# Appendix I

## Sample reception procedure

On the receipt of the samples, the laboratory sample submission data will be completed and checked against the information recorded by the sampler on the sample submission form. The information will be entered into the Laboratory Information Management System and transferred from there into a spreadsheet compatible with Microsoft Excel. Product information will be captured of each chicken in its packaging and the data will be stored, labelled with the appropriate sample number. Following examination, the product label itself will be removed and stored if intact and readable. All samples would be tested before/on their use-by dates. In addition to the original number of samples planned for Scotland an additional 220 will be included to ensure representative data.

Sample testing would usually begin within the 24 h period after sampling, and only under exceptional circumstances would testing be delayed. Samples would be examined to ensure that the packaging is intact before testing. If packaging has been perforated during transportation this will be recorded on the sampling form.

## Sample examination and testing

The outmost care would be taken to avoid cross contamination between samples and between the chicken and its packaging as well as from the surrounding environment at all stages from sample receipt to test completion. Gloves would be worn and changed between each sample of chicken. The work-surface of the bench would be sanitised before un-wrapping each chicken. Thorough cleaning of equipment and work surfaces would be undertaken regularly. There would be environmental sampling of the laboratory for during the testing period at regular intervals. The contractor will carry out examinations in areas dedicated to the examination of survey samples and clearly separated from other potentially contaminated materials.

Campylobacter enumeration testing would be performed based on the ISO method to provide consistency in approach and to facilitate the comparison of results with findings from previous surveys (and comparison with data obtained from post-chill chickens as required). The microbiological methodology for the testing of each chicken sample for Campylobacter is as follows: The quantitative analysis of Campylobacter in the chicken sample will be based on the method described in EN/ISO/TS 10272-2:2006 ‘Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of Campylobacter spp Part 2: Colony-count technique’ (as described: <https://www.food.gov.uk/sites/default/files/media/document/retail_survey_protocol_year3.pdf>) – although a small amendment reflecting imminent implementation of the improved full ISO issued in 2017 will be considered in liason with the FSA.

In short this would entail the following:

Wearing a fresh pair of disposable gloves, the chicken will be removed from its wrapping, taking care not to allow contact between the chicken and outer packaging. Using sterile instruments (e.g. scissors and tweezers) 10 g skin from the neck area (if 10 g of neck skin is not available, a range of 2 to 10 g can be used and weights would be recorded), will be aseptically removed (avoiding fat) and placed into a sterile bag. A ratio of one part chicken skin weight to nine parts diluent will be prepared and homogenised for one minute. Chickens with < 5 g neck-skin available for testing would be scheduled for re-sampling. Chickens with neck skin weights of 2 to 5 g would be analysed according to protocol but may not be published.

*Campylobacter* spp. would be enumerated by the surface plate method as described in the ISO method (‘Microbiology of food and animal feeding stuffs -- Horizontal method for detection and enumeration of Campylobacter spp. -- Part 2: Colony-count technique’) and entail the following:

‘Plating of 1 ml of the chicken skin homogenate onto three modified cefoperazone, charcoal deoxycholate agars (CCDA plates: e.g. Oxoid CM739 with Oxoid selective supplement SR155) and 100 μl onto duplicate CCDA plates. Prepare two further 10-fold dilutions in maximum recovery diluent and plate 100 μl of each of these in onto CCDA plates. Incubate CCDA plates in a microaerophilic atmosphere at 41.5 ± 1°C for 44 ± 4 h. Count plates from those with less than 150 colonies, where possible. As the bacteria rapidly deteriorate in air progress confirmation of colonies immediately. Pick 5 (or less if less present) colonies (based on typical colony morphology) and sub-culture onto Columbia Blood Agar (containing 5 % (v/v) defibrinated blood). Check that growth is absent after incubation under aerobic conditions after 48 h and check for typical growth in a microaerophilic atmosphere at 41.5 ºC. Confirm oxidase reaction of pure cultures and typical Campylobacter cell morphology (small, slim, curved or spiral, Gram-negative rods/motility (wet mount/phase contrast)). Commercially available latex agglutination test kits can be used to identify campylobacters (e.g. Microscreen® campylobacter (Microgen bioproducts) and Dryspot campylobacter test (Oxoid Ltd) consistent with accredited Standard Operating Procedures.’

Isolates of *Campylobacter* spp. will be sent, as soon as possible, to the Bacteria Reference Department (BRD, PHE London) Gastrointestinal Bacteria Reference Unit (GBRU) for archiving, whole genome sequencing and phenotypic antimicrobial resistance testing as required. One isolate from each positive sample will be sent to be archived and stored for 2 years by GBRU (further years can be agreed with the FSA as appropriate). Isolates sent to GBRU must be clearly labelled with their sample number and the name of the referring laboratory.

The use of Whole Genome Sequencing and in-house PHE bioinformatics pipelines will be used to determine the *Campylobacter* species, 7-loci MLST and, the presence of genes or specific mutations known to confer resistance to the four classes of antibiotics: fluoroquinolones (*gyrA* mutation), macrolides (*23s* mutation), tetracyclines (presence *tetO* gene) and aminoglycosides (presence of multiple genes possible). The detection of these AMR genes and mutations has been validated in-house by GBRU to correspond to phenotypic resistance to ciprofloxacin and nalidixic acid (fluroquinolones), erythromycin (macrolide), tetracycline (tetracyclines) and, gentamicin and streptomycin (aminoglycosides), as determined by the EUCAST interpretative thresholds; thereby allowing comparison with phenotypic data from the previous survey years.