A project to establish whether carry-over of meat species occurs in UK meat processing plants during the GMP production of mince meat

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1. LGC

LGC is an international science based company and is Europe's leading independent provider of analytical, forensic and diagnostic services and reference standards. Our products and services enable our customers to achieve excellence in investigative, diagnostic and measurement science and to conform to international statutory and regulatory standards. LGC is the UK’s designated National Measurement Institute for chemical and biochemical analysis and is also the host organisation for the UK’s Government Chemist function. Separately, LGC also holds a range of National Reference Laboratory duties including the National Reference Laboratory for added water in poultrymeat. With headquarters in Teddington, South West London, LGC employs over 2,000 staff, operating out of 22 countries worldwide with laboratories and centres across Europe and the US, as well as sites in Brazil, China and India.

LGC was awarded this project following the submission of a proposal to the Department for Environment, Food and Rural Affairs (Defra) and the Food Standards Agency (FSA).

2. Document Control

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Approvals

The document obtained the following approval:

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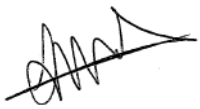
Steve Wood		Head of Regulatory Services	21 March 2014	2
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Name	Title	Date of Issue	Version
Mr John Barnes	Head, Local Delivery Division, Food Standards Agency	23 April 2014	4
Dr Lucy Foster	Food and Environmental Risks Science coordinator and Food Chain Evidence Programme Manager, Department for Environment, Food and Rural Affairs	23 April 2014	4

3. Executive Summary

Food authenticity failures and food fraud can be driven by pressures on food production and the current climate of financial constraint. The horse meat incident which emerged in early 2013 impacted on confidence in the UK food supply chain and showed that the presence of undeclared species in meat products is of concern to consumers. Including those consumers who choose to avoid certain species from their diets on the basis of faith.

The incident with the presence of horse and pork meat in processed beef products in 2013 has raised a number of questions including whether carry-over, i.e. adventitious contamination of meat species occurs during industrial production of meat products prepared according to good manufacturing practice (GMP).

A review of scientific literature showed that information on the carry-over of meat species during commercial processing was not available. As a consequence, the Food Standards Agency (FSA) and the Department for Environment, Food and Rural Affairs (Defra) commissioned a research project to assess whether carry-over of meat species occurs during the industrial production of minced meat according to GMP in the UK and, if it does, at what concentrations it occurs.

The project plan was devised by LGC with input from statisticians and was widely consulted on with industry groups and Defra's Authenticity Methods Working Group (AMWG) (Expert advisory group). Although it was acknowledged that there are many points during the meat processing process that could give rise to the carry-over of meat species, it was agreed by experts that the mincing stage was the step that had the greatest potential risk for gross contamination. The project therefore focussed on the mincing process.

Two commercial quantitative real time PCR kits were selected for use in this project; one for the analysis of carry-over of raw pork in raw beef meat samples and the other for the analysis of swab samples to test for the presence of pork. Before any samples were processed, the analytical procedures were validated at LGC using gravimetrically prepared, on a weight: weight basis, quality control materials. A reporting limit of 0.1 % raw pork in raw beef was established and further work indicated that a LOD of 0.03 % raw pork meat in raw beef was achievable. It was also established that there was no statistical difference between the results reported as % pork DNA and the actual percentage pork present determined by mass, but a consistent positive bias was observed. Evaluation of the results obtained for the 0.1 % raw pork in raw beef quality control standard estimated a reported pork content of 0.04 to 0.27 %; this variation is in line with expectations when working at the reporting limit of a method. The results for this project are reported on a quantitative weight: weight basis and represent the 'best case scenario' in that the standards and samples were made from the same authentic lean meats.

The project was conducted in two phases; phase 1 was carried out in a commercial pilot plant under controlled conditions and phase 2 trials were carried out in three working UK commercial plants. Both phases focussed on raw pork carry-over into raw beef mince. Input meat for phase 1 was sourced from single species abattoirs after an audit of the process by LGC staff. Input meat for phase 2 was provided by the plants from their intake meat used for production. All mincing and cleaning operations were conducted by plant staff and all meat samples were taken by LGC staff. A total of 1032 beef samples and 390 swab samples were analysed.

The results from phase 1 showed that both deep chemical cleaning and cleaning with high pressure water of industrial mincing equipment and conveyor belts in line with GMP were

effective in preventing carry-over of pork meat into beef (down to 0.1 % raw pork in raw beef on a weight for weight (w/w) basis). These results were confirmed in the phase 2 when the work was replicated in commercial plants using their input meats and processes.

When no cleaning was carried out between species processing, both the phase 1 and phase 2 studies showed that significant carry-over of pork meat into beef meat does occur. The level of carry-over in each was, however, different. In the pilot plant study carry-over of pork into beef was most significant in the first 0.75 kg of meat where the concentrations ranged from 99 % to 54 % pork. The estimated pork carry-over in the 100 kg of beef processed in the pilot plant trial was 653 g (0.65 %), whilst the estimated pork carry-over for experiments carried out in a commercial plant was 11.2 kg in 200 kg (5.6 %). The difference in the estimated pork carry-over between pilot plant and commercial plant studies is thought to be due primarily to the differences in the mincing equipment used, e.g. size, age, complexity, potential traps points etc…. This indicates that the amount of carry-over will vary from plant to plant based on the equipment and processes used. This report provides clear evidence that detectable levels of carry-over does occur when no cleaning is undertaken between species which needs to be considered by manufacturers when presenting information to consumers about the composition of meat products so the contents are accurately described.

To check the effectiveness of the cleaning regimes used in commercial meat plants (deep chemical and water wash), three types of swabs were taken; adenosine triphosphate (ATP)(ATP is present in all organic material and a positive reading is an indication of the presence of contamination, for example food residue, allergens and/or bacteria), protein and DNA swabs. Following a deep chemical clean, all three swabs gave equivalent negative results demonstrating that any one of the three swab methods may be used to check the effectiveness of cleaning. However, although the majority of swabs taken after the water wash also gave negative results, there were some notable differences:

- One positive protein swab result was obtained (negative after re-cleaning)
- Several very high ATP results were obtained (reduced after re-cleaning).

The results indicate that from a practical perspective, of the two cleaning methods used, the deep chemical clean is the most effective cleaning method as all swabs taken by all three swabbing methods gave negative results in both Phase 1 and 2. In addition, no carry-over was observed in any of the beef samples taken after a deep chemical clean. The water wash method did give rise to one protein failure and a number of ATP failures (in both phase 1 & 2) demonstrating that greater care is required to ensure cleaning is effective in removing all traces of organic matter. However, no carry-over was observed in any of the beef samples taken after a water wash demonstrating that it is an effective cleaning method, with regards to preventing carry-over, for equipment which is readily accessible and can be easily dismantled. Unless it is important to monitor the effectiveness of cleaning with respect to proteins, e.g. in plants producing allergen-free food or with respect to individual meat species, e.g. Kosher / Halal plants, then the protein and DNA swabs offer no further advantage to the ATP and cost more to undertake.

Thus, of the three types of swabs taken, ATP appears to be an appropriate cost-effective way to monitor the cleanliness of equipment with regards to generic traces of residue provided each plant undertakes the validation required to establish its own control limits.

The project has fulfilled its objectives and generated data that previously did not exist in the scientific literature. It has been established that when raw minced beef is produced according to GMP, either a deep chemical clean or a high pressure water wash between species is effective in preventing the carry-over of raw pork into raw beef with an associated limit of detection (LOD) of less than 0.1 % on a w/w basis. The project has also shown that when no cleaning is performed between species, carry-over does occur which needs to be

considered by manufacturers when presenting information to consumers about the composition of meat products so the contents are accurately described.

Stakeholders now have the evidence to differentiate between adventitious contamination of raw pork mince in raw beef mince and deliberate fraud. There should not be an expectation of adventitious contamination and the presence of low concentrations of undeclared species in relevant meat products, as this project has shown that it is possible to clean to <0.1 % pork w/w using GMP employed in UK meat processing plants.

The outcomes of this project are based on the determination of raw pork in raw beef only. Whilst it would not be unreasonable to assume the outcomes would be similar for other species of meat, the work needed to confirm this assumption was not within the scope of this project.

4. Customer

This project was funded by the Food Standards Agency and the Department for Environment, Food and Rural Affairs. Customer contacts for this project were:

- Mr John Barnes, Head, Local Delivery Division, Food Standards Agency, Aviation House, 125 Kingsway, London WC2B 6NH.
- Dr Lucy Foster, Food and Environmental Risks Science Coordinator and Food Chain Evidence Programme Manager, Department for Environment, Food and Rural Affairs, Nobel House, 17 Smith Square, London SW1P 3JR.

5. Introduction

5.1. Rationale for the project

Food authenticity failures and food fraud can be driven by pressures on food production and the current climate of financial constraint. The horse meat incident in early 2013 impacted on consumer confidence in the UK food supply and showed that the presence of undeclared species in meat products is of concern to consumers. Low level presence of foreign species is of particular concern to consumers who choose to avoid certain species on the basis of faith.

The 2013 incident involving the presence of horse and pork meat in processed beef products has raised a number of issues including a question of whether carry-over of meat species occurs during the industrial production of meat products manufactured according to good manufacturing practice.

There is no direct legal obligation for food manufacturers to clean equipment when changing a meat processing line from one red meat species to another so a meat processor can quite legitimately mince, e.g. pork, beef and sheep on the same line and on the same day without cleaning the equipment in between species. In this situation, it is clear that some species carry-over will occur if no cleaning is performed between species. Thus meat processing plants that process multiple species on the same line will see some carry-over of one species into another when no cleaning is performed, but how much and how does this carry-over manifest itself in terms of finished products? The issue of species carry-over is not new and has been dealt with on a small scale in terms of butchers' shops where local authorities have monitored and taken successful prosecutions in cases of gross carry-over of one

species into another. However, the question of species carry-over in commercial meat processing plants has not been previously been addressed down to DNA detectable levels.

A review of the scientific literature indicated that there is little or no information on this matter in the public domain. Therefore the Food Standards Agency and the Department of Environment Food and Rural Affairs decided to commission a research project to assess whether carry-over of meat species occurs during the industrial production of meat products according to good manufacturing practice.

The aim of this project was to establish whether carry-over of meat species occurs when minced meat is produced according to good manufacturing practice in UK meat processing plants and if it does, at what concentrations it occurs.

The project was conducted in two phases:

- Pilot plant – assessing what levels of carry-over can be achieved under controlled conditions in a pilot plant for three different cleaning scenarios.
- Industry practise – assessing what levels of carry-over occur in UK meat mincing plants. The plants were chosen to reflect different cleaning scenarios used in the industry together with a single species plant to act as a baseline.

The output from this project work will:

- Establish whether species carry-over occurs with cleaning methods most commonly used by UK industry in raw meat plants and under different meat species handling scenarios
- Provide evidence to inform policy on the difference between carry-over and deliberate food fraud, and practical monitoring approaches
- Inform consumer choice about the acceptability of trace levels of carry-over.

5.2. Regulations

In the UK, it is a criminal offence under Sections 14 and 15 of the Food Safety Act 1990 to sell food that is not of the nature, substance or quality demanded by the consumer, or to falsely or misleadingly describe or present food. If all or most of the meat in a product labelled ‘beef burger’ is a meat other than beef, the product is not of the nature or substance demanded. If low levels of meat from a species other than beef are present, and this is not indicated to the consumer, the product may be considered not to be of the quality demanded. There are also specific offences under the Food Labelling Regulations 1996, the Meat Products Regulations 2003 (which stipulate compositional criteria for burgers) or the Consumer Protection from Unfair Trading Regulations 2008. Consumers do not expect horsemeat or undeclared meat from other species to be present in a product labelled as beef burgers and for those who wish to avoid pig meat or any other meat, the description and labelling of the food must be sufficiently accurate and honest to allow them to do so.

Thus the presence of undeclared species in meat products is illegal and is of concern to the UK public. Additionally, low level presence of undeclared species is of great concern to consumers who choose to avoid certain species on the basis of faith.

5.3. Stakeholder Consultation

The outline proposal for this project was presented at an extraordinary meeting of Defra’s authenticity methods working group (AMWG) which was convened on 28 February 2013 by

Defra to seek the views of scientific and industry experts on the design and approaches needed to gather evidence to improve our understanding of whether carry-over of meat species occurred and if so, at what concentration.

The group also considered the need for additional research to improve confidence in the quantitative analysis of meat species using DNA methods. The meeting was attended by industry groups so that their input could be sought. The approaches outlined for this project were endorsed by attendees of the meeting.

The following Groups were consulted in the scoping of this project:

- Defra Authenticity Methods Working Group (AMWG) (Expert Group)
- British Retail Consortium (BRC) and its members
- Food and Drink Federation (FDF) and its members
- British Meat Processors Association (BMPA) and its members
- ABP Food Group
- Bidvest 3663
- Cranswick plc
- British Hospitality Association (BHA) and its members
- Leatherhead Food Research
- Association of Public Analysts
- Kosher and Halal Certifying Organisations
- Agriculture and Horticultural Development Board (AHDB) and its members
- Statisticians from the Food Standards Agency and Defra.

The final scope for Phase 1 was circulated for comment on 19 April 2013 to attendees of the extraordinary meeting of Defra's AMWG that was convened on 28 February 2013. A meeting of the AMWG was also held on 14 October 2013 to consider the findings from Phase 1 and to discuss the scope of Phase 2.

Key decisions were made on the scope of the project by LGC with input from FSA, Defra, AMWG and industry and these are summarised in Table 1.

No.	Decision	Rationale
1	Only the mincing stage will be studied in this project	Although it is acknowledged that there are many points during the meat processing process that could give rise to the carry-over of meat species, it is considered by experts that the mincing stage is the step that has the greatest potential for gross contamination.
2	Pork carry-over in beef to be studied	It was agreed that the carry-over of pork into beef be studied as these species are the most commonly minced.
3	Three cleaning regimes are to be studied: • No clean • Wash with water • Deep clean.	Expert and Industry input was sought to establish the most common cleaning regimes in use and to shape the design of the project: • No clean – switch from one species to another with no cleaning. • Wash with water – leave equipment assembled and flush through with water. • Deep clean – equipment dismantled and chemically cleaned (usually performed overnight)
4	Three categories of species handling are to be studied: i. Single species plant ii. Multiple species plant that runs different species on different days on the same line iii. Multiple species plant that runs different species on the same day on the same line	The reasons for choosing this these categories were because: i. It will give data on what is the best that can be achieved in terms of species carry-over and is of particular interest to consumers who exclude certain species of meat on the basis of faith ii. It will give data on what can be achieved by plants that want to handle multiple species and provide food to specialist consumers, e.g. Halal, Kosher etc. iii. It will give data on the worst case scenario in terms of species carry-over and enable an assessment on acceptability to the general consumer can be made.
5	Use the same authentic lean meat for standards and samples	By having the standards and samples made from same authentic lean meats, i.e. the exact same composition, some of the factors that make the provision of quantitative DNA results difficult are overcome, e.g. as standards and samples are of the exact same composition, the question of different cuts, age, processing and cooking do not apply. As they are the same material, they should behave similarly in the assay, e.g. with respect to being able to extract DNA. Thus, for this project, it is anticipated that it will be possible to express the results for the meat samples as % pork in beef on w/w meat basis.

No.	Decision	Rationale
6	Analyse swabs using a mitochondrial DNA based DNA kit	The swabs are not expected to contain a lot of DNA. Therefore a method with as much sensitivity as possible is required. There are typically many more copies of the mitochondrial genome per cell than there are copies of the nuclear genome (anything from 50 to 500 times more) thus a mitochondrial based assay has been selected for the swabs. The approach will be agreed with the expert Authenticity methods working group.
7	Analyse meat samples using a genomic DNA based DNA kit	With the meat samples there will definitely be DNA present and the challenge is to determine, as accurately as possible, how much pork and beef are present. As the number of nuclear genomes per cell is fixed (as opposed to mitochondrial genome copies that can vary widely between cell type, tissue type, species etc.) genomic DNA based methods are more suitable for quantitative purposes. The assay will measure the copies of gene targets specific to pork DNA compared to gene targets common to all mammalian species to estimate proportion of pork DNA present. The approach and analysis needed to assess the reliability of the data (e.g. duplicate testing, points during processing) will be agreed with the expert Authenticity methods working group.
8	Use a DNA kit with a LOD of 0.1 %	A literature search of available methods for the determination of pork showed that this was the LOD that most methods reported.
9	Assess feasibility of reliably determining down to 0.01 %	At a meeting with the Halal certification bodies, it was noted that any concentration of pork, no matter how little, would be unacceptable to a practising Muslim. This is the same for other groups excluding meat on the basis of faith. Although the 'zero tolerance' that the faith groups are requesting is not technically possible, an assessment of whether measurements down to 0.01 % w/w pork in beef meat can be reliably made will be performed using gravimetrically prepared lean authentic meat mixtures. This information will be used to inform faith groups on the reliability of detection at very low concentrations and what this means in terms of trace carry-over levels of one species in another.
10	Perform ATP, protein and DNA swabs to check effectiveness of equipment cleaning	ATP swabs are routinely used in factories to monitor bacteriological background. Protein swabs have started to be used to check for monitor for protein based allergens and since the horse meat issue, some factories are using these swabs to monitor for carry-over of species. Thus for this project we intend to clean according to the protocol and do a visual inspection followed by ATP, protein and DNA swabs.

No.	Decision	Rationale
11	Sampling plan	Was devised with input from LGC statistician and supported by FSA statistician as being a robust and fit for purpose approach.
12	Analyse samples singly	Rationale was presented at AMWG meeting on 26 April 2013 and endorsed by the group on the following basis: • Authentic samples of pork and beef being used • Each 250 g sample is thoroughly homogenised • Single analysis is 1/250 g (0.4 %) and duplicate is 2/250 g (0.8 %) so taking 0.8 % rather than 0.4 % of sample doesn't significantly increase the probability of finding low level contamination. • Analysing samples singly allows double the number of samples to be analysed and crucially, enables all of the first 10 kg post species change to be analysed as opposed to only half. • The experiment is being replicated three times so information on the consistency of mincing / carry-over across the 100 kg will be obtained which is important as the ultimate aim is to assess whether carry-over occurs and what the profile of contamination is throughout the 100 kg. • Single extraction & single PCR complies with the commercial kit instructions • ~10 % duplicates will be conducted which will give an indication of repeatability • 10 replicates of a high and low sample will be performed which will check if repeatability varies with contamination level • Initially analysing all samples singly – will give a picture – a decision can be made at any stage to reanalyse samples if required (we will have 249 g of each sample left). • Statistical input – LGC – FSA – "maximum bang for our bucks"
13	Sampling to be carried out for three different risk ratings	Samples to be taken for the following risk ratings • Chemical clean between species (risk rating 4) • Water wash between species (risk rating 5) • No cleaning between species (risk rating 6) (See section 8 of report for definition of risk ratings)
14	Duplicate experiments for Phase 2 with 50 samples per experiment	To rationalise the number of samples taken and analysed, duplicate experiments were to be carried out for each risk rating with 50 samples taken for each experiment. It was agreed that the duplicate experiments would be taken during the same visit.

No.	Decision	Rationale
15	Limited sampling for low risk rating	As in Phase 1 pork had only been detected in the high risk scenario when no cleaning had taken place, it was thought to be highly unlikely that pork would be detected in samples taken from a single species facility. However, it was decided that a limited sampling exercise should take place to obtain baseline figures.

Table 1: Key Decisions Agreed by Stakeholders

6. Literature search

Literature searches were conducted on:

- Google Scholar
- Food Standards Agency database Foodbase
- the commercial databases:
    - CAB Abstracts
    - FROSTI (Foodline®: Science)
    - Food Science and Technology Abstracts (FSTA)

The first search was to establish whether any work in relation to carry-over of meat species had been published in the scientific literature. No articles were identified directly on this subject so another search was conducted looking for evidence of persistence of DNA on surfaces of meat processing machinery, following change of meat species processed (e.g. from horsemeat to beef). Again, no articles were identified directly on the subject; the following were the most relevant to the issue:

a) Langkabel *et al* were investigating material obtained by the mincing of lymph nodes by a laboratory mill which had reusable steel and tungsten carbide grinding beads. Unexpected results in PCR analysis were attributed to carry-over from previous materials processed in the mill. Neither physical methods (three washes with distilled water, autoclaving and UV treatment) nor a procedure with Exonuclease III and the commercial DNA-removing kit DNAaway® was able to eliminate residual DNA from the steel beads. It seems reasonable to conclude that steel surfaces on commercial mincing apparatus, which is unlikely to be cleaned with such thoroughness, will also tend to retain DNA.

b) Barbiera *et al* were able to detect DNA from pistachio on surfaces of commercial equipment used in the manufacture of mortadella even following simple washing with water and from samples of mortadella prepared without pistachio nuts following earlier use of the same equipment to produce mortadella with nuts (the equipment being deliberately not washed between the batches).

The full abstracts and references are presented in Annex 1.

The literature searches showed that information on the carry-over of meat species during commercial processing was not available in the scientific literature and thus demonstrated a need for this evidence to be gathered.

Whilst protein approaches exist for the detection and potential quantitation of meat species, it is important that robust analytical methodologies can also be applied when quantifying meats species present in a mixture. There needs to be minimal cross-reactivity between

target assays to ensure confidence in detection and quantitation of a particular species, the assay must be sensitive enough to detect the target at very low concentrations, and the assay needs to be robust enough for detection of the target in processed food products. For these reasons, the Polymerase Chain Reaction (PCR) which targets DNA, is usually the method of choice as opposed to immunological assay that target proteins. The robustness of the DNA molecule can further facilitate confirmation of species identification through sequencing of the amplified PCR product and comparison to validated species databases. Recent reviews have suggested that the quantitative capability of meat speciation approaches can be best facilitated through using calibration curves based on DNA derived measurements, and this is best facilitated by real-time PCR.

In order to help make an informed decision on which analytical method should be used for the determination of meat species in this project, a survey was carried out to establish which commercial real-time PCR kits were available for the determination of pork in meat products. The results of this search are presented in Table 2.

Supplier	Brand name	LOD for pig
Primerdesign	genesig	Sensitive to <100 copies of target
Qiagen	Mericon pig kit	It can detect as few as 10 copies of the target DNA in a reaction
Congen	SureFood	Detection limit: 0.5 % depending on matrix and DNA preparation
Neogen	F.A.S.T.	1 %
Bio Line	PorkSens	0.50 %
Microsynth	AllMeat/AllHorse	Not specified
Biolytix	StripKit	Not specified
Kogene	PowerChek	Not specified
Eurofins	DNAnimal	The company's product for the detection of horse DNA has a mLOD(abs) of 10 target copies. There is no corresponding sheet online for the pig DNA product
Genomed	Meat Typing	Not specified
Profound Kestrel		Not specified
Progenus	TaqPro	10 copies

Table 2: PCR kits available for the determination of pork

Table 3 shows the results for the search for published articles describing a limit of detection for the determination of pork.

Title	Journal reference	Stated limit of detection
Semi-nested multiplex PCR enhanced method sensitivity of species detection in further-processed meats	Food Control Volume 31, Issue 2, June 2013, Pages 326–330	1 pg of DNA per reaction
Detection and quantification of meat species by qPCR in heat-processed food containing highly fragmented DNA	Food Chemistry Volume 134, Issue 1, 1 September 2012, Pages 518–523	5 % (accurately quantified) in cooked products
Quantitative detection of poultry meat adulteration with pork by a duplex PCR assay	Meat Science Volume 85, Issue 3, July 2010, Pages 531–536	Quantification of pork meat addition to poultry meat in the range of 1–75 %, with a sensitivity of 0.1 %.
A SYBR Green real-time PCR assay to detect and quantify pork meat in processed poultry meat products	Meat Science Volume 94, Issue 1, May 2013, Pages 115–120	For the development of the method, binary meat mixtures containing known amounts of pork meat in poultry meat were used to obtain a normalised calibration model from 0.1 to 25 % with high linear correlation and PCR efficiency.
Swine-Specific PCR-RFLP Assay Targeting Mitochondrial Cytochrome B Gene for Semiquantitative Detection of Pork in Commercial Meat Products	Food Analytical Methods June 2012, Volume 5, Issue 3, pp 613-623	The assay was sensitive enough to detect 0.0001 ng of swine DNA in pure formats and 0.01 % (w/w) spiked pork in extensively processed ternary mixture of pork, beef, and wheat flour.
Detection of Raw Pork Targeting Porcine-Specific Mitochondrial Cytochrome B Gene by Molecular Beacon Probe Real-Time Polymerase Chain Reaction	Food Analytical Methods June 2012, Volume 5, Issue 3, pp 422-429	The method also showed that the limit of detection of pork was 0.0001 ng. Based on the regression analysis of the standard curve, the 96 % efficiency of real-time PCR was achieved with high correlation coefficient (r 2 = 0.9989). Sensitivity of the assay in discriminating pork as low as 0.1 % (w/w) pork in pork–beef mixtures was also obtained.
Detection of Pork in High Tenperature Processed Food by Taqman Real-Time Polymerase Chain Reaction	Advanced Materials Research, 550-553, 1919	This assay was sensitive to detect 1pg of pork template DNA. Meat mixtures spiked with 1-10 % pork were successfully tested, which demonstrated the suitability of the assay for determination of swine-derived ingredient in food.
Identification of pork genome in commercial meat extracts for Halal authentication by SYBR green I real-time PCR	International Journal of Food Science & Technology Vol 46, no. 5 pp 951-955	Detection limit of the real-time PCR was down to 0.1 ng of porcine DNA. An appropriate linearity was obtained by construction of a standard curve based on Ct value and different concentrations of porcine DNA. By conventional PCR, no amplification was shown by porcine DNA less than 0.1 ng.
Nanoparticle sensor for label free detection of swine DNA in mixed biological samples	Nanotechnology 22 195503	The LOD (for genomic DNA) of the assay was 6 µg ml − 1 swine DNA in mixed meat samples.

