably different thermal signature, i.e. colder, when photographed at the pre-hide removal stage, see figure 8. Gross faecal material was readily visible, see figure 9; however as this material cooled, thermal differentiation declined quickly.

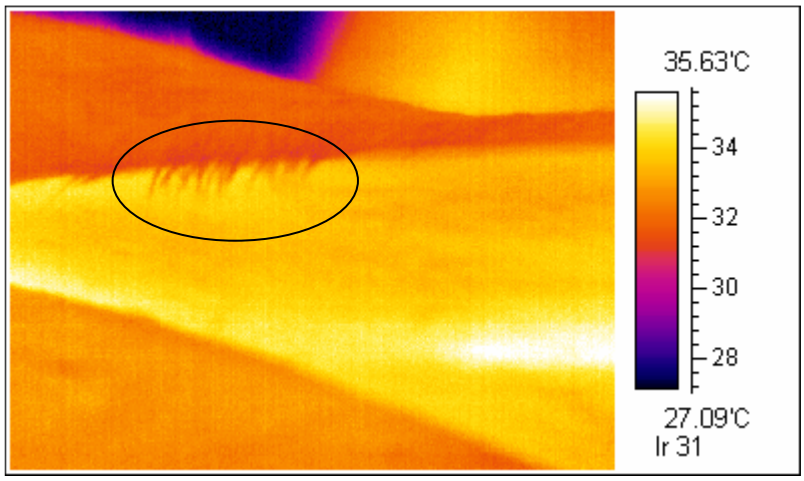

Figure 7 - Thermal image of beef brisket, showing hair against meat

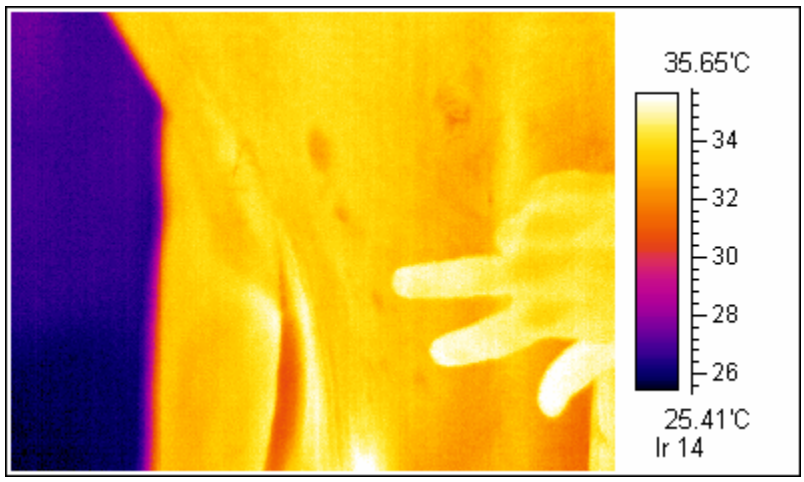

Figure 8 - Thermal image of ticks on beef hide

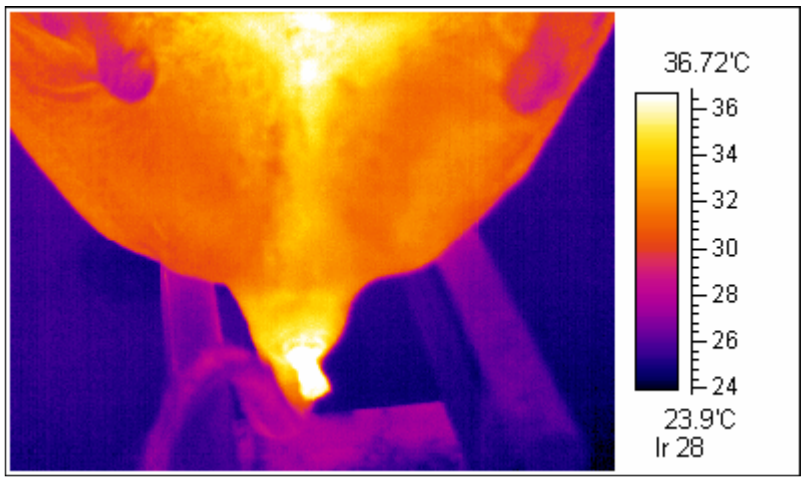

Figure 9 - Faecal material emanating from beef anus

4.5 INFRARED

Research has been carried out at the University of Manchester in relation to the real time detection of bacteria through the use of infrared light. Also pursuing this line of work is the Joint Institute for Food Safety and Applied Nutrition (JIFSAN), Chemistry and Biochemistry Department, University of Maryland College Park in the USA. Researchers have investigated the use of Fourier transform near-infrared (FT-NIR) spectroscopy along with "multivariate pattern recognition" techniques for the rapid detection and identification of bacterial contamination in liquids. In technical terms, the complex biochemical composition of bacteria yields FT-NIR vibrational transitions (overtone and combination bands) that can be used for classification and identification. (Food productiondaily, 2005)

The system developed in the United Kingdom can identify contaminated meat in seconds using infrared spectroscopy and genomic algorithms. This technology has the processing speed to enable integration into production lines. The technique incorporates infrared spectroscopy and software algorithms to identify spoiled meat in just 60 seconds, as opposed to the hours it typically takes using current methods.

Rather than detecting the bacteria itself, this method detects biochemicals produced when microbes break down food. Food spoils when the concentration of bacteria reaches a certain threshold (1 million bacteria per square centimetre for beef), and they break down nitrogenous compounds. The technology uses an emitted infrared beam, which bounces off the sample. The resulting spectrum shows an indication of the level of biochemicals. Software designed to read the spectrum then determines whether the product is acceptable.

