



A UK WIDE MICROBIOLOGICAL SURVEY OF
CAMPYLOBACTER CONTAMINATION IN FRESH
WHOLE CHILLED CHICKENS AT RETAIL SALE
(Year 3/ 4)

PROTOCOL
Revised August 2016

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ABBREVIATIONS

BPW-	Buffered Peptone Water
CCDA-	Charcoal Cefoperazone Deoxycholate Agar
DH -	Department of Health
FSA -	Food Standards Agency
h -	Hour(s)
PHE -	Public Health England
ISO-	International Standards Organisation
GBRU-	Gastrointestinal Bacteria Reference Unit
mL -	Millilitres
mm -	Millimetres
s -	Seconds
MS-	Microbiological Services
UKAS-	United Kingdom Accreditation Service

OUTLINE

Background

1. The Food Standards Agency has a key role in preventing foodborne illnesses. The Strategic Plan aims to reduce foodborne disease further and has set a target to reduce *Campylobacter* contamination in whole raw chicken.

Campylobacter is the most prominent bacterium associated with foodborne disease within the United Kingdom. Foodborne *Campylobacter* is estimated to make more than 280,000 people ill each year in the UK and is the biggest cause of food poisoning. An EFSA Opinion¹ stated that up to 80 % of cases can be attributed to raw poultry meat. It is hoped that by reducing the number of highly positive birds through effective control programmes, the number of human cases will decrease.

2. In December 2010, the Food Standards Agency, the UK poultry industry and major retailers agreed a new target that will measure efforts to reduce the levels of *Campylobacter* in chickens at the end of slaughter. The target is for the industry to reduce the numbers of most contaminated birds (>1000 cfu/g) in UK poultry houses from 27 to 10 % by the end of 2015 (this deadline has now been extended to 2016). It is estimated that achievement of this target could mean a reduction in *Campylobacter* food poisoning of up to 50 %.
3. In 2014, a UK-wide survey was established to review the levels of *Campylobacter* on fresh whole retail chickens and also on their packaging. The intention of the survey was to represent a full 12 month period (mid-February 2014 – mid February 2015) and tested a total of 4,011 samples of whole, UK-produced, fresh chicken. Over 19 % of the chickens tested were found to contain *Campylobacter* at a level above 1000 cfu/g. Just under 73 % were positive for *Campylobacter* at any level (i.e. were found to contain *Campylobacter* at a level above the detectable limit of 10 cfu/g). Just under 7 % of the samples were positive for *Campylobacter* on the outer packaging (i.e. contained *Campylobacter* at a level above the detectable limit of 10 cfu/g). For 5 out of the 4,005 samples (for which valid results were available for the outer packaging), the level on the outer packaging was found to be

¹ Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain:
<http://www.efsa.europa.eu/en/efsajournal/doc/2105.pdf>

above 1000 cfu/g. While a reduction of the most contaminated chickens to the target level of 10 % was not achieved by the end of December 2015, the evidence from retailer trials showed promising results that interventions work and the target was rolled forward to the end of 2016.

4. In July 2015, Survey Year 2 commenced under a revised sampling frame that included sampling of equal numbers of chickens from the 9 major retailers (100 chickens per retailer per sampling quarter). This equal sampling aided the ability to compare fairly between retailers. Survey Year 2 was partly completed due to the need for revising the test protocol in-year, but the data from the first 3 quarters (July 2015 – March 2016) suggested contamination rates were further decreasing (FSA 2016²).
5. Thus far, the laboratory protocol for detecting *Campylobacter* involved measuring the amount of *Campylobacter* on 25g of chicken neck skin (generally the most contaminated part of the bird) that could be topped up with breast skin if 25g was not achieved by neck skin only. During Survey Year 2, processors have increasingly trimmed back larger parts of the neck skin. While good news for the consumer, since this reduces the amount of *Campylobacter* on the bird, it also introduced a greater variation between the samples in our survey as the ratio of neck skin: breast skin in samples was variable. This hampered fair retailer to retailer comparisons and accurate comparisons with previous results.
6. The FSA therefore suspended the retail survey in April 2016 for the duration of 4 months while establishing a new testing methodology at Public Health England with the aim to restore the robustness of the survey. In the final quarter of Survey Year 2 a new protocol was developed to ensure the best possible sample was identified for the continued monitoring of campylobacters in retail chicken. The protocol presented in this document is the result of the evaluation of several methodologies and considered to provide the most robust comparison of campylobacter contamination of retail chicken.
7. Additionally, with regards to packaging contamination, recent evidence suggests a significant decline in *Campylobacter* levels on the outside of the chicken packaging. In the final quarter of Year 2, *Campylobacter* was not detected in over 95% of outer packaging (Jan 16 - Mar 16) and only 1 of the 1004 samples was found to have the highest levels of