Title	Journal reference	Stated limit of detection
Identification of meat species by TaqMan-based real-time PCR assay [Donkey, pork and horse]	Meat Science Volume 82, Issue 4, August 2009, Pages 444–449	The real-time quantitative assay used in this study allowed the detection of as little as 0.0001 ng template DNA from pure meat for each species investigated and experimental meat mixtures.
Analysis of pork adulteration in commercial meatballs targeting porcine-specific mitochondrial cytochrome b gene by TaqMan probe real-time polymerase chain reaction	Meat Science Volume 91, Issue 4, August 2012, Pages 454–459	Analysis of beef meatballs with spiked pork showed the assay can determine 100–0.01 % contaminated pork with 102 % PCR efficiency, high linear regression (r2 = 0.994) and ≤ 6 % relative errors.
Nanobioprobe for the determination of pork adulteration in burger formulations	Journal of Nanomaterials - Special issue on Advanced Nanohybrid Materials: Surface Modification and Applications archive Volume 2012, January 2012 Article No. 8	The probe quantitatively detected 1-100 % spiked pork in burger formulations with ≥90 % accuracy.
Porcine-Specific Polymerase Chain Reaction Assay Based on Mitochondrial D-Loop Gene for Identification of Pork in Raw Meat	International Journal of Food Properties Volume 15, Issue 1, 2012	The assay was able to detect as low as 0.1 % (v/v) porcine DNA spiked on DNA of cattle, sheep, goat, chicken, and deer. Furthermore, a detection limit of 0.001 ng/μL porcine DNA showed the high sensitivity conferred by the developed porcine-specific polymerase chain reaction assay.
Detection of porcine DNA in gelatine and gelatine-containing processed food products—Halal/Kosher authentication	Meat Science Volume 90, Issue 3, March 2012, Pages 686–689	Extraction and purification of DNA from gelatine were successfully achieved using the SureFood® PREP Animal system, and real-time PCR was carried out using SureFood® Animal ID Pork Sens kit. The minimum level of adulteration that could be detected was 1.0 % w/w for marshmallows and gum drops.
Meat species identification based on the loop mediated isothermal amplification and electrochemical DNA sensor	Food Control Volume 21, Issue 5, May 2010, Pages 599–605	The method is more specific and free of unwanted amplifications compared to Multiplexed PCR (M-PCR) method and gave limits of detection of ~20.33 ng/μl (3 × 104 copies/reaction), ~78.68 pg/μL (3 × 102 copies/reaction) and ~23.63 pg/μL (30 copies/reaction) for pork, chicken and bovine species, respectively.

Title	Journal reference	Stated limit of detection
Analysis of Pork Adulteration in Commercial Burgers Targeting Porcine-Specific Mitochondrial Cytochrome B Gene by TaqMan Probe Real-Time Polymerase Chain Reaction	Food Analytical Methods August 2012, Volume 5, Issue 4, pp 784-794	Analysis of beef burger formulations with spiked pork showed the assay can determine 100–0.01 % contaminated pork with a PCR efficiency (E) of 93.8 % and a correlation coefficient (R 2) of 0.991. A plot of actual value against real-time PCR-predicted value also yielded a good linear regression, R 2 0.998, and small root mean square error of calibration, RMSEC 0.42. A strong correlation was found between the partial least square (PLS)-predicted values and real-time PCR-determined values. The accuracy of the method was ≥90 % in all determinations of the standard set.
Development and validation of fast Real-Time PCR assays for species identification in raw and cooked meat mixtures	Food Control Volume 23, Issue 2, February 2012, Pages 400–404	The limit of detection of the Real-Time PCR methods ranged between 0.02 pg and 0.80 pg of template DNA, with an efficiency between 95 % and 100 %. All methods were able to detect the target species when spiked at 1 % in any other species and no relevant difference was observed between the Ct values of raw and cooked samples.
Nanobiosensor for the Detection and Quantification of Specific DNA Sequences in Degraded Biological Samples	5th Kuala Lumpur International Conference on Biomedical Engineering 2011 IFMBE Proceedings Volume 35, 2011, pp 384-387	The sensor was found to be sensitive enough to detect 0.5 pork in raw and 2.5-h autoclaved mixed samples in a single step without any separation or washing.
Identification of species (meat and blood samples) using nested-PCR analysis of mitochondrial DNA	African Journal of Biotechnology Vol. 10(29), pp. 5670-5676	Different lengths of specific nested-PCR products were detected to be 350, 570, 750 and 1000 bp for chicken, pig, cow, and crocodile, respectively. The system allowed detection with as little as 5 ng of DNA from either meat or blood sample. Detection sensitivity of individual species was improved, enabling the detection of DNA with as little as 1 pg.

Title	Journal reference	Stated limit of detection
Nanobiosensor for detection and quantification of DNA sequences in degraded mixed meats	Journal of Nanomaterials archive Volume 2011, January 2011 Article No. 32	The accuracy of the method was over 90 % and 80 % for raw and autoclaved pork-beef binary admixtures in the range of 5-100 % pork adulteration. The biosensor probe identified a target DNA sequence that was several-folds shorter than a typical PCR-template. This offered the detection and quantitation of potential targets in highly processed or degraded samples where PCR amplification was not possible due to template crisis.
Conventional multiplex polymerase chain reaction (PCR) versus real-time PCR for species-specific meat authentication	Life Science Journal, 2012;9(4)	Either analysis indicated the successful detection of as little as 0.05 pg (5 %) adulteration in cattle meat.
Effect of Heat Processing on DNA Quantification of Meat Species	Journal of Food Science Volume 77, Issue 9, pages N40–N44, September 2012	In this study, real-time polymerase chain reaction (PCR) was used for identifying the effects of different temperatures and times of heat treatment on the DNA of meat products. For this purpose, beef, pork, and chicken were baked at 200 ℃ for 10, 20, 30, 40, 50 min, and for 30 min at 30, 60, 90, 120, 150, 180, 210 ℃ and also cooked by boiling at 99 ℃ for 10, 30, 60, 90, 120, 150, 180, 210, and 240 min. The DNA was then extracted from all samples after the heat treatment. Further, a region of 374, 290, and 183-bp of mitochondrial DNA of beef, pork, and chicken, respectively, was amplified by real-time PCR. It was found that baking and boiling of the beef, pork, and chicken resulted in decreases in the detectable copy numbers of specific genes, which varied with the heating time and degree.
Nanobiosensor for the detection and quantification of pork adulteration in meatball formulation	Journal of Experimental Nanoscience	The sensor was found to be sensitive enough to detect 1 % pork in raw and cooked meatballs, prepared from the previously mixed pork and beef in specific ratios (% w/w).
Quantification of Pork, Chicken and Beef by Using a Novel Reference Molecule	Bioscience, Biotechnology, and Biochemistry Vol. 75 (2011) No. 9 P 1639-1643	Standard plasmid was constructed as a novel reference molecule for use in real-time quantitative PCR assays to verify the identity of beef, pork, chicken, mutton, and horseflesh. The calculated R2 values of the standard curves (103–107 copies per reaction) for the five species ranged between 0.998 and 0.999 in the quantification analysis.
PCR-RFLP Using BseDI Enzyme for Pork Authentication in Sausage and Nugget Products	Journal of Animal Science and Technology, Vol 34, No 1 (2011)	Assay developed during this work was able to detect 0.1 % of their respective target species

Title	Journal reference	Stated limit of detection
SYBR-Green real-time PCR approach for the detection and quantification of pig DNA in feedstuffs	Meat Science Volume 82, Issue 2, June 2009, Pages 252–259	Analysis of experimental mixtures demonstrated the suitability of the assay for the detection and quantification of porcine DNA in mixtures containing as little as 0.1 %.
A multiplex PCR assay for fraud identification of deer products	Food Control Volume 21, Issue 10, October 2010, Pages 1402–1407	The detection limit was 1 ng for porcine and ovine primers, 5 ng for poultry primers and 0.5 ng for bovine primers.
Comparison of gene nature used in real-time PCR for porcine identification and quantification: A review	Food Research International Volume 50, Issue 1, January 2013, Pages 330–338	... primers also allows detection of porcine DNA at very low level of 0.0001 ng porcine DNA and 0.1 % (w/w) pork in meat ... On the other hand, high sensitivity of the MC1R-based PCR system developed is indicated by a very low detection limit of 5 pg of DNA (Evans et al ... [Text from the Key Words In Context on Google Scholar - the abstract does not refer to sensitivity]
Quantification of beef, pork, chicken and turkey proportions in sausages: use of matrix-adapted standards and comparison of single versus multiplex PCR in an interlaboratory trial	European Food Research and Technology November 2009, Volume 230, Issue 1, pp 55-61	Regardless of the method used (either multiplex or single PCR), when using calibration sausages, it was always possible to quantify the proportions of meats in the unknown samples (in the range of 0.5–80 %) with high precision and accuracy.

Table 3: Results of literature survey for papers describing the determination of pork

It was decided that it would be most appropriate to use a commercial quantitative DNA kit for the estimation of the amount of pig meat in beef meat. The Surefood® ANIMAL QUANT Pork kit was chosen because:

a) The kit is based on nuclear DNA and thus can give quantitative results. This is because the number of nuclear genomes per cell is fixed (as opposed to mitochondrial genome copies that can vary widely between cell type, tissue type, species etc.).
b) The assay measures the copies of gene targets specific to pork DNA compared to gene targets common to all mammalian species to estimate proportion of pork DNA present.
c) LGC has previously found SureFood kits to be reliable based on validation data that is published and available with the kits.

For the analysis of the DNA swabs, a kit based on mitochondrial DNA was chosen (PrimerDesign Pork Meat Speciation). This was because the swabs were not expected to contain a significant amount of DNA and therefore a method with as much sensitivity as possible was required; as there are typically many more copies of the mitochondrial genome per cell than there are copies of the nuclear genome (anything from 50 to 500 times more), mitochondrial DNA methods are typically more sensitive than methods based on genomic DNA.

7. Validation

Before any samples were analysed, validation of the PCR kits was carried out at LGC. The following areas were evaluated:

- Precision
  - Repeatability
  - Intermediate precision
- Accuracy
  - Pork content as % DNA
  - Pork content as % weight/weight
- Limit of Detection (LOD).

7.1. Quality control standard preparation

To evaluate the performance of the method, quality control (QC) materials of known pork and beef concentrations were prepared gravimetrically. Due to the difficulties with preparing homogenous mixes of low concentrations of meat, individual aliquots (single use pots) were prepared at concentrations of 3 to 75 % pork in beef whilst bulk quantities of 0.5, 1.0 and 0.01 % pork in beef were prepared. Individual aliquots of 0.1 % pork in beef were produced by dilution of the bulk 1.0 %.

In order to ensure that the QCs were homogenous, the bulk materials were prepared by weighing the appropriate amount of homogenised beef into a small (200 ml approximate volume) kitchen food processor then weighing the appropriate weight of pork on top. The pork was added in as small pieces as possible and were spread evenly over the surface of the beef. The meat was gently mixed with a spatula, ensuring no meat adhered to the spatula, before being blended, in short bursts, repeatedly until a smooth paste was obtained. Tables 4 to 8 illustrate the weights of pork and beef used to prepare each of the QC materials. Bulk quantities of standards were also prepared at concentrations between 0.1 and 0.01 % pork in beef to establish the LOD.

% Pork	Weight of pork (g)	Weight of beef (g)	Actual % Pork	Weight taken for extraction (g)
75	0.675 - 0.825	0.225 - 0.275	71.0 - 78.6	Entire weight
50	0.45 - 0.55	0.45 - 0.55	45.0 - 55.0	Entire weight
25	0.225 - 0.275	0.675 - 0.825	21.4 - 28.9	Entire weight
10	0.09 - 0.11	0.81 - 0.99	8.3 - 12.0	Entire weight
5	0.045 - 0.055	0.855 - 1.045	4.1 - 6.0	Entire weight
3	0.027 - 0.033	0.873 - 1.067	2.5 - 3.6	Entire weight

Table 4: Preparation of 3 to 75 % pork in beef QC materials (single use pots)

% Pork	Weight of pork (g)	Weight of beef (g)	Actual % Pork	Weight taken for extraction (g)
0.5	1.05	199.08	0.52	1.0 ± 0.1g
1	2.01	197.9	1.01	1.0 ± 0.1g

Table 5: Preparation of 0.5 and 1 % pork in beef QC materials

% Pork	Weight of 1.0 % pork (g)	Weight of beef (g)	Actual % Pork	Weight taken for extraction (g)
0.1	0.09 - 0.11	0.81 - 0.99	0.08 - 0.12	Entire weight

Table 6: Preparation of 0.1 % pork in beef QC material

% Pork	Weight of 1.0 % pork (g)	Weight of beef (g)	Weight taken for extraction (g)
0.01	1.0	99.0	1.0 ± 0.1g

Table 7: Preparation of 0.01 % pork in beef QC material

% Pork	Weight of 1.0 % pork (g)	Weight of beef (g)	Weight taken for extraction (g)
0.1	10	90	1.0 ± 0.1g
0.05	5	95	1.0 ± 0.1g
0.03	3	97	1.0 ± 0.1g
0.02	2	98	1.0 ± 0.1g
0.01	1	99	1.0 ± 0.1g

Table 8: Preparation of 0.1 to 0.01 % pork in beef QC materials for LOD determination

The quality control standards were prepared from the same source meat as the samples. By using this approach the following issues, which account for why it is difficult to provide fully quantitative DNA results for meat species, were avoided:

- cut of meat
- age of animal
- matrix background
- other ingredients
- sample preparation
- temperature of processing
- level of processing
- level of DNA degradation
- DNA extraction approach and DNA recovery, PCR efficiency, DNA template amount, DNA target etc.

7.2. Validation

7.2.1 Repeatability (within plate)

To investigate the repeatability of the PCR plates, one sample extract and one QC extract were each analysed 6 times on the same plate and the results are presented in Table 9. The coefficient of variation (% CV) obtained were below the manufacturer's expectation of 30 %.

The 5 % CV observed for the 50 % pork in beef shows a much better level of precision than expected.

	Sample 311390	50 % Pork in beef
n	6	6
Mean % pork DNA	8.9	52.8
Standard deviation	2.25	2.84
% CV	25.4	5.4

Table 9: Within plate repeatability

7.2.2 Intermediate precision - between plate

One extract of 50 % pork in beef was analysed on numerous different plates to evaluate the intermediate precision. The results are presented in Table 10 and show % CVs less than the manufacturer's expected CV of 30 %.

	50 % Pork in beef	50 % Pork in beef
n	10	6
Mean % pork DNA	55.2	69.7
Standard deviation	13.73	15.10
% CV	24.9	21.7

Table 10: Intermediate precision – between plate

7.2.3 Intermediate precision – multiple extracts, plates, analysts, etc.

0.1 and 50 % pork in beef QC materials were extracted and analysed with every batch and Table 11 shows typical % CVs for these materials. The % CV for the 0.1 % pork in beef was significantly higher than that observed for the 50 % (67 % and 28 % respectively); greater variation is expected close to the limit of detection.

	0.1 % Pork in beef	50 % Pork in beef
n	62	53
Mean % pork DNA	0.16	54.0
Standard deviation	0.11	15.24
% CV	67.1	28.2

Table 11: Intermediate precision

7.2.4 Accuracy – pork content (% DNA)

The results obtained from the DNA kit are expressed as % pork DNA relative to total mammalian DNA present. In order to compare the results expressed as % DNA to the actual w/w % of pork meat in beef meat present, a range of standards were prepared gravimetrically containing 0.01 % to 75 % pork in beef, in addition to 100 % pork and 100 % beef. The results obtained are presented in Table 12 and show that the results expressed as % DNA do not differ significantly from the actual pork content determined by mass. The lower and upper confidence limits have been calculated on the standard deviation of the results multiplied by an appropriate coverage factor.

Sample	*N*	Mean corrected % pork DNA	Lower 95 % Confidence Limit	Upper 95 % Confidence Limit	Significant difference between observed and expected?
100 % Beef	9	0	0	0	No
100 % Pork	9	115.1	79.1	151.0	No
75 % Pork in beef	9	95.3	62.3	128.3	No
50 % Pork in beef	9	56.7	36.3	77.0	No
25 % Pork in beef	9	36.4	23.9	48.9	No
10 % Pork in beef	9	14.5	7.8	21.2	No
5 % Pork in beef	9	7.6	1.1	14.0	No
3 % Pork in beef	8	6.0	1.0	11.1	No
1 % Pork in beef	9	1.6	0.5	2. 8	No
0.5 % Pork in beef	8	1.4	0.3	2.5	No
0.1 % Pork in beef	9	0.18	0.02	0.3	No
0.01 % Pork in beef	4	0.026	0	0.05	No

Table 12: Pork content expressed as %DNA compared to pork content determined by mass

7.2.5 Accuracy – pork content (% m/m)

Table 13 illustrates the relationship between the % pork DNA determined and the actual mass of pork in the prepared QC materials. It can be seen that the results obtained

expressed as % DNA are greater than the actual percentage of pork present. The difference is statistically significant (Wilcoxon test, two-sided, $p<0.001$ on 11 differences). However, the difference between the expected and observed proportions of pork are all within the expected measurement uncertainty for DNA determinations at the levels examined.

Table 13 illustrates the relationship between the % pork DNA determined and the actual mass of pork in the prepared QC materials. It can be seen that the results obtained expressed as % DNA are greater than the percentage of pork present on a gravimetric basis. However, as presented in Table 12, the difference between the figures is not statistically different due to the observed analytical variability, in other words, the confidence interval based on repeated measurements using the DNA/DNA approach always encompasses the pork content of the samples prepared on a gravimetric basis.

Sample	Mean corrected % Pork content (DNA/DNA)	Determined % Pork DNA as % of expected pork meat content
100 % Pork	115.1	115
75 % Pork in beef	95.3	127
50 % Pork in beef	56.7	113
25 % Pork in beef	36.4	146
10 % Pork in beef	14.5	145
5 % Pork in beef	7.6	152
3 % Pork in beef	6.0	200
1 % Pork in beef	1.6	160
0.5 % Pork in beef	1.4	280
0.1 % Pork in beef	0.18	180
0.01 % Pork in beef	0.026	260

Table 13: Determined % Pork DNA as % of expected pork meat content

7.2.6 Limit of detection

In order to assess the lowest concentration of gravimetrically prepared authentic lean meat mixture of raw pork in raw beef meat that could be reliably determined, standard mixes of 0.1, 0.05, 0.03, 0.02 and 0.01 % pork in beef were prepared and multiple replicates analysed. The results are summarised in Table 14 and show that the lowest concentration of pork which can be reliably detected in beef is 0.03 % (where at least 95 % of replicates

showed a detectable response for the presence of pork DNA). The results from the LOD experiments are also shown graphically in Figure 1.

% Pork in beef	Number of determinations	Number of determinations where pork detected	% of determinations where pork detected
0.10 %	18	18	100
0.05 %	15	15	100
0.03 %	21	21	100
0.02 %	21	19	90
0.01 %	21	20	95

Table 14: Assessment of the limit of detection

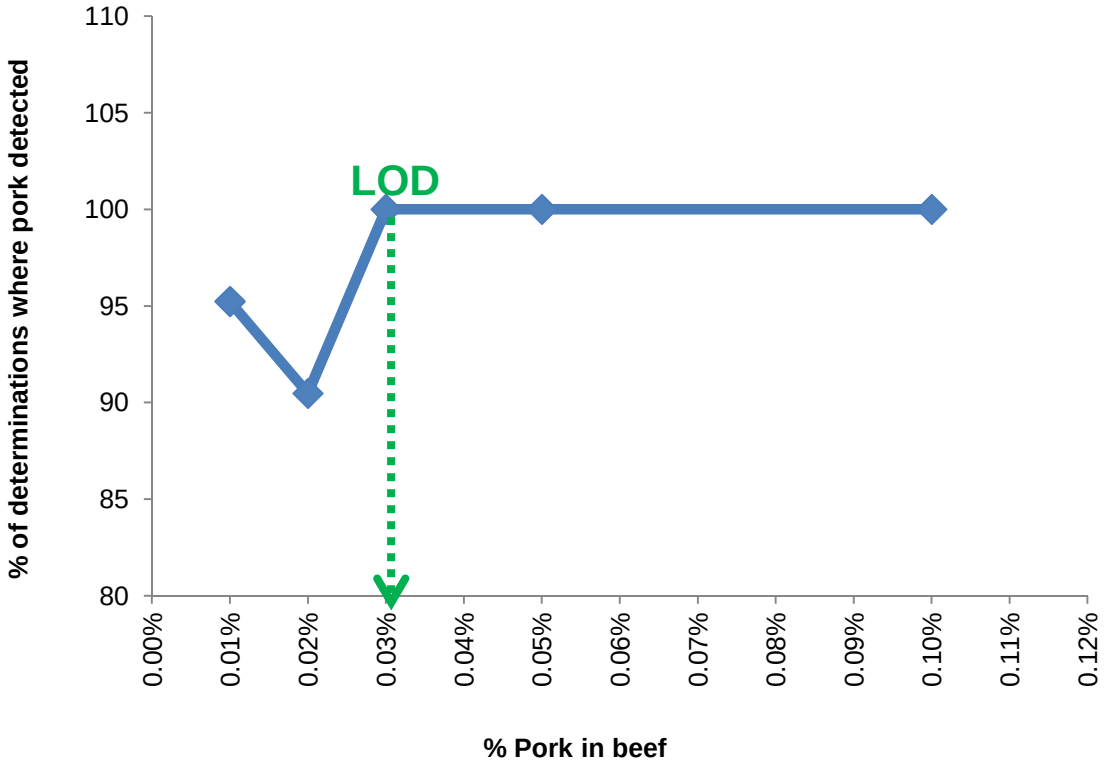

Figure 1: Limit of detection

The validation data were also used to model the likely LOD using the Probability Of Detection approach (POD). The POD approach estimated the LOD based upon a least squares model using results from the PCR experiments of the validation data set as previously described[1], and estimated that the theoretical LOD should also be around the 0.03 % level.

It is reassuring that both approaches gave the same estimate of the LOD as 0.03 %.

[1] "Modelling the Limit Of Detection in real-time quantitative PCR" M. Burns and H. Valdivia. European Food Research and Technology (2008) 226(6): 1513-1524. DOI: 10.1007/s00217-007-0683-z

7.3. Validation summary

The pork content of the meat samples was determined using a genomic DNA based DNA kit. As the number of nuclear genomes per cell is fixed (as opposed to mitochondrial genome copies that can vary widely between cell type, tissue type, species etc.) genomic DNA based methods are more suitable for quantitative purposes. The assay chosen measures the copies of gene targets specific to pork DNA compared to gene targets common to all mammalian species to estimate proportion of pork DNA present.

Validation of the method was carried out using gravimetrically prepared standards containing known amounts of raw lean pork and beef. In LGC's hands, the genomic DNA based PCR kit performed well in that the repeatability (within a PCR plate) and intermediate precision (between plate) were found to be less than the manufacturer's expected value of 30 % CV.

When multiple aliquots of 50 % raw pork in beef were extracted and analysed by different analysts, on different plates on different days, the % CV was found to be 28 %, i.e. less than the manufacturer's expectation of 30 % for the same extract analysed on the same plate. The %CV for the 0.1 % pork in beef QCs was found to be much higher (67 %) but a higher CV is to be expected when analysing samples close to the LOD.

When gravimetrically prepared mixtures of raw pork in raw beef at concentrations of 0.01 to 100 % pork in beef were analysed a consistent positive bias was observed, with all of the results expressed as % pork DNA being greater than the weight / weight % pork present. However, the observed differences were within the expanded uncertainty for individual results at the levels of interest.

A small amount of work was conducted to see whether an LOD of <0.1 % could be achieved and this showed that a LOD of 0.03 % raw pork meat in raw beef was achievable using this particular genomic real time PCR kit.

The assay performance was assessed as being satisfactory and thus the kit was considered to be appropriate for use in this project.

7.4. Swab validation

DNA was extracted from the swabs using a commercially available kit. The extracted DNA was then analysed for pork content using a commercially available quantitative real time PCR DNA assay which was based on mitochondrial DNA.

To validate the swabbing procedure and the analysis of the swabs, replicate swabs were prepared containing between 5 and 50mg of 0.1 % pork in beef. Table 15 presents the results for the quantities of meat added to extraction tubes with no swab, meat added directly to swabs, meat placed on a stainless steel square then swabbed, and blank swabs. Very low levels of beef were detected in the blank swabs; the most likely cause is low level contamination.

Mass of 0.1 % pork in beef (mg)	Sampling method	Comments	Number of PCR	Number of PCR where pork detected	Number of PCR where beef detected
50	Direct (no swab)	Meat transferred directly to eppendorf tubes, with the aid of a pipette piston, where necessary. Swabs prepared in triplicate.	6	6	6
25	Direct (no swab)		6	4	6
10	Direct (no swab)		6	0	6
5	Direct (no swab)		6	0	6
50	Direct swab	Meat transferred directly to eppendorf tubes using a water moistened swab. Swabs prepared in triplicate.	6	5	6
25	Direct swab		6	0	6
10	Direct swab		6	0	6
5	Direct swab		6	0	6
50	Swabbed onto & off stainless steel	250 µl water added to each sample pot and left to stand to moistened the meat. Meat transferred, then smeared, onto a stainless steel square, using a pipette piston. Sample allowed to air dry for approximately 1 hour then swabbed with a water moistened swab. Swabs prepared in triplicate.	6	5	6
25	Swabbed onto & off stainless steel		6	2	6
10	Swabbed onto & off stainless steel		6	0	6
5	Swabbed onto & off stainless steel		6	0	6
0 (no meat control)	Swabbed onto & off stainless steel	Clean stainless steel square swabbed with a water moistened swab. Swabs prepared in triplicate.	6	0	6

Table 15: Swab validation

In summary, the swab extracts were analysed using a mitochondrial DNA based DNA kit due to its greater sensitivity compared to genomic based DNA kits. Analysis of extracts from multiple swabs containing different weights of 0.1 % pork in beef indicated that pork could be repeatedly detected from 50 mg pork in beef which had been swabbed from stainless steel. This is equivalent to 50 µg pork per swab.

The assay performance was assessed as being satisfactory and thus the kit was considered to be appropriate for use in this project.

8. Categorisation of risk ratings

Following consultation with the AMWG, it was decided that the project would be conducted in two phases:

i. Pilot plant – assessing what level of carry-over occurs under controlled conditions in a pilot plant for three different cleaning scenarios that are commonly used in industry:
   - Scenario 1: without cleaning between species
   - Scenario 2: with 'Deep' chemical clean between species
   - Scenario 3: with 'Wash' clean (high pressure water) between species

ii. Industry practise – assessing what levels of carry-over occur in UK meat processing plants. The plants were chosen on the basis they handled multiple species and used one of the three different cleaning scenarios. In addition, limited sampling was undertaken at a single species plant to act as a baseline.

