

**ANNEXES TO THE
REPORT OF THE SURVEY OF *SALMONELLA*
CONTAMINATION OF UK PRODUCED SHELL
EGGS ON RETAIL SALE**

ANNEX A: RETAIL EGG SURVEY SAMPLING PLAN

A.1. Available Information

A.1.1. The information made available to construct the sampling plan was obtained from Taylor Nelson Sofres (TNS) and relates to the 52 weeks ending 10th Jan 2003. This is outlined below.

A.1.2. Data were available for the overall market share for:

- Each retail outlet in Great Britain (**Table A.1.**).
- Each retail outlet in Scotland (**Table A.2.**).
- Each retail outlet in Wales + Gloucestershire (**Table A.3.**).
- Each retail outlet in Northern Ireland (**Table A.4.**).

A.1.3. TNS uses the BARD TV regions in which to base their data on. One of which is an area known as “Wales and West”. By looking closely at this region, it was best to label this as Wales and Gloucester, as the “west” appeared to encompass all of the Gloucestershire area.

Market share data for each egg type were available for:

- Seven supermarket chains for all four egg types in Great Britain (**Table A.5.**).
- Northern Ireland for barn, free range and caged eggs (**Table A.6.**).

A.2. General Assumptions

A.2.1. The sampling plan was to be accessible at the country level i.e. England, Wales, Scotland and Northern Ireland, but due to the information available some assumptions had to be made.

- The market share of eggs in England is equivalent to that in Great Britain, since England represents 85% of the total egg consumption in Great Britain.

A.3. Values that need Estimating

A.3.1. The final survey sample plan could not be produced until all of the appropriate proportions for the market shares for each egg production type had been obtained or estimated. Those that had to be estimated are outlined below.

Overall market share data were estimated for:

- Each retail outlet Wales (Excluding the effect Gloucestershire has on the proportions of the Welsh market shares)

A.3.2. Market share data for each egg type were estimated for:

- The remaining outlets for Great Britain.
- All retail outlets in Scotland.
- All retail outlets in Wales.

- Organic market share for all retail outlets for Northern Ireland.

A.4. Estimation of the market share of each retail outlet in Wales (to exclude Gloucestershire)

A.4.1. The proportion of the retail market share for Wales alone (excluding Gloucestershire) were estimated and the following methodology was used. It was assumed that all information available for Great Britain was applicable to Gloucestershire. This means that retail outlet market shares, egg consumption and the pattern of consumption are assumed to be the same for Gloucestershire and Great Britain.

A.4.2. Using population figures from the 2001 Census, the overall population of *Wales + Gloucester* is 3,510,599 (2,946,000 from Wales and 564,599 from Gloucester) (**Table A.7.**).

A.4.3. On average consumers from Wales eat 1.81 eggs per week whilst those from England consume 1.73 (**Table A.7.**). Therefore, the overall weekly egg consumption for *Wales + Gloucester* is 6,309,016 (2,946,000*1.81 from Wales and 564,599*1.73 from Gloucester). Within this region, *Wales + Gloucester*, the Welsh make up 84.52% ($=2,946,000*1.81/6,309,016$) of the weekly egg consumption, whilst those from Gloucester make up the remainder of 15.48%.

A.4.4. Using the proportion of 84.52% calculated in point (1.3), the individual retail market shares are in Wales alone, rather than for *Wales + Gloucester* (as in **Table A.3.**) was determined. This was achieved by multiplying the existing information on each outlet by 84.52%. For example, for ASDA we have $17\%*0.8452 = 14.37\%$, that is ASDA has a 14.37% share of the Welsh egg market. By calculating this for all retail outlets we obtained the overall Welsh egg market, however we still need to remove the “effect” Gloucestershire has. By this we mean the individual retailers market share proportions that are observed within Gloucestershire that may not be the same as in Wales. We believe the market shares for Gloucester would have been the same as those observed in England and therefore in Great Britain.

A.4.5. A similar approach was taken as in point (A.4.4), to determine the effect that Gloucestershire may have on Wales. The proportion of weekly egg consumption in Gloucester multiplied the market in Great Britain, i.e. multiplying the Great Britain market share by 0.1548 (see point (A.4.3)). So for example, ASDA was $15.60\%*0.1548 = 2.42\%$, that is 2.42% of the ASDA market share comes from Gloucester.

A.4.6. We can remove this Gloucester “effect” by subtracting these proportions from the Wales market share calculated in (1.4). So for example, for ASDA was $14.37\% - 2.42\% = 11.95\%$ (**Table A.8.**, column 3).

A.4.7. Due to the calculations above, the total percentages do not amount to 100%; therefore it is necessary to carry out an adjustment. This was achieved by dividing each

retail's market share value by the total sum of the unstandardised figures, which was 68.87% (see **Table A.8.**, column 3). For example, ASDA was $11.95\%/68.87\% = 17.36\%$, these corrected market share figures can be found in **Table A.8.** Estimation of the market share by egg type for the remaining retail outlets in Great Britain.

A.5.1. The values for ASDA, Co-op, Morrisons, Sainsburys, Somerfield and Tesco were available from the TNS (which were labelled as the main chains throughout this report). The sum of the market shares for these seven retail outlets equals 62.40% for barn eggs, 62.40% for free range eggs, 84.20% for caged eggs and 81.10% for organic eggs (**Table A.5**). These main chains also account for 70.00% of the overall market share (derived from the summation of their overall market shares in **Table A.1.**).

A.5.2. It was assumed that the proportions observed in the overall market shares would be reflected in the remaining market share for each egg type. This means that if a retail outlet has a small percentage of the overall market share it will have an equivalent small proportion in each type of egg market share.

A.5.3. The remaining retail outlets accounted for 30% of the overall egg market and 37.6%, 37.6%, 15.8% and 18.9% for the barn, free range, caged and organic eggs respectively (derived using the information from point (A.2.1)). In order to calculate the market shares for these retailers and to make sure that these and the main chains accounted for 100%, both the overall figures and egg type figures were used. As these remaining retailers accounted for only 30% of the overall market share, each figure from Table 1 was first divided by 30% so that their summation was equal to 100%. The data was then multiplied by the total amount that these remaining retailers contributed to each egg type so in the case of free range eggs by 37.6%. This ensured that the proportions observed within the overall market share were accounted for, whilst the total amount these remaining retailers accounted for, for each egg type did not exceed the figures outlined above. So for example, the Iceland free range egg market share is, $1.8\%/30\% = 6\% * 37.6\% = 2.26\%$. This data can be found in Table A9. Estimation of the market share by egg type for all retail outlets in Scotland.

A.6.1. It was assumed that egg type market share in Scotland was equivalent to Great Britain.

A.6.2. The egg type market share for each retail outlet in Scotland was calculated by multiplying the overall Scottish market share of a retail outlet by the ratio of Great Britain egg type and overall Great Britain market share. For example, for ASDA it has an overall market share for Scotland of 21.62%, an overall market share for Great Britain of 15.60% and a market share of barn eggs of 7.40%. So the ASDA barn egg market share is equal to $21.62\% * 7.40\% / 15.60\% = 10.26\%$. The values that were calculated were then standardised to make sure the sum of all retail outlets was 100% (**Table A.10**).

A.7. Estimation of the market share by egg type for all retail outlets in Wales.

A.7.1. A repetition of the same process in section 3 was carried out for Wales. (**Table A.11**).

A.8. Estimation of the organic market share for all retail outlets in Northern Ireland.

A.8.1. It was assumed that the consumption pattern across the different retail outlets across the different types of eggs was equivalent between Northern Ireland and Great Britain.

A.8.2. It was also assumed that there is a relationship between the free range, caged and barn egg consumption market and the organic egg consumption market. Such that it was expected that the organic egg market share in a retail outlet was dependent on the market shares of the other egg types in the same retail outlet.

A.8.3. The data supplied by the TNS for the four eggs types for all different retail outlets in Great Britain (see **Table A.5.**) was used to estimate what were the optimal weights for barn, free range and caged in order to predict the organic egg consumption.

A.8.4. The consumption data from the barn, caged and free range eggs was deemed to be a good estimator of the organic egg market share across different retail outlets with an accuracy of 88%. The weights were: - 0.37 for barn eggs, 1.3 for free range eggs and - 0.14 for caged eggs.

A.8.5. By multiplying each Northern Ireland retail outlet market share for each egg type by the estimated weights the organic market share for each retail outlet could be calculated. These values were then standardised so the sum was equal to 100% (**Table A.12.**).

Table A.1.: Overall egg market share for each retail outlet in Great Britain

Retail Outlet	Market Share
Asda	15.60%
Budgen	0.40%
Butchers	2.40%
Co-op	2.90%
Farm shops	1.30%
Greengrocers	1.90%
Iceland	1.80%
Independent*	2.90%
Kwik Save	2.90%
M&S	0.90%
Markets	2.70%
Milkman	2.70%
Morrisons	6.30%
Safeway	7.00%
Sainsburys	13.20%
Somerfield	2.20%
Spar	0.40%
Tesco	22.80%
Waitrose	2.00%
Others (e.g. dept stores)**	7.70%

*Includes V.G., Mace/Wavy Line and Londis

** Includes Farm Foods, Capital Foods, Wm. Low, Aldi, Shoprite, Netto, Ed, Lidl and Normans.

Table A.2.: Overall egg market share for each retail outlet in Scotland

Retail Outlet	Market Share
Asda	21.62%
Budgen	0.00%
Butchers	2.42%
Co-op	4.92%
Farm shops	1.72%
Greengrocers	1.02%
Iceland	1.52%
Independent*	5.02%
Kwik Save	3.82%
M&S	0.72%
Markets	0.82%
Milkman	0.22%
Morrisons	0.62%
Safeway	14.52%
Sainsburys	3.12%
Somerfield	4.52%
Spar	1.32%
Tesco	23.12%
Waitrose	0.00%
Others (e.g. dept stores)**	8.92%

*Includes V.G., Mace/Wavy Line and Londis

** Includes Farm Foods, Capital Foods, Wm. Low, Aldi, Shoprite, Netto, Ed, Lidl and Normans.

Table A.3.: Overall egg market share for each retail outlet in *Wales + Gloucester*

Retail Outlet	Market Share
Asda	17.00%
Budgen	0.20%
Butchers	2.90%
Co-op	3.50%
Farm shops	0.80%
Greengrocers	2.20%
Iceland	2.60%
Independent*	3.40%
Kwik Save	6.00%
M&S	0.70%
Markets	2.30%
Milkman	2.90%
Morrisons	1.60%
Safeway	7.00%
Sainsburys	9.80%
Somerfield	3.30%
Spar	0.50%
Tesco	25.80%
Waitrose	0.40%
Others (e.g. dept stores)**	6.90%

*Includes V.G., Mace/Wavy Line and Londis

** Includes Farm Foods, Capital Foods, Wm. Low, Aldi, Shoprite, Netto, Ed, Lidl and Normans.

Table A.4.: Overall egg market share for each retail outlet in Northern Ireland

Retail Outlets	Market Share
Butchers	0.21%
Centra	0.61%
Co-op	4.21%
Cost Cutters	1.71%
Dunnes	1.71%
Greengrocers	0.51%
Iceland	6.41%
M&S	1.21%
Mace	5.51%
Safeway	5.51%
Sainsburys	11.11%
Spar/Vivo	6.81%
SuperValue L&N	8.11%
Tesco	38.21%
Others (e.g. dept stores)	8.21%

Table A.5.: Egg market share by type for the 7 largest retail outlets in Great Britain.

Retail Outlet	Barn	Free Range	Caged	Organic
Asda	7.40%	8.40%	18.90%	5.90%
Co-op	0.20%	5.00%	4.40%	4.30%
Morrisons	0.40%	3.80%	8.60%	1.20%
Safeway	17.20%	7.40%	5.90%	9.60%
Sainsburys	2.00%	16.10%	16.50%	35.40%
Somerfield	0.40%	2.80%	8.20%	0.90%
Tesco	34.80%	18.90%	21.70%	23.80%
Total	62.40%	62.40%	84.20%	81.10%

Table A.6.: Egg market share by type for all retail outlets in Northern Ireland (excluding organic eggs).

