ANNEX 1

MASTER SAMPLING PLAN

	ENGLAND				WALES			
	Fresh		Frozen		Fresh		Frozen	
	Whole	Portions	Whole	Portions	Whole	Portions	Whole	Portions
Retailer								
Asda	100	194	23	33	34	67	7	10
Budgen	4	5	0	0	0	0	0	0
Co-op	26	58	2	5	10	20	1	2
Somerfield	37	75	2	3	13	25	1	1
Kwik Save	8	24	15	18	3	8	5	6
Morrisons	36	33	4	7	0	0	0	0
Presto	0	0	0	0	0	0	0	0
Safeway	38	109	15	19	13	36	5	6
Sainsbury's	78	197	43	95	28	64	15	32
Tesco	104	275	28	84	40	93	9	27
Waitrose	5	10	1	1	0	0	0	0
Other Multiples	29	23	8	13	9	7	2	4
Symbols	0	1	3	0	0	0	1	0
Iceland	15	41	34	124	5	14	11	41
Other Freezer centres	0	0	9	19	0	0	3	6
Other Grocers	5	4	1	3	2	1	0	1
Butchers	44	131	2	21	16	43	1	7
M&S	7	54	0	0	2	18	0	0
Other	12	53	3	8	4	18	1	2
Dunnes stores	0	0	0	0	0	0	0	0
Totals	548	1287	193	453	179	414	62	145
		1835		646		593		207
				2481				800
Free range	3-28	13-52			5	11		
Corn fed	<28	26			5	8		
Organic	1-6	3-26			1	5		

	SCOTLAND				NORTHERN IRELAND			
	Fresh		Frozen		Fresh		Frozen	
	Whole	Portions	Whole	Portions	Whole	Portions	Whole	Portions
Retailer								
Asda	34	67	7	10	0	0	0	0
Budgen	0	0	0	0	0	0	0	0
Co-op	10	20	1	2	25	55	1	3
Somerfield	13	25	1	1	0	0	0	0
Kwik Save	3	8	5	6	0	0	0	0
Morrisons	0	0	0	0	0	0	0	0
Presto	0	0	0	0	0	0	0	0
Safeway	13	36	5	6	26	61	2	3
Sainsbury's	28	64	15	32	13	30	7	15
Tesco	40	93	9	27	44	99	6	14
Waitrose	0	0	0	0	0	0	0	0
Other Multiples	9	7	2	4	12	27	1	1
Symbols	0	0	1	0	14	29	1	3
Iceland	5	14	11	41	15	48	40	93
Other Freezer centres	0	0	3	6	0	0	0	0
Other Grocers	2	1	0	1	0	0	0	0
Butchers	16	43	1	7	14	31	0	1
M&S	2	18	0	0	0	0	0	0
Other	4	18	1	2	9	20	2	5
Dunnes stores	0	0	0	0	6	14	3	7
Totals	179	414	62	145	178	414	63	145
		593		207		592		208
				800				800
Free range	5	11			5	11		
Corn fed	5	8			5	8		
Organic	1	5			1	5		

ANNEX 2

GEOGRAPHICAL AREAS USED IN THE SAMPLING FRAMEWORK

ENGLAND	
CD	County
	Cornwall
	Dorset
	Devon
SX	East Sussex
	Surrey
	West Sussex
GL	Greater London
EK	Essex
	Kent
CE	Berkshire
	Hampshire & Isle of Wight
WS	Somerset & Bristol
	Wiltshire
GH	Gloucestershire
	Herefordshire
	Worcestershire
	Warwickshire
SN	Cambridgeshire
	Suffolk
	Norfolk
LN	Nottinghamshire
	Leicestershire & Rutland
	Lincolnshire
SD	Shropshire
	Derbyshire
	Staffordshire
WM	West Midlands
YO	East Riding of Yorkshire
	North Yorkshire
	South Yorkshire
	West Yorkshire
LM	Cheshire & Greater Manchester
	Merseyside
	Lancashire
CN	Cumbria
	Northumberland, Durham & Tyne & Wear
BB	Bedfordshire
	Buckinghamshire
	Hertfordshire
	Northamptonshire
	Oxfordshire

WALES	
NW	Anglesey
	Gwynedd
	Conwy
NE	Denbighshire
	Flintshire
	Wrexham
SW	Carmarthenshire
	Ceredigion
	Pembrokeshire
SE	Monmouthshire
	Powys
SC	Cardiff, Caerphilly, Bridgend, Merthyr Tydfil, Vale of Glamorgan, Blaneau Gwent, Rhondda Cynon Taff, Torfaen, Newport, Swansea, Neath Port Talbot

SCOTLAND	
	Angus inc. Dundee
	Argyll and Bute
	Aberdeenshire inc. Aberdeen
	Dumfries and Galloway
	South and East Ayrshire
	Edinburgh
	Fife inc. Clackmannanshire
	Highland
	Moray
	North Ayrshire
	Perth and Kinross
	Renfrewshire
	Scottish Borders inc. West Lothian, Midlothian, East Lothian, Glasgow
	Stirling inc. East & West Dunbartonshire, North & South Lanarkshire, Falkirk, Inverclyde, Renfrewshire, East Renfrewshire

NORTHERN IRELAND		
ANT	ANTRIM	Antrim
		Ballymena
		Ballymoney
		Carrickfergus
		Coleraine
		Larne
		Moyle
		Newtownabbey
ARM	ARMAGH	Armagh
		Craigavon
		Lisburn
BEL	BELFAST	Belfast
		Castlereagh
LON	LONDONDERRY	Derry
		Limavady
		Magherafelt
DOW	DOWN	Ards
		Banbridge
		Down
		Newry & Mourne
		North Down
FER	FERMANAGH	Fermanagh
TYR	TYRONE	Cookstown
		Dungannon
		Omagh
		Strabane

ANNEX 3

SAMPLE PREPARATION AND MICROBIOLOGICAL METHODS

1. Because of the significant amount of handling involved, and the potential for cross-contamination to occur, it is essential to keep the testing area clear and to sanitise splashes or spillages as soon as they occur.

Sample preparation – Whole carcasses

2. Wearing disposable gloves, remove the chicken from its retail wrapping, taking care not to contaminate the outer surface of the carcass with any residual liquid. Remove the bag of giblets if present, (usually in frozen chickens), noting at the same time whether the bag is intact. Weigh the bag of giblets so that the weight of the carcass can be adjusted when the data are analysed. Retain the label from the packaging.

3. Transfer the chicken to a sterile disposable tray. Wearing disposable gloves, aseptically remove 25 g of neck-skin using a sterile scalpel and place into a stomacher bag (~180mm x 300mm). Place the chicken vertically into a large stomacher bag (~ 380 x 505mm) so that the vent is uppermost. Pour 300 ml of Buffered Peptone Water (BPW) through the vent into the abdominal cavity of the chicken. Twist the bag about halfway down while ensuring that most of the air is squeezed out of the bag. Rinse the chicken carcass for 1 minute by shaking the bag, ensuring that the BPW comes into contact with all chicken surfaces. Pour the rinse into the smaller stomacher bag containing the neck-skin and stomach for 2 minutes. After stomaching, please follow testing detailed in section 6 onwards.

Sample preparation – Chicken portions

4. Remove the chicken from its retail wrapping, taking care not to contaminate the outer surface of the portions with any residual liquid. Note how many portions are present in the pack. Retain the label from the packaging.

5. Transfer the chicken to a sterile disposable tray. If skin is present, aseptically remove 25g (remove all skin if less than 25g and record the amount weighed) with a sterile scalpel and place into a stomacher bag (~180mm x 300mm). Place the remainder of the chicken into a large stomacher bag (~ 380 x 505mm) containing 300 ml of Buffered Peptone Water (BPW). Twist the bag about halfway down while ensuring that most of the air is squeezed out of the bag. Rinse the chicken portions for 1 minute by shaking the bag, ensuring that the BPW comes into contact with all chicken surfaces. Pour the rinse into the smaller stomacher bag containing the skin (if present) and stomach for 2 minutes.

6. Remove 5 ml of homogenate for enumeration of *Campylobacter*, using a sterile open-ended pipette.

7. Transfer 25ml of homogenate using a sterile open-ended pipette, to a 300ml sterile plastic container (e.g. honey jar) for enrichment of *Campylobacter*.

8. Pour the remaining contents of the stomacher bag into another sterile plastic container (e.g. 300ml honey jar) for enrichment of *Salmonella*.

9. Testing laboratories should ensure that they have pure cultures of standard reference strains of both *Salmonella* and *Campylobacter*, from which colonies can be identified correctly.

Enumeration of *Campylobacter* spp.

10. Spread plate 0.5 ml from neat homogenate and 10^{-1} and 10^{-2} dilutions (dilute using Maximum Recovery Diluent – MRD) onto Charcoal Cefoperazone Deoxycholate Agar (CCDA) plates, all plates in duplicate. Care should be taken to ensure that all CCDA plates are sufficiently dry before plating out. Incubate plates in a microaerobic atmosphere for 24h at 37°C ± 0.5°C, followed by a further 24 h at 41.5°C ± 0.5°C. *Campylobacter* will grow well if oxygen does not exceed 10% and there is at least 5% CO_2. A number of commercially available gas-generating kits fulfill these criteria. Where microaerobic atmospheres are generated by other means, e.g. using a VAIN cabinet or manual gas-mixing, a suitable gas mixture would consist of 10% CO_2, 10% H_2, 5% O_2 and 75% N_2.