Before the plants for Phase 2 of the project were chosen, discussions were held with industry for advice on the criteria to be used to identify plants which would be most suitable to assist with this project. It was agreed that the plants for Phase 2 should be chosen based on their current cleaning practices and the information presented in Tables 16 and 17 was sent to various plants who were asked if they could rate their current practices. The responses received are summarised in Table 19 and based on these results, plants were contacted to see if they were willing for sampling to take place in their factory. Whilst it was easy to identify and recruit plants which processed multiple species and carried out a chemical clean or water wash between species, a plant which currently did not clean equipment between the processing of pork and beef could not be identified. Thus, the plant which cleaned using water washing also volunteered to process pork followed directly by beef, without any cleaning. This approach was agreed with FSA and Defra.

Categorisation	Species Risk Score	Risk Rating
Single species plant	1	Low
Multiple species plant that runs different species on different days on the same line	2	Medium
Multiple species plant that runs different species on the same day on the same line	3	High

Table 16: Risk rating categorisation based on species handled

Cleaning regime	Cleaning Risk score	Risk Rating
Chemical clean between species	1	Low
Equipment wash down with water and visual inspection between species	2	Medium
No wash down, flush through with different species	3	High

Table 17: Risk rating categorisation based on cleaning regime

The species handling score and cleaning score were added to obtain an overall risk rating as described in Table 18.

Total risk score	Overall Risk Rating
1-2	Low
3-4	Medium
5-6	High

Table 18: Overall risk rating classification

Plant	Species handled	Species Risk Score	Cleaning Risk Score	Total Risk Score	Overall Risk Rating	Special Considerations ? Halal, Kosher etc..	Fresh / Frozen	Annual Mince Volume (tons)	Source
Plant 1	Beef, Pork, Lamb, Turkey	3	1	4	Medium	None	Fresh	30,000	ABP
Plant 2	Beef, Pork, Lamb, Chicken	3	1	4	Medium	None	Frozen	12,000	ABP
Plant 3	Beef	1	NA	1	Low	None	No response	23,000	ABP
Plant 4	Pork	1	NA	1	Low	Organic	No response	No response	BMPA
Plant 5	Pork, Beef, Duck & Venison	3	2	5	High	Organic	No response	No response	BMPA
Plant 6	Beef, Lamb, Pork	3	1	4	Medium	None	No response	~5000	BMPA
Plant 7	Beef	1	NA	1	low	None	No response	0	AHDB
Plant 8	Beef	1	NA	1	low	None	No response	3000	AHDB
Plant 9	Beef pork lamb poultry game	3	2	5	High	Occasional Halal	No response	40	
Plant 10	Beef, Pork, Lamb, Poultry	3	2	5	High	None	No response	120	
Plant 11	Lamb	1	NA	1	Low	Halal	No response	ca. 364	FDF/BRC/BMPA
Plant 12	Lamb	1	NA	1	Low	Halal	No response	120	BMPA
Plant 13	Beef, Lamb and Pork	3	2	5	High	Chilled	No response	30.5 Million / 15600	BMPA/ BRC
Plant 14	Beef & Lamb	2	2	4	Medium	None	Frozen	1500	BRC
Plant 15	Pork	1	NA	1	Low		No response	No response	BRC
Plant 16	Multiple species	3	3	6	High		No response	No response	FSA

Table 19: Information and risk ratings received from plants

Based on the results received from the plants and summarised in Table 19, Table 20 illustrates the possible combinations of cleaning regimes and handling of different meat species.

Handling of meat species - Categorisation	Species Risk Score	Cleaning regime	Cleaning Risk score	Overall Risk Rating	Comments
Single species plant	1		NA	1	The cleaning process is not relevant if only one species is processed and the assumption is made that a chemical clean is carried out overnight. Therefore risk ratings of 1 and 2 can be considered to be equivalent.
Single species plant	**1**	**Chemical clean**	**1**	**2**	
Multiple species plant that runs different species on different days on the same line	2	Chemical clean between species	1	3	These two scenarios are fundamentally the same as there is no difference between the chemical clean carried out overnight and the one carried out between species.
Multiple species plant that runs different species on the same day on the same line	**3**	**Chemical clean between species**	**1**	**4**	
Multiple species plant that runs different species on different days on the same line	2	Equipment wash down with visual inspection between species	2	4	This scenario would not occur if it is standard practice to carry out a chemical clean overnight.
Multiple species plant that runs different species on the same day on the same line	**3**	**Equipment wash down with visual inspection between species**	**2**	**5**	
Multiple species plant that runs different species on the same day on the same line	**3**	**No wash down, flush through with different species**	**3**	**6**	

Table 20: Summary of risk ratings

The lines in bold show the scenarios evaluated during Phase 2 of the project, i.e.

- Risk rating 4 – Multi-species plant that runs different species on the same line on the same day with a chemical clean between species
- Risk rating 5 - Multi-species plant that runs different species on the same line on the same day with a water wash with visual inspection between species
- Risk rating 6 - Multi-species plant that runs different species on the same line on the same day with no cleaning between species.

Risk rating 3 was assessed as being the same as risk rating 4 as it was confirmed by industry that a chemical clean carried out between species during the day would be the same as a chemical clean carried out overnight.

As agreed with the AMWG, in addition to the three risk ratings described above, limited samples were taken at a single species (beef only) plant to provide an estimate of baseline concentrations.

In addition to wanting to assess the different cleaning regimes used by the UK meat processing industry, these categories were chosen because:

i. It would provide data on what is the best that can be achieved in terms of species carry-over and would be of particular interest to consumers who exclude certain species of meat on the basis of faith
ii. It would provide data on what can be achieved by plants that want to handle multiple species and provide food to specialist consumers, e.g. Halal, Kosher etc.
iii. It would provide data on the worst case scenario in terms of species carry-over and enable an assessment on acceptability to the general consumer can be made.

9. Sampling plan and cleaning regimes

The following sampling plan was developed with input from LGC statisticians and supported by FSA statistician as being a robust and fit for purpose approach. The plan was also endorsed by the AMWG.

Phase 1 (Pilot plant)

- A typical industry batch size of 100 kg of pork and beef to be processed per experiment
- 3 experiments to be carried out for each so information on the consistency of mincing / carry-over across the 100 kg would be obtained which is important as the ultimate aim was to assess whether carry-over occurs and what the profile of contamination is throughout the 100 kg.
- Each sample taken to weigh 250 g ± 10 g to reflect the weight of a typical retail pack of minced meat
- 80 samples to be taken according to a sampling plan devised in discussion with LGC's statistics team on the basis that the total number of observations gave a good probability of detecting contamination affecting 1-2 % of test samples.
- Sampling to be weighted towards the production directly after the species change as this is where carry-over is most likely to be concentrated:
  - First 10 kg after species change – 40 x 250 g portions to be sampled
  - Remaining 90 kg of production – 40 x 250 g portions to be sampled
- Single extraction and single PCR to be carried out for each sample (in line with kit instructions). With a sample weight of 1g, a single analysis would be 1/250 g (0.4 %) of the total sample and duplicate analysis would be 2/250 g (0.8 %). Taking 0.8 % rather than 0.4 % of the entire sample would not significantly increase the probability of finding low level contamination, therefore all samples to be analysed singly. This decision was further substantiated by the fact that the kit specifies that samples can be analysed singly.
- 10 % of samples to be analysed in duplicate to give an indication of the repeatability of observations at different observed contamination levels.

Table 21 shows the sampling plan for Phase 1 (pilot plant).

The rationale for the sampling plan was presented at AMWG meeting on 26 April 2013 and endorsed by the group on the following basis:

- Authentic, traceable samples of pork and beef would be used
- Each 250 g sample was to be thoroughly homogenised before analysis
- Analysing the samples singly allows double the number of samples to be analysed and crucially, enables all of the first 10kg post species change to be analysed for phase 1. This was considered important as the ultimate aim was to assess whether carry-over occurs and what the profile of contamination is throughout the 100 kg so the more samples analysed, the more information obtained.
- The remaining 249 g of each sample, after 1g taken for analysis, will be stored securely so repeat analysis can be carried out if required.

For Phase 2 of the project, the commercial plants were asked to process a weight of pork and beef typical of a production run in their plant. For risk rating 4, deep clean between species, a weight of 100 kg was used, for risk ratings 5 and 6, water wash and no cleaning between species, 200 kg of pork and beef were used. Table 22 shows the sampling plan for Phase 2. Based on the results obtained for Phase 1, a sampling plan was drawn up by LGC's statistics team which involved, once again, focussing the sampling directly after the species change as this is the most likely place that carry-over would be expected to occur, the remainder of the samples being taken in increasing intervals throughout the remainder of the run.

To rationalise the number of samples taken and analysed, duplicate experiments were carried out for each of the Phase 2 scenarios with 50 samples analysed per experiment at the commercial plants. It was agreed by the AMWG that the duplicate experiments would be taken during the same visit.

A limited sampling exercise was carried out for low risk rating; in Phase 1 pork had only been detected in the high risk scenario when no cleaning had taken place between species, it was thought to be highly unlikely that pork would be detected in samples taken from a single species facility. Thus, it was decided at an AMWG meeting that a limited sampling exercise should take place to obtain baseline figures for a single species plant which is the optimum scenario if carry-over is to be avoided.

Cleaning	Overall Risk Rating	Experiment 1 - No. of samples of:			Experiment 2 - No. of samples of:			Experiment 3 - No. of samples of:			Total samples
		Input meat	After species change	Rest of batch	Input meat	After species change	Rest of batch	Input meat	After species change	Rest of batch	
Deep	Low	1	40	40	same	40	40	same	40	40	241
Environmental swabbing		10			10			10			30
Wash	Medium	1	40	40	same	40	40	same	40		241
Environmental swabbing		10			10			10			30
None	High	1	40	40	same	40	40	same	40		241
Environmental swabbing		10			10			10			30

Table 21: Sampling plan for Phase 1

Cleaning	Overall Risk Rating	Experiment 1 - No. of samples of:			Experiment 2 - No. of samples of:			Total samples taken	Total samples analysed*
		Input meat	After species change	Rest of batch	Input meat	After species change	Rest of batch		
Deep	4	1	21	38	-	21	38	118	100
Environmental swabbing		10			10			20	20
Water wash	5	1	21	38	-	21	38	118	100
Environmental swabbing		10			10			20	20
None	6	1	21	38	-	21	38	118	118
Environmental swabbing		10			10			20	20

Table 22: Sampling plan for Phase 2

*For Phase 2, the first three 250 g portions of meat processed were sampled and analysed as, if any carry-over occurred it would most likely be in the meat directly after species change. The next eighteen 250 g portions were sampled but only alternate ones analysed, the other nine samples being held in reserve in the event repeat analyses were required.

The following summarises the sampling plan for Phase 2 (commercial plants).

- A batch size of pork and beef typical for the plant to be processed for each experiment
- 2 experiments to be carried out for each scenario
- Each sample taken to weigh approximately 250 g to reflect the weight of a typical retail pack of minced meat as in Phase 1. The exact weight of each sample to be recorded at LGC.
- 50 samples per experiment to be taken according to a sampling plan devised by LGC's statistics team
- Sampling to be weighted towards the production directly after the species change
  - First 750 g after species change – 3 x 250 g portions to be sampled
  - Next 4.5 kg – 18 x 250 g portions to be sampled and alternate samples analysed
  - Remaining weight of beef – 38 x 250 g portions to be sampled, for example, based on a 200 kg production batch, 20 samples approximately 1 every 2.5 kg, then 10 samples approximately 1 every 5 kg, then 8 samples approximately 1 every 10 kg
- Single extraction and single PCR to be carried out for each sample (in line with kit instructions)

- 10 % of samples to be analysed in duplicate

Staff at a single species plant were asked to take 6 approximately 250 g samples from the first 10 kg of beef processed from the beginning of each of two runs and send the samples to LGC for analysis.

A standard operating procedure (SOP) for sample collection was prepared and discussed and agreed with the FSA and Defra officials prior to commencement of sampling. The final version of the SOPs for Phase 1 is presented in Annex 2 and for Phase 2 in Annex 3.

ATP and protein swabs were taken by plant staff, DNA swab samples were taken by LGC staff.

An industry pilot plant was used to conduct the experiments for Phase 1. All mincing and cleaning operations were performed, according to procedures used in industry, by pilot plant staff under the direct supervision of LGC staff. The cleaning procedure used for the chemical clean is summarised in Annex 4.

For the chemical clean in both Phase 1 and 2, the mincing equipment was dismantled before cleaning. On dismantling the front plate, the compacted pieces of meat were removed by hand. The individual pieces of the equipment were laid out on a trolley and were washed with a jet of water (high pressure water at approximately 50℃ in the pilot plant and hot mains pressure water in the commercial plant) until visually clean. After the equipment had been washed with water, detergent was applied with a contact time of at least 20 minutes. After the detergent had been left for at least 20 minutes it was rinsed from the equipment with water. In the pilot plant a sanitiser was then applied before the equipment was reassembled.

For the water wash in both Phase 1 and 2, the mincing equipment was dismantled as described above and washed with a jet of water until visually clean, i.e. until no traces of meat or meat residue could be seen. The remainder of the mincing equipment was then washed until no meat or meat residue was visible. When the equipment was assessed as being visually clean, for Phase 2 the equipment was reassembled ready for the next batch of meat to be processed whilst in the pilot plant a sanitiser was applied before the equipment was reassembled.

The photographs in Annex 4 give an indication of the quantity of meat present on the mincing equipment directly after it had been dismantled (photograph 1) and after it had been washed with water and detergent applied (photograph 2).

According to the cleaning protocols supplied by both the pilot plant and the commercial plant used for the chemical clean, different commercially available detergents were used for the chemical clean in Phase 1 (pilot plant) and Phase 2 (commercial plant). According to the safety data sheets for the detergents, the main active ingredient in the detergent used in Phase 1 was 10 – 30 % sodium hydroxide whilst the detergent used in Phase 2 contained 7 – 10 % potassium hydroxide and 1 – 5 % sodium hypochlorite. These concentrations are those stated for the product as purchased and do not take into account any dilutions made prior to use. Again, according to the cleaning protocols supplied by the two plants, the same disinfectant was used by both and is based on a quaternary ammonium chloride biocide. As the outcome from the trials carried out at the pilot plant and those carried out at the commercial plant was the same in that no carry-over was detected after a chemical clean, the difference in formulation of the detergents used does not appear to be significant with

respect to the effectiveness of the cleaning.

To facilitate sampling during Phase 1, a conveyor belt was placed under the mincer outlet at the pilot plant. The conveyor belt used was an interlock conveyor belt (chain) as opposed to a solid belt; with an interlock conveyor there is more potential for pieces of meat to get stuck and cause a 'memory' effect than with a solid belt and thus represented a worst case scenario in this respect. Sampling for Phase 2 was carried out directly from the outlet as, in a commercial setting, conveyor belts are not usual at this stage in the process as the meat is generally collected directly in a tote bin ready to be transferred to a hopper for either packaging or further processing.

Figure 2 illustrates the sampling process, from mincing the pork, through cleaning, where applicable, to sampling the beef. The process was carried out in triplicate for Phase 1 and in duplicate for Phase 2. For Phase 1, samples of 250 ± 10 g were taken, for Phase 2 a nominal sample weight of 250 g was taken, the minimum weight of sample was 154 g, the maximum weight was 433 g, with a mean sample weight of 263 g.

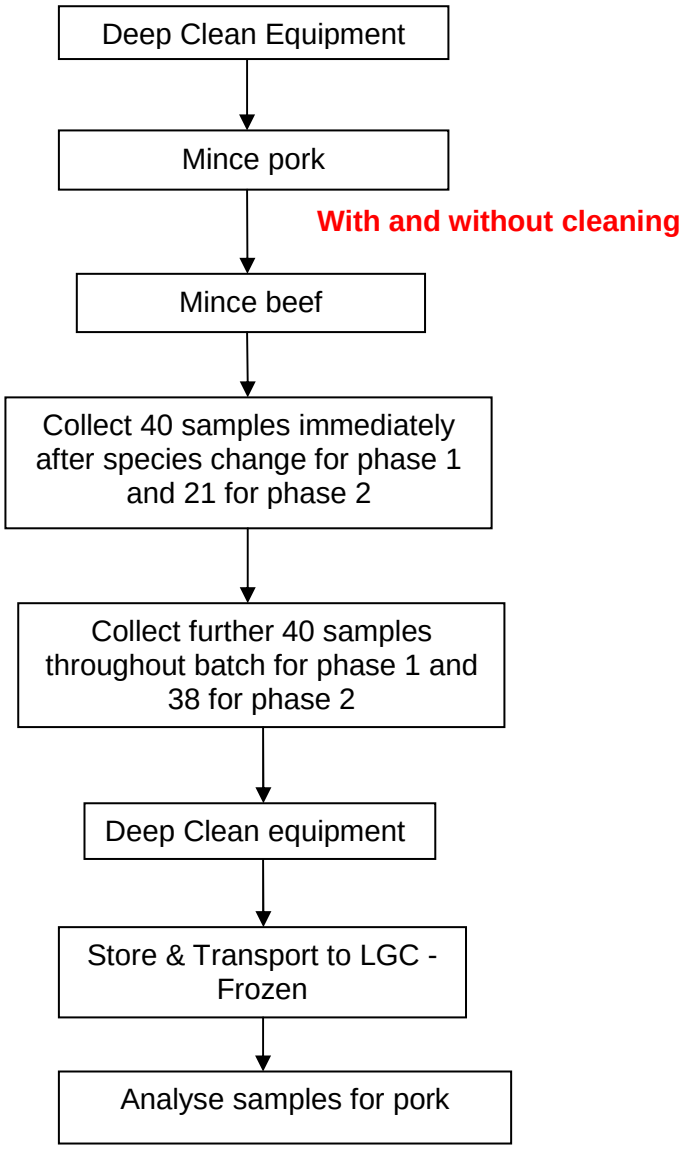

Figure 2: Sampling process low

10. Authentic Meats

For Phase 1 authentic samples of pork (~620 kg of 95 % visual lean (VL)) and beef (~620 kg labelled as 95 % visual lean) were sampled from two independent single species abattoirs. The samples were cut into pieces by abattoir staff and vacuum packed. The sealed bags were packed into boxes that were secured with tamper evident LGC tape and transported to the pilot plant under refrigeration. The process used was audited by a member of LGC staff.

The authenticity of the pork and beef meat sampled was verified using DNA sequencing.

The meat used for the Phase 2 trials was provided by the plants and was from the same suppliers who provide meat for their products.

For risk rating 4 (chemical clean between species) 100 kg pork was minced for each experiment followed by 150 kg beef for experiment 1 and 100 kg beef for experiment 2. For risk ratings 5 and 6, 200 kg each of pork and beef were used for each experiment.

Table 23 summarises the stated fat content of the meat used for each of the experiments.

	Pork	**Beef**
Phase 1 – Pilot plant	95VL	95VL
Phase 2 – Chemical clean	85VL	85VL
Phase 2 – water wash and no cleaning	95VL	85VL

Table 23: Grade of meat used for each experiment

11. Sample Transport and Storage

Once the sampling was complete at the plants, the samples were double bagged and placed in boxes or crates which were wrapped in cling film or sealed with LGC tape to prevent tampering during transit. The wrapped boxes / crates were then frozen overnight before being transported to LGC by the plant's couriers. On arrival at LGC, the samples were stored in a secure freezer until required for analysis.

12. Sample Homogenisation

The homogenisation of the frozen portions was carried out as described in standard operating procedure Homogenisation of Minced Meat which is presented in Annex 5. In summary, the procedure was as follows:

i. Homogenisation Equipment Cleaning Procedure

The sample homogenisation equipment cleaning procedures were optimised and validated as follows:

a) Twenty new food processors were purchased at the beginning of the project
b) Cleaned in dish washers
c) Dried using clean tissues
d) Cleaned with Microsol (destroys DNA residues) and ethanol
e) Dried using clean tissues

f) Swabbed using a protein swabbing kit to check for protein residues. If any traces of protein were detected, the food processors were re-cleaned and confirmed to be free of any protein residue before use.

Steps b – e were repeated for each food processor after the homogenisation of every sample.

ii. Sample Homogenisation Procedure

Each beef sample was thawed and thoroughly homogenised in a pre-cleaned (as described in i) food processor. 1 g ±0.1 g of each sample was weighed into tubes ready for DNA extraction. The remaining sample (~249 g) was returned to frozen storage.

13. Analysis and results

For each scenario and risk rating, QC mixes were prepared using the input meat processed for each scenario. For this reason, the results represent the best case scenario as the QCs match the samples as closely as is possible with respect to the animal, cut of meat, etc.
By having the standards and samples made from same authentic meats some of the factors that make the provision of quantitative DNA results difficult are overcome, in other words, as the samples and QCs are from the same material, they should behave similarly in the assay, for example, with respect to being able to extract DNA. Thus, for this project, it was anticipated that it would be possible to express the results for the meat samples as % pork in beef on w/w meat basis.

DNA was extracted from samples and QCs using an LGC proprietary method according to a standard operating procedure. The extracted DNA was analysed for pork content using a commercially available quantitative real time PCR DNA assay. The SOP used for the determination of the pork content can be found in Annex 6.

Summaries of the results for the analysis of samples from Phase 1 and 2 are presented in Tables 24 to 36, the full set of results can be found in Annex 7 to 13.

Sampling Plan			Low risk – Experiment 1			Low risk – Experiment 2			Low risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
1	2	3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
4	5	6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
7	8	9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
10	11	12	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
13	14	15	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
16	17	18	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
19	20	21	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
22	23	24	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
25	26	27	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
28	29	30	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
31	32	33	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
34	35	36	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
37	38	39	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
40	41	42	<0.1			<0.1			<0.1		

Table 24: Sample results – Phase 1, Low risk scenario (Chemical clean between species), Pork content of beef samples post species change (~10 kg)

Sampling Plan	Low risk – Experiment 1	Low risk – Experiment 2	Low risk – Experiment 3
Conveyor belt	Conveyor belt	Conveyor belt	Conveyor belt
Positions 41 - 400 (40 samples from remaining ~90 kg)	<0.1	<0.1	<0.1

Table 25: Sample results – Phase 1, Low risk scenario (Chemical clean between species), Pork Content of Beef Samples in Remaining ~90 kg

Sampling Plan			Medium risk – Experiment 1			Medium risk – Experiment 2			Medium risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
1	2	3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
4	5	6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
7	8	9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
10	11	12	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
13	14	15	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
16	17	18	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
19	20	21	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
22	23	24	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
25	26	27	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
28	29	30	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
31	32	33	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
34	35	36	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
37	38	39	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
40	41	42	<0.1			<0.1			<0.1		

Table 26: Sample Results – Phase 1, Medium risk scenario (High pressure water wash between species), Pork content of beef samples post species change (~10 kg)

Sampling Plan	Medium risk – Experiment 1	Medium risk – Experiment 2	Medium risk – Experiment 3
Conveyor belt	Conveyor belt	Conveyor belt	Conveyor belt
Positions 41 - 400 (40 samples from remaining ~90 kg)	<0.1	<0.1	<0.1

Table 27: Sample Results – Phase 1, Medium risk scenario (High pressure water wash between species), Pork content of beef samples in remaining ~90 kg

Sampling Plan			High risk – Experiment 1			High risk – Experiment 2			High risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
1	2	3	70.5	54.1	69.2	59.9	60.2	71.9	88.0	63.2	99.0
4	5	6	2.8	10.9	4.9	1.2	3.9	1.4	11.1	5.2	16.2
7	8	9	1.5	1.5	1.3	<0.1	3.7	0.9	5.6	3.2	0.7
10	11	12	0.9, 1.7	0.6	1.5	<0.1, <0.1	<0.1	<0.1	1.8, 1.7	8.0	1.1
13	14	15	0.8	3.9	1.2	<0.1	0.6	0.7	2.5	<0.1	0.2
16	17	18	0.5	0.9	0.6	1.2	0.5	0.9	1.0	<0.1	2.0
19	20	21	0.3	0.5, 0.9	0.4	0.4	0.8, 2.9	2.0	<0.1	0.5 , <0.1	<0.1
22	23	24	<0.1	<0.1	0.7	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
25	26	27	<0.1	0.2	<0.1	<0.1	1.2	3.0	<0.1	<0.1	<0.1
28	29	30	0.3	<0.1	<0.1, <0.1	2.2	3.8	0.7, 1.2	<0.1	<0.1	<0.1, <0.1
31	32	33	<0.1	<0.1	<0.1	2.1	5.4	1.4	<0.1	<0.1	2.2
34	35	36	0.5	1.9	0.2	1.1	1.8	1.2	0.8	5.2	1.5
37	38	39	<0.1	0.4	<0.1	0.4	0.8	0.9	<0.1	<0.1	0.7
40	41	42	<0.1, 0.3			0.5, <0.1			<0.1, <0.1		

Table 28: Sample Results – Phase 1, High risk scenario (No cleaning between species), Pork content of beef samples post species change (~10 kg)

Sampling Plan	High risk – Experiment 1		High risk – Experiment 2		High risk – Experiment 3	
Conveyor belt	**Conveyor belt**		**Conveyor belt**		**Conveyor belt**	
Positions 41 - 400 (40 samples from remaining ~90 kg)		**% of samples**		**% of samples**		**% of samples**
	<0.1 %	60.0	<0.1 %	80.0	<0.1 %	85.0
	<1 %	35.0	<1 %	20.0	<1 %	12.5
	1-10 %	5.0	1-10 %	0	1-10 %	2.5
	10-50 %	0	10-50 %	0	10-50 %	0
	>50 %	0	>50 %	0	>50 %	0

Table 29: Sample Results – Phase 1, High risk scenario (No cleaning between species), Pork Content of Beef Samples in Remaining ~90 kg

Experiment 1			Experiment 2		
<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
<0.1		<0.1	<0.1		<0.1
	<0.1			<0.1	
<0.1		<0.1	<0.1		<0.1
	<0.1			<0.1	
<0.1		<0.1	<0.1		<0.1
	<0.1			<0.1	

Table 30: Sample Results – Phase 2, Chemical clean between species, Pork content of beef samples post species change (~5 kg)

Sampling Plan	Experiment 1	Experiment 2
Positions 28 - 400 (38 samples from remaining ~95 kg)	<0.1	<0.1

Table 31: Sample Results – Phase 2, Chemical clean between species, Pork content in beef samples in remaining 95 kg

Experiment 1			Experiment 2		
<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
<0.1		<0.1	<0.1		<0.1
	<0.1			<0.1	
<0.1		<0.1	<0.1		<0.1
	<0.1			<0.1	
<0.1		<0.1	<0.1		<0.1
	<0.1			<0.1	

Table 32: Sample Results – Phase 2, Water wash between species, Pork content of beef samples post species change (~5 kg)

Sampling Plan	Experiment 1	Experiment 2
Positions 28 - 800 (38 samples from remaining ~195 kg)	<0.1	<0.1

Table 33: Sample Results – Phase 2, Water wash between species, Pork content in beef samples in remaining 195 kg

Experiment 1			Experiment 2		
97.2	97.8	94.7	184.4	162.9	145.2
100.3		49.9, 46.2	144.7		98
	8.4			86.9	
13.6		10.3	29.7		13.2
	5.8			6.2	
2.7		5.7	4.8		2.9
	3.3			2.2	

Table 34: Sample Results – Phase 2, No cleaning between species, Pork content of beef samples post species change (~5 kg)

Sampling Plan	Experiment 1		Experiment 2	
		% of samples		% of samples
Positions 28 - 800 (38 samples from remaining ~195 kg)	<0.1 %	44.7	<0.1 %	52.6
	<1 %	55.3	<1 %	47.4
	1-10 %	0	1-10 %	0
	10-50 %	0	10-50 %	0
	>50 %	0	>50 %	0

Table 35: Sample Results – Phase 2, No cleaning between species, Pork content in beef samples in remaining 195 kg

Production run 1 and 2	
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1

Table 36: Sample Results – Phase 2, Single species plant

In Tables 24 to 36, pork content is expressed as % pork DNA relative to total mammalian DNA present as interpreted in accordance with the instructions of the commercial quantitative real time PCR DNA assay that was used to analyse the samples. Results from comparisons of % pork DNA to % pork meat on a w/w basis showed that, in these instances, there was no statistical difference between the results expressed as % DNA and the actual pork content determined by mass. The results are reported on a quantitative weight: weight basis and represent the 'best case scenario' in that the standards and samples were made from the same authentic lean meats.