Retail Outlet	Barn	Free Range	Caged
Butchers	0.00%	0.29%	0.21%
Centra	0.00%	0.29%	0.91%
Co-op	0.00%	8.29%	3.41%
Cost Cutters	0.00%	1.79%	1.91%
Dunnes	0.00%	0.39%	2.51%
Greengrocers	0.00%	0.00%	0.81%
Iceland	0.00%	2.09%	9.31%
M&S	0.00%	4.79%	0.00%
Mace	0.00%	2.29%	7.81%
Safeway	7.30%	3.79%	5.81%
Sainsburys	0.00%	10.19%	13.61%
Spar/Vivo	0.00%	1.59%	10.11%
SuperValue L&N	0.00%	3.99%	11.21%
Tesco	92.70%	53.89%	21.91%
Others (e.g. dept stores)	0.00%	6.29%	10.51%

Table A.7.: Country weekly egg consumption and overall population.

	Eggs per wk	Population	Total Eggs
England	1.73	49,997,000	86,494,810
Wales	1.81	2,946,000	5,332,260
Scotland	1.94	5,115,000	9,923,100
NI	1.95	1,698,000	3,311,100
GB		58,058,000	101,750,170
UK		59,756,000	105,061,270
Gloucestershire	1.73	564,599	976,756
Wales + Gloucestershire		3,510,599	6,309,016

Table A.8.: Overall egg market share for each retail outlet in Wales

Retail Outlet	Wales + Gloucester	Wales Market Share	Wales Market Share (corrected)
Asda	17.00%	11.95%	17.36%
Budgen	0.20%	0.11%	0.16%
Butchers	2.90%	2.08%	3.02%
Co-op	3.50%	2.51%	3.64%
Farm shops	0.80%	0.47%	0.69%
Greengrocers	2.20%	1.57%	2.27%
Iceland	2.60%	1.92%	2.79%
Independent	3.40%	2.42%	3.52%
Kwik Save	6.00%	4.62%	6.71%
M&S	0.70%	0.45%	0.66%
Markets	2.30%	1.53%	2.22%
Milkman	2.90%	2.03%	2.95%
Morrisons	1.60%	0.38%	0.55%
Safeway	7.00%	4.83%	7.02%
Sainsburys	9.80%	6.24%	9.06%
Somerfield	3.30%	2.45%	3.56%
Spar	0.50%	0.36%	0.52%
Tesco	25.80%	18.28%	26.54%
Waitrose	0.40%	0.03%	0.04%
Others (e.g. dept stores)	6.90%	4.64%	6.74%
Total	100%	68.87%	100.00%

Table A.9.: Egg market share for remaining retail outlets in Great Britain

Retail Outlet	Barn Free Range	Caged	Organic
Budgen	0.55%	0.50%	0.23%
Butchers	3.33%	3.01%	1.40%
Farm shops	1.80%	1.63%	0.76%
Greengrocers	2.64%	2.38%	1.11%
Iceland	2.50%	2.26%	1.05%
Independent	4.02%	3.63%	1.69%
Kwik Save	4.02%	3.63%	1.69%
M&S	0.00%	1.13%	0.00%
Markets	3.75%	3.38%	1.57%
Milkman	3.75%	3.38%	1.57%
Spar	0.55%	0.50%	0.23%
Waitrose	0.00%	2.51%	0.00%
Others (e.g. dept stores)	10.68%	9.65%	4.49%
Total	37.60%	37.60%	18.90%

Table A.10.: Egg market share for all retail outlets in Scotland

Retail Outlet	Barn	Free Range	Caged	Organic
Asda	8.54%	11.75%	24.91%	9.38%
Budgen	0.00%	0.00%	0.00%	0.00%
Butchers	2.80%	3.06%	1.34%	1.75%
Co-op	0.28%	8.56%	7.10%	8.37%
Farm shops	1.99%	2.18%	0.95%	1.24%
Greengrocers	1.18%	1.29%	0.57%	0.74%
Iceland	1.76%	1.92%	0.84%	1.10%
Independent	5.80%	6.35%	2.78%	3.63%
Kwik Save	4.41%	4.83%	2.12%	2.76%
M&S	0.00%	0.91%	0.00%	0.52%
Markets	0.95%	1.04%	0.46%	0.59%
Milkman	0.26%	0.28%	0.12%	0.16%
Morrisons	0.03%	0.38%	0.81%	0.14%
Safeway	29.71%	15.49%	11.64%	22.83%
Sainsburys	0.39%	3.84%	3.71%	9.60%
Somerfield	0.68%	5.81%	16.03%	2.12%
Spar	1.53%	1.67%	0.73%	0.96%
Tesco	29.38%	19.34%	20.93%	27.67%
Waitrose	0.00%	0.00%	0.00%	0.00%
Others (e.g. dept stores)	10.31%	11.28%	4.95%	6.44%

Table A.11.: Egg market share for all retail outlets in Wales

Retail Outlet	Barn	Free Range	Caged	Organic
Asda	7.34%	9.22%	20.72%	6.87%
Budgen	0.19%	0.19%	0.09%	0.10%
Butchers	3.74%	3.73%	1.74%	1.99%
Co-op	0.22%	6.19%	5.45%	5.66%
Farm shops	0.85%	0.85%	0.40%	0.45%
Greengrocers	2.81%	2.81%	1.31%	1.50%
Iceland	3.45%	3.44%	1.60%	1.84%
Independent	4.36%	4.35%	2.02%	2.32%
Kwik Save	8.31%	8.29%	3.86%	4.43%
M&S	0.00%	0.81%	0.00%	0.43%
Markets	2.74%	2.74%	1.27%	1.46%
Milkman	3.65%	3.65%	1.70%	1.95%
Morrisons	0.03%	0.33%	0.74%	0.11%
Safeway	15.38%	7.31%	5.83%	10.08%
Sainsburys	1.22%	10.90%	11.16%	25.45%
Somerfield	0.58%	4.46%	13.06%	1.52%
Spar	0.65%	0.65%	0.30%	0.35%
Tesco	36.13%	21.69%	24.89%	29.01%
Waitrose	0.00%	0.05%	0.00%	0.03%
Others (e.g. dept stores)	8.34%	8.33%	3.87%	4.45%

Table A.12.: Egg market share for all retail outlets in Northern Ireland

Retail Outlet	Barn	Free Range	Caged	Organic
Butchers	0.00%	0.29%	0.21%	0.44%
Centra	0.00%	0.29%	0.91%	0.32%
Co-op	0.00%	8.29%	3.41%	12.97%
Cost Cutters	0.00%	1.79%	1.91%	2.60%
Dunnes	0.00%	0.39%	2.51%	0.21%
Greengrocers	0.00%	0.00%	0.81%	0.00%
Iceland	0.00%	2.09%	9.31%	1.82%
M&S	0.00%	4.79%	0.00%	7.84%
Mace	0.00%	2.29%	7.81%	2.40%
Safeway	7.30%	3.79%	5.81%	1.81%
Sainsburys	0.00%	10.19%	13.61%	14.32%
Spar/Vivo	0.00%	1.59%	10.11%	0.86%
SuperValue L&N	0.00%	3.99%	11.21%	4.59%
Tesco	92.70%	53.89%	21.91%	41.33%
Others (e.g. dept stores)	0.00%	6.29%	10.51%	8.48%

ANNEX B: GUIDANCE FOR SAMPLERS

B.1. FSA Egg Survey Sampling Instructions – England and Wales.

Please note and action the following points prior to sampling

- To avoid cross contamination of egg samples, it is essential that samplers do not visit farms on the same day as egg sampling and under no circumstances should milk recording take place prior to egg sampling.
- Samplers are reminded of standard NMR disinfection procedures between farms and are asked to abide by these at all times with no exceptions.
- It is particularly important to keep all recording gowns and related equipment in the storage box provided between disinfection and to remove all recording equipment from your vehicle prior to egg sampling.
- If you have any difficulties regarding the above then please inform the Sampling Manager.

B.2. General Instructions

- For the purpose of this exercise, **a sample is equivalent to a box of six eggs** but larger boxes (e.g. 10's or 12's), may be sampled if that is all that is on sale and the extra eggs will be discarded at the Laboratory.
- You will be responsible for ensuring that the appropriate number of samples as detailed in your pack, are collected in accordance with the sampling plan.
- Retail outlets should not be sampled **more than once** and no more than **four samples** should be collected from any one store. If unsure of the production type, the eggs should not be sampled. This should be noted on your sample detail sheet.
- Retailers should receive payment for the eggs at the time of sampling. If required we will arrange for you to have payment for eggs in advance. All receipts must be kept. Instructions on how claims are to be paid for work completed will be forwarded to you in the next few days.
- You should provide the sampler independent retail outlets with a letter (enclosed) from the FSA informing them, that samples have been taken from their premises in order to carry out the survey. (I would suggest you do this once you have paid for the eggs.) For the larger retail chains, this will not be necessary as the relevant contact at their Head Office will be sent a list of their premises from which samples have been obtained. If you have any queries or concerns from retailers please ask them to contact the Project Managers or the FSA.
- It is essential that cross- contamination be avoided during the collection of eggs. The aim should be to collect samples from the rear of shelves and not at the front of the display. It would also be useful to have a selection of sizes.

- Samples must consist of intact eggs with **NO** evidence of damage or of contamination e.g. dirty. A brief examination of each box of eggs (**without touching the contents**) should enable you to possibly eliminate any grossly damaged eggs. However, we have to accept that in some retailers such as a Farm shop/Markets for example, one would expect to see a bit of “dirt” and if this is the case, then please document it on your sample sheet in the comments box. Please discourage check out staff from handling the eggs. Simply indicate that you have already checked them.
- At some retail outlets e.g. Market stalls or Farm shops it may not be possible to buy pre – packaged eggs and retailers may pack boxes of eggs from larger trays on demand. These should also be examined as above.* Please note that it is not a legal obligation for retailers to state the production type when selling eggs loose in this way. You should endeavour to find out production type when buying these eggs but to also ensure the eggs collected are UK produced.
- Each box must be separated and placed in a separate sampling bag to avoid the risk of cross contamination during transport and storage. This of course will not be possible in the majority of retailers prior to purchase but if all precautions can be taken to keep the boxes separate before you put them in the individual bags it would be ideal.
- You must ensure samples are kept between 5 – 20°C during transportation and are kept dry and out of direct sunlight.
- All the relevant information available from the sample should be entered onto the relevant sampling form. The post-code must also be entered.
- The special peel-off label (same identity as your sample sheet) must be clearly placed on the box of eggs before enclosing and securing in the plastic bag. This is a unique identification number that links to a particular sample and will be retained throughout testing in the Laboratory.
- Once you have completed the sampling all samples must be packed securely in the cardboard boxes. For part filled boxes extra cushioning must be added to prevent damage during transit. You must also remember to enclose your completed sampling sheets preferably also in a plastic bag.
- The filled boxes must be identified with Fragile tape and be ready for collection by the Transport Division at a pre- arranged time. They will then be delivered in the “appropriate” manner direct to the Laboratory at Wolverhampton.

In the extremely unlikely event of all samples being unfit to test upon receipt in the Laboratory, you will be contacted and a re-sampling visit arranged

B.3. Samplers Check List:

- Sample forms and clip-board

- FSA letter for small retailers
- FSA sampling plan
- List of addresses and retail outlets
- Ordnance survey map
- Pens
- Polystyrene boxes for transportation of eggs (labelled)
- Temperature logging equipment
- Bubble wrap and tape
- Polythene bags for samples and for forms
- Cash for payment of eggs
- Disinfectant and disposable gloves
- Mobile phone and contact telephone numbers

ANNEX C –DIRECT LABRATORIES STANDARD OPERATING PROCEDURE FOR SALMONELLA TESTING IN RAW SHELL EGGS

STANDARD OPERATING PROCEDURE

MICRO/179

Survey of *Salmonella* contaminating UK produced shell eggs on for retail sale

Edition: 01

Last amended: 07.11.2001

C.1. INTRODUCTION

C.1.1. This SOP describes the protocol for analysis of samples for the FSA survey of *Salmonella* in shell eggs on retail sale in Great Britain.

C.1.2. The test method is based on the Direct Laboratories Standard technique for *Salmonella* detection (SOP MICRO/009, BS EN 12824). The method uses an enrichment stage, followed by selective enrichment, selective plating and confirmation using serological and biochemical tests. The method has modifications specified by the FSA to rinse the outside of the eggs, and then to homogenise the egg contents. The two broths are then tested separately.

C.1.3. Aseptic methods must be used throughout to ensure that samples are not subject to cross contamination. The environmental monitoring programme (SOP MICRO/078) will be extended to cover areas of the laboratory reserved for this project to identify any potential problems.