11. Subculture 5 typical colonies onto Columbia Blood Agar (BA) and perform the following confirmatory tests for *Campylobacter* spp: Gram-stain for morphology using carbol fuchsin for the counter stain, oxidase test, growth after 48h under microaerobic conditions at 37°C ± 0.5 °C, and growth after 48h in air at 20°C ± 0.5 °C.

12. Following confirmation that colony types on CCDA plates are *Campylobacter* spp., count the number on the duplicate plates to determine the number per ml of the dilution plated. Multiply this by the dilution factor and then by the total rinse volume, to give the number per carcass, portions and weight (g). Plates containing only a few colonies should be included in the count to improve the cell detection limit.

13. After confirmation of *Campylobacter*, remove a heavy inoculum from 1/4 of the blood plate and emulsify this in the liquid supplied with each container of beads (e.g. Mast or ProLab). Mix by inversion and remove the liquid phase using a disposable Pasteur pipette. Freeze *Campylobacter* isolates on beads at –70°C or lower (preferably -80°C). One confirmed isolate from each chicken sample and 5 isolates from every 5th *Campylobacter*-positive chicken sample should be frozen.

14. For the purpose of typing, only *Campylobacter* colonies isolated from enumeration should be sent to the reference laboratory.

15. To send isolates for typing, transfer the bead to a blood agar plate. Streak and incubate for 24-48 hours in microaerobic conditions at 37°C ± 0.5°C. The plate should be checked for purity. Swab the culture with a charcoal swab (e.g. Amies Transport) and send to: PHLS Laboratory of Enteric Pathogens (LEP), CPHL, 61 Colindale Avenue, London NW9 5HT (Tel: 020 8200 4400) for confirmation, serotyping, phage typing, antibiotic susceptibility testing and archiving. *Campylobacter* HS serotyping, phage typing and antimicrobial

resistance testing are described in the literature (Thwaites and Frost 1999; Frost *et al*., 1998; 1999; Oza *et al*., 2002).

Enrichment culture for *Campylobacter* spp.

16. Add 225 ml Exeter Modified *Campylobacter* Broth (ECB) to the 25g sample in the sterile plastic container (e.g. honey jar).

17. Incubate for 48 h at 37°C ± 0.5°C

18. After incubation, streak 10 µl of the enrichment broth onto CCDA. Incubate at 37°C ± 0.5°C. for a further 48h in a microaerobic atmosphere.

19. Subculture 3 typical colonies of *Campylobacter* spp. on to Columbia Blood Agar (BA) and perform confirmatory tests: Gram-stain for morphology using carbol fuchsin for the counter stain, oxidase test and growth after 48h under microaerobic conditions at 37°C ± 0.5°C, and growth after 48h in air at 20°C ± 0.5°C. If none were confirmed from the chicken sample by the enumeration procedure then store confirmed isolates as described previously (para. 13).

Enrichment culture for *Salmonella*

20. Incubate sample in a sterile plastic container for 18-20 h at 37°C ± 0.5°C for non-selective pre-enrichment.

21. Add 0.1 ml of the pre-enriched culture to 10 ml Rappaport-Vassiliadis Soya Peptone Broth (RVS) and incubate for selective enrichment at 41.5°C ± 0.5°C for 24 h in an incubator. Also, add 10 ml of the pre-enriched cultures to 100 ml Selenite Cystine Broth with added Sodium Biselenite (SCB), (4g/l) and incubate for selective enrichment at 37°C ± 0.5°C for 24 h.

22. After selective enrichment, streak a 10µl loop from the selective enrichment broths onto modified Brilliant Green Agar (mBGA) and Xylose Lysine Desoxycholate agars (XLD). Incubate plates for 24 h at 37°C ± 0.5°C. Colonies on mBGA: red/pink or white opaque colonies with brilliant red/pink zone, on XLD: red with black centre. Plates should not be incubated for longer than 24 hours, as this will encourage growth of other flora.

23. Perform appropriate biochemical tests for *Salmonella* on typical or suspect colonies (3 from each sample) from both mBGA and XLD plates. Isolates showing typical *Salmonella* biochemical reactions should be tested with polyvalent antisera for typical O and H antigens.

24. Send one isolate of each *Salmonella* type on a nutrient agar slope to PHLS Laboratory of Enteric Pathogens, CPHL, 61 Colindale Avenue, London NW9 5HT Tel: (020 8200 4400) for confirmation, serotyping, phage typing, antibiotic susceptibility testing and archiving.

Media

25. Full details of the microbiological methods used together with methodology flow charts and media ingredients are available from the literature (Jørgensen *et al.,* 2002).

ANNEX 4

SAMPLER RECORDING SHEET

LAB REF No:	WEEK No:	SAMPLER REF. No:	CHICKEN REF No:	
SURVEY LEAFLET ISSUED TO RETAILER? (PLEASE CIRCLE)			YES	NO

TYPE OF CHICKEN – PLEASE CIRCLE ALL THAT APPLY						
Type:	Fresh	Frozen	Whole	Portion		
Production Type:	Standard (broiler)	Organic	Free-range	Corn-fed	Other	
Portion Type:	Breast	Leg	Quarter	Drumstick	Thigh	Wing
Store Type:	Supermarket	Butcher	Grocer	Market/Farm Stall	Other	
Packaging:	Pre-wrapped (on shelf)		Wrapped (at sale)			
Type of packaging:	Cling film + Tray	Plastic bag	Greaseproof paper	Other		
Boneless:	Yes	No	**Skinless:**	Yes	No	

Name and Address of Retailer (including post code)	…… …… …… ……		
Date Purchased		**Time Purchased***	
Temperature of sample at purchase		**Use by Date**	
Country of origin (if known)		**Packing number**	
Producer Number (EC number or health mark)		**Pack price + price per kg**	
No. of portions in pack		**Declared pack weight (kg)**	
Date received at laboratory		**Time received at laboratory**	
Temperature of sample on arrival at lab		**Date sample tested**	
Details of basic cooking instructions (if given)			

Section 2

	Salmonella	***Campylobacter***
Detected? (Y/N)		
Total colony count on carcass	**N/A**	
No. of colonies sent for typing		

Details of *Campylobacter* typing and *Campylobacter* and *Salmonella* antimicrobial resistance results should be recorded in the Excel Spreadsheet

*** Please attach original packaging (or photocopy) to this form**

ANNEX 5

STATISTICAL ANALYSIS OF THE DATA

1. The statistical summary pertains to a survey of UK retail raw chicken conducted between April – June 2001. The survey covered all parts of the UK and planned to take 2481 samples from locations throughout England, with 800 from each of Wales, Scotland and N. Ireland. The fieldwork adhered very closely to this plan and achieved 4866 samples, whose coverage, in terms of 10 classification factors, is summarised below.

2. Three prime classifiers were deliberate stratification factors: country of purchase (England, Wales, Scotland, N. Ireland); fresh versus frozen produce; and whole chicken versus portions. Representation of fresh/frozen and whole/portion produce was strictly controlled on the basis of estimated share of the UK market. For country, however, representation departed from strict market share, with boosting applied to the quota drawn from Wales, Scotland and N. Ireland. This required re-weighting of the data when computing prevalence estimates representative of the whole UK and, for this particular purpose, reduced the actual sample size of 4866 to an effective sample size of 3424.

3. To control collection costs samplers were permitted to purchase up to 5 items at a single visit when sampling large supermarkets. This modest within-store clustering, which incurs a negligible reduction in effective sample size, was offset by requiring that items purchased simultaneously from a store should span a range of different product types (fresh/frozen, whole/portion etc), with no repeats of the same product. Overall, the 4866 samples purchased were drawn from approximately 1500 different retail outlets.

4. The report aimed to go beyond baseline estimation to investigate factors that may be associated with variation in the prevalence of *Salmonella* and *Campylobacter* in retail, raw chicken in the UK. The study data contain 13 potential main explanatory variables on which to base such exploration. The full set is itemised in Table A5.1.