The reporting limit is 0.1 % pork DNA relative to total mammalian DNA present as established by method validation at LGC. Experiments to assess the reliability of detecting concentrations of pork below 0.1 % pork DNA relative to total mammalian DNA were conducted and showed that a Limit of Detection of 0.03 % pork DNA relative to total mammalian DNA present was achievable in some instances.

14. Quality Assurance

The instructions received with each PCR kit recommended that the following be carried out in each batch of analysis:

- For each run, reactions needed for reference-gene (mammalian) detection
  - 5 reactions of standard curve
  - 1 reaction for no-template control
  - 2 reactions for positive control
  - 1 reaction per sample
- For each run, reactions needed for pork detection
  - 5 reactions of standard curve
  - 1 reaction for no-template control
  - 2 reactions for positive control
  - 1 reaction per sample

Following the validation it was, however, determined to be more appropriate to analyse the following quality controls within each batch:

- No template controls (x2)
- Negative control (100 % beef x1)
- Extraction blanks (one per extraction batch)
- Kit standards (five standards x1)
- Kit positive control (x2)
- Samples (x1)

- In-house w/w QCs:
  - 0.1 % pork in beef (x1)
  - 50 % pork in beef (x1)

Even though it was recommended that the above quality controls be analysed in each batch, no performance criteria were provided by the kit manufacturer. To ensure consistency between batches LGC drew up the following acceptability criteria (Table 37) which were endorsed by the AMWG. Where results were obtained outside the acceptable ranges, the analysis was repeated.

QC Material	**Expected Result**
No template controls	No pork detected
100 % beef	No pork detected
Extraction blanks	No pork detected
Kit positive control	Corrected pork content: 70 – 130 %
Kit standards:	
PCR efficiency	85 -115 %
R2	>0.98
In-house w/w QCs:	
0.1 % pork in beef	Pork detected
50 % pork in beef	25 – 100 % pork
100 % beef	No pork detected

Table 37: Criteria for Acceptability

In every batch, duplicate aliquots of one sample were extracted and analysed. Table 38 shows examples of duplicate results obtained.

	% Pork DNA	
Sample number	**Result A**	**Result B**
311177	0.9	1.7
311187	0.5	0.9
311197	<0.1	<0.1
311207	0.9	0.4
311217	<0.1	0.2
311227	0.04	<0.1
311237	<0.1	<0.1
311355	1.4	4.5
311365	0.7	1.2
311375	0.5	<0.1
320041	0.2	0.1
320051	<0.1	<0.1
320061	<0.1	0.3
320071	<0.1	<0.1
311395	1.8	1.7
311435	<0.1	<0.1
311437	<0.1	<0.1
311445	<0.1	<0.1
311465	<0.1	<0.1

Table 38: Examples of duplicate sample results

As described in section 11, in every batch, checks on the kit's performance were made. Table 39 summarises the performance of the first 28 kits.

Plate number	Kit correction value (k)	Sus efficiency	Ref efficiency	Sus R-squared	Ref R-squared
1	0.695	92.2 %	108.2 %	0.998	0.994
2	0.749	96.1 %	98.1 %	0.998	0.999
3	0.746	99.5 %	101.6 %	0.998	0.999
4	0.604	100.0 %	97.1 %	0.998	0.999
5	0.597	98.7 %	100.1 %	0.999	0.999
6	0.618	99.5 %	91.9 %	0.999	0.997
7	0.916	89.6 %	95.8 %	0.998	0.995
8	0.777	94.6 %	94.8 %	0.995	1.000
9	0.785	104.4 %	99.7 %	0.989	0.990
10	1.251	86.8 %	94.9 %	0.993	1.000
11	0.610	110.0 %	92.8 %	0.975	0.986
12	0.573	97.8 %	103.8 %	0.999	0.997
13	0.763	100.0 %	100.0 %	0.999	0.999
14	0.656	93.0 %	96.0 %	0.999	0.999
15	0.775	97.6 %	93.9 %	1.000	0.999
16	0.535	99.1 %	95.9 %	0.998	0.998
17	0.580	111.7 %	106.0 %	0.999	0.994
18	0.993	98.7 %	97.8 %	0.999	1.000
19	0.572	94.0 %	97.8 %	0.999	0.999
20	0.610	102.0 %	101.0 %	1.000	0.996
21	0.621	101.6 %	106.0 %	1.000	0.999
22	0.635	92.3 %	89.4 %	1.000	0.999
23	0.760	94.3 %	93.9 %	1.000	0.999
24	0.620	94.2 %	104.0 %	0.999	0.998
25	1.038	92.1 %	99.5 %	0.998	0.994
26	0.616	102.0 %	98.7 %	0.997	0.998
27	1.100	96.5 %	94.6 %	1.000	0.999
28	1.430	104.9 %	99.6 %	0.996	0.999

Table 39: Example of Kit QCs

Every batch of analysis also included a gravimetrically prepared QC standard of 0.1 % pork in beef and another of 50 % pork in beef. Table 40 summarises the results obtained for the QCs analysed in the first 28 PCR plates; the results highlighted in red were outside the acceptable range and the analysis was repeated.

Plate number	0.1 % Pork in beef	50 % Pork in beef	100 % Beef
1	0.28	47.8	0
1	0.12	62.0	0
1	0.08	60.3	0
2	0.08	53.0	0
2	0.14	56.0	0
2	0.17	51.8	0
3	0.07	62.2	0
3	0.13	64.6	0
4	0.17	39.0	0
4	0.09	41.1	0
5	0.33	55.9	0
5	0.13	49.6	0
6	0.10	68.0	0
6	0.16	57.2	0
7	0.21	83.3	0
7	0.29	68.8	0
7	0.28	72.9	0
8	0.09	40.8	0
8	0.26	30.0	0
9	0.09	59.1	0
9	0.07	55.6	0
10	0.68	134.6	0
10	0.20	115.5	0
10	0.19	103.0	0
11	0.07	60.0	0
11	0.06	49.5	0
12	0.16	13.4	0
13	0.13	45.6	0
13	0.13	58.9	0
14	0.12	58.9	0
14	0.15	31.9	0
15	0.15	109.6	0
15	0.06	52.4	0
16	0.16	49.7	0
16	0.51	48.9	0
17	0.11	0.0	0
17	0.06	23.0	0
18	0.21	92.0	0
18	0.11	116.3	1.6
19	0.13	48.1	0
19	0.08	29.9	0
20	0.07	26.8	0

Plate number	0.1 % Pork in beef	50 % Pork in beef	100 % Beef
20	0.09	60.0	0
20	0.11	1.2	0
21	0.07	46.8	0
21	0.15	34.4	0
22	0.31	36.8	0
22	0.06	34.2	0
23	0.13	56.1	0
23	0.12	43.5	0
24	0.21	64.4	0
24	0.13	33.0	0
25	0.11		0
25	0.26		0
26	0.00	83.3	0
26	0.17	114.8	0
27	0.09	46.7	0
27	0.10	40.0	0
28	0.33		0

Table 40: QC data for 0.1 % and 50 % pork in beef

From the acceptable results of the 0.1 % QC a 95 % confidence interval based on logged data was calculated giving an estimated concentration for the 0.1 % standard of between 0.04 to 0.27 % pork DNA relative to total mammalian DNA present.

14.1. Quality assurance summary

Included in every extraction and PCR batch were a 0.1 % and 50 % gravimetrically prepared pork in beef QCs. If the results for these QCs, or those of the kit performance checks, did not fall within the agreed acceptable ranges, the results for the samples in that batch were not accepted for reporting and the samples were reanalysed. For the 0.1 % pork in beef QC the acceptance criteria was that pork must have been detected.

The results for the 0.1 % pork in beef QCs included in the first 28 extraction and analysis batches were evaluated by LGC's statistics team. Based on the variation in results, due to preparation, extraction and analysis, it was estimated that the pork content of a sample containing 0.1 % pork in beef could be determined to be between 0.04 and 0.27 % pork DNA.

15. Interpretation of results

The results reported in section 12 are the pork content of the individual 250 g beef samples taken and represent what a consumer would get if they purchased a 250 g retail packet of minced meat.

As minced meat is used in the manufacture of a wide variety of processed meat products, it was considered useful to show the results interpreted to reflect what the theoretical

concentration of pork would be, in a number of end products, if the beef was used in the manufacture of meat products.

In order to ensure the calculations performed reflected the proportions and volumes used by UK industry, the following information was requested from four of the UK's leading members based organisations representing the UK food industry. The organisations contacted were:

- British Retail Consortium (BRC)
- Food and Drink Federation (FDF)
- British Meat Processors Association (BMPA
- Agriculture and Horticultural Development Board (AHDB)

a. Proportion of meat in products for:

Proportion of ingredient	High Value Burger	Low Value Burger	High Value Sausage	Low Value Sausage	High Value Lasagne	Low Value Lasagne	?	?
Meat								
Other ingredients								

b. What is the size of a batch for:

Scale of production	Size of batch / kg
Small	
Medium	
Large	

The information received, summarised in Tables 41 and 42, was applied to the observed carry-over of pork, reported in section 13 and used to calculate the theoretical carry-over of pork in certain meat products for different quality of products and different sized operations.

Company	Proportion of ingredient (%)	High Value Burger	Low Value Burger	High Value Sausage	Low Value Sausage	High Value Mince	Low Value Mince
A	Meat	99	49	72	32	100	65
B	Meat			61	42		
C	Meat	95					
D	Meat	90	75	75	54		
E	Meat			85	56		
F	Meat	85	63	85	43		
G	Meat	62	47	60	30		
H	Meat	99	50	98	42		

Table 41: Proportion of meat added to various products

		Typical batch sizes (kg)			
		High Value Burger	**Low Value Burger**	**High Value Sausage**	**Low Value Sausage**
A	Small	20	20	200	200
	Medium	820	820	400	400
	Large	1500	1500	600	600
B	Small			200	200
	Medium				
	Large			1200	1200
C	Small	100			
	Medium	200			
	Large				
D	Small	30	30	30	30
	Medium	100	100	100	100
	Large	150	150	150	150
E	Small			50	50
	Medium			200	200
	Large			400	400
F	Small	500	500	500	500
	Medium	1000	1000	1000	1000
	Large	5000	5000	5000	5000

Table 42: Typical production batch sizes

The weight of a typical retail pack of minced beef is 500 g. Based on the results obtained from Phase 1 high risk scenario, where the equipment was not cleaned between species, Table 43 presents the quantity and percentage of pork that would theoretically be present in 500 g of mince packed sequentially from beef processed directly after pork.

Pack number	Weight of pork in 500 g retail pack (g)	% Pork DNA in retail pack
1	330.0	66.0
2	212.6	42.5
3	35.4	7.1
4	12. 9	2.6
5	6.1	1.2
6	9.3	1.9
7	6.8	1.4
8	4.6	0.9
9	5.2	1.0
10	4.3	0.9
11	2.0	0.4
12	1.0	0.2
13	1.3	0.3
14	4.6	0.9
15	4.2	0.8
16	6.3	1.3
17	4.9	1.0
18	10.9	2.2
19	2.4	0.5
20	6.5	1.3

Table 43: Pork carry-over in minced beef 500 g retail packs containing 100 % meat, where no cleaning of the mincing equipment was carried out between species

From the samples analysed, the total mass of pork present in the entire 100 kg of processed beef for Phase 1 high risk scenario was estimated using a zero-end point model and found to be 653 g, as illustrated in Figure 3.

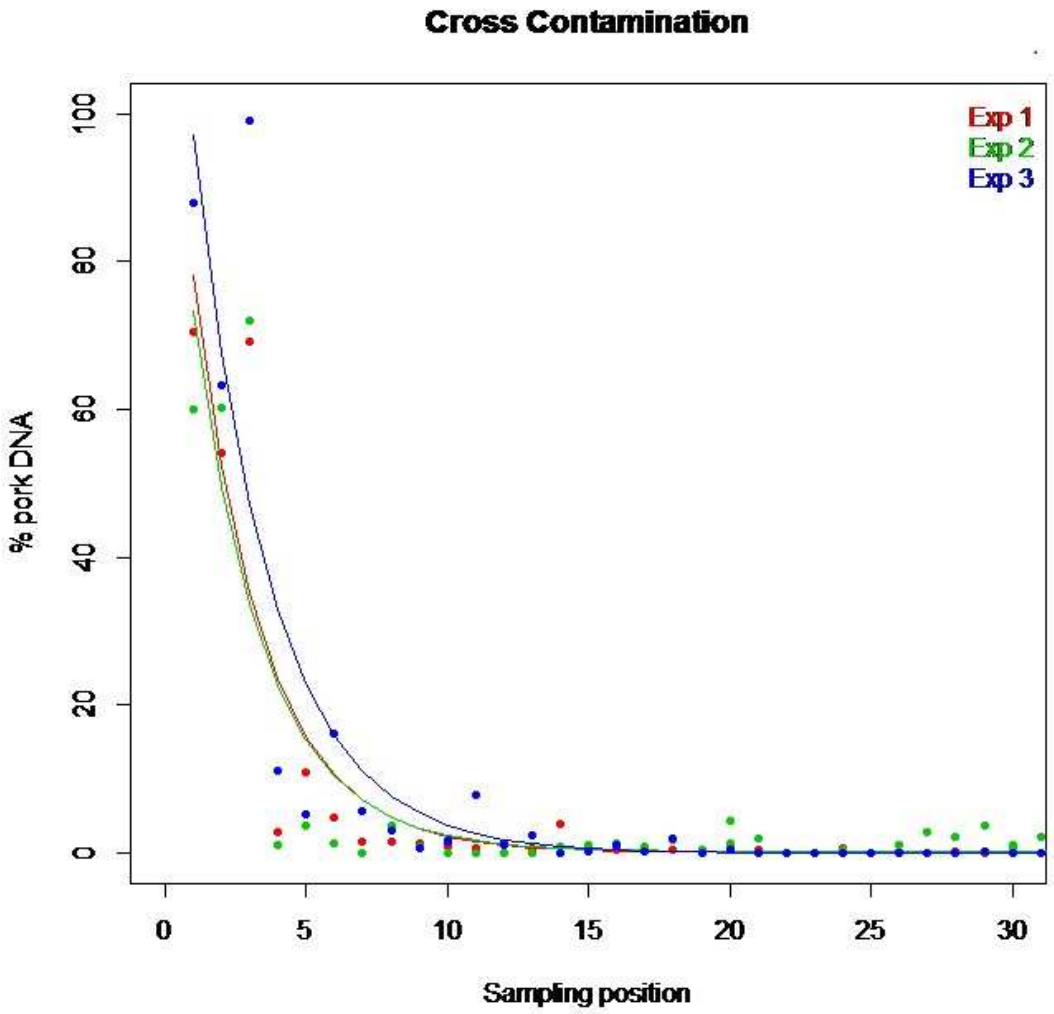

Figure 3: Calculation of pork content of 100 kg beef using a zero-end point model

Using the carry-over figure of 653 g of pork in 100 kg beef (0.65 %), the approximate weight and percentage of pork expected to be found in a finished product was calculated. It should be noted though that the calculated concentration of pork in the finished products shown in the tables below is due to dilution effects only i.e. weight of pork in relation to the total weight of the production batch and no allowance has been made for the effect of other ingredients or additional processing on the determination of pork content.

From the information received from industry it was noted that a 'typical' batch size could be anything from 20 to 5000 kg. Table 44 shows the pork content of a 90 % meat product prepared with beef containing 653 g of pork in 100 kg beef. As, for the Phase 1 experiments, no pork was detected after approximately 16 kg of beef had been processed so it was assumed that for batch sizes of greater than 100 kg, only 653 g of pork would be present.

Percentage meat	Batch size (kg)	Wt meat in batch (kg)	Wt pork (g) in batch	Weight pork in 454g retail pack (g)	% Pork in retail pack (g)
90	20	18	653.5	14.83	3.27
90	30	27	653.5	9.89	2.18
90	100	90	653.5	2.97	0.65
90	150	135	653.5	1.98	0.44
90	200	180	653.5	1.48	0.33
90	500	450	653.5	0.59	0.13
90	820	738	653.5	0.36	0.08
90	1000	900	653.5	0.30	0.07
90	1500	1350	653.5	0.20	0.04
90	5000	4500	653.5	0.06	0.01

Table 44: Pork carry-over in a 90 % meat product for different batch sizes where no cleaning of the mincing equipment was carried out between species

In addition to ‘typical’ batch sizes varying greatly, there can also be a significant difference in the percentage of meat in different products. Tables 45 to 47 present the weight and percentage of pork that would be present in burgers, sausages or lasagne if beef containing a carry-over of 653 g pork was used to prepare quantities of each product.

Percentage meat in product	Batch size (kg)	Weight pork in 454 g retail pack(g)	% Pork in retail pack	Detectable to LOD of 0.1 % DNA
47 - 99	20	14.83	3.27	Yes
47 - 99	30	9.89	2.18	Yes
47 - 99	100	2.97	0.65	Yes
47 - 99	150	1.98	0.44	Yes
62 - 99	200	1.48	0.33	Yes
47 - 99	500	0.59	0.13	Yes
47 - 99	820	0.36	0.08	No
47 - 99	1000	0.30	0.07	No
47 - 99	1500	0.20	0.04	No
47 - 99	5000	0.06	0.01	No

Table 45: Carry-over extrapolated to Burgers where no cleaning of the mincing equipment was carried out between species

Percentage meat in product	Batch size (kg)	Weight pork in 454 g retail pack(g)	% Pork in retail pack	Detectable to LOD of 0.1 % DNA
30 - 98	30	9.89	2.18	Yes
30 - 98	50	5.93	1.31	Yes
30 - 98	100	2.97	0.65	Yes
30 - 98	150	1.98	0.44	Yes
30 - 98	200	1.48	0.33	Yes
30 - 98	400	0.74	0.16	Yes
30 - 98	500	0.59	0.13	Yes
30 - 98	600	0.49	0.11	Yes
30 - 98	1000	0.30	0.07	No
30 - 98	1200	0.25	0.05	No
30 - 98	5000	0.06	0.01	No

Table 46: Carry-over extrapolated to Sausages where no cleaning of the mincing equipment was carried out between species

Percentage meat in product	Batch size (kg)	Weight pork in 400 g retail pack(g)	% Pork in retail pack	Detectable to LOD of 0.1 % DNA
15 - 30	30	8.71	2.18	Yes
15 - 30	50	5.23	1.31	Yes
15 - 30	100	2.61	0.65	Yes
15 - 30	150	1.74	0.44	Yes
15 - 30	200	1.31	0.33	Yes
15 - 30	400	0.65	0.16	Yes
15 - 30	500	0.52	0.13	Yes
15 - 30	600	0.44	0.11	Yes
15 - 30	1000	0.26	0.07	No
15 - 30	1200	0.22	0.05	No
15 - 30	5000	0.05	0.01	No

Table 47: Carry-over extrapolated to Lasagne where no cleaning of the mincing equipment was carried out between species

It can be seen from these tables that the batch size, rather than the proportion of meat added, is the defining factor as to whether pork would be theoretically detected in a finished product. Assuming that a limit of detection of 0.1 % pork in beef could be reliably achieved in each of these products, which is as yet not established, carry-over could be detected in batch sizes of up to 600 kg; for batch sizes >600 kg, the pork carry-over would effectively be diluted to below the detection limit assuming the batch has been mixed homogeneously. The figures highlighted in red illustrate the batch sizes where pork would not be detected, assuming a LOD of 0.1 %.

The results presented in section 15 have been derived from calculations based on results obtained for the Phase 1 – pilot plant high risk scenario (no cleaning between species); they are given for illustration only and did not involve the analysis of processed products.

Similar calculations were carried out on the results obtained for Phase 2, risk rating 6 (commercial plant with no cleaning between species). Statistical evaluation of the results obtained for the two experiments estimated a total pork content of 5.6 %, equivalent to 11.2 kg of pork carry-over in the 200 kg beef processed.

If 200 kg of processed beef containing an estimated 11.2 kg of pork was added to a production batch of burgers, for example, weighing 5000 kg, the maximum batch size stated by industry, the pork content would be 0.2 %. A production batch size of greater than 11,000 kg would be needed for 11.2 kg pork to be 'diluted' to such an extent that the content would be below the limit of detection of 0.1 %. Therefore, based on the results obtained from one set of mincing equipment in a commercial setting, making no allowance for any possible effect on the LOD from added ingredients, carry-over would be detected in all batch sizes up to a maximum of 11,000 kg.

The difference in the estimated pork content from Phase 1 and Phase 2 is thought to be due, in part, to the differences in the equipment used. The mincing equipment used in Phase 2 of the project being greater in volume and utilising more mincing plates, for example, than the mincer used in the pilot plant, and therefore having more trap points for meat debris to collect. This was highlighted by the fact that, for the pilot plant trials, no significant quantities of pork were detected after approximately 16 kg of beef had been processed, whilst for Phase 2 0.3 and 0.1 % pork were detected in the last of the samples taken from the 200 kg beef for experiments 1 and 2 respectively.

As an example, Table 48 shows the quantity of pork needed to be present in various production batch sizes to give a final concentration of pork at the LOD of 0.1 %. As can be seen, 1 kg of pork would need to be included in a batch size of 1000 kg for the pork content to be 0.1 % and therefore be detected using the analytical method described in this report. However, it is not the exact amount of carry-over that is considered important as it will vary with batch size and type of equipment used and this needs to be considered by manufacturers when presenting information to consumers about the composition of meat products so the contents are accurately described.

Batch size (beef) (kg)	Weight of pork (kg)
100	0.1
1000	1.0
5000	5.0

Table 48: Examples of the quantity of pork needed to be present to be detected at 0.1 % in different production batch sizes

16. Swabs

In order to check the effectiveness of the cleaning regimes employed by UK industry and used in this project, it was decided to swab surfaces after cleaning. It was decided to take a number of different swabs:

- Swabs for detection of adenosine triphosphate (ATP) - ATP is present in all organic material and a positive reading for a swab is an indication of the presence of contamination, for example food residue, allergens and/or bacteria. ATP swabs are cost effective and widely used by the UK meat industry as a means for validating and checking cleaning regimes. ATP swabs are cost effective at around £2 per swab after the initial cost of the luminometer needed to read the swabs, and can be performed by trained factory staff.

- Swabs for the detection of protein - Protein swabs are cost effective and are more recently being used by the UK meat industry in addition to or instead of ATP swabs as a means for validating and checking cleaning regimes where removal of protein residues is important. These swabs detect any protein residue to a detection limit of 50 ug. Protein swabs are cost effective at £2 per swab and can be performed by trained factory staff.
- Swabs for the detection of pork DNA – DNA swabs are desirable as they are very specific to the target so in this instance pork was detected to a limit of 50 ug. DNA swabs are the most expensive of the three swabs taken as they require analysis at a laboratory by analysts trained in DNA analysis; DNA has to be extracted from the swab before the PCR can be performed thus cost per sample can range from £100 - £200 / sample.

During each of the experiments for Phase 1, swabs for ATP, protein and DNA were taken. The swabs were taken after cleaning with water, and detergent where applicable, but before any sanitiser was applied as per the manufacturer's instructions for the protein and ATP swabs.

The results from Phase 1 are presented in Tables 49 to 51.