² *Campylobacter* contamination in fresh whole chilled UK-produced chickens at retail: January – March 2016: <http://www.food.gov.uk/sites/default/files/campy-survey-report-jan-mar-2016.pdf>

Campylobacter (more than 1000 cfu/swab) contamination. Hence, detection of Campylobacter levels on the chicken packaging was suspended and does not form part of the new protocol.

8. This new survey will extend the previous surveys for up to an additional 24 months, including a breakpoint after 12 months, and will investigate the prevalence and levels of Campylobacter contamination in fresh whole chilled chickens at retail using the enumeration method. Although the survey will take into consideration seasonal fluctuations in Campylobacter prevalence in retail chickens, this is secondary to the survey's primary function to analyse prevalence among retailers. To draw any definitive conclusions regarding seasonality, we will require data from a number of separate years. The continuation of the retail survey for up to another 24 months additionally intends to identify trends as a result of specific retailer initiatives, such as improvements in biosecurity on farm or processing interventions.

Objectives

1. To determine the prevalence and levels of *Campylobacter* spp. contamination on fresh whole chilled chickens produced in the UK and sold at UK retail outlets by sampling up to a 24 month period, including annual breakpoints.
2. To identify *Campylobacter* spp. present and determine susceptibility of an agreed percentage of isolates to a defined range of antimicrobial agents.
3. To collect information from each sample on a range of factors including bird weight, rearing method and type of packaging and determine any correlation with Campylobacter contamination.

Publication of results

9. The survey results will be published under the Code of Practice for Official Statistics. This entails restricted access to raw data and pre-release publication documents.
At the end of the survey, the results and all the information that has been collected about all of the samples taken for the whole survey period will be published on the Agency's website.
In addition, sample data and results will be reported within four weeks following the end of a sampling month to the Agency. At predefined time points the Agency will publish interim reports.

Timetable

10. The survey will consist of a 12 month sampling period plus a further 2 months for data analysis and report preparation.

SURVEY DESIGN

11. Based on information available, the UK core sample per 12 month period will be 4000 samples. These samples will be taken evenly over a 12-month period unless agreed otherwise with the FSA to address additional objectives.
12. An equal number of chickens (100) will be sampled from each of the nine main retailers each quarter. This design, informed by the knowledge gained during the Year 1 and 2 baseline study, increases statistical confidence in the validity of results obtained during the survey. It also enables to report earlier, more robustly, and with greater confidence, on any improvements which are observed in samples from individual retailers. The market share data, obtained from Kantar in March 2015, will be applied to re-weight the dataset when producing estimates of the average prevalence of *Campylobacter* within the UK market.
13. The contractor will be responsible for ensuring that the appropriate number of samples is collected in accordance with the sampling plan agreed with the FSA. The number of chickens to be sampled from each UK country will be proportional to retailer market share figures of the respective country. If any deviations are necessary these will be noted in the final report. The contractor will ensure that sampling is evenly distributed throughout the period of the survey and is responsible for selecting and collecting samples at random within these criteria. If possible and in agreement between the contractor and the Agency, a maximum of 4 different chicken types (e.g. different size, brand or rearing) will be collected from any one store on any one occasion; the number of samples collected should be reduced if the sampler is unable to collect 4 different chicken types. A maximum of 2 samples should be taken from butchers and smaller independent stores/grocers at any one time.
14. The aim of this survey is to obtain a total of 4000 samples of whole UK produced raw fresh chilled chicken within any 12 month period. Sample numbers should be reviewed every month to ensure that chickens are being sampled according to the agreed sampling plan.
15. The contractor will provide smaller independent retail outlets with a letter from the Agency informing them that samples have been taken from their premises in order to carry out a survey (Annex 1). For larger retail chains (i.e. Tesco, Asda, Sainsbury's etc.) this is not necessary,

as the relevant contact at head office will be sent a list of the premises from which samples have been obtained by the Agency.