Table A5.1 - Variables investigated for their association with variation in *Salmonella* prevalence in raw, retail UK chicken

Classification Factors	1. Country of Purchase
	2. Fresh/Frozen
	3. Whole/Portion
	4. Place of purchase
	5. Wrapped (Yes/No)
	6. Country of Origin
	7. Production Type
	8. Week number (1…8)
	9. Boneless (Yes/No)
	10. Giblets (Yes/No)
Quantitative Variables	Pack weight (kg)
	Giblets weight
	Number of portions

5. Investigation of factors associated with variability in the prevalence of *Salmonella* and *Campylobacter* in retail raw chicken was put on a statistical footing by using logistic regression modelling. Logistic regression is a natural, commonly applied technique when attempting to model or predict dichotomous response variables such as presence/absence of a pathogen. It provides a framework with the potential to simultaneously assess the impact of multiple explanatory or risk factors on a single response variable. However, no statistical method can provide an unequivocal apportionment of relative explanatory power when the candidate explanatory variables are themselves correlated (or confounded) one with another. Some key variables in Table A5.1 are relatively easy to disentangle, by virtue of deliberate study design: in particular the key stratification factors of country (of purchase), fresh (or frozen), and whole (or portions). For others, we are at the mercy of the structure of the market: e.g. all 24 Thai samples purchased were of frozen chicken, while 219 out of 222 unwrapped chicken samples were fresh. It would have been logistically, if not physically, impossible to ensure that all factor combinations were neatly balanced and the effects of different factors perfectly separable. However, we can go some way towards disentangling factor effects by applying the following principles:-

- sensibly prioritising inclusion of terms in a model to explain variation in *Salmonella* and *Campylobacter* prevalence,

- interpreting all factor effects in the context of a "full" model that allows each to be gauged after eliminating effects due to other important factors.

6. The inclusion or non-inclusion of explanatory variables in the logistic regression model was ultimately dictated by the statistical significance of their association with *Salmonella* and *Campylobacter* prevalence in retail raw chicken. A forward selection (FS) strategy was employed, using reductions in the unexplained model deviance to gauge the explanatory power of successive factors. The inclusion or non-inclusion of terms was based on a 1% significance rule, assuming deviance reductions to be approximately χ^2 distributed in the case of non-association. The three key stratifiers (factors 1-3 in Table A5.1) were given first refusal, followed by classification factors 4-8 and the quantitative variable *pack weight*. This strategy was designed to minimise spurious entry of less well-conditioned variables into the model: for example it could be misleading to judge the importance of *wrap* without first taking account of the factor *fresh* (with which *wrap* is confounded). The factor *boneless* and the variable *number of portions* were only fitted for chicken portions, while *giblets* and *giblets weight* were fitted only for whole chickens.

7. Another modelling principle was to test all 2-factor interactions between terms accepted into the model. Thus, if *country* and *fresh* were both found to be related to prevalence the *country.fresh* interaction term was also tested to see whether the two factors exert independent effects or whether one effect is modified by the other. One of the benefits of such interaction testing is to assess the extent to which any phenomenon observed at a whole-UK level can be assumed to be applicable separately to each of its four constituent countries.

ANNEX 6

CHARACTERISATION AND DISTRIBUTION OF THE MAIN VARIABLES IN THE SURVEY

The explanatory variable data were coded and distributed as follows:-

1. Country of purchase

Country	Code	Count
England	1	2475
Wales	2	800
Scotland	3	794
N. Ireland	4	797
		4866

2. Fresh/Frozen

Fresh	Code	Count
Yes	1	3614
No	2	1252
		4866

3. Whole/Portions

Whole	Code	Count
Yes	1	1467
No	2	3399
		4866

4. Place of purchase

Place	Code	Count
Shelf	1	4461
Butcher	2	322
Other	3	72
Market	4	11
		4866

Note: “Other” includes four samples entered as “Grocer” and one sample entered as “Farm”.

5. Wrapped (Yes/No)

Wrap	Code	Count
Yes	1	4644
No	2	222
		4866

6. Country of Origin

Origin	Code	Count
UK	1	4075
France	2	259
Holland	3	151
Denmark	4	42
Germany	5	109
Thailand	6	24
Rep. Ireland	7	56
Brazil	8	49
Not specified	9	101
		4866

Note: A single Italian chicken sample was coded as ”Not specified”.
A sample labelled as “Thailand and Brazil” was coded as ”Thailand”.

7. Production Type

Type	Code	Count
Broiler	1	4537
Free Range	2	182
Organic	3	62
Corn Fed	4	85
		4866

Note: Samples declared as “Free range and corn fed” or “Free range and organic” were coded as simply “Free range”. Three samples declared as “Naturally farmed” were coded as “Organic”.

8. Week Number

Samples were coded 1–8 depending on which of the 8 weeks of fieldwork they fell into.

9. Boneless (Yes/No)

Whole	Code	Count
Yes	1	1324
No	2	3542
		4866

10. Giblets

Place	Code	Count
Yes	1	194
Yes – Not intact	2	74
No	3	1197
Not applicable	4	3401
		4866

ANNEX 7

DEFINITION OF STATISTICAL TERMS USED

logistic regression model – a logistic regression model is used where the response variable can take one of two values e.g. yes/no, present/absent. Instead of using the expected value of the response variable in the regression model, e.g. $E(y) = a + bx$, it uses a logistic transformation of p, $\log(p/(1-p)) = a + bx$, where p is the probability of a positive response – in this case, the presence of *Salmonella* or *Campylobacter*. [NB $(\log(p/(1-p))$ is known as logit(p)]

explanatory variables – variables which may help to explain variation in the response variable (the response variable in this case being prevalence of *Salmonella* or *Campylobacter* in raw retail chicken).

deviance – it is unlikely that any model based on the explanatory variables available will exactly fit the observed data. (If it did it would be a 'perfect' model and the fitted values of the response variable would equal those observed.) Deviance is a measure of how much a proposed model differs from the 'perfect' model. If the proposed (current) model is good, the fitted values will be close to the observed values and the deviance will be low.

deviance reduction - the addition of an extra explanatory variable to a non-perfect model will always reduce the deviance by a certain amount, even if only due to the vagaries of chance. The greater the deviance reduction the less likely it is to be attributable to chance and the more likely to be symptomatic of a genuine association. **Statistical significance** (**p-values**), based on the size of the deviance reduction, help to weigh the evidence in this respect. For example $p < 0.01$ implies a probability of less than one per cent that the observed deviance reduction has arisen by chance (giving greater assurance that a repeatable relationship has been revealed).

forward selection (FS) strategy – starting from a model with no parameters included (other than a constant term, if required), variables are added to the model one at a time. If they are found to make a statistically significant improvement to the model fit (deviance reduction) they are retained. In this case variables were included if the decrease in deviance brought about by including the variable was found to be significant at the 1% level (i.e. $p < 0.01$).

statistical significance (or **p-value**) **–** see "deviance reduction" for context-specific explanation.

standard error – standard deviation of the sampling distribution of an estimate.

confounded - two variables are said to be confounded in a particular study if the study design makes it impossible to disentangle their separate contribution to the model. For example, if all frozen samples were of non-UK origin and all imports were frozen then any difference between fresh and frozen might be due to the contrast between UK and non-UK produce. In practise, there tend to be degrees of, rather than absolute, confounding.

2-factor interactions –one factor may modify the effect of another. For example the model $y = ax_1 + bx_2$ does not contain any factor interactions and assumes that the two explanatory variables x_1 and x_2 act independently. However, the model $y = ax_1 + bx_2 + cx_1x_2$, which does contain the interaction between x_1 and x_2, allows for the possibility that the effect of x_1 is modified by x_2 and vice-versa.

degrees of freedom (d.f.) – the number of extra parameters included when a variable is added to the model. For categorical explanatory variables (e.g. production type) the d.f. will be one less than the number of categories, fitting the level in the remaining production type as differences from the level in the first type in the list.

stratifiers - acknowledged features of a population to be surveyed, which should be controlled by analysis or design to ensure balanced and representative results.

ANNEX 8

DISTRIBUTION OF FRESH AND FROZEN SAMPLES PURCHASED FROM MAJOR RETAILERS

Table A8.1 - Number of fresh and frozen chicken samples purchased from the major retailers during the survey.

Store	Fresh	Frozen	Total
ASDA	494	88	582
CO-OP	227	18	245
ICELAND	154	400	554
KWIK SAVE	40	47	87
LIDL	25	19	44
MARKS & SPENCER	110	0	110
MORRISONS	70	11	81
SAFEWAY	333	60	393
SAINSBURY'S	507	255	762
SOMERFIELD	192	15	207
TESCO	782	203	985
WAITROSE	18	3	21

ANNEX 9

CAMPYLOBACTER SUBTYPES ISOLATED DURING THE SURVEY

Campylobacter jejuni

HS Type	No. Isolates	% of Total Isolates
31	183	15.1
13	154	12.7
50	84	6.9
37	48	4.0
18	35	2.9
4	26	2.2
2	25	2.1
9	22	1.8
12	22	1.8
27	22	1.8
1	18	1.5
6	18	1.5
21	16	1.3
60	15	1.2
5	13	1.1
8	12	1.0
19	11	0.9
44	11	0.9
30	8	0.7
55	8	0.7
3	7	0.6
7	7	0.6
16	6	0.5
23	6	0.5
57	5	0.4
67	5	0.4
10	3	0.2
52	3	0.2
11	2	0.2
68	2	0.2
69	2	0.2
28	1	0.1
33	1	0.1
40	1	0.1
42	1	0.1
56	1	0.1
61	1	0.1
Untypeable	404	33.4
Total isolates	**1209**	