		Phase 1 - Low risk scenario								
		Experiment 1			**Experiment 2**			**Experiment 3**		
	Surface type	**ATP**	**Protein**	**Pork DNA**	**ATP**	**Protein**	**Pork DNA**	**ATP**	**Protein**	**Pork DNA**
Mincer top	Stainless steel	5	Negative	Not detected	8	Negative	Not detected	0	Negative	Not detected
Underside of guard	Stainless steel	6	Negative	Not detected	6	Negative	Not detected	0	Negative	Not detected
Infeed sheet to auger	Stainless steel	7	Negative	Not detected	5	Negative	Not detected	2	Negative	Not detected
Auger barrel	Stainless steel	8	Negative	Not detected	9	Negative	Not detected	0	Negative	Not detected
Collar	Stainless steel	15	Negative	Not detected	4	Negative	Not detected	1	Negative	Not detected
Knife	Stainless steel	4	Negative	Not detected	5	Negative	Not detected	5	Negative	Not detected
Auger	Stainless steel	7	Negative	Not detected	4	Negative	Not detected	1	Negative	Not detected
Mincing plate	Stainless steel	6	Negative	Not detected	4	Negative	Not detected	2	Negative	Not detected
Belt drive wheel on conveyor belt	Plastic	0	Negative	Not detected	4	Negative	Not detected	17	Negative	Not detected
Belt	Stainless steel	3	Negative	Not detected	4	Negative	Not detected	22	Negative	Not detected

Table 49: ATP, protein and DNA swab results for Phase 1, low risk scenario

		Phase 1 - High risk scenario								
		Experiment 1			Experiment 2			Experiment 3		
	Surface type	ATP	Protein	Pork DNA	ATP	Protein	Pork DNA	ATP	Protein	Pork DNA
Mincer top	Stainless steel	10	Negative	Not detected	6	Negative	Not detected	1	Negative	Not detected
Underside of guard	Stainless steel	4	Negative	Not detected	3	Negative	Not detected	4	Negative	Not detected
Infeed sheet to auger	Stainless steel	4	Negative	Not detected	8	Negative	Not detected	25	Negative	Not detected
Auger barrel	Stainless steel	7	Negative	Not detected	9	Negative	Not detected	0	Negative	Not detected
Collar	Stainless steel	6	Negative	Not detected	13	Negative	Not detected	2	Negative	Not detected
Knife	Stainless steel	18	Negative	Not detected	23	Negative	Not detected	5	Negative	Not detected
Auger	Stainless steel	6	Negative	Not detected	11	Negative	Not detected	2	Negative	Not detected
Mincing plate	Stainless steel	4	Negative	Not detected	24	Negative	Not detected	8	Negative	Not detected
Belt drive wheel on conveyor belt	Plastic	4	Negative	Not detected	17	Negative	Not detected	2	Negative	Not detected
Belt	Stainless steel	3	Negative	Not detected	13	Negative	Not detected	2	Negative	Not detected

Table 50: ATP, protein and DNA swab results for Phase 1, high risk scenario

		Phase 1 - Medium risk scenario								
		Experiment 1			Experiment 2			Experiment 3		
	Surface type	ATP	Protein	Pork DNA	ATP	Protein	Pork DNA	ATP	Protein	Pork DNA
Mincer top	Stainless steel	131	Negative	Not detected	38	Negative	Not detected	871	Negative	Not detected
Underside of guard	Stainless steel	152	Negative	Not detected	100	Negative	Not detected	583	Negative	Not detected
Infeed sheet to auger	Stainless steel	172	Negative	Not detected	28	Negative	Not detected	8	Negative	Not detected
Auger barrel	Stainless steel	58	Negative	Not detected	20	Negative	Not detected	7	Negative	Not detected
Collar	Stainless steel	13	Negative	Not detected	84	Negative	Not detected	31	Negative	Not detected
Knife	Stainless steel	40	Negative	Not detected	7	Negative	Not detected	75	Negative	Not detected
Auger	Stainless steel	10	Negative	Not detected	3	Negative	Not detected	45	Negative	Not detected
Mincing plate	Stainless steel	85	Negative	Not detected	2	Negative	Not detected	15	Negative	Not detected
Belt drive wheel on conveyor belt	Plastic	42	Negative	Not detected	36	Negative	Not detected	2	Negative	Not detected
Belt	Stainless steel	85	Negative	Not detected	72	Negative	Not detected	1	Negative	Not detected

Table 51: ATP, protein and DNA swab results for Phase 1, medium risk scenario

Photographs of the swab locations for Phase 1 can be found in Annex 14.

Table 52 gives the ATP limits established by staff at the pilot plant where the swabs for Phase 1 were taken.

Surface	Pass	Cautionary result	Fail
Stainless steel	≤50	51 – 99	≥100
Plastic	≤75	76 – 124	≥125

Table 52: ATP limits for swabs taken at pilot plant

All of the results obtained for the high and low risk scenarios were deemed acceptable as no significant concentrations of ATP, protein or DNA were detected. The swabs for both these scenarios were taken after a full chemical clean, the low risk scenario after the processing of pork and the high risk scenario after the processing of beef.

The swabs for the medium risk scenario were taken after a water wash following processing of pork and some ATP swab failures (highlighted in red in Table 51) and cautionary results were obtained in locations adjacent to areas that gave negative results for the protein swabs. This indicates the presence of either non-protein residues or protein below the LOD of the protein swabs after cleaning with water but before sanitisation. The limit of detection stated by the manufacturer for the protein swabs is 50 µg.

The swabs for Phase 2 risk rating 4 were taken following the chemical clean after the processing of the pork. Protein swabs were not taken as the plant did not routinely carry out this type of screening and therefore did not have the necessary equipment. Photographs of the swabbing locations can be found in Annex 15; the results for the ATP and DNA swabs are summarised in Table 53.

Swab location	Risk rating 4			
	Experiment 1		Experiment 2	
	ATP	Pork DNA	ATP	Pork DNA
Inspection conveyor	142	Not detected	109	Not detected
Mincer screw	7	Not detected	13	Not detected
Mincer plate	237	Not detected	3	Not detected
Outfeed	85	Not detected		Not detected
Ring plate	71	Not detected	12	Not detected
Inner screw	12	Not detected	12 and 583	Not detected
Ring	18	Not detected	6	Not detected
Locking screw	903 and 388	Not detected	14 and 159	Not detected
Inside of plate	12	Not detected		
Plate	490	Not detected	203	Not detected
Species tote bin	392	Not detected	13	

Table 53: ATP and DNA swab results for Phase 2, risk rating 4

The limit's used by the plant to assess the ATP results were as follows:

- Pass <500
- Caution 500 – 1000
- Fail >1000

Based on these limits, all of the ATP results were considered to be satisfactory apart from the result obtained for the locking screw (ATP result of 903). This piece of equipment was re-cleaned and the result for the repeat swab was 388, which was deemed satisfactory and allowed the processing of the beef to proceed. DNA swabs were taken from the locking screw at the same time as both of the ATP swabs, pork DNA was not detected on either swab.

QC swabs containing 50 mg of 0.1 % pork in beef, equivalent to 50 µg of pork, were analysed alongside the sample swabs and gave positive results for pork demonstrating that the swabbing procedure and kit were functioning as expected.

For risk rating 5, swabs were taken following the water wash after the pork had been processed and the results are summarised in Table 54. Photographs of the swabbing locations can be found in Annex 16.

Exceptionally high ATP results were obtained for the locking ring; whilst the protein result from the same location for experiment one was negative, the protein result for experiment two was positive. Following the positive protein result, the locking ring was re-cleaned, after which the protein swab was negative and the ATP result had decreased from around 386,000 (mean of duplicate results) to 59,000. ATP screening is not generally carried out by staff at the plant used for risk rating 5 and 6 experiments, the ATP equipment had been borrowed from another site. Therefore acceptable thresholds for ATP results relating to the mincing equipment used were not available and a judgement could not be made by plant staff as to whether the ATP results obtained would be deemed satisfactory. As the protein results were all negative, plant staff decided that the once the locking ring had been re-cleaned, processing of the beef could be started.

Whilst it was of interest to know the effect of sanitiser on the level of ATP present it was not possible to evaluate this at the plant used to carry out the experiments for risk rating 5 as the plant did not use sanitiser in their facility and it was not possible to introduce another chemical into the factory environment.

Swab location	Experiment 1			Experiment 2		
	ATP	Protein	Pork DNA	ATP	Protein	Pork DNA
5mm plate	52	Negative	Not detected	77	Negative	Not detected
9mm plate	5451, 4079	Negative	Not detected	102	Negative	Not detected
Kidney plate	9227	Negative	Not detected	27	Negative	Not detected
Straight knife	2632	Negative	Not detected	364	Negative	Not detected
Serrated knife	5248	Negative	Not detected	515	Negative	Not detected
Locking ring	116000	Negative	Not detected	542000, 230000 (59000 after re-clean)	Positive (Negative are re-clean)	Not detected
Screw	3603	Negative	Not detected	478	Negative	Not detected
De-gristler	800	Negative	Not detected	84	Negative	Not detected
Outlet	46	Negative	Not detected	223	Negative	Not detected

Table 54: ATP, protein and DNA swab results for Phase 2, risk rating 5

As no protein or DNA was detected in the swabs taken in areas adjacent to those where high ATP results were obtained it is assumed that the high ATP results were due to non-protein residue from the raw meat or traces of protein below the limit of detection of the swabs (50 µg).

The same equipment used for risk rating 5 was also used for risk rating 6 (water wash between species and no cleaning between species respectively). The swabs for risk rating 6 were taken following a full chemical clean after the processing of beef and the results are presented in Table 55. As the results for the ATP swabs were low, it was not felt necessary by plant staff to carry out protein swabs.

	Experiment 1		
Swab location	**ATP**	**Protein**	**Pork DNA**
5mm plate	7	No protein swabs taken as all ATP results very low	Not detected
9mm plate	9		Not detected
Kidney plate	4		Not detected
Straight knife	5		Not detected
Serrated knife	33		Not detected
Locking ring	2, 7		Not detected
Screw	6		Not detected
De-gristler	6		Not detected
Outlet	39		Not detected

Table 55: ATP and DNA swab results for Phase 2, risk rating 6

16.1. Summary of swab results

The QC used for the analysis of the swabs consisted of 50 mg 0.1 % pork in beef which is equivalent to 50 µg pork; the QCs were successfully detected in every batch demonstrating that the DNA method chosen to analyse the kits was functioning to at least this sensitivity.

The LOD for the protein swabs was stated by manufacturer as being 50 µg.

No pork DNA was detected in any of the swabs taken after either a chemical clean or a water wash.

After a chemical clean, following the processing of either pork or beef, no protein was detected. This is as expected considering the harshness of the cleaning.

After a water wash one positive protein result was obtained. It was standard practice for the plant that if a positive protein result was obtained, processing could not continue. Therefore, the locking ring, the piece of equipment where the positive result had been obtained, was cleaned again and the repeat protein swab gave a negative result and therefore processing could continue.

For both Phase 1 and Phase 2, high ATP results were observed after a water wash; the highest result being observed in the area adjacent to the area where the positive protein result was seen.

Depending on the type of equipment used to assess ATP levels, acceptable ranges were set by each individual plant.

The water wash, for both Phase 1 and Phase 2, was carried out to a 'visually clean' standard, i.e. the equipment was dismantled in that the auger, plates and knives were removed, and the entire machine washed with water until no meat debris or residue could be seen. Whilst this was generally sufficient to remove traces of protein and DNA down to the respective LODs of these tests, high levels of ATP still remained in certain areas. If ATP is to be used as a practical indicator of the cleanliness of a piece of equipment, acceptable limits will need to be set by each facility based on validation data obtained using their specific ATP instrument in their own factory setting. When taking swabs to assess the cleanliness of any piece of equipment, factory staff should be encouraged to focus on the pinch points of the equipment where meat residue can get trapped in addition to the easily accessible, flat, smooth areas of the equipment such as the hopper or tote bins.

17. Discussion

The results from the experiments carried out in a pilot plant under controlled conditions showed that carry-over of pork was not detected down to a reporting limit of 0.1 % raw pork in raw beef on a weight for weight basis when 'deep' chemical cleaning of industrial mincing equipment and conveyor belts, in line with GMP, was carried out. These results were confirmed when trials were carried out in working commercial plants. In commercial plants, a chemical deep clean is performed overnight and is a very effective cleaning procedure with respect to preventing carry-over of species.

Results from both the pilot plant and commercial plant showed that when mincing equipment was cleaned with high pressure water between species carry-over of pork was not detected down to a reporting limit of 0.1 % raw pork in raw beef on a weight for weight basis demonstrating that this too is an effective cleaning method. The water wash, in both the pilot plant and commercial plant, was carried out to a 'visually clean' standard, i.e. the equipment was dismantled in that the auger, plates and knives were removed, and the entire machine washed with water until no meat debris or residue could be seen.

When no cleaning of industrial mincing equipment and conveyor belts are performed between species, then as expected, significant carry-over of meat species does occur. The estimated pork carry-over in the 100 kg beef processed in the pilot plant trials was 653 g, whilst the estimated pork carry-over for the experiments carried out in a commercial setting was 11.2 kg. The difference in the estimated pork carry-over between the pilot plant trials and those carried out in a commercial plant is thought to be due to differences in the type, age and size of the equipment used. Thus the amount of pork carry-over is likely to vary from plant to plant depending on the equipment and processes used. The actual amount of carry-over is not considered important provided that manufacturers take it into consideration when presenting information to consumers about the composition of meat products so the contents are accurately described.

Based on the results from Phase 1 a limited sampling exercise was carried out at a single species, beef only, plant. No pork was detected in any of the 12 samples taken confirming that any pork detected during the other experiments was due to contamination during the mincing process rather than contamination of the meat itself.

The results for this project are reported on a quantitative weight: weight basis and represent the 'best case scenario' in that the standards and samples were made from the same authentic lean meats which overcomes many of the factors that make the provision of quantitative DNA results difficult; if standards and samples are of the exact same composition, the question of different cuts of meat, age of animal, processing and cooking do not apply as the standards and samples should behave similarly in the assay for

example, with respect to the DNA extraction efficiency. Whilst this approach was chosen for this project so that all variations were minimised as much as possible, it is acknowledged that it would be impossible to replicate in a real life situation where analysis would most likely be required on a finished retail product so it would be highly unlikely that portions of the exact input meat could be sourced to make a direct comparison to the meat in the sample. However, as the focus of this project was to establish whether meat species carry-over occurred and if so at what concentration then this approach to quantitation was deemed desirable.

The accuracy of the quantitative results obtained using the genomic DNA based commercial test kit was assessed by preparing and analysing mixtures of raw pork in raw beef at concentrations of 0.01 to 75 % and 100 % pork and beef. When gravimetrically prepared mixtures of raw pork in raw beef were analysed a consistent positive bias was observed, with all of the results expressed as % pork DNA being greater than the weight / weight % pork present. However, the observed differences were within the expanded uncertainty for individual results at the concentrations of interest so the observed bias is not statistically significant.

For all of the samples taken for the scenarios which involved either a chemical clean or water wash between species, in addition to the samples taken at the single species plant, all of the results were reported as <0.1 % and in fact, no signal was detected for pork in any of the samples, whilst in every analysis batch pork was detected in the 0.1 % QC demonstrating that the assay was functioning. For the scenarios where no cleaning was carried out between species the results reported as <0.1 % indicate a determined pork concentration of <0.1 %, whilst the vast majority of these also gave rise to no signal, traces of pork i.e. > 0.03 <0.1 % were detected in some samples.

All of the samples were reported against a detection limit of 0.1 % but limited analysis has indicated that a LOD of 0.03 % was achievable in this instance which is why it is possible to detect concentrations > 0.03 < 0.1 % i.e. between the detection and reporting limit. This LOD is based solely on the analysis of raw pork in raw beef and the effect of processing on the LOD was outside the scope of this project and so was not studied.

To check the effectiveness of the cleaning regimes used (deep chemical and water wash), three types of swabs were taken; adenosine triphosphate (ATP) (ATP is present in all organic material and a positive reading is an indication of the presence of contamination, for example food residue, allergens and/or bacteria), protein and DNA swabs. Following a deep chemical clean, all three swabs gave equivalent negative results demonstrating that any one of the three swabs methods may be used to check the effectiveness of cleaning. However, although the majority of swabs taken after the water wash also gave negative results, there were some notable differences:

- One positive protein swab result was obtained (negative after re-cleaning)
- Several high ATP results were obtained (reduced after re-cleaning).

The results informative on a number of points:

i) Of the two cleaning methods used, the deep chemical clean is the most effective cleaning method as all swabs taken by all three swabbing methods gave negative results in both Phase 1 and 2. In addition, no carry-over was observed in any of the beef samples taken after a deep chemical clean. However, it is recognised that to deep chemical clean a line takes anywhere from 2 – 6 hours depending on the complexity of the line which is why plants undertake the deep chemical clean overnight. Thus the deep chemical cleaning method is not considered a viable procedure to use to clean a line within a day.

ii) The water wash method did give rise to one protein failure and a number of ATP failures (in both phase 1 & 2) demonstrating that greater care is required to ensure

effective cleaning. However, no carry-over was observed in any of the beef samples taken after a water wash demonstrating that it is an effective cleaning method. It also has the advantage that it is quick (1 – 2 hours depending on the complexity of the line) and so can be used to clean lines within a day between species. It is recognised that using a high pressure water wash in certain plant environments would not be desirable due to the production of aerosols which could cause microbial contamination of neighbouring processes.

iii) ATP swabs did give some failures after a water wash indicating the presence of traces of organic material It is thought most likely that these residues are due to bacteria naturally present in raw meat so it is not surprising that washing with water alone does not eliminate all traces of bacteria, especially since swab samples are taken prior to sanitisation as per the kit instructions. Different types of equipment are available to monitor and assess the presence of ATP, thus each plant should validate their processes in their own facility following advice from the swab manufacturer to establish control limits for ATP swabs.

iv) Unless it is important to monitor the effectiveness of cleaning with respect to proteins, e.g. in plants producing allergen-free food or with respect to individual meat species, e.g. Kosher / Halal plants, then the protein and DNA swabs offer no further advantage to ATP swabs and cost more to undertake (the relative cost of undertaking the three swabbing methods is DNA>>> Protein~ ATP). In fact, the ATP swabs detected residues more often than the other approaches thus offering a greater degree of cleanliness and protection to the consumer.

Thus, of the three types of swabs taken, ATP appears to be an appropriate cost effective way to routinely monitor the cleanliness of equipment with regards to generic traces of residue provided each plant undertakes the validation required to establish its own control limits. Also, when taking swabs to assess the cleanliness of any piece of equipment, factory staff should be encouraged to focus on the pinch points of the equipment where meat residue can get trapped in addition to the easily accessible, flat, smooth areas of the equipment such as the hopper or tote bins.

18. Conclusions

This project has been successful in fulfilling its objectives and generating data that previously did not exist in the scientific literature and establishes that when raw minced beef is produced according to GMP, either a deep chemical clean or a high pressure water wash clean between species is effective in preventing the carry-over of raw pork into raw beef with an associated LOD of less than 0.1 % on a w/w basis.

The project has also shown that when no cleaning is performed between species significant carry-over does occur. The amount of carry-over measured varied between Phase 1 (pilot plant) and Phase 2 (commercial plant) which is most likely due to differences in the mincing equipment used such as type, age, complexity, potential trap points, etc….This report provides clear evidence that that detectable levels of carry-over does occur when no cleaning is undertaken between species which needs to be considered by manufacturers when presenting information to consumers about the composition of meat products so the contents are accurately described.

No pork was detected in any of the 12 samples taken at the single species, beef only, plant confirming that any pork detected during the other experiments was due to contamination during the mincing process rather than contamination of the input meat itself. These results

are reassuring in that contamination of foreign meat species would not be expected in a single species plant.

From a practical perspective, unless it is important to monitor the effectiveness of cleaning with respect to proteins, e.g. in plants producing allergen-free food or with respect to individual meat species, e.g. Kosher / Halal plants, then protein and DNA swabs offer no further advantage to ATP swabs in terms of assessing the effectiveness of cleaning and cost more to undertake. In fact, the research showed that the ATP swabs detected residues more often than the other approaches which lead to an action of re-cleaning thus offering a greater degree of cleanliness and protection to the consumer. Thus, of the three types of swabs taken, ATP appears to be an appropriate cost effective way to monitor the cleanliness of equipment with regards to generic traces of residue provided each plant undertakes the validation required to establish its own control limits.

To summarise, the data from this project has shown that:

- Deep chemical cleaning is an effective cleaning procedure in preventing the carry-over of meat species. Due to the length of time required to execute it, it is usually applied as an overnight cleaning method.
- High pressure water washing is also an effective cleaning method in preventing the carry-over of meat species, although greater care is required to remove visible debris, and because of its relatively fast application time, it can be used to clean lines between species within a working day.
- ATP, Protein or DNA swabs can be used to monitor the effectiveness of cleaning; ATP swabs offer a rapid cost effective method of monitoring the effectiveness of cleaning, provided each plant validates its processes to establish control limits.
- The ‘push through’ method (no cleaning between species) is a legitimate procedure that can still be employed provided the level of carry-over is considered by manufacturers when presenting information to consumers about the composition of meat products so the contents are accurately described.

This project has provided robust data on the levels of species carry-over that occurs with different cleaning regimes and different species handling processes in UK meat processing plants. The research has provided information that can be used to inform consumers, industry and regulators, and allow evidence based differentiation between deliberate fraud and adventitious contamination. Stakeholders now have the evidence to differentiate between adventitious contamination of raw pork in raw beef and deliberate fraud. There should not be an expectation of adventitious contamination and a presence of low concentrations of undeclared species in meat products as this project has shown that it is possible to clean to <0.1 % pork w/w using GMP employed in UK meat processing plants.

It should be noted, however, that these results / outcomes relate only to minced raw pork in raw beef and may not be indicative of all species. It may not be unreasonable to make the assumption that the similar outcomes would be observed for other meat species, however the work needed to confirm this assumption has not been carried out.

19. Recommendations

The data from this project has provided the evidence for stakeholders to differentiate between adventitious contamination of minced raw pork in minced raw beef and deliberate fraud. In fact, unless the push-through method (no cleaning between species) is undertaken, the study has shown that adventitious contamination should not occur

However, it must be recognised that

i) The experiments studied the carry-over of raw pork into raw beef only. The scope of the project did not extend to studying the effects in other species so no data is available to confirm whether carry-over in other species would be similar, for example, in species with differing fat content, for example, lamb.

ii) The mincing stage of processing was the focus of this study as the AMWG agreed that this was the stage that was most likely to be prone to gross carry-over of meat species. Whilst it has been shown that cleaning the equipment used for mincing is effective in preventing carry-over, and this process was considered to present the highest risk, it is not known whether the remainder of equipment would add significant carry-over if the minced meat had fed directly into process lines for the production of meat products such as burgers, sausages etc….

iii) Experiments to show carry-over into heavily processed retail products, such as ready meals, were not conducted.

iv) Equipment design, age and variety were not studied but all work was conducted in commercial plants to reflect current UK industry practise.

v) LGC demonstrated that it could reliably attain a reporting limit of 0.1 % and a LOD of 0.03 % pork in beef was achievable on a w/w basis using one commercial PCR kit. It is not known whether all laboratories using a variety of different methods for the determination of pork could achieve the same limits.

vi) The LOD and LOQ in processed retail products have not been studied and it is not known what effect, if any, processing and added ingredients would have on the LOD.

Thus in order to support guidance, it is recommended that the following work is considered:

1. Effect of different species / fat content on the LOD/LOQ.

Conduct a limited trial with, for example lamb, to assess the effect of different species and higher fat contents on our ability to reliably determine beef in lamb, using a different assay, down to a reporting limit of 0.1 w/w as was possible for pork in beef. This will provide information on the way different species behave during extraction and analysis but without processing trials it would not be possible to say whether the carry-over would be the same for high fat content meats.

2. Effect of processing on the LOD/LOQ.

As stated above, this project has focussed on the determination of raw meat with no additional ingredients. Whilst this determination has now been well characterised, the effect of processing or added ingredients on the sensitivity of the analysis is unknown. To provide increased confidence in the processes it is recommended that the effects of different ingredients and cooking / processing conditions on the LOD/LOQ is studied by producing two processed beef products, e.g. a simple product such as a beef burger and a complex product such as lasagne, from beef containing known amounts of pork in order to establish the LOD/LOQ in processed products as follows:

a. **Added ingredients** - A known amount of pork to be added to a starting quantity of beef then ingredients added stepwise. Each combination of ingredient(s) and meat to be analysed to establish a reliable LOD.
b. **Cooking / processing** – the products will be cooked using a number of different cooking / processing methods as used by industry. As some products are likely to undergo multiple processes, for example mincing, cooking then freezing or canning. It is recommended that the processes be evaluated singly and in combinations typically used by industry.

3. Inter-laboratory comparison to confirm that a LOD of 0.1 % is achievable using different kits across different laboratories

In 2013, method verification of the LOD associated with the Defra/FSA Study protocol for detection of horse DNA in food samples was carried out by LGC. The results obtained show that three methods (PCR-CE (FSA); Neogen BioKits; PrimerDesign) had the capability of reaching a LOD of less than 0.1 % w/w raw horse-meat in a raw beef (meat) background if Quality Procedures and Good Laboratory Practice for molecular biology methods were adhered to. Whilst an LOD of <0.1 % (w/w) of raw horse-meat in a raw beef (meat) background was shown to be achievable with these three methods, it is not known if the outcomes would be the same for pork in beef as it is well known that the analysis of pork is more difficult than other meats. Therefore it is recommended that an interlaboratory comparison be carried out encompassing the following areas:

a. Quality control materials containing 0.1 % pork in beef with varying fat contents to be prepared and analysed using a number of reputable PCR kits by a single laboratory to establish the kits' performance in experienced hands. This trial should also compare the determination of pork content using genomic DNA versus mitochondrial DNA.
b. A proficiency test to be carried out with gradient concentrations of pork in beef to assess the ability of different laboratories to reliably detect pork in beef. In addition, a selection of products, such as beef burgers and lasagne, containing known quantities of pork that have been processed in a variety of ways, for example cooked and/ or frozen, should also be included in the trial. Existing PT data should be analysed first to ensure that this data doesn't already exist.

20. Acknowledgements

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21. Annexes

21.1. Annex 1 – Results of literature search

Identification of hidden allergens. The case of detection of pistachio traces in mortadella.
G. Barbieria; G. Frigeria
Journal: Food Additives and Contaminants, Volume 23, Issue 12, 2006, pp 1260-1264

An analytical method based on the detection of specific DNA was developed, and was applied to mortadella samples with and without pistachio (*Pistacia Vera*). The method is proposed for detection of traces of pistachio deriving from previous processes or from accidental contamination, since in predisposed individuals pistachios can cause allergic reactions leading to anaphylactic shock. Three pairs of primers were identified and tested by PCR on mortadella samples prepared with pistachio. Accidental contamination was also simulated. The optimised PCR was able to detect the presence of pistachio even at low concentrations. The primers pair PSTC 1-2 is suggested for unambiguous identification of pistachio in mortadella. The limit of detection for this primers pair was 100 mg/kg. No interference was observed from other spices or ingredients utilised in the formulation of the mortadella. The method enabled the identification of possible traces of pistachio remaining in the production plant after less than thorough washing.