C.2. REFERENCE DOCUMENTS

BS EN 12824:1998 - Microbiology of food and animal feedingstuffs - horizontal method for the detection of *Salmonella*

SOP MICRO/009 - *Salmonella* by enrichment and plating

SOP MICRO/035 - Oxidase test (direct colony method)

SOP MICRO/067 - Sample handling in microbiology

SOP MICRO/073 - LIMS procedures in Microbiology.

SOP MICRO/078 - Environmental Monitoring of the Laboratory using settle plates and swabs

SOP MICRO/111 - Use of positive and negative control organisms

SOP MICRO/131 - Sample preparation.

SOP MICRO/132 - Allocation of Laboratory Numbers.

C.3. MATERIALS AND EQUIPMENT

C.3.1. Reagents

- Buffered Peptone Water (BPW).
- Rappaport Vassiliadis Soya Peptone Broth (RVS) in 10 ml volumes.
- Selenite Cystine Broth with added Sodium Biselenite (SCB) (4g/l) in 90 ml volumes.

- Pre - poured & dried modified Brilliant Green Agar plates (BGA).

Note: pre-poured plates have a limited shelf-life. DLS has validated storage of plates in the fridge for up to 1 month without problems. However, if the positive control fails to give satisfactory performance tests, the media must be replaced and the analysis repeated.

- Pre - poured & dried plates of Xylose Lysine Desoxycholate agar (XLD).
- Pre - poured & dried Nutrient Agar plates (NUT).
- Biochemical identification kit (e.g. API20E).
- Poly O, Poly H & Poly Vi antisera.
- Bacteriological saline 0.85%.

C.3.2. Apparatus

- Sample record form reference MIC/029
- Laboratory record forms reference MIC/030 & MIC/031
- Stomacher & triple thickness stomacher bags.
- Sterile pipettes and loops.
- Incubators running at $37 \pm 0.5^{\circ}\text{C}$ and $41.5 \pm 0.5^{\circ}\text{C}$.
- Microscope slide

C.5. PROCEDURES

C.5.1. Sample receipt

C.5.1.1. Samples will be submitted to the laboratory accompanied by a sample record form (MIC/029) detailing relevant information. On arrival at the laboratory, enter this data onto the survey spreadsheet. Follow routine procedures for receipt and registration of samples (SOPs MICRO/067, MICRO/073, MICRO/132). The test date will be no more than 1 day after the date sampled. Label the samples clearly with the test date.

C.5.1.2. Remove any eggs damaged in transit and note this information on the laboratory record form (MIC/030) together with details of date received, condition of the samples, appearance, reference numbers. Examine eggs to confirm the absence of cracks and the number of eggs that were clean, dirty or visibly soiled with adherent organic matter. Eggs bearing any marks other than natural markings or printed marks will be considered dirty.

C.5.2. Sample preparation and pre-enrichment for detection of *Salmonella*.

Samples will consist of 6 eggs from one box. It is essential that handlers take care to avoid cross contamination between boxes at all stages.

C.5.2.1. For contents: Take each of the six eggs and separate the contents from the shell by breaking them aseptically into a double stomacher bag, taking care to avoid contaminating the contents with pieces of shell. Hold the shells in a separate stomacher

bag. Add 100mls of BPW to the contents and stomach for 1 mins \pm 10 seconds. Add further BPW to create a 50:50 dilution and stomach again.

C.5.2.2. For shells: Crush the shells down and add 20 mls of BPW (enough to ensure they are completely covered). Shake gently and add further BPW to create a 50:50 dilution.

C.5.2.3. Incubate both samples at $37 \pm 0.5^{\circ}\text{C}$ for 18 - 20 hours. Set up positive and negative controls with each batch of samples following SOP MICRO/111.

C.6. Selective enrichment culture for *Salmonella*

C.6.1. A – Transfer (by Finnpiquette) 0.1 ml of the pre-enriched sample into 10 ml of RVS medium. Incubate at $41.5 \pm 0.5^{\circ}\text{C}$ for 24 hours \pm 1 hour.

C.6.2. B - Transfer (by pipette) 10 ml of the pre-enriched sample into 90 ml of SCB. Incubate at $37 \pm 0.5^{\circ}\text{C}$ for 24 hours \pm 1 hour.

C.7. Plating Out & incubation

C.7.1. After selective enrichment streak a 10 μl loop from the selective enrichment broths onto modified Brilliant Green Agar (BGA) and Xylose Lysine Desoxycholate agars (XLD).

C.7.2. Incubate plates for 24 hours \pm 1 hour at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.

C.7.3. Colonies on BGA: red/pink or white opaque colonies with brilliant red/pink zone, on XLD: red with black centre.

C.7.4. Plates should not be incubated for longer than 24 h, as this will encourage growth of other flora.

C.7.5. Any typical or suspect colony should be confirmed. A slide agglutination test may be performed at this stage to aid selection of suspect colonies.

5. Confirmation

C.8.1. Perform appropriate biochemical tests for *Salmonella* on 3 typical or suspect colonies from each sample, taken from both BGA and XLD plates. If there are fewer than 3 suitable colonies, take all available colonies through confirmation.

C.8.2. Isolates showing typical *Salmonella* biochemical reactions should be tested with polyvalent antisera for typical O and H antigens.

C.8.3. Streak these colonies onto Nutrient plates to obtain well isolated colonies. Incubate at 37°C for 18 - 24 hours, and use these cultures for confirmatory tests.

C.9. Serological confirmation

Test for the presence of *Salmonella* antigens by slide agglutination with the appropriate sera, from pure colonies after auto-agglutinable stains have been eliminated.

This method relies on the antibody/antigen reaction between a test culture and commercially prepared antisera.

C.9.1. Elimination of auto - agglutinable strains

- Place one drop of saline onto a clean glass slide.
- Disperse in this drop part of the colony to be tested or a colony from a pure culture, so as to obtain a homogenous and turbid suspension.
- Rock the slide gently for 30→60 seconds.
- Observe the result against a dark background, preferably with the aid of a magnifying glass. If the bacteria have clumped together into more or less distinct units, the strain is considered auto - agglutinable, and the detection of antigens will be impossible.
- In practice, auto - agglutinating strains of *Salmonella* are rare; it is more economical to perform poly O, H and Vi serology first.

C.9.2. Examination for O antigen

- Using one pure colony, recognised as non - autoagglutinable, proceed as above, using one drop of the anti O serum instead of saline solution.
- If agglutination occurs, the reaction is considered positive for the presence of that antigen.

C.9.3. Examination for H antigens

- Inoculate a Nutrient Agar slope with a pure non - autoagglutinable colony. Incubate at 37°C for 20→24h.
- Use the liquid at the base of the slope for examination for H antigens, proceeding as above, but using one drop of the anti H serum instead of saline solution.
- If agglutination occurs, the reaction is considered positive for the presence of H antigen.

C.10. Biochemical confirmation

Perform an oxidase test following SOP MICRO/035 (*Salmonella* is oxidase negative). On oxidase negative colonies, use an API20E biochemical test kit (or equivalent) following the Manufacturer's instructions. Only one API 20E need be used for each typical or

suspect colony type. It is important when using the API 20E system that a pure culture has been used.

C.11. Results

Biochemical Reactions	Auto agglutination -	Serological reactions	Interpretation
Typical	No	O, Vi or H antigen positive	Considered <i>Salmonella</i>
Typical	No	All reactions negative	May be <i>Salmonella</i>
Typical	Yes	Not tested	May be <i>Salmonella</i>
Not typical	No	O, Vi or H antigen positive	May be <i>Salmonella</i>
Not typical	No	All reactions negative	Not considered <i>Salmonella</i>

C.11.1. Send one isolate of each *Salmonella* type on a nutrient agar slope to the reference laboratory nominated by FSA for confirmation, serotyping, phage typing, antibiotic susceptibility testing and archiving.

C.11.2. Record all results on the laboratory record forms MIC/030 & MIC/031, enter results into LIMS as per SOP MICRO/073 and enter the data onto the survey spreadsheet.

C.12. Data and reporting

C.12.1. It is the responsibility of the Project Manager to ensure that every two weeks a summary report is sent to the FSA containing details of the samples collected to date.

C.12.2. An interim report (electronic and hard copy) containing a summary of the results to date will be submitted to the Agency at monthly intervals. The report will be updated to incorporate data on serotyping and phage typing for the isolates sent for typing.

C.12.3. At the end of the survey, the Project Manager will prepare a final report summarising statistics on the prevalence of *Salmonella* together with a breakdown of the serotype and phage types.

C.12.4. As required by the Laboratory's UKAS accreditation, all forms, records and electronic files related to the survey will be retained in Document Store after completion of the work. These documents will be copied to the client if requested.

C.13. Quality assurance

Samples from this project are subject to all routine Quality Control measures. All temperatures are monitored daily. Media are checked for sterility and performance. Equipment is maintained and calibrated as required by the laboratory's UKAS accreditation. All data is transcription checked. The laboratory is subject to routine

cleaning and disinfection procedures and the environmental monitoring programme will be extended to identify potential sources of cross contamination. DLS Participates in the FEPAS external proficiency scheme for *Salmonella* in food.

C.14. Zoonoses Order 1989

Under the Zoonoses Order 1989, laboratories which isolate *Salmonella* from foodstuffs must provide DEFRA (and DARDNI in Northern Ireland) with a listing of subtype found together with the name of the packing station from which the egg originated.

ANNEX D: SAMPLING FORM

Sample Ref:	
Type:	
Sampler name:	

Sample taken from:

Outlet Address:

--

Postcode:

--

Sample details

Purchase Date		Purchase Time	
Outlet Storage	Chilled	Ambient	Temp °C
Production type	Caged	Barn	Free Range
Egg Size	Small	Medium	Large
Pack Size		Extra Large	Other
Brand name	Purchase price		
Comments	Best before date		

Packing details

Packing station no. (if available):	9/ /	Lion Code:	Yes/No
-------------------------------------	------	------------	--------

Other markings:

--

Packer/producer name (if available):

--

Address:

--

Lab use only.

Appearance/condition	Acceptable	Unacceptable
Date received/checked		
LIMS Number		

Results

<i>Salmonella</i> spp. Positive		<i>Salmonella</i> spp. Negative	
Sent for serotype		Date	

ANNEX E: PROJECTED SAMPLING PLAN AT THE START OF THE SURVEY

RETAILER	ENGLAND				NORTHERN IRELAND				SCOTLAND				WALES			
	Caged	Barn	Free range	Organic	Caged	Barn	Free range	Organic	Caged	Barn	Free range	Organic	Caged	Barn	Free range	Organic
Asda	282	37	42	29	n/a	n/a	n/a	n/a	97	11	15	12	19	2	3	2
Budgen	3	3	2	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Butchers	21	17	15	8	1	0	0	1	5	4	4	2	2	1	1	1
Centra+	-	-	-	-	4	0	1	1	-	-	-	-	-	-	-	-
Co-op	66	1	25	21	13	0	11	17	28	0	11	11	5	0	2	2
Cost Cutters+	-	-	-	-	7	0	2	3	-	-	-	-	-	-	-	-
Dunnes	n/a	n/a	n/a	n/a	10	0	1	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Farm shops	11	9	8	4	0	0	0	0	4	3	3	2	0	0	0	0
Greengrocers	17	13	12	6	3	0	0	0	2	2	2	1	1	1	1	0
Iceland	16	12	11	6	36	0	3	2	3	2	3	1	1	1	1	1
Independent*	25	20	18	9	0	0	0	0	11	8	8	5	2	1	1	1
Kwik Save	25	20	18	9	n/a	n/a	n/a	n/a	8	6	6	4	3	3	3	1
M&S	n/a	n/a	6	3	n/a	n/a	6	10	n/a	n/a	1	1	n/a	n/a	0	0
Mace+	-	-	-	-	30	0	3	3	-	-	-	-	-	-	-	-
Markets	24	19	17	9	0	0	0	0	2	1	1	1	1	1	1	0
Milkmen	24	19	17	8	0	0	0	0	1	0	1	0	2	1	1	1
Morrisons	128	2	19	6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1	0	0	0
Others**	67	53	48	24	41	0	8	11	19	13	15	8	4	3	3	1
Safeway	88	85	37	48	23	9	5	2	45	38	20	30	5	5	2	3
Sainsbury	247	10	80	175	53	0	13	19	15	1	5	12	10	0	3	8
Somerfield	123	2	14	5	n/a	n/a	n/a	n/a	63	1	8	3	12	0	1	0
Spar/Vivo	3	3	2	1	39	0	2	1	3	2	2	1	0	0	0	0
Supervalue L&N	n/a	n/a	n/a	n/a	43	0	5	6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Tesco	324	171	93	118	85	121	70	54	82	38	25	36	22	11	7	9
Waitrose	n/a	0	12	6	n/a	n/a	n/a	n/a	n/a	0	0	0	n/a	0	0	0
TOTAL	1494	496	496	496	388	130	130	130	388	130	130	130	90	30	30	30

n/a – not available

* Independents include V.G, Mace/Wavy Line, Londis and other symbols (and outside of Northern Ireland may include those marked +)

**Others include Aldi, Netto, Lidl, Farm Foods, Woolworths, bakers, fishmongers, garage shops, cash and carry, department stores, other multiples, other freezer centres, chemists/drugstores, all other outlets.