SAMPLING

Sample collection

16. It is essential that cross-contamination be avoided during the collection of chicken samples. Precautions will therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage is not contaminated with the pathogens investigated in the survey.
17. Contractors will aim to collect samples at random from the refrigerator cabinet and not necessarily from the front of the display. The surface temperature of the chicken should be recorded using an appropriately calibrated Infra-red thermometer, as should information on whether it was displayed in a temperature-controlled environment e.g. chillers and the overall condition of shelving e.g. was there any visible meat juices on the shelf.

Only packaged whole fresh chilled UK produced birds should be purchased. Unwrapped chickens may be bought but it should be noted on the sampling form and if available, with an indication of the Use-by Date. Samples, which are packaged, must not show evidence of damage. Each sample then should be placed in a separate sampling bag to avoid the risk of cross-contamination during transport and until testing can take place. For chickens collected from retail premises, the sample should not be purchased if the label on the chicken is not clear, does not include the approval number of the slaughterhouse, or is damaged. Chickens from butchers without labels may be sampled only if approval code can be obtained and must be noted down.

18. **Only unseasoned, fresh whole UK produced chilled whole chickens** should be sampled.

The chickens sampled may be labeled such as: Whole fresh chilled pre-packed UK-produced chicken and may include ones named as "Standard", "Value", "Ex Large", "Large", "Medium", "Roaster", "Small", "Barn-reared", "Free-range", "Roast-in-bag" etc.

Samples NOT included are:

- **Frozen whole chickens, portions (whether fresh or frozen) including legs, breast, thigh and wing portions.**

- **Any ready basted, marinated, seasoned, herbed, stuffed or pre-prepared whole birds.**
 - **Cooked chickens.**
 - **Processed chicken products including goujons, nuggets etc..**
19. Standard produced chickens will be sampled as well as a smaller number of free range and organic chickens (sampling of free range and organic chickens is structured to reflect their market share as outlined in Annex 2). A range of chicken weights will be sampled and weights should be noted down and logged in a separate column of the sample detail spreadsheet. Each sample should, at the point of sampling, have at least 2 days remaining on its Use-by Date.
20. When chilled un-packaged chicken is purchased from butchers/independents the sampling officer may need to enquire about the country of origin; if the bird is/or may not be UK-produced it should not be included in the survey. The sampler should ask the butcher for the approval number which should be present on the bulk packaging. Only samples where approval code can be obtained are to be included in the survey.
21. Each sample should be placed in a plastic bag, which is then sealed. Contractors will ensure that samples are kept at between 1 to 8 °C ($\pm 1^{\circ}\text{C}$) during transportation and kept dry and out of direct sunlight. A data logger should be placed (not in contact with or close to the cool pack) with the samples to monitor compliance with these requirements. If cool packs are used, samples shall not come into direct contact with their surfaces. Samples should not be frozen. Internal air temperature of the temperature controlled unit and package integrity shall be recorded on receipt at the laboratory.
22. It is essential to identify the approval number (used to be known as the health mark) from each sample so that the origin of the chicken can be determined retrospectively.

Sample information

23. All relevant information available from the sample should be recorded on the sample submission form (Annex 3). As far as possible this information should include the name (please ensure consistency throughout all laboratories) and postcode of the retailer, date and time of purchase, the approval number, weight (in a separate column), use-by date, price, product name, packaging information and display

temperature. The sampling sheet is completed with the addition of the results from the microbiological testing. This data are then entered onto a spreadsheet compatible with Microsoft Excel (please see Annex 4 for model spreadsheet with required information).

24. Sampling and results should be reviewed every month to ensure that the chickens sampled could generate statistically valid/meaningful results. The samplers should co-ordinate their sampling with the testing laboratory, project manager and the Agency.

TESTING

Receipt of samples

25. On receipt of the samples, laboratories will check the information recorded by the sampler and complete the relevant sections of the laboratory 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 or entered directly onto an Excel sheet. Following examination, the product label itself will be removed and stored if intact and readable.
26. Product information will be captured with digital photographs of each chicken in its packaging and the file will be stored and labelled with the appropriate sample number. Photos or scans are to be stored on suitable digital media under the appropriate sample number separated by sampling months. This will be shared with the Agency via Dropbox or other resources. The scan/photograph will be of a high resolution so that all the relevant labelling details are clear. Following examination, the product label itself shall be removed, cleaned and stored if intact and readable.
27. Chickens sampled should reach the laboratory within 24 hours of sampling. In exceptional situations (e.g. long journeys from the Northern Scottish Isles) this period may be extended to within 48 hours; if the transport period was 48 hours from sampling, the sampler must instruct the laboratory to test on receipt. All samples should always be tested before/on their use-by dates.