Dekontamination von Stahl- und wiederverwendbaren Wolframcarbidkugeln zur Prävention einer DNA-Verschleppung im PCR-Labor [Decontamination of steel and reusable tungsten carbide beads for prevention of DNA deportation in a PCR-laboratory]
N Langkabel; S Meyer; R Grosspietsch; L Bräutigam; V Eckert-Funke; J Plendl; R Fries

In order to mince lymph nodes for MAIC PCR-analysis, the Mixer Mill MM200 (RETSCH GmbH, Haan, Germany) was used with reusable tungsten carbide grinding beads. Some of unexpected PCR results indicated the carry-over of DNA contamination among different samples. Hence, several decontamination procedures were used and the surface of steel and tungsten carbide beads was examined for remaining and intact DNA. Physical methods (three washes with distilled water, autoclaving and UV treatment) did not eliminate the DNA from both type of surface; this was also true for a procedure with Exonuclease III and the commercial DNA-removing kit DNAaway® (only used for steel beads). Chemical methods 0.25 % peracetic acid (PAA) (pH 5 and pH 7) and sodium hypochlorite (NaClO) (concentraction of active chlorine 4.7 % or 5.4 %) removed DNA from tungsten carbide, but caused cracking of the surface of the beads. In conclusion, for grinding beads, the use of disposable material is suggested, as also employed for reagents and equipment in the PCR laboratory.
Revue / Journal Title
Archiv für Lebensmittelhygiene ISSN 0003-925X CODEN ALMHAO
Source / Source
2012, vol. 63, no2, pp. 47-52 [6 page(s) (article)] (3/4 p.)

21.2. Annex 2 – Sampling SOP for Phase 1

Commercial in Confidence

LGC Limited

STANDARD OPERATING PROCEDURE

Title: **Sample Collection for Phase 1 (Pilot Plant) of the FSA Carry-over Project**

Issue number: 1

Issue date: 09 April 2013

Authorised by: S Elahi

Authorising signature:

UNCONTROLLED IF NOT SIGNED BY STATED AUTHORISED PERSON OR SOP CO-ORDINATOR

Sample Collection for Phase 1 (Pilot Plant) of the FSA Carry-over Project

Safety - All factory safety procedures must be adhered to at all times.

1. SCOPE AND FIELD OF APPLICATION

This standard operating procedure (SOP) describes the procedures that are to be used to prepare and collect samples for Phase 1 (Pilot Plant) of the Food Standard Agency's Carry-over Project using three different cleaning regimes for the equipment between the mincing of authentic lean pork and beef.

2. PRINCIPLE

This standard operating procedure (SOP) describes the procedures that are to be used to prepare and take samples at the pilot plant.

3. SAMPLING PLAN

Samples will be taken according to a plan which was drawn up in consultation with LGC's statistics team and has been widely circulated to the UK Food Industry, FSA and Defra for consultation. Input into the study design was also received from Industry and the FSA statistician. The sampling plan is presented in Appendix 1.

In 250 g portions, the entire first 10 kg of beef minced after 100 kg pork will be sampled, then 40 further samples will be taken at specified regular intervals during the mincing of the remaining 90 kg of beef.

4. MATERIALS

4.1 Plastic bags to be sealed by pressing, size A5 (to be provided by LGC)

4.2 Individual sample number labels (to be provided by LGC)

4.3 Sterile water ('Double processed tissue culture water' (Sigma, W3500))

4.4 Swabs (sterile wooden applicator cotton tipped individually wrapped)

4.5 Disposable gloves (to be provided by LGC)

4.6 Bin (to be provided by plant staff)

4.7 Cardboard boxes for transport of minced beef (to be provided by plant staff)

4.8 Formatted spreadsheet, with C-LIMS sample numbers and sample description.

4.9 100 kg authentic lean (95 % visual lean) pork, for each experiment, sourced from a pig only abattoir.

4.10 100 kg authentic lean (95 % visual lean) beef, for each experiment, sourced from a cow only abattoir.

4.11 Biro Model 552 meat grinder and conveyor belt

5. SAMPLE PREPARATION / MINCING

5.1 Low risk scenario – Experiment 1

5.1.1 The mincer is to be 'deep cleaned' before use using the plant's deep cleaning standard operating procedure. This process will be undertaken by trained staff.

5.1.2 100 kg of pork (4.9) to be placed into the hopper of the mincer by staff trained to use the mincing equipment. The minced pork will be collected by staff and used for the production of meat products. The mincer will be deep cleaned using the plant's deep cleaning standard operating procedure by trained staff.

5.1.3 After cleaning but before the sanitiser is applied to the equipment, 10 areas of the mincer will be selected for swabbing and the location of the areas recorded and photographed.

5.1.4 Swabbing of the areas to be carried out according to the SOP for environmental monitoring for meat processing plants. ATP and protein swabs to be taken by plant staff at similar areas to the DNA swabs.

5.1.5 100 kg of beef (4.10) will be placed in the hopper of the mincer by trained staff. After the first 10 kg has passed through, calculated from the speed of operation of the mincer, the mincer will be stopped and samples will be taken according to the sampling plan (Appendix 1) from the 10 kg of minced beef on the conveyor belt using the following process:

5.1.5.1 Before sampling begins, a new pair of disposable gloves is to be put on.

5.1.5.2 250 g ± 10 g of mince to be taken by hand and placed in a push seal bag (4.1) which has been sequentially numbered.

5.1.5.3 A second person will then place this bag into second push seal bag (4.1).

5.1.5.4 A third person will record details of where in the minced beef the sample was taken from according to the sampling plan (Appendix 1) and will record the corresponding C-LIMS sample number (4.8) on the outside of this bag.

5.1.5.5 Dispose of gloves in bin provided by plant staff (4.6).

5.1.5.6 Repeat 5.1.5.1 – 5.1.5.5 until the entire first 10 kg has been sampled.

5.1.6 The mincer will be restarted and the remaining 90 kg of beef minced. The mincer will be stopped intermittently to allow the sampling of a further forty 250 g portions of minced beef according to the sampling plan (Appendix 1) in the same manner as described above 5.1.5.1 – 5.1.5.5.

5.1.7 Once all eighty minced beef samples have been taken, all of the bags are to be to be placed in a box (4.7) and stored in a freezer until being transported to LGC.

5.1.8 Swabs are to be placed in a separate box (4.7) to the minced beef samples and also stored in a freezer until being transported to LGC.

5.1.9 After the entire 100 kg of beef has been minced, the equipment will be deep cleaned using the plant's deep cleaning standard operating

5.2 Low risk scenario – Experiment 2

Repeat 5.1 entirely.

5.3 Low risk scenario – Experiment 3

Repeat 5.1 entirely.

5.4 ATP and ELISA Swabs

Ensure that ATP and ELISA swabs are taken and analysed at the end of each working day. This will be done by trained plant staff and analysed using the plant's normal procedures. Results will be provided to LGC.

The process flow diagram for the low risk experiments is illustrated in Figure 1 of Appendix 2.

5.5 High risk scenario – Experiment 1

5.5.1 The mincer is to be 'deep cleaned' before use using the plant's deep cleaning standard operating procedure . This process will be undertaken by trained staff.

5.5.2 100 kg of pork (4.9) to be placed into the hopper of the mincer by staff trained to use the mincing equipment. The minced pork will be collected by staff and used for the production of meat products. The mincer should NOT be cleaned.

5.5.3 100 kg of beef (4.10) will be placed in the hopper of the mincer by trained staff. After the first 10 kg has passed through, calculated from the speed of operation of the mincer, the mincer will be stopped and samples will be taken according to the sampling plan (Appendix 1) from the 10 kg of minced beef on the conveyor belt using the following process:

5.5.4.1 Before sampling begins, a new pair of disposable gloves is to be put on.

5.5.4.2 250 g ± 10 g of mince to be taken by hand and placed in a push seal bag (4.1) which has been sequentially numbered.

5.5.4.3 A second person will then place this bag into second push seal bag (4.1).

5.5.4.4 A third person will record details of where in the minced beef the sample was taken from according to the sampling plan (Appendix 1) and will record the corresponding C-LIMS sample number (4.8) on the outside of this bag.

5.5.4.5 Dispose of gloves in bin (4.6) provided by the plant.

5.5.4.6 Repeat 5.5.4.1 – 5.5.4.5 until the entire first 10 kg has been sampled.

5.5.5 The mincer will be restarted and the remaining 90 kg of beef minced. The mincer will be stopped intermittently to allow the sampling of a further forty 250g portions of minced beef according to the sampling plan (Appendix 1) in the same manner as described above 5.5.4.1 – 5.5.4.5.

5.5.6 Once all eighty minced beef samples have been taken, all of the bags are to be to be placed in a box (4.7) and stored in a freezer until being transported to LGC.

5.5.7 After the entire 100 kg of beef has been minced, the equipment will be deep cleaned using the plant's deep cleaning standard operating procedure by trained staff.

5.5.8 After cleaning, 10 areas of the mincer will be selected for swabbing and the location of the areas recorded. ATP and protein swabs to be taken by plant staff at similar areas to the DNA swabs.

5.5.9 Swabbing of the areas to be carried out according to the SOP for environmental monitoring for meat processing plants.

5.5.10 Swabs are to be placed in a separate box (4.7) to the minced beef samples and also stored in a freezer until being transported to LGC.

5.6 High risk scenario – Experiment 2

Repeat 5.5 entirely.

5.7 High risk scenario – Experiment 3

Repeat 5.5 entirely.

5.8 ATP and ELISA Swabs

Ensure that ATP and ELISA swabs are taken and analysed at the end of each working day. This will be done by trained staff and analysed using the plant's normal procedures. Results will be provided to LGC.

The process flow diagram for the high risk experiments is illustrated in Figure 2 of Appendix 2.

5.9 Medium risk scenario – Experiment 1

5.9.1 The mincer is to be 'deep cleaned' before use using the plant's deep cleaning standard operating procedure. This process will be undertaken by trained staff.

5.9.2 100 kg of pork (4.9) to be placed into the hopper of the mincer by staff trained to use the mincing equipment. The minced pork will be collected by staff and used for the production of meat products. The mincer to be cleaned using hot water and a high pressure hose. The equipment is to be cleaned so that all visible traces of meat are removed.

5.9.3 After cleaning but before the sanitiser is applied to the equipment, 10 areas of the mincer will be selected for swabbing and the location of the areas recorded. ATP and protein swabs to be taken by plant staff at similar areas to the DNA swabs

5.9.4 100 kg of beef (4.10) will be placed in the hopper of the mincer by trained staff. After the first 10 kg has passed through, calculated from the speed of operation of the mincer, the mincer will be stopped and samples will be taken according to the sampling plan (Appendix 1) from the 10 kg of minced beef on the conveyor belt using the following process:

5.9.5.1 Before sampling begins, a new pair of disposable gloves is to be put on.

5.9.5.2 250 g ± 10 g of mince to be taken by hand and placed in a push seal bag (4.1) which has been sequentially numbered.

5.9.5.3 A second person will then place this bag into second push seal bag (4.1).

5.9.5.4 A third person will record details of where in the minced beef the sample was taken from according to the sampling plan (Appendix 1) and will record the corresponding C-LIMS sample number (4.8) on the outside of this bag.

5.9.5.5 Dispose of gloves in bin (4.6) provided by the plant.

5.9.5.6 Repeat 5.9.5.1 – 5.9.5.5 until the entire first 10 kg has been sampled.

5.9.6 The mincer will be restarted and the remaining 90 kg of beef minced. The mincer will be stopped intermittently to allow the sampling of a further forty 250 g portions of minced beef according to the sampling plan (Appendix 1) in the same manner as described above 5.9.5.1 – 5.9.5.5.

5.9.7 Once all eighty minced beef samples have been taken, all of the bags are to be to be placed in a box (4.7) and stored in a freezer until being transported to LGC.

5.9.8 After the entire 100 kg of beef has been minced, the equipment will be deep cleaned using the plant's deep cleaning standard operating procedure by trained staff.

5.9.10 Swabs are to be placed in a separate box (4.7) to the minced beef samples and also stored in a freezer until being transported to LGC.

5.10 Medium risk scenario – Experiment 2

Repeat 5.9 entirely.

5.11 Medium risk scenario – Experiment 3

Repeat 5.9 entirely.

5.12 ATP and ELISA Swabs

Ensure that ATP and ELISA swabs are taken and analysed at the end of each working day. This will be done by trained staff and analysed using the plant's normal procedures. Results will be provided to LGC.

The process flow diagram for the high risk experiments is illustrated in Figure 3 of Appendix 2.

5.13 Transport of Samples to LGC

All samples and swabs will be transported to LGC using a courier with the capability to transport under frozen conditions.

Appendix 1: Sampling plan

100 kg of beef to be sampled per experiment

250 g weight of each sample

400 potential samples

Numbers below relate to each 250 g portion on the conveyor belt

The 250 g portions denoted by the red/pink coloured cells will be sampled:

First ten kilograms - sample 40 x 250 g portions

Remaining 90 kg - sample 40 x 250 g portions

.....Conveyor belt		
1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18
19	20	21
22	23	24
25	26	27
28	29	30
31	32	33
34	35	36
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67	68	69

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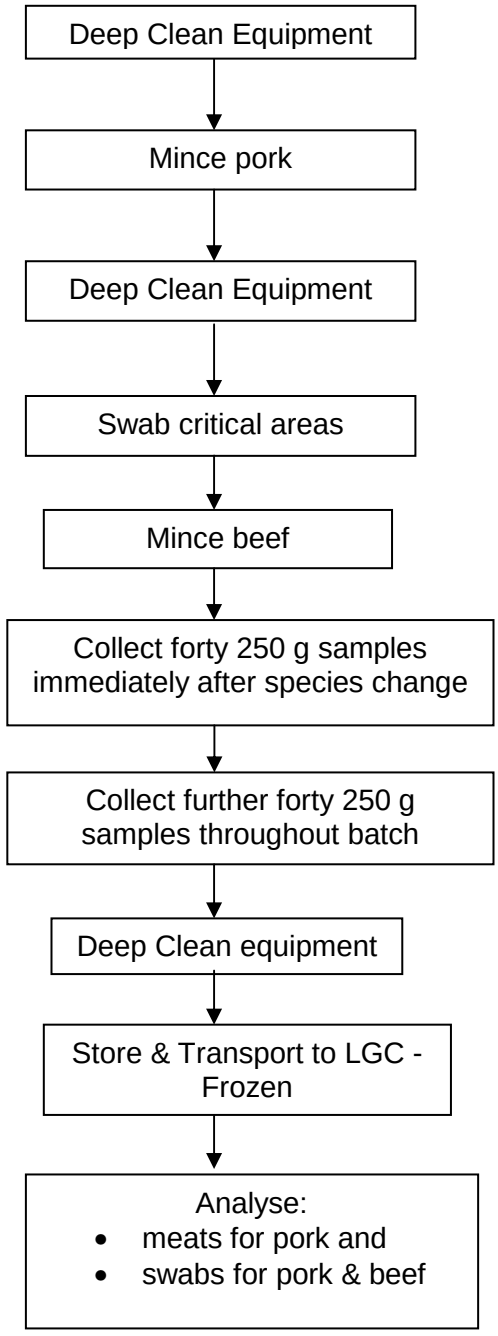
400		

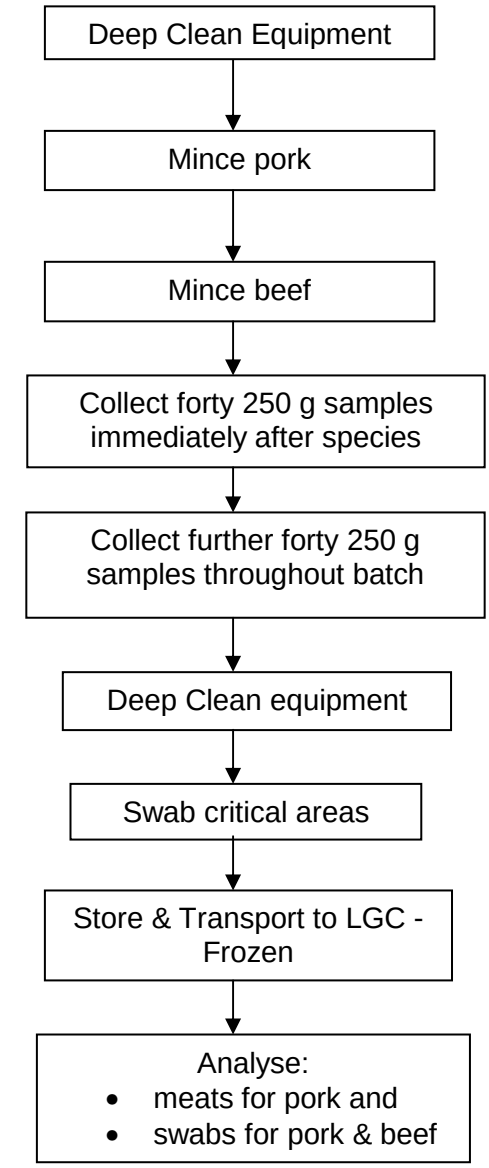
Appendix 2: Process Flow Diagrams

Figure 1: Low risk scenario



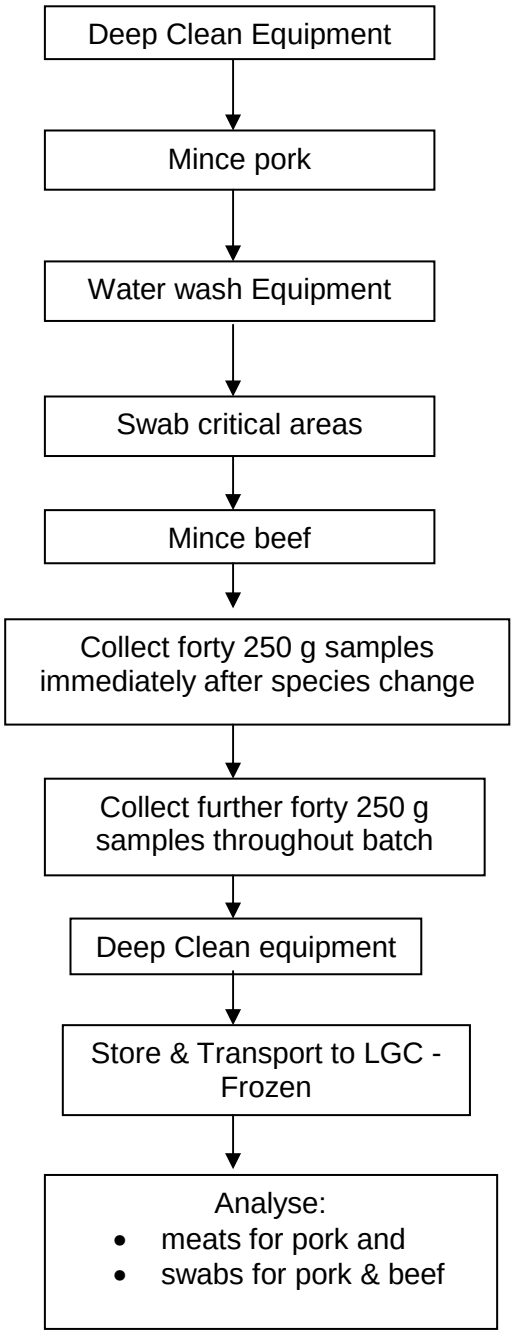
Undertake this experiment in triplicate

Figure 2: High risk scenario



Undertake this experiment in triplicate

Figure 3: Medium risk scenario



Undertake this experiment in triplicate

21.3. Annex 3 – Sampling SOP for Phase 2

Commercial in Confidence

LGC Limited

STANDARD OPERATING PROCEDURE

Title: **Sample Collection for Phase 2 (Commercial Plants) of the FSA Carry-over Project**

Issue number: 1

Issue date: 1 November 2013

Authorised by: S Elahi

Authorising signature:

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Sample Collection for Phase 2 (Commercial Plants) of the FSA Carry-over Project

Safety - All factory safety procedures must be adhered to at all times.

1. SCOPE AND FIELD OF APPLICATION

This standard operating procedure (SOP) describes the procedures that are to be used to prepare and collect samples for Phase 2 (Commercial Plant) of the Food Standard Agency's Carry-over Project using different cleaning regimes for the equipment between the mincing of authentic lean pork and beef.

2. PRINCIPLE

This standard operating procedure (SOP) describes the procedures that are to be used to prepare and take samples at various commercial plants.

3. SAMPLING PLAN

Samples will be taken according to a plan which was drawn up in consultation with LGC's statistics team. The sampling plan is presented in Appendix 1.

21 samples will be taken immediately after species change, 38 further samples will be taken at specified regular intervals during the mincing of the remaining beef.

4. MATERIALS

4.1 Plastic bags to be sealed by pressing, size A5 (to be provided by LGC)

4.2 Individual sample number labels (to be provided by LGC)

4.3 Sterile water ('Double processed tissue culture water' (Sigma, W3500))

4.4 Swabs (sterile wooden applicator cotton tipped individually wrapped)

4.5 Disposable gloves (to be provided by the plants)

4.6 Bin (to be provided by the plants)

4.7 Boxes for transport of minced beef (to be provided by plants)

4.8 Formatted spreadsheet, with C-LIMS sample numbers and sample description

4.9 Pork (to be provided by the plants). Information on the source of the meat to be supplied to LGC.

4.10 Beef (to be provided by the plants). Information on the source of the meat to be supplied to LGC.

4.11 Meat grinder and conveyor belt or other suitable means to allow sampling to take place.

5. SAMPLE PREPARATION / MINCING

5.1 Risk rating 4 (chemical clean between species) – Experiment 1

5.1.1 The mincer and associated equipment is to be 'deep cleaned' before use by trained staff following the plant's standard operating procedures. If possible, a copy of the cleaning protocol to be supplied to LGC.

5.1.2 Pork (4.9) to be minced by staff trained to use the mincing equipment. The minced pork will be collected by plant staff and used for the production of meat products. The mincer and associated equipment will be deep cleaned using the plant's standard operating procedure.

5.1.3 After cleaning but before the sanitiser is applied to the equipment, 10 areas of the mincer will be selected for swabbing and the location of the areas recorded and photographed.

5.1.4 ATP and protein swabs to be taken by plant staff. At similar areas on the equipment DNA swabs to be taken by LGC staff.

5.1.5 Beef (4.10) to be minced by staff trained to use the mincing equipment. After the first 6 – 10 kg has passed through, calculated from the speed of operation of the mincer, the mincer will be stopped and samples will be taken according to the sampling plan (Appendix 1) from the minced beef on the conveyor belt using the following process:

5.1.5.1 Before sampling begins, a new pair of disposable gloves is to be put on.

5.1.5.2 Approximately 250 g of mince to be taken by hand and placed in a push seal bag (4.1) which has been sequentially numbered.

5.1.5.3 A second person will then place this bag into second push seal bag (4.1).

5.1.5.4 A record of where in the minced beef the sample was taken from according to the sampling plan (Appendix 1) will be made and the corresponding C-LIMS sample number (4.8) will be placed on the outer bag.

5.1.5.5 Dispose of gloves in bin provided (4.6).

5.1.5.6 Repeat 5.1.5.1 – 5.1.5.5 until the entire first 21 samples have been taken.

5.1.6 The mincer will be restarted and the remaining beef minced. The mincer will be stopped intermittently to allow the sampling of a further 38 x 250 g portions of minced beef according to the sampling plan (Appendix 1) in the same manner as described above 5.1.5.1 – 5.1.5.5.

5.1.7 Once all 59 minced beef samples have been taken, all of the bags are to be to be placed in a box (4.7) and stored in a freezer until being transported to LGC.

5.1.8 DNA swabs are to be placed in a separate box (4.7) to the minced beef samples and also stored in a freezer until being transported to LGC.

5.1.9 After the entire batch of beef has been minced, the equipment will be deep cleaned following the plant's standard operating procedures

5.2 Risk rating 4 (chemical clean between species) – Experiment 2

Repeat 5.1 entirely.

The process flow diagram for the risk rating 4 experiments is illustrated in Figure 1 of Appendix 2.

5.3 Risk rating 6 (no clean between species) – Experiment 1

5.3.1 The mincer and associated equipment is to be 'deep cleaned' before use by trained staff following the plant's standard operating procedures. If possible, a copy of the cleaning protocol to be supplied to LGC.

5.3.2 Pork (4.9) to be minced by staff trained to use the mincing equipment. The minced pork will be collected by plant staff and used for the production of meat products. The mincer should NOT be cleaned.

5.3.3 Beef (4.10) to be minced by staff trained to use the mincing equipment. After the first 6 – 10 kg has passed through, calculated from the speed of operation of the mincer, the mincer will be stopped and samples will be taken according to the sampling plan (Appendix 1) from the minced beef on the conveyor belt using the following process:

5.3.3.1 Before sampling begins, a new pair of disposable gloves is to be put on.

5.3.3.2 Approximately 250 g of mince to be taken by hand and placed in a push seal bag (4.1) which has been sequentially numbered.

5.3.3.3 A second person will then place this bag into second push seal bag (4.1).

5.3.3.4 A record of where in the minced beef the sample was taken from according to the sampling plan (Appendix 1) will be made and the corresponding C-LIMS sample number (4.8) will be placed on the outer bag.

5.3.3.5 Dispose of gloves in bin provided (4.6).

5.3.3.6 Repeat 5.3.3.1 – 5.3.3.5 until the entire first 21 samples have been taken.

5.3.4 The mincer will be restarted and the remaining beef minced. The mincer will be stopped intermittently to allow the sampling of a further 38 x 250 g portions of minced beef according to the sampling plan (Appendix 1) in the same manner as described above 5.3.3.1 – 5.3.3.5.

5.3.5 Once all 59 minced beef samples have been taken, all of the bags are to be to be placed in a box (4.7) and stored in a freezer until being transported to LGC.

5.3.6 After the entire batch of beef has been minced, the equipment will be deep cleaned following the plant's standard operating procedures

5.3.7 After cleaning, 10 areas of the mincer will be selected for swabbing and the location of the areas recorded. ATP and protein swabs to be taken by plant staff , DNA swabs to be taken by LGC staff.

5.3.8 Swabs are to be placed in a separate box (4.7) to the minced beef samples and also stored in a freezer until being transported to LGC.

5.4 Risk rating 6 (no clean between species) – Experiment 2

Repeat 5.3 entirely.

The process flow diagram for the high risk experiments is illustrated in Figure 2 of Appendix 2.

5.5 Risk rating 5 (water wash between species) – Experiment 1

5.5.1 The mincer and associated equipment is to be 'deep cleaned' before use by trained staff following the plant's standard operating procedures. If possible, a copy of the cleaning protocol to be supplied to LGC.

5.5.2 Pork (4.9) to be minced by staff trained to use the mincing equipment. The minced pork will be collected by plant staff and used for the production of meat products. The mincer and associated equipment will be cleaned using hot water and a high pressure hose following the plant's standard operating procedure. The equipment is to be cleaned so that all visible traces of meat are removed.

5.5.3 After cleaning but before the sanitiser is applied to the equipment, 10 areas of the mincer will be selected for swabbing and the location of the areas recorded. ATP and protein swabs to be taken by plant staff, DNA swabs to be taken by LGC staff.