Source: Sampling plan derived from data supplied by Taylor Nelson Sofres for the 52 weeks ending 10th Jan 2003.

ANNEX F : DISTRIBUTION OF SAMPLES TAKEN DURING THE SURVEY

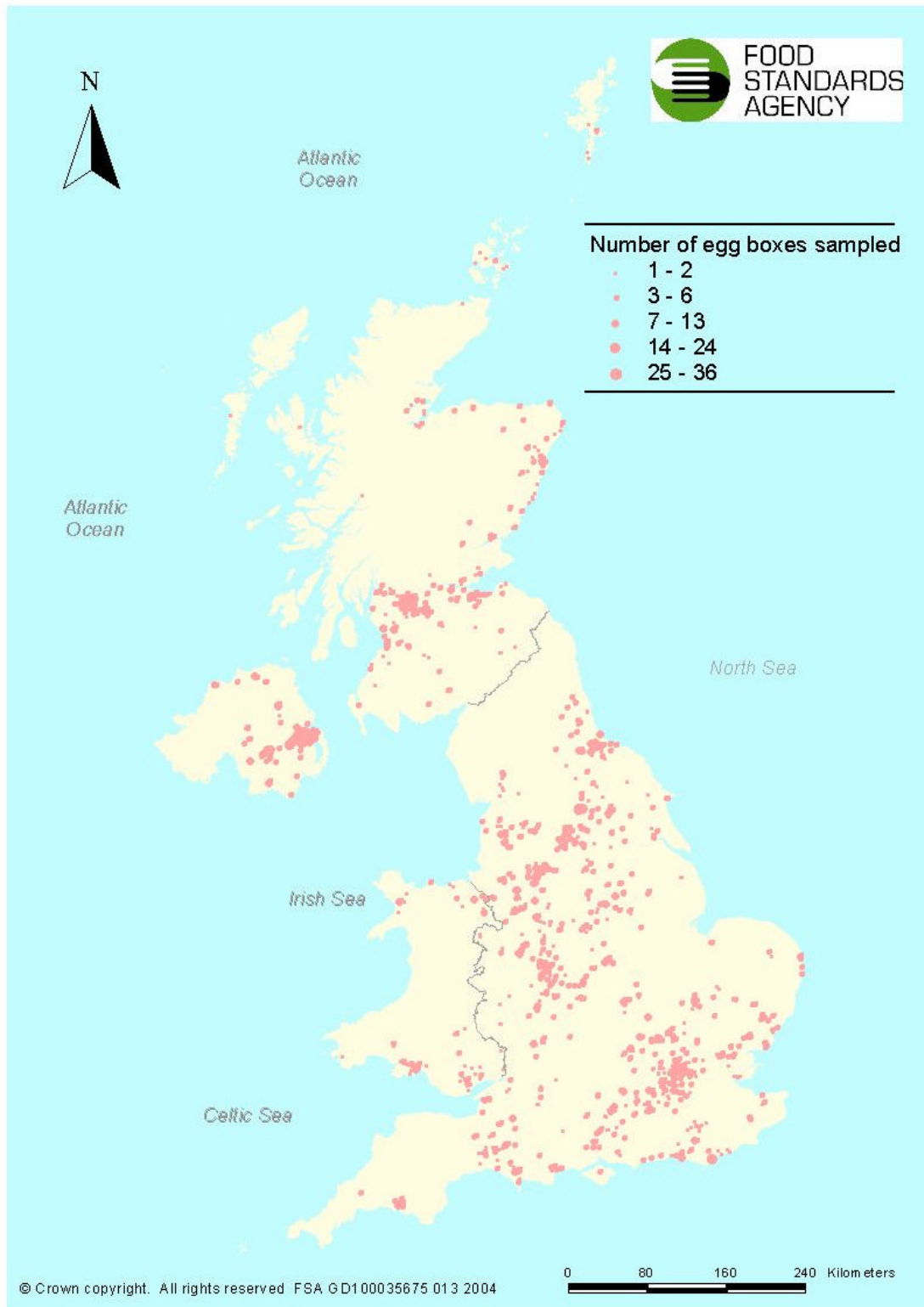
RETAILER	ENGLAND				NORTHERN IRELAND				SCOTLAND				WALES			
	Caged	Barn	Free range	Organic	Caged	Barn	Free range	Organic	Caged	Barn	Free range	Organic	Caged	Barn	Free range	Organic
Asda	282	37	42	30	n/a	n/a	n/a	n/a	97	15	15	13	19	3	3	2
Budgen	3	3	2	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Butchers	21	17	15	8	1	0	0	0	5	1	4	0	2	1	1	0
Centra+	-	-	-	-	4	0	1	0	-	-	-	-	-	-	-	-
Co-op	66	0	25	22	13	0	11	19	28	0	11	12	5	0	2	2
Cost Cutters+	-	-	-	-	7	0	2	0	-	-	-	-	-	-	-	-
Dunnes	n/a	n/a	n/a	n/a	10	0	1	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Farm shops	11	9	8	4	0	0	0	0	4	4	3	2	0	0	0	0
Greengrocers	17	13	12	6	3	0	0	0	2	1	2	0	1	1	1	1
Iceland	16	13	11	0	36	0	3	0	3	0	3	0	1	0	1	0
Independent*	25	20	18	9	0	0	0	0	11	1	8	5	2	2	1	1
Kwik Save	25	20	18	9	n/a	n/a	n/a	n/a	8	0	6	0	3	0	3	0
M&S	n/a	n/a	6	3	n/a	n/a	6	12	n/a	n/a	1	1	n/a	n/a	0	0
Mace+	-	-	-	-	30	0	3	0	-	-	-	-	-	-	-	-
Markets	24	19	17	9	0	0	0	0	2	2	1	0	1	1	1	0
Milkmen	24	19	17	9	0	0	0	0	1	0	1	0	2	1	1	0
Morrisons	128	0	19	6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	1	0	0	0
Others**	67	54	48	24	41	0	8	13	19	3	15	9	4	3	3	1
Safeway	88	85	37	48	23	9	5	3	45	52	20	32	5	5	2	3
Sainsbury	247	10	80	178	53	0	13	21	15	0	5	14	10	1	3	9
Somerfield	123	2	14	5	n/a	n/a	n/a	n/a	63	0	8	3	12	0	1	1
Spar/Vivo	3	3	2	0	39	0	2	0	3	0	2	0	0	0	0	0
Supervalu L&N	n/a	n/a	n/a	n/a	43	0	5	1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Tesco	324	172	93	119	85	121	70	61	82	51	25	39	22	12	7	10
Waitrose	n/a	0	12	6	n/a	n/a	n/a	n/a	n/a	0	0	0	n/a	0	0	0
TOTAL	1494	496	496	496	388	130	130	130	388	130	130	130	90	30	30	30

n/a not available

* Independents include V.G, Mace/Wavy Line, Londis and other symbols (and outside of Northern Ireland may include those marked +)

**Others include Aldi, Netto, Lidl, Farm Foods, Woolworths, bakers, fishmongers, garage shops, cash and carry, department stores, other multiples, other freezer centres, chemists/drugstores, all other outlets

ANNEX G: MAP OF THE UK SHOWING AREAS SAMPLED IN THE SURVEY



Draft

ANNEX H: VALIDATION STUDY TO DETERMINE THE BEST METHOD FOR ISOLATING *SALMONELLA* FROM EGG SHELLS

H.1. INTRODUCTION

H.1.1. The Food Standards Agency required that before commencing the UK survey of *Salmonella* contamination of eggs an initial validation study should be undertaken to determine the best method for isolating *Salmonella* from egg shells. The purpose of this was to be able to distinguish external from internal *Salmonella* contamination. The evaluation work was undertaken by Direct Laboratories, Wolverhampton.

H.1.2. Using a solution of Crystal Violet dye a range of swabs were evaluated in terms of giving good coverage of the egg shells. The two most promising swabs namely cotton tipped and sterile sponge were then further evaluated and compared with a standard ADAS rinse method using eggs inoculated with a *Salmonella* Enteritidis PT4.

H.2. PROCEDURE

H.2.1. A stock culture of *Salmonella* Enteritidis PT4 was cultured in buffered peptone water (BPW) at 37°C for 12 hours. Based on previous work it was estimated there was then 5.0×10^8 cfu/ml. This stock suspension was diluted with sterile BPW to give two working solution one with an estimated 100,000 cfu/ml (Solution A - high inoculum) and the second with an estimated 10,000 cfu/ml (Solution B low inoculum).

H.2.2. 720 fresh eggs and the working solutions were stabilised at the same temperature of 22°C. 360 eggs were dipped into solution A for 1 minute (high inoculum) and the remaining 360 eggs were dipped into solution B for 1 minute (low inoculum). All eggs were then removed from the solutions and air-dried on sterile plastic trays for one hour.

H.2.3. The inoculated eggs were divided into 12 batches of 60 eggs (batches 1-6 inoculated with solution A high level of inoculum and batches 7-12 inoculated with solution B low level of inoculum). Each batch of 60 eggs was then divided into 10 replicate samples of 6 eggs. Total *Salmonella* were recovered from each sample as follows.

H.2.4. Testing shell surfaces. Batches 1-6 - high level of inoculum.

H.2.4.1. Batch 1: Control

Draft

The 6 eggs were broken into a stomacher bag and the shells placed into a second stomacher bag. The egg contents were weighed, an equal weight of BPW added and the mixture stomached for 2 minutes. The shells were crushed by hand, added to the egg contents/BPW and thoroughly mixed in by hand manipulation of the bag. The suspension and serial dilutions were plated out on XLD agar, incubated at 37°C for 24 hours and counted. Finally, the numbers of *Salmonella* per egg were calculated.

H.2.4.2. Batch 2: Swab procedure 1 – individual eggs

Individual eggs were swabbed using cotton tipped swabs dampened immediately before use with BPW. Each egg was held by hand wearing fresh sterile gloves during the swabbing procedure. After swabbing the entire egg shell surface, the swab was placed in 10 ml of BPW. The next egg in the sample was swabbed with a fresh swab and the process repeated until all 6 eggs were swabbed. The container with swabs and BPW was mixed thoroughly using a vortex mixer. The resulting solution and serial dilutions were plated on XLD as above and *Salmonella* per egg calculated.

H.2.4.3. Batch 3: Swab procedure 2 – individual eggs

The eggs were swabbed as described above except a sterile sponge (Sterilab hydrasponge) was used instead of the cotton tip swab.

H.2.4.4. Batch 4: Swab procedure 1, batches of 6 eggs

The eggs were tested as described above under batch 2 except only one swab was used to recover *Salmonella* from all 6 eggs.

H.2.4.5. Batch 5: Swab procedure 2, batches of 6 eggs

The eggs were tested as described above under batch 3 except only one swab was used to recover *Salmonella* from all 6 eggs.

H.2.4.6. Batch 6: Rinsing batches of 6 eggs

100 mls of sterile BPW were placed in a stomacher bag. One egg from the sample was added and the *Salmonella* removed by gentle hand manipulation of the bag. The egg was removed, the next egg from the sample added and the process repeated until all 6 eggs had been treated. *Salmonella* levels in the BPW were determined using XLD agar as described above.

H.2.4.7. Batches 7-12: Low level of inoculum

Batches 7-12 were treated as batches 1-6.

H.2.5. Testing egg contents

H.2.5.1. All eggs from batches 2-6 and 8-12 were immediately placed in 70% ethanol for 5 minutes. They were then prepared as batches 1 and 7 described above and tested for *Salmonella* presence/absence by the Standard FSA method supplied in the draft protocol.