Initially, the researchers collected the spectra from meat samples kept at room temperature every hour for a total of 24 hours, and simultaneously measured the actual bacterial count. They analysed the data with a computer, where genetic algorithms distilled the thousands of wavelengths to a small number that would best indicate that the bacterial concentration has risen to spoilage threshold levels. The software senses contamination by searching for these wavelengths in meat samples. The technique has detected bacteria concentrations as low as 10,000 on beef samples, but ideal wavelengths for beef have not yet been defined.

The technology to create handheld spectroscopic detectors for food inspection is currently in use to sense explosives and toxic material detection. It would be questionable if enough microbial break-down would occur during the slaughter process for this type of sensor to detect bacterial growth on a carcass.

4.6 ELECTRONIC NOSE

Several versions of a so-called “Electronic Nose”, systems developed for the detection of odours, have been released in recent times. One of these is produced in Australia by E-Nose Pty Ltd. This type of technology can be utilised to determine odour concentrations above prescribed levels. Information released by the company indicated that very little research has been performed worldwide in the area of carcass contamination detection. One of the main problems is the directionality factor involved with airborne particles. Any detected odour would not necessarily have reached the probe via any certain path. Hence, the technology would probably only be suitable for detecting the presence of contaminants, without providing an indication of specific locations.

Another key factor is that odours emanating from bacteria-caused degradation take a certain period of time to develop. These smells are from the breakdown of tissue by the action of the microbes. It would be unlikely that these odours would have sufficient time to develop on a carcass on the slaughter floor. There would be a more appropriate application for this technology in the case where there is a signature odour from a substance itself, such as faeces, urine or bile.

4.7 EMERGING TECHNIQUES

University of Rochester Medical Centre scientists have demonstrated a technology based on reflective interferometry, which accurately and rapidly detects the meat-spoiling and potentially dangerous *E. coli* bacteria. This novel technology uses a protein from the suspect bacteria as part of the sensing system that also uses a silicon chip and a digital camera. The technology could potentially used to detect any biological entity.