5.9.4 Beef (4.10) to be minced by staff trained to use the mincing equipment. After the first 6 – 10 kg has passed through, calculated from the speed of operation of the mincer, the mincer will be stopped and samples will be taken according to the sampling plan (Appendix 1) from the minced beef on the conveyor belt using the following process:

5.5.3.1 Before sampling begins, a new pair of disposable gloves is to be put on.

5.5.3.2 Approximately 250 g of mince to be taken by hand and placed in a push seal bag (4.1) which has been sequentially numbered.

5.5.3.3 A second person will then place this bag into second push seal bag (4.1).

5.5.3.4 A record of where in the minced beef the sample was taken from according to the sampling plan (Appendix 1) will be made and the corresponding C-LIMS sample number (4.8) will be placed on the outer bag.

5.5.3.5 Dispose of gloves in bin provided (4.6).

5.5.3.6 Repeat 5.5.3.1 – 5.5.3.5 until the entire first 21 samples have been taken.

5.5.4 The mincer will be restarted and the remaining beef minced. The mincer will be stopped intermittently to allow the sampling of a further 38 x 250 g portions of minced beef according to the sampling plan (Appendix 1) in the same manner as described above 5.5.3.1 – 5.5.3.5.

5.5.5 Once all 59 minced beef samples have been taken, all of the bags are to be to be placed in a box (4.7) and stored in a freezer until being transported to LGC.

5.5.6 Swabs are to be placed in a separate box (4.7) to the minced beef samples and also stored in a freezer until being transported to LGC.

5.5.7 After the entire batch of beef has been minced, the equipment will be deep cleaned following the plant's standard operating procedures

5.6 Risk rating 5 (water wash between species) – Experiment 2

Repeat 5.9 entirely.

The process flow diagram for the high risk experiments is illustrated in Figure 3 of Appendix 2.

5.7 Transport of Samples to LGC

All samples and swabs will be transported to LGC using a courier with the capability to transport under frozen conditions.

Appendix 1: Sampling plan

...Conveyor belt ...		
1	2	3
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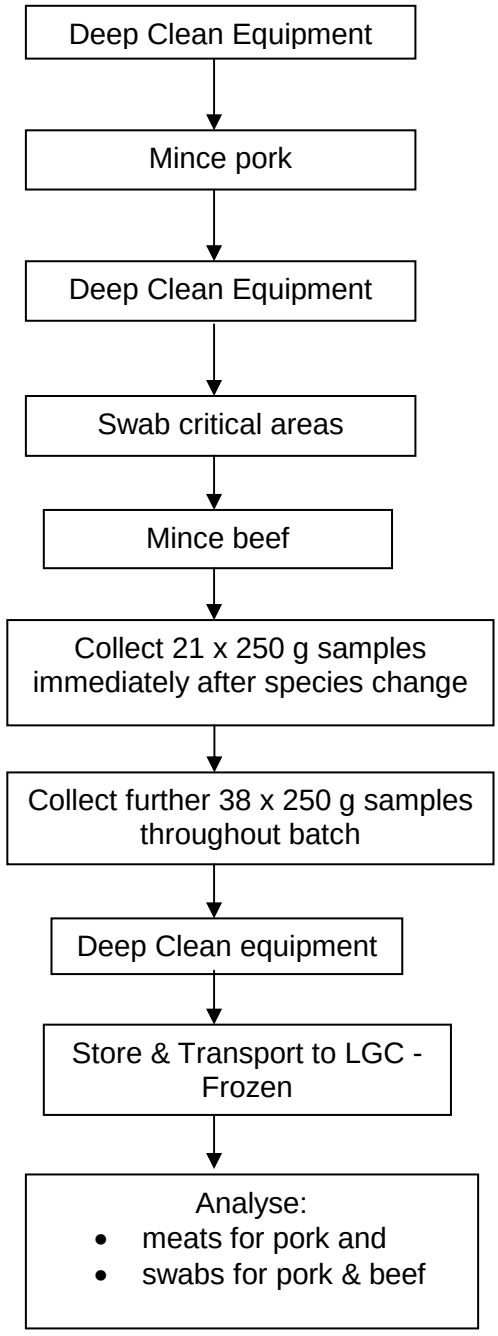
823 - 1218		
1219	1220	1221
1222 - 1617		
1618	1619	1620
1621 - 2019		
2020	2021	2022
2023 - 2418		
2419	2420	2421
2422 - 2817		
2818	2819	2820
2821 - 3219		
3220	3221	3222
3223 - 3618		
3619	3620	3621
3622 - 4000		

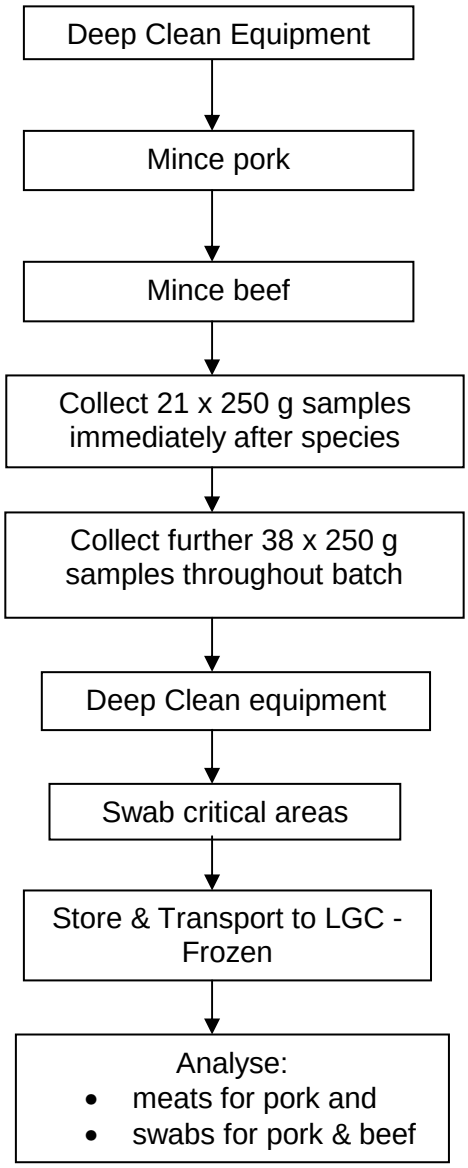
Appendix 2: Process Flow Diagrams

Figure 1: Risk rating 4 scenario



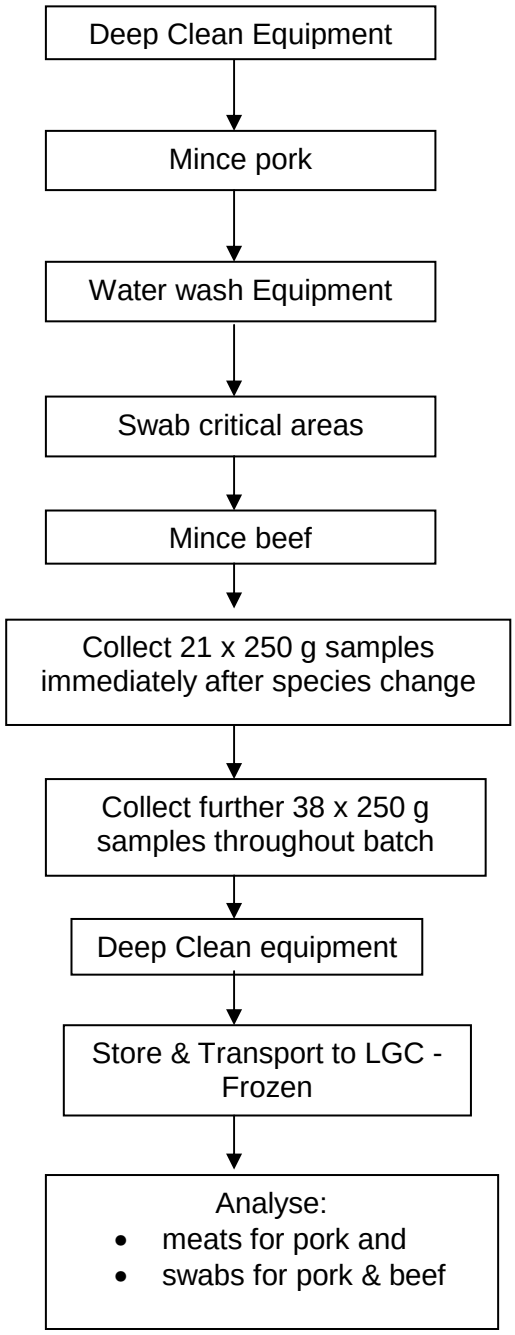
Undertake this experiment in duplicate

Figure 2: Risk rating 6 scenario



Undertake this experiment in duplicate

Figure 3: Risk rating 5 scenario



Undertake this experiment in duplicate

21.4. Annex 4 – Cleaning procedure

PREPARATION

1. When production has finished, the operative should make sure that the augers are in the correct position for stripping down and the grinder has been isolated.
2. Unlock the safety ring.
3. Screw in the supplied handle and remove the auger onto the supplied trolley rack (wearing safety gloves).
4. Remove the locking wheel from the grinder section and take out all tooling and place onto the trolley rack.
5. Use the auger handle to fully remove the small auger onto the trolley rack. (Photograph 1)
6. The machine is now ready for cleaning.

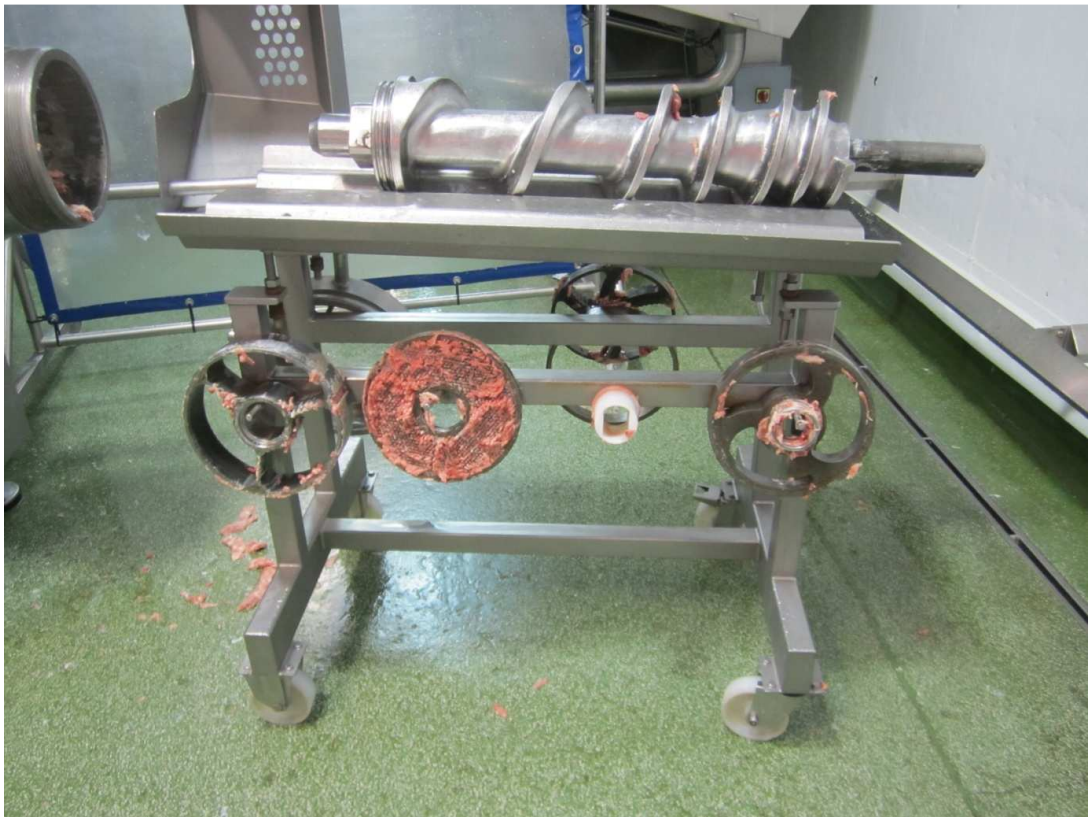
WASHING

7. Fully rinse off all of the debris from all surfaces of the machine and conveyor (including undersides and cables/cable trunking) and removed parts.
8. Foam all surfaces (including undersides & cable/cable trunking) using a suitable detergent, ensuring an even coverage. (Photograph 2)
9. Hand pad all surfaces (including undersides) to remove stubborn soil. Attention should be paid to the key inspection points as listed below.
10. Allow a minimum of 20 minutes contact time.
11. Rinse off all foam/debris from all surfaces (including undersides & cables/cable trunking) using fresh clean water.
12. Inspect all surfaces visually to ensure all debris and foam has been removed.
13. If debris is visible the equipment should be re-cleaned as described in points 7 to 12.
14. Spray a suitable disinfectant solution on to all surfaces.
15. Allow a 15 minute contact time
16. Allow to air dry
17. Reassemble machine

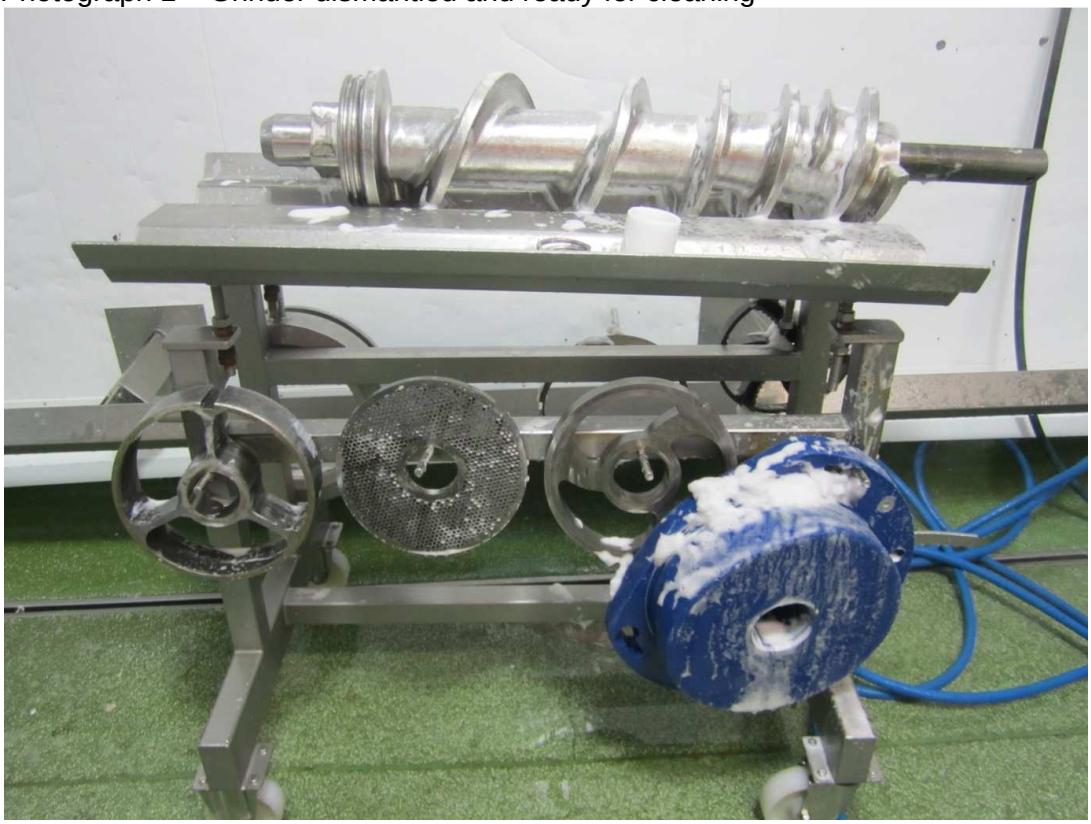
KEY INSPECTION POINTS

1. Hopper.
2. Auger.
3. Cables and cable trunking
4. Die plate and tooling.
5. Safety guards and control panels

6. Threads and small outlets



Photograph 1 – Grinder dismantled and ready for cleaning



Photograph 2 – Application of foam detergent

21.5. Annex 5 - Homogenisation SOP

Commercial in Confidence

LGC Limited

STANDARD OPERATING PROCEDURE

Number:

Title: **HOMOGENISATION OF MINCED MEAT PORTIONS (PROJECT REF: FSA/DEFRA STUDY ON CARRY-OVER IN MEAT PROCESSING PLANTS)**

Issue number: **DRAFT**

Author: **Joanna Topping and Kirstin Gray**

Issue date:

Next Review Date	Reviewed by	Date

Authorised by: Liam Gormley

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METHOD: HOMOGENISATION OF MINCED MEAT PORTIONS (Project REF: FSA/DEFRA STUDY ON CARRY-OVER IN MEAT PROCESSING PLANTS)

1. SCOPE

This method is to be used to homogenise samples of minced meat (250g ±10g) prior to DNA analysis to determine the quantity of pork meat present.

2. HEALTH AND SAFETY

2.1 Staff should wear all of the personal protective equipment (PPE) supplied. The wearing of this equipment is mandatory. This PPE includes:

- Lab coat and safety specs
- Suitable gloves, either latex or nitrile.

3 METHOD PRINCIPLE

Homogenisation of minced meat samples.

It is imperative that no cross contamination of the samples is allowed to occur either from other samples being homogenised or from other meat samples that may be present in the laboratory.

4 REAGENTS

4.1 70 % ethanol or industrial methylated spirits for cleaning equipment and lab bench.

4.2 MicroSol3+™, Anachem. The Microsol should be diluted as directed on the bottle prior to use for cleaning equipment and lab bench.

4.3 Rapid protein residue test, Hygiena part number: PRO-100.

5 APPARATUS

5.1 Analytical balance accurate to at least 0.001 g.

5.2 Sample tracking spreadsheet – one per day. Refer to Appendix 1 for example.

5.3 Small kitchen blenders. Each blender should have been cleaned through one dishwasher cycle, sprayed with Microsol, allowed to air dry for several minutes and wiped with ethanol using a clean tissue before use.

For the first three batches, at the beginning of each day, after the blenders have been removed from the dishwasher and before being treated with Microsol, each blender should be checked for the presence of protein using a Rapid Protein Residue test. The inside of the blender and the blades should be wiped over with the swab. After replacing the swab in the tube the Snap Valve should be broken by bending the bulb backward and forward. The bulb should then be squeezed twice to expel the liquid down the shaft and the tube shaken gently for 5-10 seconds. After leaving to stand for 10 minutes the colour of the solution should be noted on the daily record of blender cleaning (see Appendix 2). If the solution turns any shade of purple the blender from which the swab was taken must be taken through the cleaning procedure again and re-checked for traces of protein before use. If any traces of protein are detected either Joanna Topping or Kirstin Gray should be notified immediately.

After the first three batches, providing that all of the tests have been negative for the presence of protein, one blender only should be picked at random to be tested before use. If traces of protein are detected, before use, all of the blenders should be taken through the cleaning procedure again.

5.4 Spoons, cleaned using the same procedure as the blenders (5.3).

5.5 Roll of pre-printed small LIMS labels (one per sample)

5.6 Sheet of pre-printed 'SAMPLED' labels

5.7 Spatulas, cleaned using the same procedure as the blenders (5.3).

5.8 Nunc EZ Flip conical centrifuge tubes, 50 ml, Thermo Scientific

6 HOMOGENISATION

At the end of each day the bench should be wiped with Microsol. The following morning, before starting any blending, the bench should be wiped with ethanol.

Before starting, a list of sample numbers and homogenisation order will be supplied by the project manager.

6.1 The day prior to homogenisation, collect an appropriate number of samples from the freezer and leave in the fridge overnight. Note the numbers of these samples, from both the inner and outer bags, and the date on the sample tracking sheet (example given in Appendix 1). Samples are to be prepared in the order specified by the project manager.

6.2 On the day of homogenisation, collect further samples from the freezer and allow them to defrost at room temperature in a tray, cleaned according to 5.3, (for processing later in the day). Note the numbers of these samples, and the date, on the sample tracking sheet.

6.3 Gloves should be changed between each sample and a clean blender (5.3) and spoon (5.4) used for each homogenisation.

6.4 Each sample is double bagged. Check for any damage / leakage from inner bag to the outer bags; record any damage to the bag on the sample tracking sheet. If damaged, do not homogenise but contact either Joanna Topping or Kirstin Gray or other nominated person.

6.5 Remove the outer bag and the place the contents of the inner bag inside a kitchen blender that has been cleaned and then sterilised as described in 5.3. Run the blender for 10 seconds then, using a clean spoon mix the sample by hand to ensure the top portion of the sample comes into contact with the blades. Run the blender for a further 10 seconds, mix the sample again by hand then run the blender for a final 10 seconds. If required, repeat the mixing by hand using a spoon and running the blender for 10 seconds until the sample has become homogenous and resembles a smooth paste. When mixing the sample by hand it is important to ensure that any sample sticking to the lid is re-incorporated into the bulk of the sample.

6.6 Transfer the homogenised sample back into the initial inner bag using the spoon used to mix the sample.

6.7 Using a clean spatula (5.7) weigh 1 g ± 0.1 g of sample into the conical centrifuge tubes provided (5.8). Record the exact weight on the tube and on the sample tracking form, the DNA extraction tube should be labelled with the correct CLIMS number. One sample in every batch should be weighed out in duplicate and the tubes labelled A and B.

6.8 Place a 'SAMPLED' sticker on the main sample, with the date of homogenisation and return this bag to its original outer bag. Ensure that both bags are fully sealed.

6. 9 Once all samples have been homogenised and a subsample prepared, the samples should be returned to the freezer and the sub sample stored in a fridge until being transferred to the DNA extraction team.

6.10 After each use the blenders, spoons and spatulas should be rinsed in hot soapy water then washed in a dishwasher. NOTE: the dishwasher should be set to 70℃. After the dishwasher cycle is complete the blenders should be dried with clean paper towel, sprayed with Microsol then wiped with ethanol as described in 5.3. Sufficient blenders have been provided to allow sample homogenisation to continue whilst 'used' blenders are being washed in the dishwasher.

6.11 As stated in 6.1, remove an appropriate number of samples from the freezer and allow to thaw overnight in the fridge.

7 REFERENCES

None Applicable

Document history

Document changes and acknowledgment of staff awareness to these changes:

Issue:	Change:	Date:

All staff carrying out this procedure should sign below (on the master copy – usually Copy 2) to record that they have read and understood this SOP.

Staff Name:	Signature:	Date:

Appendix 1: Example of Sample Tracking Sheet

FSA/Defra Cross-contamination project

Date: _____

Sample taken from freezer (Sample ID, project customer number & CLIMS number)	Defrost area (fridge no/lab bench)	Sample homogenised (date, initial)	Weight taken for sub sample(g)	Sample returned to freezer number	Sub sample transferred to DNA extraction team (date, initials)	Sub sample received by DNA extraction team (date, initials)

Approved:

Appendix 2: Example of Daily Record of Blender Cleaning

FSA/Defra Cross-contamination project

Date: _____

Blender	Dishwasher	Microsol & Ethanol clean	Result of Hygiena Protein Swab

21.6. Annex 6 – Analysis SOP

Commercial in Confidence

LGC Limited

STANDARD OPERATING PROCEDURE

Number:

Title: **ANALYSIS OF MINCED MEAT PORTIONS FOR MEAT SPECIES BY DNA (PROJECT REF: FSA/DEFRA STUDY ON CARRY-OVER IN MEAT PROCESSING PLANTS)**

Issue number: **1**

Author: **Kirstin Gray**

Issue date:

Next Review Date	Reviewed by	Date

Authorised by: Selvarani Elahi

Authorising signature:
UNCONTROLLED IF NOT SIGNED BY STATED AUTHORISED PERSON OR SOP CO-ORDINATOR

Copy Control:	Copy Number: 1 of 1
UNCONTROLLED IF NOT COMPLETE	

No unauthorised copying of this procedure

METHOD: ANALYSIS OF MINCED MEAT PORTIONS FOR MEAT SPECIES BY DNA

1. SCOPE

This method is to be used to determine the concentration of pork present in samples of minced beef that have been prepared according to the SOP titled Homogenisation of minced meat portions.

2. HEALTH AND SAFETY

2.1 Staff should wear all of the personal protective equipment (PPE) supplied. The wearing of this equipment is mandatory. This PPE includes:

- Lab coat and safety specs
- Suitable gloves, either latex or nitrile.

3 METHOD PRINCIPLE

DNA is to be extracted from the samples using SOP HMT/SOP/002, DNA extraction from meat and processed meat based food samples. The quantity of pork present is then to be determined using the SureFood Animal Quant Pork real-time PCR kit from R-Biopharm (product code S1011).

It is imperative that no cross contamination of the samples is allowed to occur from other meat samples that may be present in the laboratory.

4 REAGENTS

See extraction method and kit instructions for details of reagents required.

5 APPARATUS

See extraction method and kit instructions for details of reagents required.

6 SAMPLE EXTRACTION

Before starting, a list of sample numbers and extraction order will be supplied by the project manager. To help minimise the possibility of cross contamination the samples must be extracted in the order specified by the project manager.

6.1 The pre-weighed 1 g aliquots of samples are to be collected from the fridge. The appropriate tracking log must be signed to confirm receipt.

6.2 In each batch, samples are to be extracted together with a negative control (100 % beef), a positive control (50 % pork in beef w/w), a LOD check (0.1 % pork in beef) and one sample in duplicate for the high and medium risk scenario samples.

The maximum number of samples that can be extracted per batch is ideally 38, depending on the experience of staff and availability of equipment. Each batch should include three extraction controls (100 % beef, 50 % pork in beef and 0.1 % pork in beef) together with the appropriate number of samples and two extraction negatives.

The samples are to be extracted singly (one duplicate per homogenisation batch for the high and medium risk scenarios) and analysed with one PCR. Results for the low risk scenario are to be evaluated and a decision made as to duplicate extractions (relevant samples and number).

6.3 All samples and quality controls are to be extracted singly using SOP HMT/SOP/002, DNA extraction from meat and processed meat based food samples.

6.4 Once the DNA has been extracted it should be stored at 5ºC ± 3ºC until required.

7 PCR

7.1 An example of how a PCR plate should be filled is as follows:

	No of wells (Mammalian)	No of wells (Pork)	No of extracts
Kit Controls			
No template controls	2	2	
SureFood pork gene calibration		5	
SureFood mammalian gene calibration	5		
SureFood 100 % pork control	2	2	
Extraction QCs			
Negative control (100 % beef)	1	1	1
Positive control (50 % pork in beef w/w)	1	1	1
LOD control (0.1 % pork in beef w/w)	1	1	1
Extraction negative	2	2	2
Samples			
Sample duplicate	1	1	1
Total	48	48	38

The Kit Controls should be followed exactly as detailed in the above table.