Draft

H.3. RESULTS AND DISCUSSION

H.3.1. Results for the *Salmonella* recovered from the external surfaces of the eggs are shown in Table 1 for the eggs with a high level of inoculum and in Table 3 for the eggs with a low level of inoculum. Presence/absence results for the eggs after shell disinfection with 70% alcohol are shown in Tables 2 and 4 for the eggs with high and low levels of inoculum respectively.

H.3.2. In Tables H1 and 3 the % recovery of *Salmonella* are shown based on the totals determined from the respective controls. These clearly indicate that rinsing recovers more *Salmonella* from the egg shells than either of the swabbing techniques. Rinsing is no more difficult than swabbing to undertake in the laboratory. Therefore, it is recommended that the rinse procedure described in this report be used to determine external shellborne *Salmonella* in the forthcoming FSA survey.

H.3.3. Finally, it is interesting to note that some *Salmonella* were isolated from the eggs after shell disinfection as shown in Tables H2 and H4. This suggests that the disinfection procedure using 70% ethanol cannot be relied on to kill all *Salmonella* present on eggshells.

Table H.1. Comparison of test methods to determine shellborne *Salmonella*. High level of inoculum.

Test Procedure	<i>Samonella</i> cfu/egg – 10 replicates											% recovery
	1	2	3	4	5	6	7	8	9	10	Geometric Mean	
Control	160000	190000	140000	130000	210000	160000	150000	160000	190000	150000	160000	100
Swab procedure 1 Individual eggs	5800	3700	6000	4300	12000	7300	5000	6200	8500	5800	6500	4.1
Swab procedure 2 Individual eggs	57000	73000	72000	60000	90000	42000	72000	73000	57000	83000	68000	42.5
Swab procedure 1 batches of 6 eggs	30000	16000	9800	4200	2300	4500	5800	3500	6700	8000	9500	5.9
Swab procedure 2 batches of 6 eggs	25000	16000	25000	22000	25000	25000	23000	22000	22000	23000	23000	14.4
Rinsing – batches of 6 eggs	140000	140000	120000	170000	140000	160000	170000	140000	160000	140000	150000	93.7

Table H.2. Comparison of test methods for internal *Salmonella*. High level of inoculum.

Test Procedure	<i>Salmonella</i> presence/absence in 6 eggs – 10 replicates										
	1	2	3	4	5	6	7	8	9	10	Average result
Swab procedure 1 Individual eggs	Present	Present	Present	Present	Present	Present	Present	Absent	Present	Present	Present
Swab procedure 2 individual eggs	Present	Present	Present	Present	Present	Present	Present	Absent	Present	Present	Present
Swab procedure 1 batches of 6 eggs	Present	Absent	Present	Present	Present	Present	Present	Present	Absent	Present	Present
Swab procedure 2 batches of 6 eggs	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Rinsing batches of 6 eggs	Present	Present	Absent	Present	Present	Present	Present	Present	Present	Present	Present

Table H.3. Comparison of test methods to determine shellborne *Salmonella*. Low level of inoculum.

Test Procedure	<i>Salmonella</i> cfu/egg – 10 replicates											% recovery
	1	2	3	4	5	6	7	8	9	10	Geometric Mean	
Control	5200	7700	11000	7900	11000	8700	9900	12000	10000	10000	9300	100
Swab procedure 1 Individual eggs	980	380	350	330	300	330	280	400	500	500	630	6.8
Swab procedure 2 Individual eggs	6200	4500	9700	6800	6700	4800	6000	5000	7500	3700	6100	66
Swab procedure 1 Batches of 6 eggs	430	500	800	680	580	720	450	500	430	820	590	6.3
Swab procedure 2 Batches of 6 eggs	1400	1400	1600	1700	1000	1200	1900	1700	1200	1400	1500	16
Rinsing – Batches of 6 eggs	3200	6500	4500	6800	9300	5200	8100	6700	8300	9000	6800	73

Table H.4. – Comparison of test methods for internal *Salmonella*. Low level of inoculum.

Test Procedure	<i>Salmonella</i> presence/absence in 6 eggs – 10 replicates										
	1	2	3	4	5	6	7	8	9	10	Average result
Swab procedure 1 individual eggs	Absent	Absent	Present	Absent	Present	Present	Present	Absent	Absent	Absent	Absent
Swab procedure 2 individual eggs	Present	Absent	Present	Present	Present	Absent	Present	Present	Present	Absent	Present
Swab procedure 1 batches of 6 eggs	Present	Present	Present	Present	Absent	Present	Present	Present	Absent	Present	Present
Swab procedure 2 batches of 6 eggs	Present	Present	Present	Present	Present	Present	Present	Absent	Present	Present	Present
Rinsing batches of 6 eggs	Present	Present	Present	Present	Absent	Present	Present	Present	Absent	Present	Present

ANNEX I: ACMSF COMMENTS ON THE SWABBING TEST METHOD VALIDATION STUDY

I.1. The Advisory Committee on the Microbiological Safety of Food (ACMSF) Surveillance Working Group were invited to comment on the swabbing test method in Annex 7. The Working Group's overall comment was that the results do not accord with methods from previous trials. Their detailed comments are as follows:

I.2. Shell penetration

I.2.1. In general, when eggs are contaminated naturally, the source is either faecal contamination or, rarely, contamination in the shell gland. Chicken faeces are quite a dry material and *Salmonella* is unlikely to get into the pores of eggs with any regularity. The protocol chosen by ADAS appears unrealistic, given that immersion in broth would have allowed *Salmonella* to invade deeply into the pores. Moreover, the use of such high numbers of organisms would also have facilitated the crossing of the shell membrane. If salmonellae were present in the pores, they may have been protected from the alcohol. Table H2 data show that 45 of 50 eggs were contaminated internally after alcohol immersion. Even with "low" levels of inoculum, 36 of 50 eggs were positive for *Salmonella* (Table H4). It should also be borne in mind that shell contamination would occur as a discrete event on one area of the shell, rather than all over the shell. (Nascimento *et al*, 1992) showed that egg shells have defined areas which will differ in shell quality and membrane cover. The literature quoted in Annex A suggests that the ADAS technique would have allowed the contamination of egg contents during egg immersion, thus explaining why so many eggs were found to be contaminated in their contents after disinfection. In addition to the problems caused by the method of contamination, the numbers used were much too high at c. 100,000 and c. 10,000 cells of *Salmonella* per egg. These levels of contamination would not be seen on the shells of naturally - contaminated eggs because the environment is quite hostile.

I.3. Shell disinfection

I.3.1. This is a tricky area. The issue is not whether all of the *Salmonella* from the shell can be recovered but whether enough can be removed to allow subsequent detection. Thus, the numbers given in the ADAS report are misleading. Surprisingly, there are relatively few published papers on this. Professor Humphrey's initial work at Exeter used sterile faeces contaminated with *S. Enteritidis*. The faeces were allowed to dry, as much faecal material as possible was removed, and the egg was swabbed and then immersed in

70% ethanol for 5 minutes. *S. Enteritidis* was not obtained from either egg contents or disinfected shells.

I.3.2. Himathongkham *et al.* (1999) compared a variety of methods for shell disinfection and used essentially the same method as ADAS. Perhaps not surprisingly, they also found that ethanol was not wholly efficient and that the only method that disinfected all shells was immersion in boiling water for 3 seconds. However, this sometimes caused eggs to crack.

I.3.3. Gast (1993) also explored this and compared immersion in iodine with immersion in boiling water for 5 seconds. As with the Himathongkham study, boiling eliminated all *Salmonella* on egg shells. In Gast's study, eggs were also contaminated by dipping in broth containing *Salmonella*.

I.3.4. If ADAS are to sample eggs by immersion, they will need to disinfect by immersion in boiling water, as this will reach bacterial cells in the pores. A better technique would be to swab the eggs, as the aim is to establish presence/absence, not to recover large numbers.

I.3.5. There remains the problem of what to do with the shells post-swabbing and disinfection. Disregarding them could result in *Salmonella* being missed, although safeguarding against that eventuality would mean that the study would be out of step with other similar studies undertaken in the past. A possible compromise would be to add disinfected shells to the shell swabs.

I.3.6. Another option would be to swab the surface and test this separately from the contents (and discard the shell). Considering the problem pragmatically, one risk comes from the outside of the egg when handled (this will be assessed by testing the swabbed surface). A second risk comes from what might be consumed when the egg contents are eaten (which will be assessed by testing the contents). What is left in the pores and membrane at the time of eating the egg is not likely to impact on its safety, and it is unlikely that this will occur without the contamination also being found on the external shell or in the contents. That brings into question the justification for testing the shell after swabbing.

I.3.7. To summarise, the Surveillance Working Group's preference would be to go for swabbing of the exterior shell using a sterile sponge or ball of cotton wool. Under no circumstances should a rinse technique be used as these risks introducing *Salmonella* into the egg contents. The Group is confident that using a big swab for each batch of 6 eggs will recover *Salmonella* from the exterior shell. Immersion in 70% ethanol will successfully eliminate *Salmonella* naturally present on egg shells. As an alternative, the Group would be content with the use of immersion in boiling water for 5 seconds. Shells should be discarded rather than cultured.

I.4. References

Gast R K. Immersion in boiling water to disinfect egg shells before culturing egg contents for *Salmonella enteritidis*. J Food Protect 1993; **56(6)** : 533-535.

Himathongkham S, Riemann H, Ernst R. Efficacy of disinfection of shell eggs externally contaminated with *Salmonella enteritidis* : implications for egg testing. Int J Food Microbiol 1999; **49** : 161-167.

Javed T, Hameed A, Siddique M. Egg shell penetration tendency of different *Salmonella* serotypes by attached ring colour method. Acta Microbiol Pol 1994; **43(1)** : 67-72.

Miyamoto T, Horie T, Baba E, Sasai K, Fukata T, Arakawa A. *Salmonella* penetration through egg shell associated with freshness of laid eggs and refrigeration. J Food Prot 1998; **61(3)** : 350-353.

Nascimento V P, Cranstoun S, Solomon S E. Relationship between shell structure and movement of *Salmonella enteritidis* across the egg shell wall. Br Poult Sci 1992; **33(1)** : 37-48.

Padron M. Egg dipping in hydrogen peroxide solution to eliminate *Salmonella typhimurium* from egg shell membranes. Avian Dis 1995; **39(3)** : 627-630.

Peel B, Simmons G C. The effect of dazomet on salmonellas on artificially contaminated eggs. Aust Vet J 1976; **52(5)** : 220-223.

Sauter E A, Petersen C F, Parkinson J F, Steele E E. Effect of pH on egg shell penetration by salmonellae. Poult Sci 1979; **58(1)** : 135-138.

ANNEX J : MICROBIOLOGICAL METHODS USED IN THE SURVEY

J.1 Microbiological methods

J.1.1. The Agency considered the comments raised by the ACMSF (Annex I) and also consulted with the relevant industry stakeholders on the methodology. The conclusion was that there are no satisfactory techniques that could effectively differentiate between *Salmonella* contamination on the shell and that in the contents and the methods used in the validation study (Annex H) had the potential to give false positives or negative results. In view of this, the Agency decided that the same method be used as in the previous egg surveys (de Louvois 1993; ACMSF 2001). This would give an indication as to the relative proportion of contamination on the shell or in the contents and was considered to offer the best chance of picking up all positives. There was also the advantage that the method would allow a direct comparison to be made with the previous egg surveys.

J.1.2. As there would be a significant amount of handling involved in testing the eggs, a potential for cross-contamination to occur, it was essential to keep the testing area in the laboratories clear and to sanitise splashes or spillage as soon as they occurred.

J.1.3. Testing laboratories ensured that they had pure cultures of standard reference strains of *Salmonella* from which colonies can be identified correctly.

J.2. Sample preparation & non-selective pre-enrichment for *Salmonella*

J.2.1. A box of 6 eggs pooled together represented a sample. Wear disposable gloves for handling eggs and change gloves after each batch of six eggs. Inspect eggs for gross faecal contamination and or presence of cracks. Use duplicate contingency sample if necessary.

J.2.2. Aseptically break open the eggs using a spatula flamed in alcohol and separate shell from contents, taking care to avoid contamination of contents with shell. If a portion of shell contaminates the contents, the sample should be discarded and duplicate contingency sample used.