Phage type	No. isolates	% of Total Isolates
1	385	31.84
2	181	14.97
44	103	8.52
5	57	4.71
33	40	3.31
8	32	2.65
39	27	2.23
21	25	2.07
35	25	2.07
34	22	1.82
14	21	1.74
19	19	1.57
36	17	1.41
11	10	0.83
67	10	0.83
58	9	0.74
38	7	0.58
25	6	0.50
6	5	0.41
9	4	0.33
17	4	0.33
23	4	0.33
40	4	0.33
80	4	0.33
62	3	0.25
72	3	0.25
7	2	0.17
16	2	0.17
18	2	0.17
20	2	0.17
29	2	0.17
32	2	0.17
41	2	0.17
43	2	0.17
48	2	0.17
4	1	0.08
24	1	0.08
51	1	0.08
63	1	0.08
64	1	0.08
65	1	0.08
68	1	0.08
69	1	0.08
73	1	0.08
75	1	0.08
78	1	0.08
82	1	0.08
RDNC	104	8.60
Untypeable	48	3.97
Total isolates	**1210**	

Campylobacter coli

HS Type	No. Isolates	% of Total Isolates
56	161	38.2
61	44	10.5
28	41	9.7
14	30	7.1
66	24	5.7
59	14	3.3
48	9	2.1
49	6	1.4
26	5	1.2
51	5	1.2
30	4	1.0
34	4	1.0
24	3	0.7
39	1	0.2
42	1	0.2
50	1	0.2
Untypeable	68	16.2
Total isolates	**421**	

Phage type	No. isolates	% of Total Isolates
44	134	31.8
2	134	31.8
7	51	12.1
1	41	9.7
17	5	1.1
8	3	0.7
32	3	0.7
5	2	0.4
19	1	0.2
24	1	0.2
25	1	0.2
33	1	0.2
34	1	0.2
36	1	0.2
39	1	0.2
RDNC	28	6.6
Untypeable	13	3.0
Total isolates	**421**	

ANNEX 10

Table A10.1 - BREAKPOINT CONCENTRATIONS OF ANTIMICROBIALS

Antimicrobial	Abbreviation	Concentration Used (mg/l)	
		Salmonella	*Campylobacter*
Ampicillin	A	8	32
Chloramphenicol	C	8	8
Colistimethate	Co	8	-
Erythromycin	E	-	4
Gentamicin	G	4	4
Kanamycin	K	16	16
Streptomycin	S	16	-
Spectinomycin	Sp	64	-
Sulphonamides	Su	64	-
Tetracycline	T	8 and 128	8
Trimethoprim	Tm	2	-
Neomycin	Ne	4	4*
Nalidixic Acid	Nx	16	16
Ciprofloxacin	Cp	0.125 and 1.0	1.0
Furazolidone	Fu	8**	-

* For *C. jejuni*. Other species of *Campylobacter* have different breakpoints: *C. coli* (8 mg/l), *C. lari* (8 mg/l)
** PHLS LEP Standard Operating Procedure (SOP), January 2001

ANNEX 11. Table A11.1 - Summary of antimicrobial resistance for *Salmonella* isolates from raw retail chicken (April-June 2001)

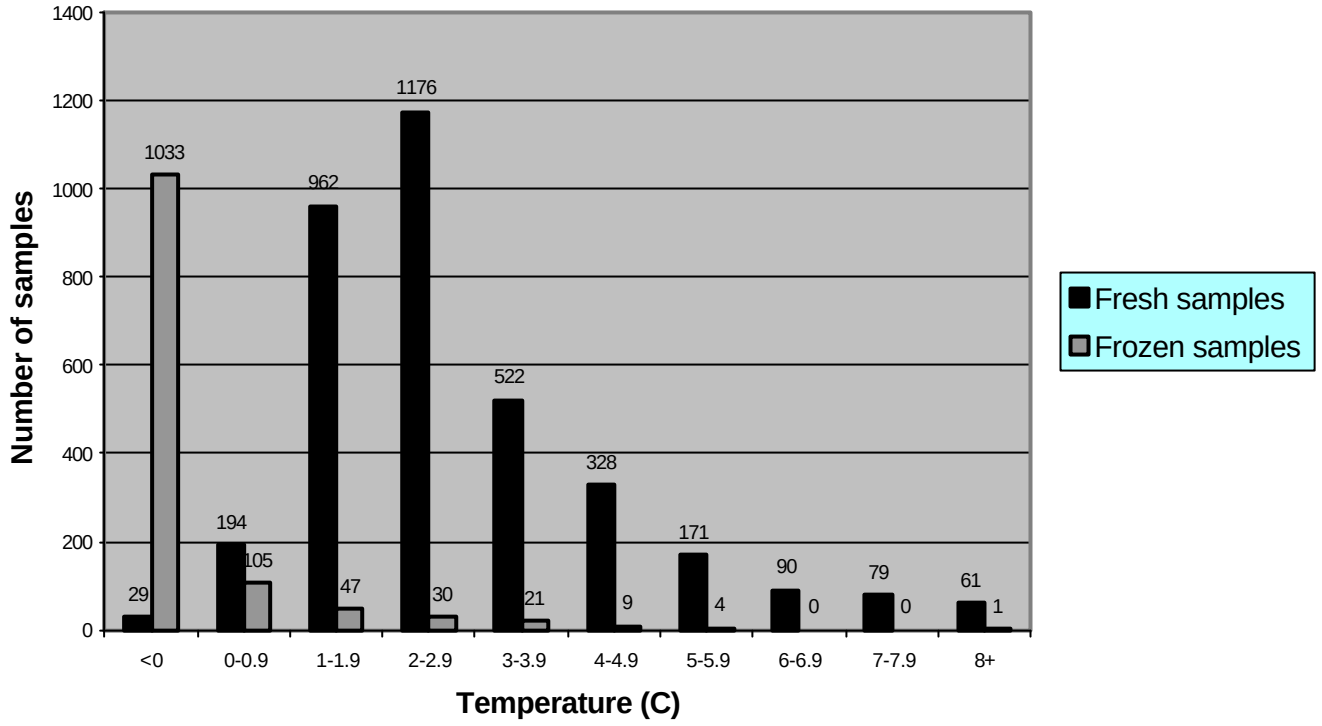
Salmonella			**Antimicrobials tested (number of isolates resistant)**												
SEROTYPE	**Number found**	**Number sensitive**	**A**	**C**	**Co**	**Fu**	**K**	**Ne**	**S**	**Sp**	**Su**	**T**	**Tm**	**Nx**	**Cp**
S. Typhimurium	38	0	36	35	1		1	1	38	38	38	36	5		
S. Heidelberg	34	18	16	2					4	1	8	4	8		
S. Infantis	21	14	2			5			3	2	4	1	2	1	1
S. Ohio	20	7					2	2			12	2	12		
S. Enteritidis	20	16	2											2	2
S. Thompson	18	4	1								13		13	2	2
S. Bovis-morbificans	16	16													
S. Java	11	0	7			11			11	11	5	1	11	2	2
S. Agona	10	5	2	1					2	2	5	3	3		
S. Indiana	8	3							3	3	4	4	3		
S. Kentucky	8	3	1						3	3	5		3		
S. Montevideo	8	8													
S. Virchow	8	1	1			7								3	3
S. Livingstone	7	2							4		5	4	1		
S. Mbandaka	7	6									1			1	1
S. Brandenburg	6	5									1		1		
S. Bredeney	6	5	1	1			1	1	1		1	1	1		
S. Hadar	5	0	4						5			5	1	4	4
S. Derby	4	3									1		1		
S. Senftenberg	3	3													
S. Tennessee	3	3													
S. Kottbus	2	1												1	1
S. Liverpool	2	1									1				
S. Saint-paul	2	0	2						2		2	2	2	2	2
S. Binza	1	0							1	1			1		
S. Cerro	1	1													
S. Manhattan	1	1													
S. Schwarzengrund	1	0												1	1
S. Wagenia	1	0	1			1			1	1	1		1		
S. unnamed	7	4				1			2	2	2	1	1	1	1
Total isolates	**279**	**129**	**76**	**39**	**1**	**25**	**4**	**4**	**80**	**64**	**109**	**64**	**70**	**20**	**20**
% of Total isolates		46.4	27.3	14.0	0.4	9.0	1.4	1.4	28.8	23.0	39.2	23.0	25.2	7.2	7.2

Table A11.2 - Summary of antimicrobial resistance for *Campylobacter jejuni* and *C. coli* isolates from raw retail chicken (April-June 2001).