The above table gives maximum sample numbers but the number of samples should be adjusted according to the number of samples extracted in each batch and the relevant number of extraction QCs.

The sample extracts must be run on the same plate as the QCs extracted at the same time. An example of a suitable plate layout is presented in Appendix 1.

7.2 The quantity of pork is to be determined according to the instructions included with the SureFood Animal Quant Pork real-time PCR kit from R-Biopharm (product code S1011) kit.

In contrast to the product datasheet, the kit standard template should be diluted in nuclease-free water. Additionally, the master mix preparation and template addition should also be carried out on ice to avoid non specific polymerisation.

7.3 When analysing the qPCR data, wells should be grouped by assay, and a constant threshold value should be set at **200 RFU** for both assays, as well as the '**all wells'** group.

8 REPORTING

All results are to be reported as % pork DNA/DNA basis. The spreadsheet in Appendix 2 should be used for reporting the results.

9 REFERENCES

None Applicable

Document history

Document changes and acknowledgment of staff awareness to these changes:

Issue:	Change:	Date:

All staff carrying out this procedure should sign below (on the master copy – usually Copy 2) to record that they have read and understood this SOP.

Staff Name:	Signature:	Date:

Appendix 1: Example PCR plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	Assay
1	Sus Assay
2	Ref Assay

Appendix 2: Reporting sheet

Well	Fluor	Target	Content	Sample	Biological Set Name	Cq	Cq Mea	Cq Std. Dev	Starting Quantity (SQ)	Log Starting Quantity	SQ Mean	SQ Std. Dev	dCt between Std dilutions **(& Average)**	Qty Sus/Qty Ref	Relative Pork content (% DNA/DNA)	Run-run variation correction value (k)	Corrected Pork content (%)
														#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
														#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
														#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
														#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
														#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
														#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
														#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
														#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!

21.7. Annex 7 – Results for Phase 1, Pilot Plant - Low Risk Scenario

Key to tables 23 to 32

- The green cells in the sampling plan indicate points at which samples were taken from the beef that was minced for each experiment.
- Pork content is expressed as % pork DNA relative to total mammalian DNA present as interpreted in accordance with the instructions of the commercial quantitative real time PCR DNA assay that was used to analyse the samples. Results from comparisons of % pork DNA to % pork meat on a weight / weight basis showed that, in these instances, there was no statistical difference between the results expressed as % DNA and the actual pork content determined by mass.
- The results are reported on a quantitative weight: weight basis and represent the 'best case scenario' in that the standards and samples were made from the same authentic lean meats.
- The reporting limit is 0.1 % pork DNA relative to total mammalian DNA present as established by method validation at LGC. Experiments to assess the reliability of detecting concentrations of pork below 0.1 % pork DNA relative to total mammalian DNA were conducted and showed that a Limit of Detection of 0.03 % pork DNA relative to total mammalian DNA present was achievable in some instances.

Sampling Plan			Low risk – Experiment 1			Low risk – Experiment 2			Low risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
1	2	3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
4	5	6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
7	8	9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
10	11	12	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
13	14	15	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
16	17	18	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
19	20	21	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
22	23	24	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
25	26	27	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
28	29	30	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
31	32	33	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
34	35	36	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
37	38	39	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
40	41	42	<0.1			<0.1			<0.1		

Pork Content of Beef Samples Post Species Change (~10 kg) Against Sampling Plan for Phase 1, Pilot Plant - Low Risk Scenario

Sampling Plan			Low risk – Experiment 1			Low risk – Experiment 2			Low risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
43	44	45									
46	47	48									
49	50	51	<0.1			<0.1			<0.1		
52	53	54									
55	56	57									
58	59	60		<0.1			<0.1			<0.1	
61	62	63									
64	65	66									
67	68	69			<0.1			<0.1			<0.1
70	71	72									
73	74	75									
76	77	78	<0.1			<0.1			<0.1		
79	80	81									
82	83	84									
85	86	87		<0.1			<0.1			<0.1	
88	89	90									
91	92	93									
94	95	96			<0.1			<0.1			<0.1
97	98	99									
100	101	102									
103	104	105	<0.1			<0.1			<0.1		
106	107	108									
109	110	111									
112	113	114		<0.1			<0.1			<0.1	
115	116	117									
118	119	120									
121	122	123			<0.1			<0.1			<0.1
124	125	126									
127	128	129									
130	131	132	<0.1			<0.1			<0.1		
133	134	135									
136	137	138									
139	140	141		<0.1			<0.1			<0.1	
142	143	144									
145	146	147									
148	149	150			<0.1			<0.1			<0.1
151	152	153									
154	155	156									
157	158	159	<0.1			<0.1			<0.1		
160	161	162									
163	164	165									
166	167	168		<0.1			<0.1			<0.1	
169	170	171									
172	173	174									

Sampling Plan			Low risk – Experiment 1			Low risk – Experiment 2			Low risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
175	176	177			<0.1			<0.1			<0.1
178	179	180									
181	182	183									
184	185	186	<0.1			<0.1			<0.1		
187	188	189									
190	191	192									
193	194	195		<0.1			<0.1			<0.1	
196	197	198									
199	200	201									
202	203	204			<0.1			<0.1			<0.1
205	206	207									
208	209	210									
211	212	213	<0.1			<0.1			<0.1		
214	215	216									
217	218	219									
220	221	222		<0.1			<0.1			<0.1	
223	224	225									
226	227	228									
229	230	231			<0.1			<0.1			<0.1
232	233	234									
235	236	237									
238	239	240	<0.1			<0.1			<0.1		
241	242	243									
244	245	246									
247	248	249		<0.1			<0.1			<0.1	
250	251	252									
253	254	255									
256	257	258			<0.1			<0.1			<0.1
259	260	261									
262	263	264									
265	266	267	<0.1			<0.1			<0.1		
268	269	270									
271	272	273									
274	275	276		<0.1			<0.1			<0.1	
277	278	279									
280	281	282									
283	284	285			<0.1			<0.1			<0.1
286	287	288									
289	290	291									
292	293	294	<0.1			<0.1			<0.1		
295	296	297									
298	299	300									
301	302	303		<0.1			<0.1			<0.1	
304	305	306									

Sampling Plan			Low risk – Experiment 1			Low risk – Experiment 2			Low risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
307	308	309									
310	311	312			<0.1			<0.1			<0.1
313	314	315									
316	317	318									
319	320	321	<0.1			<0.1			<0.1		
322	323	324									
325	326	327									
328	329	330		<0.1			<0.1			<0.1	
331	332	333									
334	335	336									
337	338	339			<0.1			<0.1			<0.1
340	341	342									
343	344	345									
346	347	348	<0.1			<0.1			<0.1		
349	350	351									
352	353	354									
355	356	357		<0.1			<0.1			<0.1	
358	359	360									
361	362	363									
364	365	366			<0.1			<0.1			<0.1
367	368	369									
370	371	372									
373	374	375	<0.1			<0.1			<0.1		
376	377	378									
379	380	381									
382	383	384		<0.1			<0.1			<0.1	
385	386	387									
388	389	390									
391	392	393			<0.1			<0.1			<0.1
394	395	396									
397	398	399									
400			<0.1			<0.1			<0.1		

Pork Content of Beef Samples Post Species Change (~90 kg) Against Sampling Plan for Phase 1, Pilot Plant - Low Risk Scenario

21.8. Annex 8 – Results for Phase 1, Pilot Plant - Medium Risk Scenario

Sampling Plan			Medium risk – Experiment 1			Medium risk – Experiment 2			Medium risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
1	2	3	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
4	5	6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
7	8	9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
10	11	12	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
13	14	15	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
16	17	18	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
19	20	21	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
22	23	24	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
25	26	27	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
28	29	30	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
31	32	33	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
34	35	36	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
37	38	39	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
40	41	42	<0.1			<0.1			<0.1		

Pork Content of Beef Samples Post Species Change (~10 kg) Against Sampling Plan for Phase 1, Pilot Plant - Medium Risk Scenario

Sampling Plan			Medium risk – Experiment 1			Medium risk – Experiment 2			Medium risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
43	44	45									
46	47	48									
49	50	51	<0.1			<0.1			<0.1		
52	53	54									
55	56	57									
58	59	60		<0.1			<0.1			<0.1	
61	62	63									
64	65	66									
67	68	69			<0.1			<0.1			<0.1
70	71	72									
73	74	75									
76	77	78	<0.1			<0.1			<0.1		
79	80	81									
82	83	84									
85	86	87		<0.1			<0.1			<0.1	
88	89	90									
91	92	93									
94	95	96			<0.1			<0.1			<0.1
97	98	99									

Sampling Plan			Medium risk – Experiment 1			Medium risk – Experiment 2			Medium risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
100	101	102									
103	104	105	<0.1			<0.1			<0.1		
106	107	108									
109	110	111									
112	113	114		<0.1			<0.1			<0.1	
115	116	117									
118	119	120									
121	122	123			<0.1			<0.1			<0.1
124	125	126									
127	128	129									
130	131	132	<0.1			<0.1			<0.1		
133	134	135									
136	137	138									
139	140	141		<0.1			<0.1			<0.1	
142	143	144									
145	146	147									
148	149	150			<0.1			<0.1			<0.1
151	152	153									
154	155	156									
157	158	159	<0.1			<0.1			<0.1		
160	161	162									
163	164	165									
166	167	168		<0.1			<0.1			<0.1	
169	170	171									
172	173	174									
175	176	177			<0.1			<0.1			<0.1
178	179	180									
181	182	183									
184	185	186	<0.1			<0.1			<0.1		
187	188	189									
190	191	192									
193	194	195		<0.1			<0.1			<0.1	
196	197	198									
199	200	201									
202	203	204			<0.1			<0.1			<0.1
205	206	207									
208	209	210									
211	212	213	<0.1			<0.1			<0.1		
214	215	216									
217	218	219									
220	221	222		<0.1			<0.1			<0.1	
223	224	225									
226	227	228									
229	230	231			<0.1			<0.1			<0.1

Sampling Plan			Medium risk – Experiment 1			Medium risk – Experiment 2			Medium risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
232	233	234									
235	236	237									
238	239	240	<0.1			<0.1			<0.1		
241	242	243									
244	245	246									
247	248	249		<0.1			<0.1			<0.1	
250	251	252									
253	254	255									
256	257	258			<0.1			<0.1			<0.1
259	260	261									
262	263	264									
265	266	267	<0.1			<0.1			<0.1		
268	269	270									
271	272	273									
274	275	276		<0.1			<0.1			<0.1	
277	278	279									
280	281	282									
283	284	285			<0.1			<0.1			<0.1
286	287	288									
289	290	291									
292	293	294	<0.1			<0.1			<0.1		
295	296	297									
298	299	300									
301	302	303		<0.1			<0.1			<0.1	
304	305	306									
307	308	309									
310	311	312			<0.1			<0.1			<0.1
313	314	315									
316	317	318									
319	320	321	<0.1			<0.1			<0.1		
322	323	324									
325	326	327									
328	329	330		<0.1			<0.1			<0.1	
331	332	333									
334	335	336									
337	338	339			<0.1			<0.1			<0.1
340	341	342									
343	344	345									
346	347	348	<0.1			<0.1			<0.1		
349	350	351									
352	353	354									
355	356	357		<0.1			<0.1			<0.1	
358	359	360									
361	362	363									

Sampling Plan			Medium risk – Experiment 1			Medium risk – Experiment 2			Medium risk – Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
364	365	366			<0.1			<0.1			<0.1
367	368	369									
370	371	372									
373	374	375	<0.1			<0.1			<0.1		
376	377	378									
379	380	381									
382	383	384		<0.1			<0.1			<0.1	
385	386	387									
388	389	390									
391	392	393			<0.1			<0.1			<0.1
394	395	396									
397	398	399									
400			<0.1			<0.1			<0.1		

Pork Content of Beef Samples Post Species Change (~90 kg) Against Sampling Plan for Phase 1, Pilot Plant - Medium Risk Scenario

21.9. Annex 9 – Results for Phase 1, Pilot Plant - High Risk Scenario

Sampling Plan			High risk - Experiment 1			High risk - Experiment 2			High risk - Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
1	2	3	70.5	54.1	69.2	59.9	60.2	71.9	88.0	63.2	99.0
4	5	6	2.8	10.9	4.9	1.2	3.8	1.4	11.1	5.2	16.2
7	8	9	1.5	1.5	1.3	<0.1	3.7	0.9	5.6	3.2	0.7
10	11	12	0.9, 1.7	0.6	1.5	<0.1, <0.1	<0.1	<0.1	1.8, 1.7	8.0	1.1
13	14	15	0.8	3.9	1.2	<0.1	0.9	1.2	2.5	<0.1	0.2
16	17	18	0.5	0.9	0.6	1.5	0.8	1.8	1.0	0.2	2.0
19	20	21	0.3	0.5, 0.9	0.4	0.6	1.4, 4.5	2.0	<0.1	0.5, <0.1	<0.1
22	23	24	<0.1	<0.1	0.7	<0.1	<0.1	0.5	<0.1	<0.1	<0.1
25	26	27	<0.1	0.2	<0.1	0.1	1.2	3.0	<0.1	<0.1	<0.1
28	29	30	0.3	<0.1	<0.1, <0.1	2.2	3.8	0.7, 1.2	<0.1	0.3	<0.1, <0.1
31	32	33	<0.1	<0.1	<0.1	2.1	5.4	1.4	<0.1	<0.1	2.2
34	35	36	0.4	2.9	0.4	1.1	1.8	1.2	0.8	5.2	1.5
37	38	39	<0.1	1.7	5.3	0.4	0.8	0.9	<0.1	<0.1	0.7
40	41	42	0.9, 0.4			0.5, <0.1			<0.1, <0.1		

Pork Content of Beef Samples Post Species Change (~10 kg) Against Sampling Plan for Phase 1, Pilot Plant - High Risk Scenario

Sampling Plan			High risk – Experiment 1			High risk - Experiment 2			High risk - Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
43	44	45									
46	47	48									
49	50	51	0.5			<0.1			<0.1		
52	53	54									
55	56	57									
58	59	60		0.2			0.2			0.2	
61	62	63									
64	65	66									
67	68	69			0.3			<0.1			0.9
70	71	72									
73	74	75									
76	77	78	0.3			<0.1			<0.1		

Sampling Plan			High risk – Experiment 1			High risk - Experiment 2			High risk - Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
79	80	81									
82	83	84									
85	86	87		0.5			0.4			<0.1	
88	89	90									
91	92	93									
94	95	96			0.7			0.1			0.3
97	98	99									
100	101	102									
103	104	105	0.2			<0.1			<0.1		
106	107	108									
109	110	111									
112	113	114		1.2			0.3			0.1	
115	116	117									
118	119	120									
121	122	123			1.3			0.2			<0.1
124	125	126									
127	128	129									
130	131	132	<0.1, 0.3			0.2, 0.1			<0.1, <0.1		
133	134	135									
136	137	138									
139	140	141		0.2			0.1			<0.1	
142	143	144									
145	146	147									
148	149	150			0.1			<0.1			<0.1, <0.1
151	152	153									
154	155	156									
157	158	159	<0.1			<0.1			3.2		
160	161	162									
163	164	165									
166	167	168		<0.1			0.1			<0.1	
169	170	171									
172	173	174									
175	176	177			<0.1			0.1			<0.1
178	179	180									
181	182	183									
184	185	186	<0.1			0.1			<0.1		
187	188	189									
190	191	192									
193	194	195		0.2			<0.1			<0.1	

Sampling Plan			High risk – Experiment 1			High risk - Experiment 2			High risk - Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
196	197	198									
199	200	201									
202	203	204			<0.1			<0.1			<0.1
205	206	207									
208	209	210									
211	212	213	<0.1			0.1			<0.1		
214	215	216									
217	218	219									
220	221	222		<0.1, <0.1			<0.1, <0.1			<0.1, <0.1	
223	224	225									
226	227	228									
229	230	231			<0.1			<0.1			<0.1
232	233	234									
235	236	237									
238	239	240	<0.1			<0.1			<0.1		
241	242	243									
244	245	246									
247	248	249		<0.1			<0.1			0.1	
250	251	252									
253	254	255									
256	257	258			<0.1			<0.1			<0.1
259	260	261									
262	263	264									
265	266	267	<0.1			<0.1			<0.1		
268	269	270									
271	272	273									
274	275	276		0.1			<0.1			<0.1	
277	278	279									
280	281	282									
283	284	285			<0.1			<0.1			<0.1
286	287	288									
289	290	291									
292	293	294	<0.1			<0.1			<0.1		
295	296	297									
298	299	300									
301	302	303		0.2			<0.1			<0.1	
304	305	306									
307	308	309									
310	311	312			<0.1, <0.1			<0.1, 0.3			<0.1, <0.1

Sampling Plan			High risk – Experiment 1			High risk - Experiment 2			High risk - Experiment 3		
Conveyor belt			Conveyor belt			Conveyor belt			Conveyor belt		
313	314	315									
316	317	318									
319	320	321	<0.1			<0.1			<0.1		
322	323	324									
325	326	327									
328	329	330		<0.1			<0.1			<0.1	
331	332	333									
334	335	336									
337	338	339			0.4			<0.1			0.2
340	341	342									
343	344	345									
346	347	348	<0.1			<0.1			<0.1		
349	350	351									
352	353	354									
355	356	357		<0.1			<0.1			<0.1	
358	359	360									
361	362	363									
364	365	366			<0.1			<0.1			<0.1
367	368	369									
370	371	372									
373	374	375	<0.1			<0.1			<0.1		
376	377	378									
379	380	381									
382	383	384		<0.1			<0.1			<0.1	
385	386	387									
388	389	390									
391	392	393			<0.1			<0.1			<0.1
394	395	396									
397	398	399									
400			<0.1, <0.1			<0.1, <0.1			<0.1, <0.1		

Pork Content of Beef Samples Post Species Change (~90 kg) Against Sampling Plan for Phase 1, Pilot Plant - High Risk Scenario

Key to Annex 9 tables

<0.1 % Pork DNA relative to total mammalian DNA

0.1 – 1 % Pork DNA relative to total mammalian DNA

1 -10 % Pork DNA relative to total mammalian DNA

10 – 20 % Pork DNA relative to total mammalian DNA

>50 – 100 % Pork DNA relative to total mammalian DNA

21.10. Annex 10 – Results for Phase 2, Commercial Plant – Risk rating 4 (Multispecies plant with a chemical clean between species)

Experiment 1		
<0.1	<0.1	<0.1
<0.1		<0.1
	<0.1	
<0.1		<0.1
	<0.1	
<0.1		<0.1
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	
Approximately 3.5 kg, equivalent to 14 x 250g samples		
	<0.1	

Experiment 2		
<0.1	<0.1	<0.1
<0.1		<0.1
	<0.1	
<0.1		<0.1
	<0.1	
<0.1		<0.1
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1, <0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	

Experiment 1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1, <0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples

Experiment 2
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25kg, equivalent to 9 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples

Experiment 1
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1
Approximately 3.5 kg, equivalent to 14 x 250g samples
<0.1

Experiment 2
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1, <0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1

21.11. Annex 11 – Results for Phase 2, Commercial Plant – Risk rating 5 (Multispecies plant with a high pressure water wash between species)

Experiment 1		
<0.1	<0.1	<0.1
<0.1		<0.1
	<0.1	
<0.1		<0.1
	<0.1	
<0.1		<0.1
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	

Experiment 2		
<0.1	<0.1	<0.1
<0.1		<0.1
	<0.1	
<0.1		<0.1
	<0.1	
<0.1		<0.1
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1, <0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	

Experiment 1	Experiment 2
Approximately 2.25 kg, equivalent to 9 x 250g samples	Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1	<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples	Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1	<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples	Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1	<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples	Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1	<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples	Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1	<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples	Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1	<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples	Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1, <0.1	<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples	Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1	<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples	Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1	<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples	Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1	<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples	Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1	<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples	Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1	<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples	Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1	<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples	Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1	<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples	Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1	<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples	Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1	<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples	Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1	<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples	Approximately 9.75 kg, equivalent to 39 x 250g samples

Experiment 1
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1

Experiment 2
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1, <0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1

21.12. Annex 12 – Results for Phase 2, Commercial Plant – Risk rating 6 (Multispecies plant with no cleaning between species)

Experiment 1		
97.2	97.8	94.7
100.3		49.9, 46.2
	8.4	
13.6		10.3
	5.8	
2.7		5.7
	3.3	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.4	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.6	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.2	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.4	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.2	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.2	

Experiment 2		
184.4	162.9	145.2
144.7		98.0
	86.9	
29.7		13.2
	6.2	
4.8		2.9
	2.2	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.5	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.3	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.2	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.3	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1, 0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.5	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	<0.1	
Approximately 2.25 kg, equivalent to 9 x 250g samples		
	0.1	

Experiment 1
Approximately 2.25 kg, equivalent to 9 x 250g samples
0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
0.2
Approximately 2.25 kg, equivalent to 9 x 250g samples
0.2
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
0.2
Approximately 4.75 kg, equivalent to 19 x 250g samples
0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
0.1, 0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples

Experiment 2
Approximately 2.25 kg, equivalent to 9 x 250g samples
0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
0.2
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
0.1
Approximately 2.25 kg, equivalent to 9 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
0.2
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 4.75 kg, equivalent to 19 x 250g samples
<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples

Experiment 1	Experiment 2
<0.1	<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples	Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1	<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples	Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1	<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples	Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1	<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples	Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1	0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples	Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1	<0.1
Approximately 9.75 kg, equivalent to 39 x 250g samples	Approximately 9.75 kg, equivalent to 39 x 250g samples
<0.1	<0.1
±Approximately 9.75 kg, equivalent to 39 x 250g samples	Approximately 9.75 kg, equivalent to 39 x 250g samples
0.3	0.1

<u>Key to Annex 12 table</u>

- <0.1 % Pork DNA relative to total mammalian DNA
- 0.1 – 1 % Pork DNA relative to total mammalian DNA
- 1 -10 % Pork DNA relative to total mammalian DNA
- 10 – 20 % Pork DNA relative to total mammalian DNA
- 20 – 50 % Pork DNA relative to total mammalian DNA
- 50 – 100 % Pork DNA relative to total mammalian DNA

21.13. Annex 13 – Results for Phase 2, Commercial Plant – Risk rating 1 (Single species plant with chemical clean overnight)

Experiments 1 and 2	
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1
<0.1	<0.1, <0.1

21.14. Annex 14 – Swab locations for Phase 1

Swab 1

Swab 2

Swab 3

Swab 4

Swab 5

Swab 6

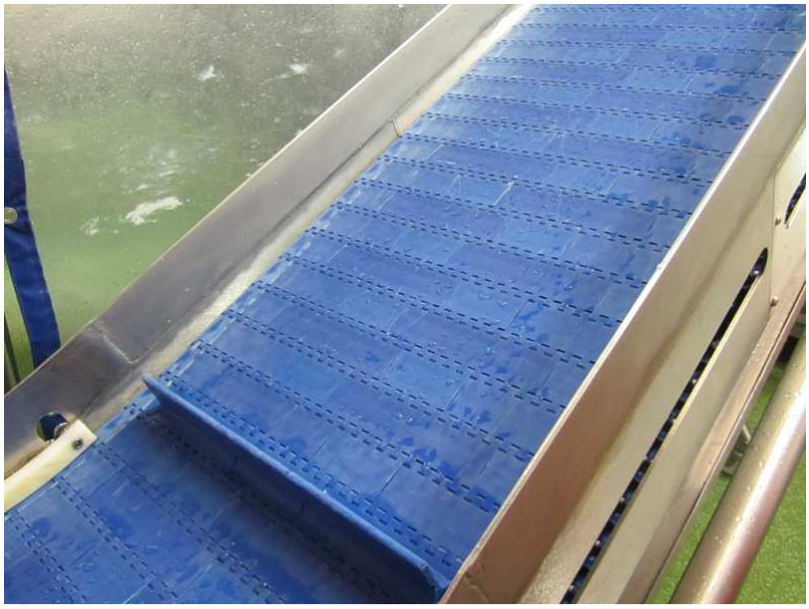
Swab 7

Swab 8

Swab 9

Swab 10

21.15. Annex 15 – Swab locations for Phase 2, risk rating 4



Swab 1

Swab 2

Swab 3

Swab 4

Swab 5

Swab 6

Swab 7

Swab 8

Swab 9

Swab 10

Experiment 1 – Swab 11

Experiment 1 – Swab 12– Repeat swab after locking screw re-cleaned.

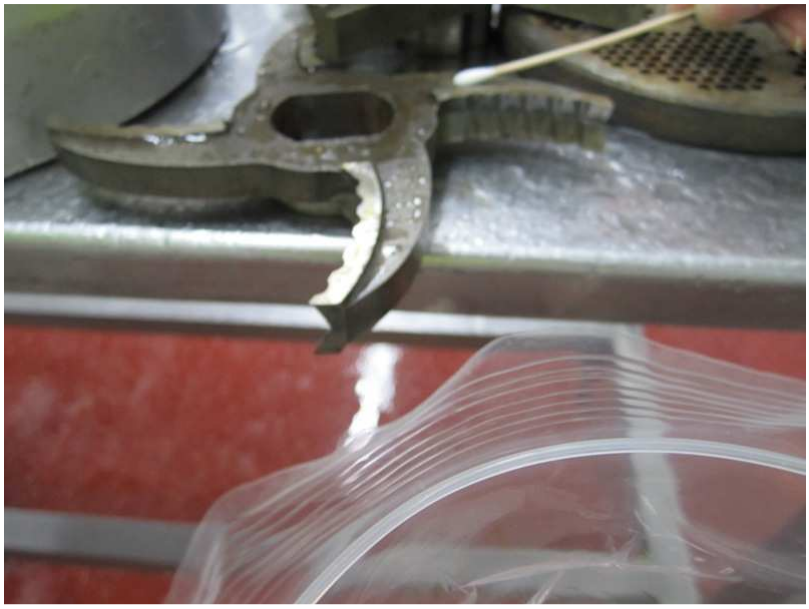
21.16. Annex 16 – Swab locations for Phase 2, risk rating 5 and 6

Swab 1

Swab 2

Swab 3

Swab 4



Swab 5

Swab 6

Swab 7

Swab 8

Swab 9