Examination

28. Samples of chicken will be examined to ensure that the packaging is intact before testing. If packaging has been damaged during

transportation this should be noted on the sampling form before testing. The temperature of the samples will also be recorded on receipt. Satisfactory samples will consider the integrity of packaging as well as sample temperature on receipt and only samples deemed satisfactory on receipt will be considered eligible for testing. Satisfactory sample receipt may be assigned if samples are within the temperature range of 1 to 8 °C (± 1 °C). If the temperature data logger records temperatures below 1 °C at receipt, the temperature of the sample itself would be measured and if this temperature was below 1 °C the sample would only be assigned as satisfactory if the sample temperature was below 1 °C (± 1 °C) when collected. Similarly, if the temperature data logger records temperatures above 8 °C, the sample itself would be measured and only deemed acceptable if the temperature at receipt is equal to or lower (± 1 °C) than the temperature when sampled. Sample receipt procedures would also take into account temperature probe uncertainty and transport time. All samples will be delivered before their use-by date.

29. It is essential that handlers take care to avoid cross contamination between samples and between the chicken and its packaging as well as from the surrounding environment at all stages. Gloves must be worn and changed between each sample of chicken. The work-surface of the bench must be sanitised before unwrapping each chicken. Thorough cleaning of equipment and work surfaces will be undertaken regularly. There must be environmental sampling of the laboratory for the test bacteria (*Campylobacter*) 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.

Methodology

30. The microbiological methodology for the testing of each sample for *Campylobacter* is as follows:
The quantitative analysis of *Campylobacter* in a 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' (please see Annex 5 for detailed method).

Data handling and reporting

31. Within four weeks after each sampling month concludes, the contractor will submit to the Agency a progress report that provides details of the

samples taken and the *Campylobacter* counts. The data for antimicrobial resistance profiles for strains isolated will be submitted independently in agreement with the Agency.

32. The contractor is responsible for collating all results and submitting a final report to the Agency. The report will present summary statistics on the prevalence of *Campylobacter*, together with a breakdown of the species where appropriate. The results should be subjected to detailed statistical analysis by the contractor; these analyses will be agreed with the Agency's Statistics team prior to commencement.
33. All forms, documentation and electronic files must be retained by the contractor until further notice from the Agency in case of issues arising after the completion of the survey. These should be retained for at least 12 months after completion of the current survey. It is not necessary to provide the Agency with hard copies of forms. However, this information must be readily available to the Agency if required.
34. Every month, the sampling numbers will be assessed to ensure that representative samples are being tested to obtain statistically valid/meaningful results. The contractor is responsible for adjusting the sampling plan every month according to any deviations occurring in the previous month(s) while the FSA is responsible for instructing the contractor on any major changes to the sampling strategy e.g. changes in market-share predictions.

Quality Assurance

35. In order to ensure a high level of accuracy in data entry, data checking and backup, the contractor has to be accredited to the relevant ISO methods by an appropriate organisation (e.g. UKAS). The EN/ISO/TS 10272-2 method is currently being revised to become a full standard and any proposed changes in the final draft may be incorporated providing they are within the scope of the accreditation. The contractor must also be able demonstrate satisfactory performance in the testing of food for *Campylobacter* spp. through participation in an independent proficiency testing scheme. The measurement of uncertainty for enumeration of *Campylobacter* spp. must also be determined and the FSA will visit the contractors during the course of the survey to assess how the work is being carried out.

ANNEX 1: LETTER TO RETAILERS

Letter to be sent to Retailers during Sampling

Insert Council Logo &/or Name

<Date>

Dear

This letter has been given to you by an Environmental Health Officer (EHO)/ Sampling Officer (SO) from [\[insert name of Contractor\]](#).

The EHO / SO is authorised by the [\[Contractor\]](#) to carry out food sampling work, and has purchased chicken from your premises as a food sample, which is to be used for a food surveillance survey.

The aim of this particular survey is to ascertain the incidence and contamination level of *Campylobacter* in raw UK produced chicken available to consumers at retail in the UK. Whole chickens are being sampled and tested during a 12 month period.

This survey is being funded by the Food Standards Agency which has commissioned [\[name of Contractor\]](#) to carry out the sampling.

Your premise has been visited as one of the retail outlets where people may buy raw chicken - the subject of this survey. The raw chicken purchased from your premises will be taken to [\[insert name of lab\]](#) for testing, and you will be provided with the results of this testing by a letter from the Food Standards Agency. Please note that the survey is not for enforcement purposes.