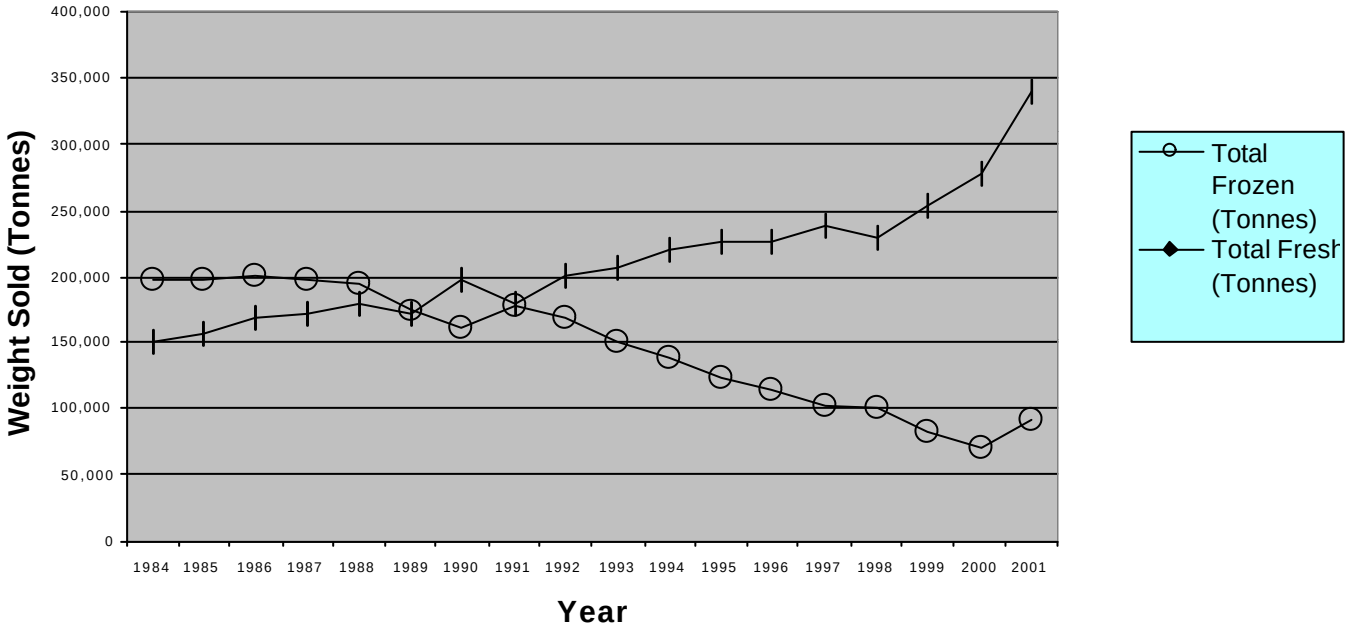
Antimicrobial tested	No. isolates sensitive/resistant (% of total)	
	C. jejuni	***C. coli***
Total number of isolates tested	1210	421
Sensitive to all antimicrobials	605 (50)	240 (57)
Ampicillin **A**	439 (36)	95 (23)
Chloramphenicol **C**	7 (0.1)	2 (0.5)
Erythromycin **E**	3 (0.02)	24 (6)
Gentamicin **G**	0 (0)	2 (0.5)
Kanamycin **K**	65 (5)	15 (4)
Neomycin **Ne**	65 (5)	13 (3)
Tetracycline **T**	333 (26)	107 (25)
Nalidixic Acid **Nx**	192 (16)	72 (17)
Ciprofloxacin **Cp**	158 (13)	64 (15)

ANNEX 12
GRAPHS TO ILLUSTRATE VARIOUS FINDINGS FROM THE SURVEY

Graph 1 - Temperature on receipt for fresh and frozen chicken

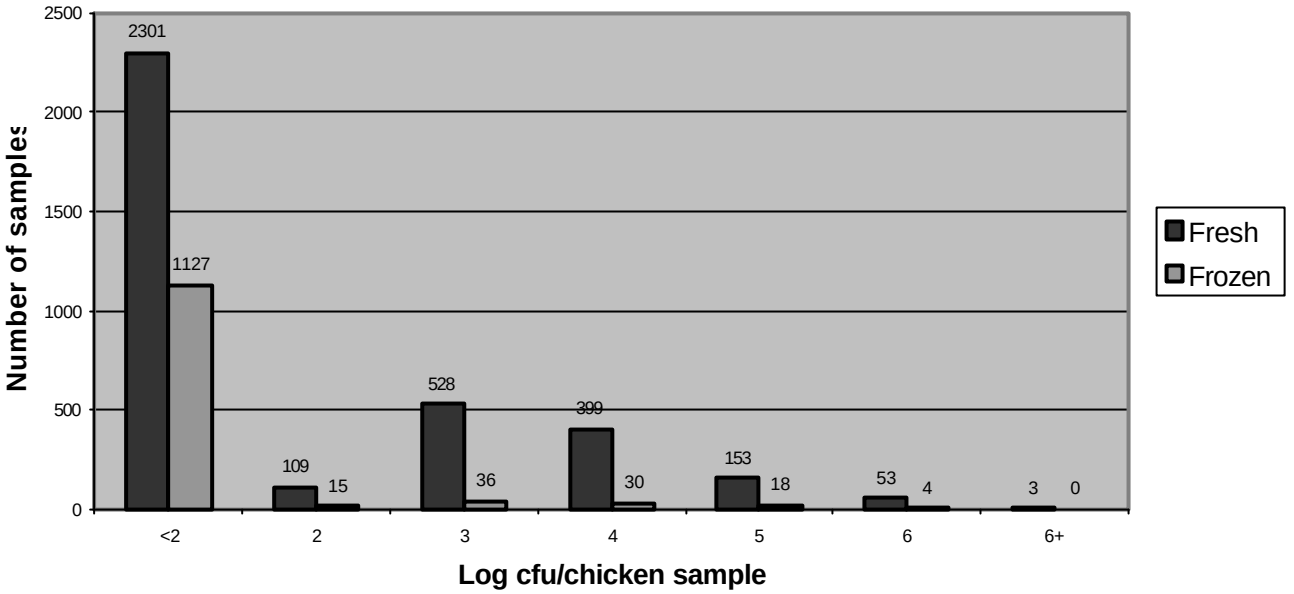


Graph 2 - Sales of Fresh and Frozen Chicken 1984-2001



Source: British Poultry Council *formerly* British Poultry Meat Federation

Graph 3 - Campylobacter Counts on Fresh and Frozen Retail Chicken



ANNEX 13

FSA Retail Chicken Survey Quality Assurance Programme

1. Introduction

The laboratories contracted to undertake the FSA UK wide survey of retail poultry all participate in proficiency testing and achieve satisfactory results. They are accredited for testing Campylobacter and Salmonella, although not by the method or for the matrix specified in the FSA protocol. In surveys where chemical analysis is undertaken it is common practice to include an internal quality control sample with every batch of samples as part of a quality assurance programme to demonstrate the test system is in control. As a check on the performance of laboratories contracted to carry out the chicken survey, and to provide information on the accuracy and precision of the test methods, it was decided to incorporate a Quality Assurance programme into the laboratories during the sample testing period.

2. Preparation of quality assurance check samples

Central Science Laboratory (CSL) York who prepare samples for the Food Examination Performance Assessment Scheme (FEPAS) were contracted to prepare and code the following 6 sets of freeze-dried cultures.

1	Campylobacter for presence/absence	**CP**
2	Salmonella for presence/absence	**SP**
3	Salmonella and Campylobacter for presence/absence	**SC**
4	Campylobacter for enumeration, high level ✳	**CE**
5	Campylobacter for enumeration, low level ✳	**CL**
6	Blank not containing Salmonella or Campylobacter	**B**

✳ The difference between the high and low level was of the order of 2 logs.

All samples contained background microbiological flora and a small amount of chicken solids to simulate a poultry carcass rinse and neck flap sample. Details of how to randomly code the samples was supplied by the Agency and the samples were numbered accordingly by CSL into 25 identical sets of 15. Each set contained duplicates of samples 1, 2 and 3 and triplicates of samples 4, 5 and 6. The samples were stored at 4 °C until dispatch.

3. Testing QA samples

The laboratories undertaking testing of the survey samples were sent a protocol detailing the coding system for the QA samples, and how they were to be tested. A copy of the protocol is given in Annex A. Each laboratory was asked the number of operators who were involved in the laboratory testing of chicken samples and then two sets of samples per operator, QA1 and QA2, were send by overnight courier. QA1 was tested according to the protocol together with chicken survey samples, and QA2 was stored at 4 °C and tested after completion of the survey. QA1 was tested during a period of peak workload compared to QA2, which was tested during a period of lower workload. A set of samples was also tested independently by CSL York at the same time as QA2, as a crosscheck.

The laboratories that took part in the survey were ADAS, SAC Aberdeen and SAC Inverness.

4. Results

4.1 Presence or absence

The results reported for QA1 are given in Annex B and for QA2 in Annex C. Results that did not agree with the sample specification were recorded as false positive or false negative results.

4.1.1 ADAS

The two sets of results for QA1 carried out during the survey recorded the following 5 results that did not agree with the sample specification out of a total of 30 samples examined (17%):

- 2 Salmonella samples gave false negative results (samples A6 and B8)
- 2 blanks gave false positive results for Campylobacter (samples A8 and B6)
- 1 Campylobacter sample gave a false positive result for Salmonella (sample A2)

The two sets of results for QA2 carried out after the completion of the survey were both correct with respect to presence or absence of Salmonella and Campylobacter in both positive and blank samples (total 30 samples).