J.3. Pre-enrichment of egg contents

J.3.1. Add the contents of six eggs to a sterile stomacher bag (~180mm x 300mm) placed on a gravitational diluter (programmed for 50:50 dilution with buffered peptone water (BPW). Add a small amount of BPW and stomach the eggs for 1 minute. Add a further BPW to create 50:50 dilution. Holding the top

of the stomacher bag closed, mix the sample well, seal the bag with clip and incubate the stomached sample for 18-20 h at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.

J.4. Pre- enrichment of egg shells

J.4.1. Add the shells to a doubled stomacher bag (~180mm x 300mm)
Place on the gravitational diluter (programmed for 50:50 dilution with BPW)
Add BPW to create a 50:50 dilution Crush the shells down gently. Mix the sample, seal the bag and incubate the stomached sample for 18-20 h at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.

J.5. Enrichment culture for *Salmonella*

J.5.1. Add 0.1 ml of the pre-enriched culture to 10 ml Rappaport-Vassiliadis Soya Peptone Broth (RVS) and incubate for selective enrichment at $41.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 h in an incubator. Also, add 10 ml of the pre-enriched cultures to 90ml Selenite Cystine Broth with added Sodium Biselenite (SCB), (4g/l) and incubate for selective enrichment at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 h.

J.5.2. After selective enrichment streak a 10 μl loop from the selective enrichment broths onto modified Brilliant Green Agar (mBGA) and Xylose Lysine Desoxycholate agars (XLD). Incubate plates for 24 h at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. 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.

J.5.3. If suspect colonies are present, perform appropriate biochemical tests for *Salmonella* notes below 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.

J.5.4. Send one isolate of each *Salmonella* type on a nutrient agar slope to the Health Protection Agency's Laboratory of Enteric Pathogens, Colindale, London for confirmation, serotyping, phage typing, antibiotic susceptibility testing and archiving.

J.6. Selective Media

J.6.1. The following list describes the media required to carry out the tests. Equivalent media from commercial manufacturers may be used.

J.6.1.1. Buffered Peptone Water (to make 1 litre)

Peptone	10.0g
Sodium chloride	5.0g
Disodium hydrogen phosphate	3.5g
Potassium dihydrogen phosphate	1.5g
Adjusted to pH 7.2 ± 0.2	

J.6.1.2. Selenite Cysteine Broth (to make 1 litre)

Tryptone	5.0g
Lactose	4.0g
Disodium phosphate	10.0g
L-Cysteine	0.01g
Sodium biselenite	4.0g
Adjusted to pH 7.0 ± 0.2	

J.6.1.3. Rappaport-Vassiliadis medium (to make 1 litre)

Soya peptone	4.5g
Sodium chloride	7.2g
Potassium dihydrogen phosphate	1.26g
Magnesium chloride (anhydrous)	13.58g
Malachite green	0.036g
Di-potassium hydrogen phosphate	0.18g
Adjusted to pH 5.2±0.2	

J.6.1.4. Brilliant Green Agar (Modified) (to make 1 litre)

'Lab-Lemco' powder	5.0g
Proteose peptone	10.0g
Yeast extract	3.0g
Disodium hydrogen phosphate	1.0g
Sodium dihydrogen phosphate	0.6g
Lactose	10.0g
Sucrose	10.0g
Phenol red	0.09g
Brilliant green	0.0047g
Agar	12.0g
Adjusted to pH 6.9±0.2	

J.6.1.5. Xylose-lysine-desoxycholate (XLD) agar (to make 1 litre)

Yeast extract	3.0g
L-Lysine HCl	5.0g
Xylose	3.75g
Lactose	7.5g
Sucrose	7.5g
Sodium desoxycholate	1.0g
Sodium chloride	5.0g
Sodium thiosulphate	6.8g
Ferric ammonium citrate	0.8g
Phenol red	0.08g
Agar	12.5g
Adjusted to pH 7.4.±0.2	

J.6.1.6. Urea Broth (to make 1 litre)

Peptone	1.0g
Glucose	1.0g
Disodium phosphate	1.2g
Potassium dihydrogen phosphate	0.8g
Sodium chloride	5.0g
Phenol red	0.004g
40% Urea solution	5ml
Adjusted to pH 6.8 ± 0.2	

J.6.1.7. Triple Sugar Iron Agar (to make 1 litre)

'Lab-Lemco' powder	3.0g
Yeast extract	3.0g
Peptone	20.0g
Sodium chloride	5.0g
Lactose	10.0g
Sucrose	10.0g
Glucose	1.0g
Ferric citrate	0.3g
Sodium thiosulphate	0.3g
Phenol red	q.s
Agar	12.0g
Adjusted to pH 7.4 ± 0.2	

J.6.1.8. Lysine Iron Agar (to make 1 litre)

Bacteriological peptone	5.0g
Yeast extract	3.0g
Glucose	1.0g
L-lysine	10.0g
Ferric ammonium citrate	0.5g
Sodium thiosulphate	0.04g
Bromocresol purple	0.02g
Agar	14.5g
Adjusted to pH 6.7 ± 0.2	

J.6.1.9. Nutrient Agar (to make 1 litre)

'Lab-Lemco' powder	1.0g
Yeast extract	2.0g
Peptone	5.0g
Sodium chloride	5.0g
Agar	15.0g
Adjusted to pH 7.4 ± 0.2	

J.7. *Salmonella* Confirmatory Tests

Biochemical test	Reaction (typical +ve)	% <i>Salmonella</i> inoculations showing the reaction
Acid formation on glucose in TSI	Yellow butt (red or unchanged shows -ve)	100 +ve
Gas formation on glucose in TSI	Bubbles or cracks in butt	91.9 +ve
Lactose or sucrose fermentation in TSI	Yellow slant	Lactose - 99.2 -ve Sucrose - 91.6 -ve
Hydrogen sulphide formation in TSI	Black butt	91.6 +ve
Lysine decarboxylation	Purple colour in lysine decarboxylation medium	94.6 +ve
Urea broth	No colour change (+ve) Red (-ve)	

**ANNEX K: BREAKPOINT CONCENTRATIONS FOR
ANTIMICROBIAL DRUGS**

Antimicrobial	Abbreviation	Concentration (mg/l)
Ampicillin	A	8
Chloramphenicol	C	8
Streptomycin	S	16
Sulphonamides	Su	64
Spectinomycin	Sp	64
Tetracycline	T	8
Trimethoprim	Tm	2
Furazolidone	Fu	8
Nalidixic Acid	Nx	16
Ciprofloxacin	Cp	0.125; 1
Rifampicin	Rf	64

ANNEX L: METHODOLOGY FOR EXTERNAL QUALITY ASSURANCE SAMPLES

L1 Method and organisms used.

L.1.1. The organisms used in the preparation of the quality assurance samples were *Salmonella* Poona PHLS 188742 and *Escherichia coli* NCDO 2328.

L.1.2. 25ml of pasteurised liquid egg samples was inoculated with a low level of *Salmonella* Poona obtained from the Public health Laboratory (PHLS) and the method used to verify the samples was based on BS EN ISO 6579:2002. A 3-tube MPN technique was used to verify the cell concentration. Buffered peptone water was incubated at 37°C for 18-20 h. The pre-enriched samples (0.1ml) were inoculated into 10ml Rappaport Vassiliadis Soya Peptone Broth (RVS), incubated at 41.5°C for 24h. This was followed by plating onto selective agar Xylose Lysine Desoxycholate (XLD), incubated at 37°C for 24 h. Biochemical and serological confirmation procedures were also undertaken using API 20E (bioMerieux) and polyvalent antisera for typical O and H antigens (Murex, ZC01 and ZD01), respectively.

L.1.3. Verified cell concentration for each batch were Batch 1: 23 cfu/ml, Batch 2: 23 cfu/ml and Batch 3: 20 cfu/ml.

L.1.4. Direct Laboratory Services, Charis and Queen's University of Belfast analysed 10 coded samples for each dispatch. All participants recovered the target organism from all the 'positive' samples and none from the 'negative' samples.

ANNEX M: CODING FOR EXTERNAL QUALITY ASSURANCE SAMPLES SENT TO PARTICIPATING LABORATORIES

M.1. Round 1 of EQA samples: 3rd March 2003

FSA1	FSA2	FSA3	FSA4	FSA5	FSA6	FSA7	FSA8	FSA9	FSA10
+	-	+	-	-	-	-	+	-	-

A decision was made after this to change the coding system as follows:

M.2. Round 2 of EQA samples: 14th April 2003

Samples to be sent to Direct Laboratories

FSA11	FSA12	FSA13	FSA14	FSA15	FSA16	FSA17	FSA18	FSA19	FSA20
-	-	-	+	-	+	+	-	-	+

Samples to be sent to Charis

FSA21	FSA22	FSA23	FSA24	FSA25	FSA26	FSA27	FSA28	FSA29	FSA30
-	-	+	+	-	-	+	-	+	-

Samples to be sent to QUB

FSA31	FSA32	FSA33	FSA34	FSA35	FSA36	FSA37	FSA38	FSA39	FSA40
+	-	+	-	-	+	-	+	-	-

Samples to be tested at CSL (blind trial)

FSA41	FSA42	FSA43	FSA44	FSA45	FSA46	FSA47	FSA48	FSA49	FSA50
+	-	-	+	-	-	+	-	-	+

M.3. Round 3 of EQA samples: 28th May 2003

Samples to be sent to Direct Laboratories

FSA51	FSA52	FSA53	FSA54	FSA55	FSA56	FSA57	FSA58	FSA59	FSA60
+	+	-	+	-	-	+	+	-	+

Samples to be sent to Charis

FSA61	FSA62	FSA63	FSA64	FSA65	FSA66	FSA67	FSA68	FSA69	FSA70
+	-	-	+	+	-	-	+	+	+

Samples to be sent to QUB

FSA71	FSA72	FSA73	FSA74	FSA75	FSA76	FSA77	FSA78	FSA79	FSA80
-	+	+	-	-	+	+	+	-	+

Samples to be tested at CSL (blind trial)

FSA81	FSA82	FSA83	FSA84	FSA85	FSA86	FSA87	FSA88	FSA89	FSA90
-	+	+	+	+	-	+	+	-	-

ANNEX N: EXTERNAL QUALITY ASSURANCE RESULTS

Table N.1. Results for round one – March 2003 sent to participating laboratories

Charis Food Research (Scotland) – Received 5th March 2003

Laboratory Number	Sample Description	Results
FSA 1	FSA1	Detected
FSA 2	FSA2	Not detected
FSA 3	FSA3	Detected
FSA 4	FSA4	Not detected
FSA 5	FSA5	Not detected
FSA 6	FSA6	Not detected
FSA 7	FSA7	Not detected
FSA 8	FSA8	Detected
FSA 9	FSA9	Not detected
FSA 10	FSA10	Not detected

Table N.2. Direct Laboratories (England and Wales) – Received 5th March 2003

Laboratory Number	Sample Description	Results
23008761	FSA 1	Detected
23008762	FSA 2	Not detected
23008763	FSA 3	Detected
23008764	FSA 4	Not detected
23008765	FSA 5	Not detected
23008766	FSA 6	Not detected
23008767	FSA 7	Not detected
23008768	FSA 8	Detected
23008769	FSA 9	Not detected
23008770	FSA 10	Not detected

Table N.3. QUBelfast (Northern Ireland) – Received 5th March 2003

Laboratory Number	Sample Description	Results
FSA 1	FSA 1	Detected
FSA 2	FSA 2	Not detected
FSA 3	FSA 3	Detected
FSA 4	FSA 4	Not detected
FSA 5	FSA 5	Not detected
FSA 6	FSA 6	Not detected
FSA 7	FSA 7	Not detected
FSA 8	FSA 8	Detected
FSA 9	FSA 9	Not detected
FSA 10	FSA 10	Not detected

Table N.4. EQA results for round 2 - April 2003

Charis Food Research (Scotland) – Samples received on 15th April 2003

Laboratory Number	Sample Description	Results
FSA 21	FSA 21	Not detected
FSA 22	FSA 22	Not detected
FSA 23	FSA 23	Detected
FSA 24	FSA 24	Detected
FSA 25	FSA 25	Not detected
FSA 26	FSA 26	Not detected
FSA 27	FSA 27	Detected
FSA 28	FSA 28	Not detected
FSA 29	FSA 29	Detected
FSA 30	FSA 30	Not detected

Table N.5. Direct Laboratories (England and Wales) – Received on 16th April 2003

Laboratory Number	Sample Description	Results
23015651	FSA 11	Not detected
23015652	FSA 12	Not detected
23015653	FSA 13	Not detected
23015654	FSA 14	Detected
23015655	FSA 15	Not detected
23015656	FSA 16	Detected
23015657	FSA 17	Detected
23015658	FSA 18	Not detected
23015659	FSA 19	Not detected
23015660	FSA 20	Detected