The results of the samples taken in this survey will be collated and will form part of a report on the incidence and contamination level of the pathogen *Campylobacter* spp. on fresh whole chilled UK chicken on retail sale within the UK. This report will be published by the Food Standards Agency. At the end of the survey, in line with Food Standards Agency policy on openness and transparency in relation to food safety and matters of interest to consumers relative to food, individual retailers/producers of the chicken sampled will be published on the Agency's website www.food.gov.uk as part of this report.

Should you have any queries, please contact Dr Bettina Mavrommatis, Foodborne Disease Control, Food Safety Policy on the following telephone number:

020 7276 8045 or send an E-mail to

Bettina.Mavrommatis@foodstandards.gsi.gov.uk

Yours sincerely

Updated 01.08.2016

ANNEX 2: SAMPLING PLAN

A UK core sample size of approximately 4000 samples of fresh whole UK produced chilled chicken are needed to achieve the precision required. The sample numbers should be reviewed periodically to ensure that statistically meaningful analyses can be carried out. The sampling will aim to take place evenly over a 12-month period. The sampling plan is structured to reflect market share data sourced from Kantar. Sampling will be kept under review and can, as agreed with the FSA, be revised to accommodate any further survey objectives e.g. over-sample during certain periods.

Table 2 Samples in relation to retailer and country

	England			Scotland			Wales			NI			All
	Standard	Free Range	Organic	Standard	Free Range	Organic	Standard	Free Range	Organic	Standard	Free Range	Organic	
Tesco	283	30	1	21	5	0	45	4	0	10	1	0	400
Sainsbury's	307	28	5	12	1	0	28	3	1	14	1	0	400
Morrisons	299	33	1	30	4	0	30	3	0	0	0	0	400
Aldi	270	43	0	34	3	0	44	6	0	0	0	0	400
Asda	280	40	1	22	3	0	38	3	0	11	2	0	400
The Co-Operative	288	25	0	44	5	0	34	4	0	0	0	0	400
Lidl	271	13	0	27	2	0	64	2	0	20	1	0	400
Waitrose	301	56	12	5	1	0	19	6	0	0	0	0	400
Marks & Spencer	287	22	1	52	4	0	30	3	1	0	0	0	400
Butchers¹	186			4			10			0			200
Others²	101	66	0	7	2	0	15	3	0	4	2	0	200

¹Samples from butchers can be collected from either category (Standard/ Free Range/ Organic) depending on availability. ² e.g Iceland, Budgens, Costcutter, Nisa, independents, farm shops, markets stalls.

Updated 01.08.2016

ANNEX 3: SAMPLING FORM



FOOD WATER AND ENVIRONMENTAL MICROBIOLOGY LABORATORY, xxxxxx
 a UKAS accredited Testing Laboratory No. xxxxx
 Tel: 0xxxxxxx
 E-mail: LabFwexxxxx@phe.gov.uk



FSA Campylobacter in chickens 2016-2017

Premises name: (circle which)	ALDI / ASDA / CO-OP / LIDL / M&S MORRISONS / SAINSBURYS TESCO / WAITROSE / BUTCHERS*	AFFIX LABORATORY NUMBER HERE	
Post Code:	OTHER*		
*If BUTCHERS or OTHER give premises name and address:	Sender contact tel #	CRCE <input type="checkbox"/> AF BT116 <input type="checkbox"/>
		Cool Box ID:

Sampling officer ID:	Date collected:	Time collected (24 hour clock):	Temperature at collection:
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Sample Details

Sample description (enter full name of product as it appears on the label)

.....

Use by date: dd/mm/yyyy

Batch Number (Approval Number) 

Additional sample details: Enter answers to the questions and TICK ONE BOX per question

Q#	Question	Response
Q1	Weight of product as shown on packagingGrams
Q2	Price	£.....
Q3	Type of chicken (as shown on packaging – Tick 'Standard' if not known)	Standard <input type="checkbox"/> Free range <input type="checkbox"/> Organic <input type="checkbox"/>
Q4	Other chicken details	Halal <input type="checkbox"/> Com fed <input type="checkbox"/> Other <input type="checkbox"/> (give details):
Q5	Assurance scheme	Red Tractor only <input type="checkbox"/> Other <input type="checkbox"/> (e.g. Freedom Foods):
Q6	Packaging atmosphere	Modified atmosphere <input type="checkbox"/> NOT modified atmosphere <input type="checkbox"/>
Q7	Packaging other details	Roast-in-bag <input type="checkbox"/> Other <input type="checkbox"/> (e.g. wrapped tight /tray present):
Q8	Visible liquid inside pack?	Yes <input type="checkbox"/> No <input type="checkbox"/>
Q9	Other sampling comments
Q10	For Lab Use Only	Weight of neck skin in sampleGrams