4.1.2 SAC Aberdeen

The two incomplete sets of results for QA1 recorded the following 3 results that did not agree with the sample specification out of a total of 20 samples examined (15%):

- 1 Campylobacter sample gave a false negative result (sample J7)
- Sample J9 gave a false negative result for Campylobacter and a false positive for Salmonella
- Sample J10 gave a false negative result for Salmonella and a false positive for Campylobacter

One set of results for QA2 was completely correct, the second set recorded the following 2 results that did not agree with the sample specification (total 30 samples):

- Sample L8 gave a false negative result for Campylobacter and a false positive for Salmonella
- Sample L9 gave a false negative result for Salmonella and a false positive for Campylobacter

The results suggest that the two samples J9 and J10 in QA1, and L8 and L9 in QA2, may have been mixed up on examination and this would explain the false positives and false negatives recorded.

4.1.3 SAC Auchincruive

The single set of results for QA1 recorded the following result that did not agree with the sample specification out of a total of 15 samples examined (7%):

- 1 blank sample gave a false positive result for Campylobacter (sample R5)

The single set of results for QA2 was completely correct (total 15 samples).

4.1.4 CSL York

The 3 sets of results from CSL York, carried out at the same time as QA2, recorded the following result that did not agree with the sample specification out of the total of 45 samples tested (2%):

- 1 Campylobacter sample gave a false positive result for Salmonella (sample U5).

4.2 Campylobacter enumeration

The enumeration of Campylobacter appeared to give very variable results in both rounds of QA testing.

When considering data from all three laboratories together, there appeared to be little or no difference between the two Campylobacter enumeration samples CE and CL, even though different levels of the order of 2 log differences were added. In QA1 results were reported for CL from <300 to 4.8 x 10^5 cfu/g (3.5 mean log) and for CE from <300 to 2.3 x 10^5 cfu/g (3.91 mean log). In QA2 results were reported for CL from <300 to 1.2 x 10^4 cfu/g (3.05 mean log) and for CE from <300 to 1.8 x 10^5 cfu/g (3.06 mean log).

Inter- and intra-laboratory differences were seen in the mean counts for the same sample tested by the three laboratories and tested in QA1 and QA2. The data expressed as log and mean log are given in Annex D, but there are too few samples to draw meaningful conclusions. However, it is clear that investigation of the reasons for the variability and further methodological development is required if Campylobacter enumeration is to be carried out in future surveys.

Observations from QA2 in comparison to QA1:

- ADAS failed to isolate Campylobacter for enumeration in either CE or CL samples. However, all samples were positive by enrichment.
- SAC Aberdeen enumerated on average fewer Campylobacter than in the samples tested in QA1.
- Both ADAS and SAC Aberdeen reported difficulties in dissolving the freeze-dried samples in QA2.
- In contrast, SAC Auchincruive enumerated similar numbers on average to those in QA1.
- The average levels enumerated by CSL York were slightly lower than the average levels recorded by SAC Auchincruive but higher than the other two laboratories.

The differences observed between QA1 and QA2 for ADAS and SAC Aberdeen, but not by SAC Auchincruive, could be due to the QA sample shelf life, method of storage (possibly leading to difficulties in preparing initial test suspensions) or, possibly, differences in laboratory methodology. Further work in this area would need to be undertaken to explain the observed differences.

5. Conclusions

- Although a number of apparent errors were recorded in QA1, no systematic error could be detected which would affect the overall results of the poultry survey samples.

- Fewer false positives and false negatives were recorded in the QA2 samples tested after the survey had finished compared to the QA1 samples tested while the survey was ongoing and the laboratory was working at very high capacity (2 compared to 9).

- No false positives were recorded for QA2 blank samples tested after the survey had finished by all four laboratories (including CSL York). In comparison 3 out of 14 blanks examined were recorded as false positives for the QA1 samples tested while the survey was ongoing.

- There is an indication that SAC Aberdeen mixed up 2 samples, possibly by mislabelling, in both rounds of testing.

- The results for both QA1 and QA2 indicate that Campylobacter enumeration of the QA samples was unreliable. Only 0.5 mean log difference was seen between the two levels added to CE and CL despite a difference in the order of 2 logs in the number of Campylobacter added to the samples. The range of values recorded when the same number of Campylobacter were added was considerable (<300 to 4.8 x 10^5 cfu/g). This observation leads to the conclusion that the results of enumeration of Campylobacter in the poultry samples measured in the UK wide survey may be also be unreliable.

- Storage conditions of the samples may have affected the viability of the freeze dried organisms examined by ADAS and SAC Aberdeen who showed a reduction in the level of Campylobacter in QA2 compared to QA1. This was not seen by SAC Auchincruive.

The difference in the level of false positive and false negative results between QA1 and QA2 support the hypothesis that mistakes are more likely to occur in a laboratory examining a high throughput of poultry samples. Similar observations are unlikely to be apparent from normal proficiency testing samples where only one sample is tested and the examination is unlikely to be carried out in a high sample throughput situation.

6. Discussion

The results of this QA exercise highlight the shortcomings of only relying on standard proficiency test schemes to assess the accuracy of laboratories whilst undertaking microbiological surveillance. This would appear to be particularly relevant when a laboratory is processing large numbers of samples where target organisms are being isolated, compared to when only QA test materials are examined. Surveys such as the present one are competitively tendered and these results have highlighted the need to ensure that in the tendering process there is a realistically resourced work plan that includes time to ensure errors due to high throughput are minimised.

The QA results did not indicate any systematic error and do not affect the overall conclusions drawn from the poultry survey. **They do, however, highlight that not every individual result can be guaranteed to be correct. It is recommended that the data are not over-interpreted and are primarily considered in terms of results for groups of products and not at the individual sample level.**

The laboratories were consulted following the disclosure of their QA results and invited to comment.

- ADAS welcomed the results and felt they confirmed their suspicion that under conditions of high throughput there will be a tendency for more errors to occur during testing. In some follow up work they have been able to pinpoint some potential routes of cross contamination and have taken the appropriate corrective actions.
- SAC Aberdeen were convinced the false positive and false negative results that looked like mislabelling would only occur during the resuscitation of freeze-dried vials and not with poultry samples. However, since all procedures are undertaken within the QA procedures of the laboratory, any mislabelling indicates a serious problem that needs addressing.
- SAC Auchincruive were concerned that the cross contaminating isolates in the QA samples were not typed and therefore the possibility that they had been introduced during preparation of the QA samples could not be ruled out. However, there were clear differences between QA1 and QA2 and this tended to rule out the possibility that the samples were contaminated during preparation.

The Agency is unaware of any other published surveillance where a comparable QA programme has been incorporated. It is hoped that the results of this study will raise awareness of the potential difference between results from proficiency testing schemes and the accuracy of a laboratory when undertaking a high throughput of samples where the target organism is being isolated.

The Agency does not have any evidence to suggest that the laboratories concerned were under performing, and has no reason to believe that other laboratories would return different results. This is illustrated by the three sets of data from CSL, which were produced during a short period of time simulating a relatively high throughput but without a workload of product samples where the target organisms were being isolated. Nevertheless, one false positive result was recorded.

This exercise has proved to be very useful and educational in terms of how surveillance studies should be designed, resourced and undertaken. The balance between using a larger number of laboratories with the variation that entails and ensuring no laboratory is over stretched in terms of capacity must be considered.

Annex A

Protocol for the examination of QA test materials for the Food Standards Agency Retail Poultry Survey 2001

Sets of QA test materials will be dispatched from CSL York by overnight courier on 23rd May to arrive in participating laboratories on 24th May.

- On receipt the test materials must be stored in a refrigerator at 4 ºC until examination. Materials stored in this way will be stable for at least one month.
- Each set of 15 test materials is coded with an alphabetic number followed by a numerical number 1-15.
- Each operator must examine all 15 test materials in **one** alphabetic set i.e. operator one test materials A1 - A15. Operator 4 test materials D1-D15.
- Each Laboratory will be sent two sets of test materials per operator but each operator will initially examine **one** set of test materials. The additional set is supplied in case of problems. Please contact Mary Howell at the Food Standards Agency mary.howell@foodstandards.gsi.gov.uk if problems arise.
- The test materials must be **examined in numerical order over a period of several days together with survey chicken samples**. The period of examination should include days with high sample throughput.
- Ideally 1-3 test materials will be examined per day but **on no account must all 15 test materials be examined on the same day.**

Laboratory procedure

The test materials contain freeze dried organisms prepared to be added to 300ml Buffered Peptone Water (BPW) as described at step 28 of the survey protocol. The following details the steps to be followed to introduce the test materials to the test protocol.

- Record the test material codes, and identify the operator and date of examination on the results sheet.
- Remove the metal cap from the vial containing the test material by lifting the metal tab at the base of the arrow on the cap. A pair of small pliers can be used if required.
- Aseptically remove the grey vial stopper and add 5 ml of BPW taken from 300 ml BPW. Recap the vial and leave to resuscitate at room temperature for 5 minutes.
- Aseptically pour the vial contents into the remaining 295 ml BPW then rinse out the vial with at least 3 x 5 ml volumes taken from the 300 ml BPW.
- Pour the 300 ml BPW containing the test material into a stomacher bag and stomach for 2 minutes as described at step 29 of the survey protocol.
- Proceed with steps 30-32 of the protocol.