Table N.6. QUB (Northern Ireland) – 15th April 2003

Laboratory Number	Sample Description	Results
FSA 31	FSA 31	Detected
FSA 32	FSA 32	Not detected
FSA 33	FSA 33	Detected
FSA 34	FSA 34	Not detected
FSA 35	FSA 35	Not detected
FSA 36	FSA 36	Detected
FSA 37	FSA 37	Not detected
FSA 38	FSA 38	Detected
FSA 39	FSA 39	Not detected
FSA 40	FSA 40	Not detected

Table N.7. Results for CSL 'blind' testing - EQA egg samples dispatched 14th April 2003

Inoculation level: 23cfu/ml

Results reported as *Salmonella* detected or not detected in 25 ml

Sample Number	Sample status	RVS/XLD	RVS/BGA	SC/XLD	SC/BGA
FSA 41	Positive	detected	detected	detected	detected
FSA 42	Negative	not detected	not detected	not detected	not detected
FSA 43	Negative	not detected	not detected	not detected	not detected
FSA 44	Positive	detected	detected	detected	detected
FSA 45	Negative	not detected	not detected	not detected	not detected
FSA 46	Negative	not detected	not detected	not detected	not detected
FSA 47	Positive	detected	detected	detected	detected
FSA 48	Negative	not detected	not detected	not detected	not detected
FSA 49	Negative	not detected	not detected	not detected	not detected
FSA 50	Positive	detected	detected	detected	detected

Table N.8. EQA results for round 3 – May 2003**Charis Food Research (Scotland) – Received 20th May 2003**

Laboratory Number	Sample Description	Result
FSA 61	FSA 61	Detected
FSA 62	FSA 62	Not detected
FSA 63	FSA 63	Not detected
FSA 64	FSA 64	Detected
FSA 65	FSA 65	Detected
FSA 66	FSA 66	Not detected
FSA 67	FSA 67	Not detected
FSA 68	FSA 68	Detected
FSA 69	FSA 69	Detected
FSA 70	FSA 70	Detected

Table N.9. Direct Laboratories (England and Wales)

Laboratory Number	Sample Description	Results
FSA 51	FSA 51	Detected
FSA 52	FSA 52	Detected
FSA 53	FSA 53	Not detected
FSA 54	FSA 54	Detected
FSA 55	FSA 55	Not detected
FSA 56	FSA 56	Not detected
FSA 57	FSA 57	Detected
FSA 58	FSA 58	Detected
FSA 59	FSA 59	Not detected
FSA 60	FSA 60	Detected

Table N.10. QUB (Northern Ireland)

Laboratory Number	Sample Description	Results
FSA 71	FSA 71	Not detected
FSA 72	FSA 72	Detected
FSA 73	FSA 73	Detected
FSA 74	FSA 74	Not detected
FSA 75	FSA 75	Not detected
FSA 76	FSA 76	Detected
FSA 77	FSA 77	Detected
FSA 78	FSA 78	Detected
FSA 79	FSA 79	Not detected
FSA 80	FSA 80	Detected

Table N.11. Results for CSL 'blind' testing - EQA egg samples dispatched 19th May 2003

MPN: 20 cfu/ml

Results reported as *Salmonella* detected or not detected in 25 ml

Sample Number	Sample status	RVS/XLD	RVS/BGA	SC/XLD	SC/BGA
FSA 81	Negative	not detected	not detected	not detected	not detected
FSA 82	Positive	detected	detected	detected	detected
FSA 83	Positive	detected	detected	detected	detected
FSA 84	Positive	detected	detected	detected	detected
FSA 85	Positive	detected	detected	detected	detected
FSA 86	Negative	not detected	not detected	not detected	not detected
FSA 87	Positive	detected	detected	detected	detected
FSA 88	Positive	detected	detected	detected	detected
FSA 89	Negative	not detected	not detected	not detected	not detected
FSA 90	Negative	not detected	not detected	not detected	not detected

ANNEX O: MEASURES TAKEN BY DIRECT LABORATORIES AND NATIONAL MILK RECORDS TO PREVENT CROSS-CONTAMINATION

O.1. All staff involved in the sampling and testing of the eggs were experienced in undertaking microbiological work and therefore had been trained in using aseptic procedures. Standard operating procedures (SOPs) specific to this survey included precautions to prevent cross-contamination and all staff were trained in their specific SOPs before the project commenced. These specific measures can be summarised as follows.

O.1.1. In the retailers, the samplers took packs of eggs which were least likely to have been handled by the public by, wherever possible, selecting those towards the back of the shelf and showing no signs of having been previously opened. Before purchase the samplers opened the boxes and rejected any which contained eggs that were broken or showed gross contamination and they avoided touching the eggs at all times.

O.1.2. Immediately after purchase, all samples were given pre-allocated sample reference numbers and placed in individual, clean plastic bags, which were folded over and sealed with elastic bands to prevent unwrapping during transit. They were placed in appropriate bulk containers containing bubble wrap and ice packs during warm weather for transport to the laboratory. The sampling forms with the reference numbers were placed in separate plastic bags in the container. The containers were sealed once sampling had been completed.

O.1.3. To avoid cross contamination of egg samples, NMR samplers were advised not to visit milk farms on the same day as egg sampling and under no circumstances should they carry out any milk recording prior to egg sampling.

O.1.4. NMR samplers were also reminded of standard NMR disinfection procedures between farms and were asked to abide by these at all times with no exceptions. It was particularly important that all milk recording gowns and related equipment were kept safely in the storage box provided after disinfection and they were also advised to remove all recording equipment from their vehicle prior to egg sampling.

O.1.5. After collection of samples, samplers ensured that the eggs were wrapped in separate polythene bags and placed in large polystyrene boxes, which were cushioned with bubble wrap to prevent damage in transit. The temperature of the cool boxes, which were used to carry the eggs, was also monitored during transit. The closed container was sealed with tape, and labelled with the address of the testing laboratory.

O.1.6. On arrival at the laboratory, the unopened containers were held in a temperature controlled sample reception area at <18°C. The storage

temperature was monitored and recorded throughout the survey. These records show storage temperatures were always satisfactory. Within 12 hours the eggs were transported to the testing laboratory for analysis. Seals on the containers were unbroken until the samples arrived in the laboratory.

O.1.7. Once samples arrived in the testing laboratory, the outer containers were opened and the samples placed in numerical order based on their sample reference number. Each individual sample was labelled with a unique laboratory reference number. These numbers were transferred to the associated paperwork, which was immediately transferred to sample registration and database entry.

O.1.8. The layout of the laboratories at Wolverhampton is shown in Figures O.1 and O.2. Initial sample preparation took place in a separate containment level 2 laboratory. This was used exclusively for the egg survey samples. This meant that no contaminated samples, *Salmonella* cultures or media after incubation ever entered this laboratory during the period of the survey. Environmental swabs were taken daily from various surfaces and items of equipment in both laboratories and routinely analysed for *Salmonella*. No environmental swab gave a positive result for *Salmonella* during the survey. In addition, staff entering the laboratory put on regularly cleaned protective clothing, which was taken off when leaving the facility. This ensured *Salmonellae* could not enter the laboratory via staff. All testing was undertaken by fully trained project specific staff. Each member of staff undertook specific tasks using separate equipment on separate areas of bench within the laboratory.

O.1.9. At the beginning and end of each working day all sample preparation areas were wiped down with a suitable sanitiser. Disposable latex gloves, which had been sanitised before handling the eggs, were worn. Samples were removed from the sealed plastic bags and opened as individual boxes. Each of the six eggs was removed and carefully broken open using a sterile spoon. The egg contents were placed into one sterile stomacher bag and the shells into another bag. Sample preparation was carried out as per the agreed protocol with the final solutions being transferred into sterile disposable screw top containers. Gloves were changed between each sample of eggs and preparation areas sanitised before the next sample was prepared. Negative and positive controls were set up with each daily batch of eggs, these being inoculated into sterile, disposable, screw cap containers in a separate room at the end of the day's preparation. Laboratory coats were changed and hands were washed whenever staff entered or left the laboratory.

O.1.10. After the initial sample preparation all waste including containers, boxes and used gloves were immediately placed into plastic bags which were sealed and sent to the council for incineration. Waste generated during testing including agar plates, enrichment media, stomacher bags, control cultures, confirmatory test strips etc was also placed into plastic bags, which were then

sealed. These bags were autoclaved and then sent to the council for incineration.

O.1.11. All enrichment media in securely sealed containers were transferred to another containment level two laboratory where they were incubated in a specific section of a large walk in incubator on shelves reserved specifically for this project.

O.1.12. All sub-culturing of suspect isolates was carried out in a second containment level 2 laboratory so that no enriched cultures ever entered the sample preparation area.

O.1.13. Both negative and positive controls were always sub-cultured at the end of the run; i.e. the positive control was the last container to be opened during the sub-culturing process. The positive control was always *S. Poona*. Benches were thoroughly sanitised at the end of each run.

O.1.14. Laboratory sample reference numbers remained with all samples throughout the laboratory processes to avoid the possibility of any sample being miss-identified.

Figure O.1. Layout of laboratory facilities at Direct laboratories, Wolverhampton. Block D is approximately 90m walk from the main Microbiology corridor.