The technology is referred to as “arrayed imaging reflectometry.” The system utilises a silicon chip that is made so that laser light reflected off the chip is invisible unless the target bacteria are present. For example in the case of *E. coli*, a protein from that bacteria, Translocated Intimin Receptor (Tir), is placed on the chip. The Tir can be seen as a “molecule harpoon”. (Horner, 2003)

Another technology that has found use in some HACCP programs is Impedimetry. This is based on the monitoring of electrical changes caused by the growth of micro-organisms Impedance is a vector parameter consisting of two components: conductance (G) and capacitance (C). The changes can be measured either directly or indirectly. In direct impedance measurement, nutrient macromolecules are broken down into smaller high-charged units as a result of microbial metabolism. The conductivity change of the medium is measured. Indirect impedance is based on the production of carbon dioxide by growing micro-organisms. The carbon dioxide is absorbed into an alkaline solution and the reduction of conductivity of the solution is measured. Detection time is reduced from the direct method. The indirect measurement can be carried out without specific growth media.

A further development involves a handheld sensor that could help food companies quickly detect within 10 minutes whether their products are laden with Escherichia *coli* or Listeria -- before they are shipped out of the plant. The device works by detecting how the mass of a few *E. coli* cells changes the vibration of a miniature glass beam. Despite the obvious benefit of this unit as part of a HACCP system, there does not appear to be any correlation with the equipment to be developed into an in-line, real time sensing system.

A company known as AgroMicron, Ltd is working on a bioluminescence detection test that could be a quick way of spotting dangerous contamination such as E. *coli* and salmonella on food. They are attempting to create a nano-scale test which should provide an instant verdict and be easy to use. The “Nano Bioluminescent Spray”, when applied, will react with the target pathogen strain and produce a luminescent glow to allow detection. The system is to be labelled 'Bio Mark'. (AgroMicron, 2006)

The plan is to use a small, luminescent protein molecule which has been modified so that it attaches itself to the surface of the target bacterium. This would work in a similar fashion to an immune system antibody, designed to lock on to a particular feature on the "coat" of the microbe. In this case the higher the number of connections between bacteria and molecules, the more intense the glow produced.

5. CONCLUSION

Many losses are encountered in the meat industry due to the presence and subsequent removal of contaminants and contaminated product. Investigation has shown there is considerable scope for further research into the application of several of the sensing techniques identified for the detection of physical contamination as well as micro-organisms.

A wide range of specific trials would be required to ascertain the efficacy of the selected techniques, in particular:

- Spectroscopic technologies appear to be very promising in selection of materials such as chlorophyll. Further trials should concentrate on light sources of greater intensity as well as utilising a comprehensive range of grids, aperture settings and filters. So-called “pulsed light” sources should also be tested under a wide range of samples and contaminants to specifically quantify fluorescence properties. The effect of different surfaces such as fat and selvedge on spectrometer readings should also be looked at.

- The use of an “electronic nose” has potential as a means of detecting information concerning the general occurrence of surface contamination. The main drawbacks with this technology are the relatively low levels of research being undertaken in the field of carcass contamination, the lack of directionality, and the low sensitivity of the instruments availability (when compared with what would be required in this application). Stronger smelling materials such as faeces, bile and milk would probably be more appropriate for application of this technology.

- Vision sensing using CCD cameras would also be worth further investigation, particularly for material such as faecal smears, ingesta spillage and seeds in sheep, as well as defects such as bruising. The limit of this method would always be the “visibility” or contrast of any contaminants, bearing in mind that most contamination cannot be detected by the naked eye. The use of light sources from the non-visual range (wavelength <400 nm, or >700 nm) in conjunction with matching detection apparatuses may be worth pursuing.

- Thermal imaging would not appear to offer a wide scope for contamination and defect detection, with the possible exception of a small number of specific items such as seeds, wool, hair or ticks, or for “internal” contamination where the bodies physical/immuno-compromised response (e.g. abscess) may be defected

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