LABORATORY USE ONLY (Record details of unsatisfactory findings in comments)

Date received: Data logger/probe ID: Comments:
 Air/ In between pack (delete as appropriate)
 Time received: Temp. on receipt:
 Received by: On STARLIMS associate with Study:
 Received from: Samples & Receipt: SATIS *Campylobacter in chicken study (2016-17)*
 UNSATIS

FSA Campy in chickens 2015 submittal form v1

ANNEX 4: MODEL DATA SUBMISSION TEMPLATE (AS EXCEL)

Country	NI lab ref	Post code	Sample Number	Temp at Collection (Store) °C	Temp on Receipt (Lab) °C	Date Examined	Retailer store	Retailer details (if Others/ Butchers)	Premises Address (Others/ butcher)	Sample Details	chicken weight (g)	Other Packaging Details	Other packaging comments	Chicken type	Specify other production types	Cost of chicken (£)	Approval Number	Use by date	Neck skin weight (g)	Result	Units	Speciation	Result	
	<i>as before</i>	<i>as before</i>	<i>as before</i>	<i>please specify accurate (without characters)</i>	<i>please specify accurate (without characters)</i>	<i>consistent date format</i>	<i>consistency throughout</i>			<i>please specify here</i>	<i>number in gram without characters</i>	<i>please specify here</i>	<i>please specify here</i>	<i>specify consistently</i>	<i>specify if described by following</i>	<i>specify (number only; no characters)</i>	<i>specify (number only; no characters)</i>	<i>consistent date format</i>	<i>please specify neck skin taken for analysis (g) Value between 2g<neck skin>10g</i>					
				5	6.2	12/08/2015	Co-op			Small whole chicken Fresh Class A	4269	Roast-in-bag	liquid visible in pack	STD	Halal	2.99	8005	12/08/2015						
							Asda					No tray, modified atmosphere packed		FR	cornfed									
							Morrisons					Tray present, modified atmosphere packed.		O										
							Sainsbury's					No tray. Plastic, lightly wrapped.												
							Tesco					Loose bag. No tray.												
							Lidl																	
							Waitrose																	
							Aldi																	
							M&S																	
							Others	NISA																
								Iceland																
								etc.																
							Butchers	Pat The Butcher	1 Little Aston Lane, Little Aston, Birmingham															

ANNEX 5: LABORATORY METHODOLOGY

Overview

Chicken samples will be analysed for *Campylobacter*. Wear suitable single-use gloves for handling the packaged chicken, changing gloves after each sample.

Chicken skin sampling

Wearing a fresh pair of disposable gloves, remove the chicken from its wrapping, taking care not to allow contact between the chicken and outer packaging. Using sterile instruments (e.g. scissors and tweezers) aseptically remove 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, but weight needs to be accurately recorded), avoiding fat and place this into a sterile bag. Add BPW so that a ratio of 1 part chicken skin weight to 9 parts BPW weight is achieved and homogenise for one minute. Remove > 3 ml for enumeration as described below. Chickens with < 5 g neck-skin available for testing must be re-sampled and tested. Chickens with neck skin weights of 2g-5g will be analysed according to protocol, but results not published.

Enumeration of *Campylobacter* spp.

Enumerate *Campylobacter* spp. by the surface plate method as described in the PHE Methods - Detection and enumeration of *Campylobacter* spp.: *FNES15* (F21) v2. This method is based 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 and entails 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 MRD and plate 100 µl of each of these in duplicate onto CCDA plates.

Incubate CCDA plates in a microaerophilic atmosphere at $41.5 \pm 1^\circ\text{C}$ for 44 ± 4 h.

Counting and confirmation of suspect/typical colonies

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°C . 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 local Standard Operating Procedures.

Isolates of *Campylobacter* spp. will be sent, as soon as possible, to the Gastrointestinal Bacteria Reference Unit (GBRU) CampyLab, PHE London (Hays DX number DX653008) for speciation and antibiotic resistance testing. One isolate from each positive sample will be sent and archived by GBRU. Isolates sent to GBRU must be clearly labelled with their sample number and the name of the referring laboratory.