Campylobacter

Enumeration

- Follow steps 33-35 of the survey protocol and report any enumeration on the standard hard copy results sheet for the survey. For calculation of results use a test material equivalent weight of 1.5 kg.

Enrichment

- Follow steps 39-42 of the survey protocol but do not store isolates.

***Salmonella* Enrichment**

- Follow steps 43-46 of the survey protocol

Enter all results obtained on the standard results form designed to be used in the survey. Copy the results and send to the Food Standards Agency. **Do not enter QA results onto the excel spreadsheet used for chicken survey results**

Mary Howell
Data Quality Unit
Food Standards Agency
Room 715C
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ANNEX B

FSA Chicken Survey Quality Assurance Programme Results Set One – QA1

ADAS Laboratories

Sample ref.	Sample content	Agreement P/A	Salmonella P/A	Campylobacter P/A	Campylobacter cfu/carcass
A1	CP	Y	Absent	Present	300
A2	CE	**N**	**Present ***	Present	27000
A3	SC	Y	Present	Present	<300
A4	CL	Y	Absent	Present	<300
A5	CL	Y	Absent	Present	900
A6	SC	**N**	**Absent ***	Present	<300
A7	CP	Y	Absent	Present	18000
A8	B	**N**	Absent	**Present ***	**900**
A9	CE	Y	Absent	Present	<300
A10	CL	Y	Absent	Present	480000
A11	SP	Y	Present	Absent	<300
A12	B	Y	Absent	Absent	<300
A13	SP	Y	Present	Absent	<300
A14	CE	Y	Absent	Present	230000
A15	B	Y	Absent	Absent	<300
B1	CL	Y	Absent	Present	68000
B2	SC	Y	Present	Present	<300
B3	B	Y	Absent	Absent	<300
B4	SP	Y	Present	Absent	<300
B5	CP	Y	Absent	Present	<300
B6	B	**N**	Absent	**Present ***	**2400**
B7	CE	Y	Absent	Present	<300
B8	SC	**N**	**Absent ***	Present	<300
B9	CP	Y	Absent	Present	3600
B10	CE	Y	Absent	Present	<300
B11	CE	Y	Absent	Present	93000
B12	CL	Y	Absent	Present	16000
B13	SP	Y	Present	Absent	<300
B14	B	Y	Absent	Absent	<300
B15	CL	Y	Absent	Present	6900

SAC Aberdeen

Sample ref.	Sample content	Agreement P/A	Salmonella P/A	Campylobacter P/A	Campylobacter cfu/carcass
I1	SC	Y	Present	Present	3000
I2	B	Y	Absent	Absent	<300
I3	SP	Y	Present	Absent	<300
I4	CL	Y	Absent	Present	1500
I5	B	Y	Absent	Absent	<300
I6	CP	Y	Absent	Present	5700
I7	CE	Y	Absent	Present	22800
I8	B	Y	Absent	Absent	<300
I9	SP	Y	Present	Absent	<300
I10	CE	Y	Absent	Present	11400
J1	B	Y	Absent	Absent	<300
J2	CL	Y	Absent	Present	2100
J3	SC	Y	Present	Present	300
J4	B	Y	Absent	Absent	<300
J5	SC	Y	Present	Present	600
J6	B	Y	Absent	Absent	<300
J7	CP	**N**	Absent	**Absent ***	**<300**
J8	CE	Y	Absent	Present	600
J9	CL	**N**	**Present***	**Absent ***	**<300**
J10	SP	**N**	**Absent ***	**Present ***	**300**

SAC Auchincruive

Sample ref.	Sample content	Agreement P/A	Salmonella P/A	Campylobacter P/A	Campylobacter cfu/carcass
R1	CE	Y	Absent	Present	60000
R2	SP	Y	Present	Absent	<300
R3	B	Y	Absent	Absent	<300
R4	CL	Y	Absent	Present	1800
R5	B	**N**	Absent	**Present ***	**53400**
R6	CE	Y	Absent	Present	17700
R7	SC	Y	Present	Present	<300
R8	CP	Y	Absent	Present	<300
R9	CL	Y	Absent	Present	3300
R10	SP	Y	Present	Absent	<300
R11	B	Y	Absent	Absent	<300
R12	SC	Y	Present	Present	900
R13	CL	Y	Absent	Present	<300
R14	CP	Y	Absent	Present	<300
R15	CE	Y	Absent	Present	30000

In the tables samples where the results are not as expected are in bold and marked with *****

ANNEX C

FSA Chicken Survey Quality Assurance Programme Results – Set Two (QA2)

ADAS Laboratories

Sample ref.	Sample content	Agreement P/A	Salmonella P/A	Campylobacter P/A	Campylobacter cfu/carcass
C1	CE	Y	Absent	Present	<300
C2	SP	Y	Present	Absent	<300
C3	SP	Y	Present	Absent	<300
C4	CL	Y	Absent	Present	<300
C5	CL	Y	Absent	Present	<300
C6	CP	Y	Absent	Present	<300
C7	SC	Y	Present	Present	<300
C8	B	Y	Absent	Absent	<300
C9	CE	Y	Absent	Present	<300
C10	CP	Y	Absent	Present	<300
C11	SC	Y	Present	Present	<300
C12	CE	Y	Absent	Present	<300
C13	B	Y	Absent	Absent	<300
C14	CL	Y	Absent	Present	<300
C15	B	Y	Absent	Absent	<300
D1	SC	Y	Present	Present	<300
D2	CE	Y	Absent	Present	<300
D3	CP	Y	Absent	Present	<300
D4	B	Y	Absent	Absent	<300
D5	SP	Y	Present	Absent	<300
D6	CL	Y	Absent	Present	<300
D7	CE	Y	Absent	Present	<300
D8	SC	Y	Present	Present	<300
D9	B	Y	Absent	Absent	<300
D10	SP	Y	Present	Absent	<300
D11	B	Y	Absent	Absent	<300
D12	CL	Y	Absent	Present	<300
D13	CE	Y	Absent	Present	<300
D14	CP	Y	Absent	Present	<300
D15	CL	Y	Absent	Present	<300

SAC Aberdeen

Sample ref.	Sample content	Agreement P/A	Salmonella P/A	Campylobacter P/A	Campylobacter cfu/carcass
K1	CE	Y	Absent	Present	300
K2	SP	Y	Present	Absent	<300
K3	CE	Y	Absent	Present	300
K4	SC	Y	Present	Present	<300
K5	CP	Y	Absent	Present	600
K6	CL	Y	Absent	Present	<300
K7	B	Y	Absent	Absent	<300
K8	CP	Y	Absent	Present	<300
K9	SC	Y	Present	Present	900
K10	B	Y	Absent	Absent	<300
K11	CL	Y	Absent	Present	<300
K12	SP	Y	Present	Absent	<300
K13	CE	Y	Absent	Present	1800
K14	B	Y	Absent	Absent	<300
K15	CL	Y	Absent	Present	<300
L1	B	Y	Absent	Absent	<300
L2	CE	Y	Absent	Present	300
L3	SC	Y	Present	Present	<300
L4	CL	Y	Absent	Present	300
L5	B	Y	Absent	Absent	<300
L6	SP	Y	Present	Absent	<300
L7	CL	Y	Absent	Present	<300
L8	CE	**N**	**Present***	**Absent***	**<300**
L9	CP	Y	Absent	Present	<300
L10	SP	**N**	**Absent***	**Present***	**<300**
L11	B	Y	Absent	Absent	<300
L12	CL	Y	Absent	Present	300
L13	CP	Y	Absent	Present	300
L14	CE	Y	Absent	Present	3300
L15	SC	Y	Present	Present	300

SAC Auchincruive

Sample ref.	Sample content	Agreement P/A	Salmonella P/A	Campylobacter P/A	Campylobacter cfu/carcass
T1	CP	Y	Absent	Present	4500
T2	CE	Y	Absent	Present	27000
T3	CL	Y	Absent	Present	9900
T4	B	Y	Absent	Absent	<300
T5	CE	Y	Absent	Present	60000
T6	CP	Y	Absent	Present	300
T7	CL	Y	Absent	Present	9300
T8	B	Y	Absent	Absent	<300
T9	SC	Y	Present	Present	3000
T10	CL	Y	Absent	Present	12000
T11	SP	Y	Present	Absent	<300
T12	CE	Y	Absent	Present	180000
T13	B	Y	Absent	Absent	<300
T14	SC	Y	Present	Present	3000
T15	SP	Y	Present	Absent	<300

CSL York

Sample ref.	Sample content	Agreement P/A	Salmonella P/A	Campylobacter P/A	Campylobacter cfu/carcass
U1	B	Y	Absent	Absent	<300
U2	CP	Y	Absent	Present	<300
U3	SC	Y	Present	Present	<300
U4	CE	Y	Absent	Present	15600
U5	CL	**N**	**Present***	Present	2700
U6	SP	Y	Present	Absent	<300
U7	SP	Y	Present	Absent	<300
U8	CL	Y	Absent	Present	1500
U9	B	Y	Absent	Absent	<300
U10	SC	Y	Present	Present	1200
U11	CE	Y	Absent	Present	13200
U12	CP	Y	Absent	Present	2400
U13	CE	Y	Absent	Present	13800
U14	B	Y	Absent	Absent	<300
U15	CL	Y	Absent	Present	2100
V1	CL	Y	Absent	Present	2100
V2	SC	Y	Present	Present	<300
V3	CE	Y	Absent	Present	4800
V4	B	Y	Absent	Absent	<300
V5	SP	Y	Present	Absent	<300
V6	CL	Y	Absent	Present	3000
V7	B	Y	Absent	Absent	<300
V8	CE	Y	Absent	Present	1200
V9	CP	Y	Absent	Present	3600
V10	SP	Y	Present	Absent	<300
V11	CP	Y	Absent	Present	10200
V12	CL	Y	Absent	Present	<300
V13	SC	Y	Present	Present	600
V14	CE	Y	Absent	Present	57000
V15	B	Y	Absent	Absent	<300

Sample ref.	Sample content	Agreement P/A	Salmonella P/A	Campylobacter P/A	Campylobacter cfu/carcass
W1	B	Y	Absent	Absent	<300
W2	B	Y	Absent	Absent	<300
W3	SP	Y	Present	Absent	<300
W4	CE	Y	Absent	Present	7500
W5	CP	Y	Absent	Present	600
W6	CL	Y	Absent	Present	900
W7	CE	Y	Absent	Present	4800
W8	SC	Y	Present	Present	1800
W9	CL	Y	Absent	Present	1500
W10	CE	Y	Absent	Present	1800
W11	SC	Y	Present	Present	600
W12	CL	Y	Absent	Present	7200
W13	SP	Y	Present	Absent	<300
W14	CP	Y	Absent	Present	<300
W15	B	Y	Absent	Absent	<300

In the tables samples where the results are not as expected are in bold and marked with *****

ANNEX D

FSA Chicken Survey Quality Assurance Enumeration of Campylobacter Results

QA1

ADAS Laboratories

	CE		CL	
	cfu/g	log cfu/g	cfu/g	log cfu/g
	27000	4.43	<300	2.48
	<300	2.48	900	2.95
	230000	5.36	480000	5.48
	<300	2.48	68000	4.83
	<300	2.48	16000	4.20
	93000	4.96	6900	3.84
MEAN		3.70		3.58
Difference			0.12	

SAC Aberdeen

	CE		CL	
	cfu/g	log cfu/g	cfu/g	log cfu/g
	600	2.78	1500	3.17
	11400	4.06	2100	3.32
	22800	4.36	<300	2.48
MEAN		3.73		2.99
Difference			0.74	

SAC Auchincruive

	CE		CL	
	cfu/g	log cfu/g	cfu/g	log cfu/g
	30000	4.48	1800	3.26
	17700	4.25	3300	3.52
	60000	4.78	<300	2.48
MEAN		5		3.08
Difference			1.42	

Overall Mean	3.91	3.50
Overall Difference	0.41	

QA2

ADAS Laboratories

	CE		CL	
	cfu/g	log cfu/g	cfu/g	log cfu/g
	<300	2.48	<300	2.48
	<300	2.48	<300	2.48
	<300	2.48	<300	2.48
	<300	2.48	<300	2.48
	<300	2.48	<300	2.48
	<300	2.48	<300	2.48
MEAN		2.48		2.48
Difference			0.00	

SAC Aberdeen

	CE		CL	
	cfu/g	log cfu/g	cfu/g	log cfu/g
	<300	2.48	<300	2.48
	<300	2.48	300	2.48
	1800	3.26	<300	2.48
	<300	2.48	300	2.48
	3300	3.52	300	2.48
	<300	2.46	<300	2.48
MEAN		2.78		2.48
Difference			0.30	

SAC Auchincruive

	CE		CL	
	cfu/g	log cfu/g	cfu/g	log cfu/g
	27000	4.43	9900	4.00
	60000	4.78	9300	3.97
	180000	5.26	12000	4.01
MEAN		4.82		3.99
Difference			0.83	

Overall Mean	3.06	3.05
Overall Difference	0.01	

CSL York

	CE		CL	
	cfu/g	log cfu/g	cfu/g	log cfu/g
	15600	4.19	7200	3.86
	57000	4.76	1500	3.18
	1200	3.08	900	2.95
	4800	3.68	<300	2.48
	13800	4.14	3000	3.48
	1800	3.26	2100	3.32
	4800	3.68	2100	3.32
	7500	3.88	1500	3.18
	13200	4.12	2700	3.43
MEAN		3.86		3.24
Difference			0.62	

REFERENCES

Advisory Committee on the Microbiological Safety of Food (ACMSF). Report on Poultry Meat. HMSO, London. 1996.

Anon. Parallel food testing in the EU Part 1: Main Report. Chicken. International Consumer Research and Testing. London. September 1994.

Atanassova V and Ring C. Prevalence of *Campylobacter* spp. in poultry and poultry products in Germany. Int J Food Microbiol 1999; **51(2-3)**: 187-190.

Dominguez C, Gomez I and Zumalacarregui J. Prevalence of *Salmonella* and *Campylobacter* in retail chicken meat in Spain. Int J Food Microbiol 2002; **72(1-2)**: 165-168.

Dufrenne J, Ritmeester W, Delfgou-van Asch E, van Leusden F and de Jonge R. Quantification of the contamination of chicken and chicken products in the Netherlands with *Salmonella* and *Campylobacter*. J Food Protect 2001; **64(4)**: 538-541.

Flynn OMJ, Blair IS and McDowell DA. Prevalence of *Campylobacter* spp. on fresh retail chicken wings in Northern Ireland. J Food Protect 1994; **57(4**): 334-336.

Frost JA, Oza AN, Thwaites RT and Rowe B. Serotyping scheme for *Campylobacter jejuni* and *Campylobacter coli* based on direct agglutination of heat stable antigens. J Clin Microbiol 1998; **36**: 335-339.

Frost JA, Kramer JM and Gillanders SA. Phage typing of *C. jejuni and C. coli.* Epidemiol Infect 1999; **123**: 47-55.

Harrison WA, Griffith CJ, Tennant D and Peters AC. Incidence of *Campylobacter* and *Salmonella* isolated from retail chicken and associated packaging in South Wales. Lett Appl Microbiol 2001; **33(6)**: 450-454.

Hood AM, Pearson AD and Shahmat M. The extent of surface contamination of retailed chickens with *Campylobacter jejuni* serogroups. Epidemiol Infect 1988; **100(1)**: 17-25.

Kramer JM, Frost JA, Bolton FJ and Wareing DRA. *Campylobacter* contamination of raw meat and poultry at retail sale: Identification of multiple types and comparison with isolates from human infection. J Food Protect 2000; **63 (12)**: 1654-1659.

Jørgensen F, Bailey R, Williams S, Henderson P, Wareing DRA, Bolton FJ, Frost JA, Ward L and Humphrey TJ. Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. Int J Food Micro 2002; **76**: 151-164.

Moore JE, Wilson TS, Wareing DR, Humphrey TJ and Murphy PG. Prevalence of thermophilic *Campylobacter* spp. in ready-to-eat foods and raw poultry in Northern Ireland. J Food Protect 2002; **65(8)**: 1326-1328.

Ono K and Yamamoto K. Contamination of meat with *Campylobacter jejuni* in Saitama, Japan. Int J Food Microbiol 1999; **47(3)**: 211-219.

Oza AN, Thwaites RT, Wareing DRA, Bolton FJ, and Frost JA. Detection of heat stable antigens of *Campylobacter jejuni* and *Campylobacter coli* by direct agglutination and passive haemagglutination. J Clin Microbiol 2002; **40**: 996-1000.

Pearson AD, Greenwood M, Healing TD, Rollins D, Shahamat M, Donaldson J and Colwell RR. Colonization of broiler chickens by waterborne *Campylobacter jejuni*. Appl Environ Microbiol 1993; **59(4)**: 987-996.

Threlfall EJ, Fisher IS, Ward LR, Tschape H and Gerner-Smidt P. Harmonization of antibiotic susceptibility testing for *Salmonella*: results of a study by 18 national reference laboratories within the European Union-funded Enter-net group. Microb Drug Resist 1999; **5(3)**: 195-200.

Thwaites RT and Frost JA. Drug resistance in *C. jejuni, C. coli and C. lari* isolated from humans in England and Wales. J Clin Pathol 1999; **52**: 812-814.

Uyttendaele M, de Troy P and Debevere J. Incidence of *Salmonella*, *Campylobacter jejuni*, *Campylobacter coli* and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgium retail market. J Food Protect 1999; **62(7)**: 735-740.

Zhao C, Ge B, De Villena J, Sudler R, Yeh E, Zhao S, White DG, Wagner D and Meng J. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. Appl Environ Microbiol 2001; **67(12)**: 5431-5436.