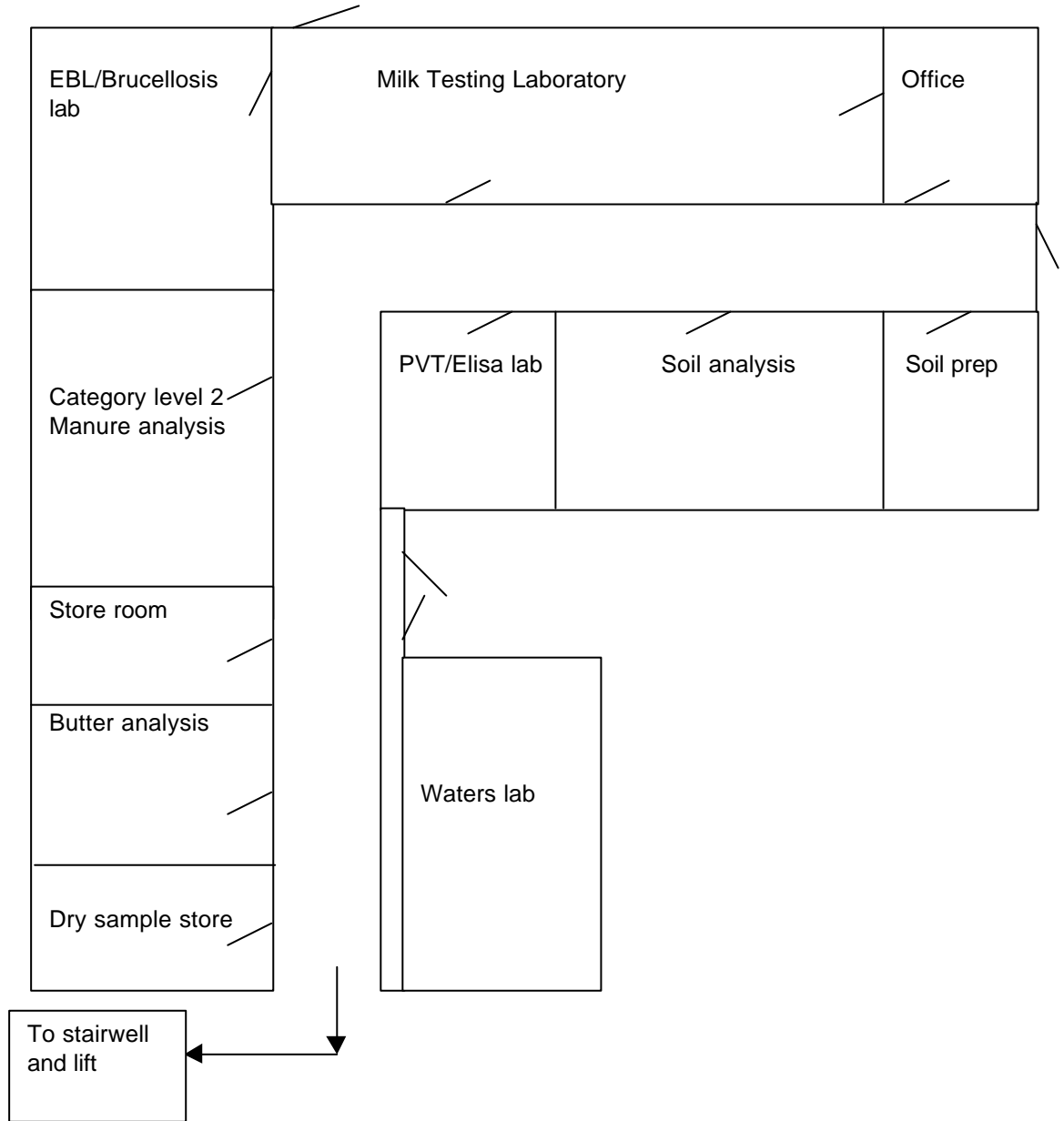
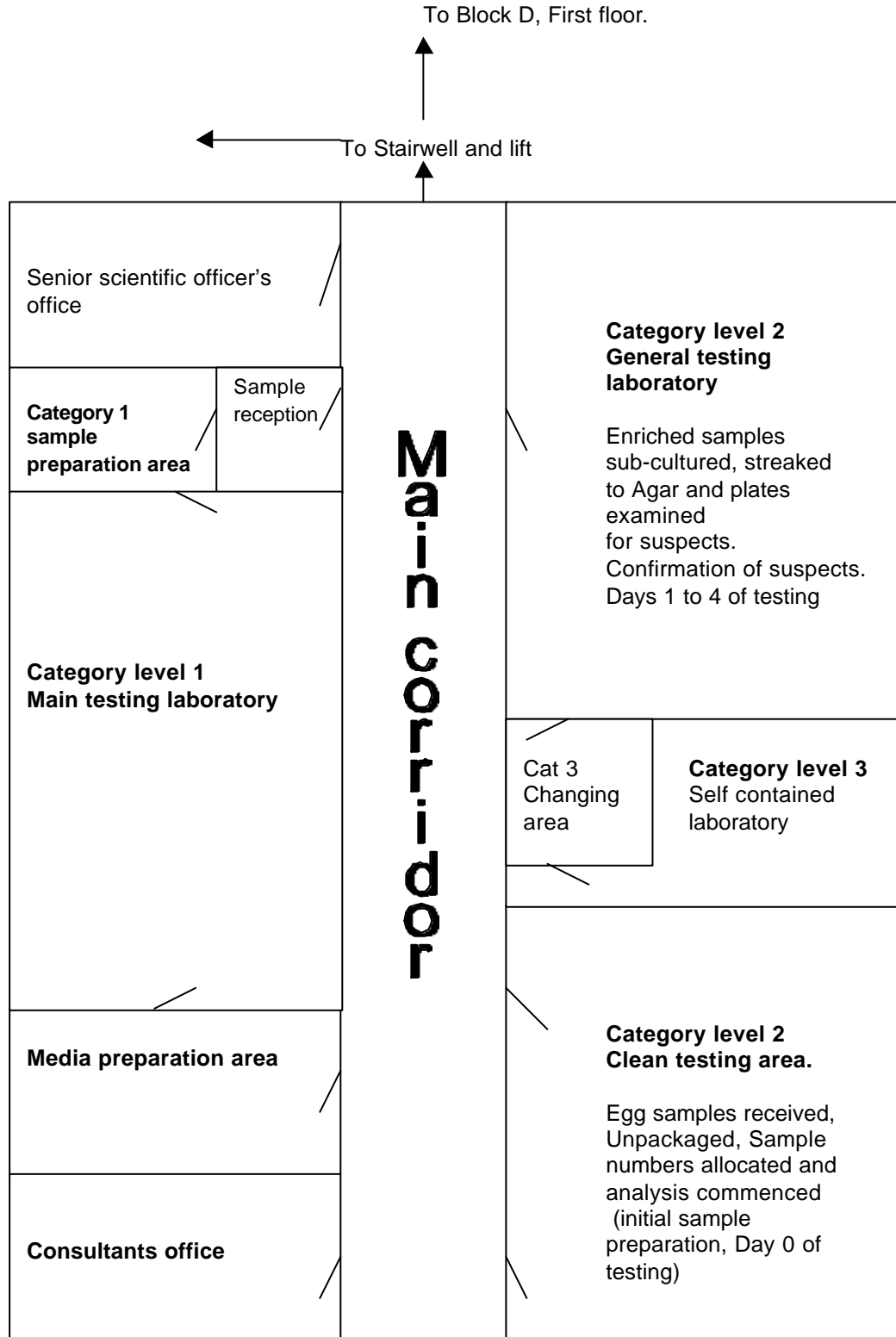


Figure O.2. Layout of laboratory facilities at Direct laboratories, Wolverhampton. Block C is the Microbiology department located on the first floor.



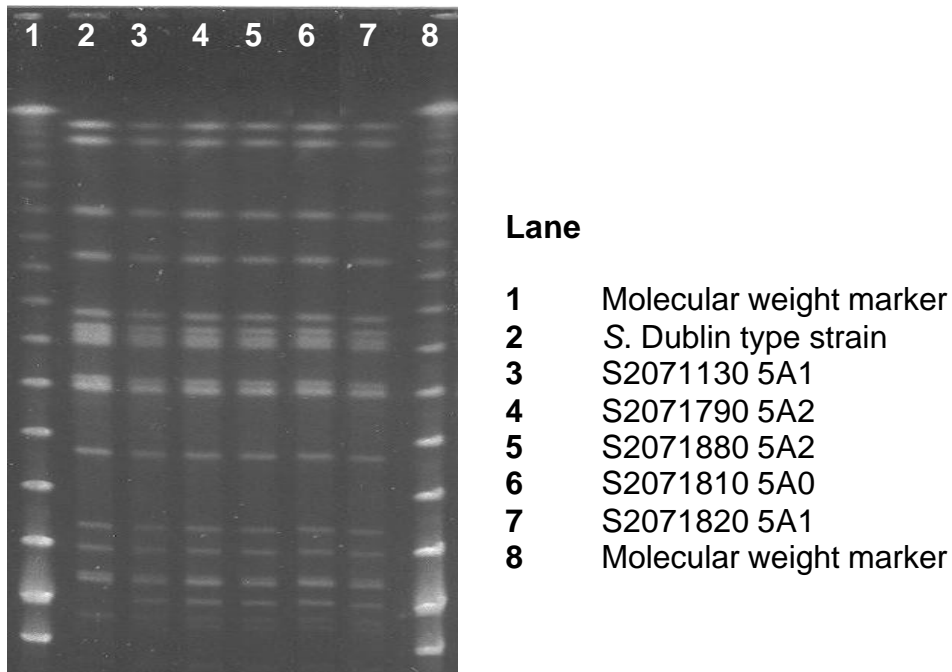
ANNEX P: ADDITIONAL WORK CARRIED OUT ON THE *SALMONELLA* DUBLIN ISOLATES

P.1. This annex reports the findings from molecular comparison of the 5 *S. Dublin* isolates by the Health Protection Agency (HPA) laboratory of Enteric Pathogens following repeated checking of the serotype of the submitted isolates. Although *S. Enteritidis* is in the same serogroup as *S. Dublin* isolates react in a different way and the plasmid profiles are very different.

P.2. DNA fingerprinting carried out in the Laboratory of Enteric Pathogens

Isolates of *Salmonella* Dublin from the egg-related samples were molecularly subtyped on the basis of plasmid profile and macrorestriction fingerprint analysis following Pulsed-Field Gel Electrophoresis (PFGE) of *Xba*1-digested DNA, as requested by the Food Standards Agency. The results are summarised below and the profiles shown in the photograph of the PFGE gel.

Figure P.1. PFGE gel of *Salmonella* Dublin type strain and isolates found from egg-related samples.



P.2.1. Plasmid profile

The method for plasmid profile was based on that of Kado and Liu (1981). All isolates were characterised by possession of a single plasmid of 50 megadaltons (MDa), which corresponds in molecular mass to the *S. Dublin* serovar-specific plasmid.

P.2.2. Macrorestriction fingerprinting

For macrorestriction fingerprinting the method for the preparation of total genomic DNA was a modification of that described by Powell *et al.* (1994). Following digestion with restriction enzyme *Xba* I, the linearised fragments were resolved by pulsed-field gel electrophoresis (PFGE) using the CHEF DR II system (Biorad UK Ltd) at 5.4 v/cm for 48 hours with pulse times of 8-64 seconds. Fragments were sized in relation to a lambda 48.5 kilobase ladder (Sigma UK). Using these methods, with one exception the macrorestriction fingerprints of all isolates tested were indistinguishable and corresponded to the pattern exhibited by the *S. Dublin* type strain. The one strain which differed in its PFGE profile (SDUBXB0002) had extra bands which were not seen in repeat runs suggesting that all 5 isolates were of the same PFGE type. This PFGE type has been designated SDUBXB0001 and was characterised by 18 bands ranging from c. 800 to 18 kilobases (kb).

P.3. Conclusions

All isolates were indistinguishable on the basis of the plasmid profiling and the macrorestriction fingerprint. From this, it was concluded that all five isolates were identical.

ANNEX Q: *SALMONELLA* DUBLIN FINDINGS AND TRACEBACK OF POSITIVE EGG SAMPLES

Q.1. Five of the egg samples were positive for *S. Dublin*. This was an unusual and unexpected finding and required follow-up to assess the basis for the results. This annex provides information on *S. Dublin* in animals, humans and the food chain together with comments on the sampling and testing and the findings from tracing the five positive samples back to the farm and subsequent investigations.

Q.2. *Salmonella* Dublin in animals

Q.2.1. *Salmonella* Dublin is the most frequent *Salmonella* serotype in adult cattle and calves in Great Britain and is associated with sporadic cases of animal disease as well as outbreaks, including enteric or reproductive disease in adults and pneumonia or septicaemia in calves. In cattle it is associated with septic abortion with a 70% mortality and prolonged carriage. It is the second most frequent cause of bovine foetopathy reported from cattle herds in the UK and is associated with dysentery in the pre and post calving periods (Richardson 1973; Counter and Gibson 1980).

Q.2.2. Cattle-associated incidents of *S. Dublin* in Great Britain have been rising over the last few years (391 in 1998, 768 in 2002) (Veterinary Laboratories Agency 2002) and show a strong seasonality with a peak in October and November coinciding with the main calving period (Veterinary Laboratories Agency 2002). *Salmonella* Dublin infections occur less frequently in small ruminants (sheep, goats) and is uncommon or rare in other animal species. Its occurrence in poultry is very unusual with only single incidents in turkeys and chickens and 3 incidents in game birds between 1998-2002 (Veterinary Laboratories Agency 2002). In cases where it appears to be a transient infection in young birds, cattle herds have always been near by (Rob Davies VLA personal communication). Although incidents of *S. Dublin* are rare in poultry, in situations where cattle and poultry are in close proximity it is possible that occasional, adventitious contamination could occur through contact with cattle faeces.

Q.3. *Salmonella* Dublin and eggs

Q.3.1. We were unable to find any reports in the literature of *S. Dublin* being isolated from eggs either in UK or elsewhere. It was not found in any of the previous surveys of UK-produced or non-UK eggs or in reports from an extensive trawl of the literature. The VLA have one pre-1993 report of *S. Dublin* from an egg, although the supporting information suggests this is doubtful. It remains plausible that if layers are in close proximity to cattle

infected with *S. Dublin* then contamination of the shell could occur. The fact that we were unable to find a well-documented report of *S. Dublin* contamination of eggs suggests that if such contamination events do occur, they are extremely rare.

Q.4. *Salmonella* Dublin in humans

Q.4.1. *Salmonella* Dublin infection in humans is relatively rare with only 27 laboratory-confirmed cases in England and Wales in 2003 (HPA-LEP provisional data). Since 1991 the number of laboratory-confirmed cases has fluctuated from between 17 and 44 per year (Liebana *et al.* 2002) with no apparent change in response to the changes since in incidents in cattle. Although *S. Dublin* infections in humans are rare, it is frequently invasive and with a high mortality rate.

Q.4.2. Although outbreaks linked to *S. Dublin* are rare they have been associated with raw milk and with soft/semi soft cheeses made from unpasteurised milk (Samll and Sharp 1979; Maguire *et al.* 1992). We are not aware of any outbreaks linked to eggs or egg products. As with all zoonotic foodborne pathogens, carriage of *S. Dublin* in a herd will always present a risk through faecal contamination. The fact that human cases of illness are relatively rare indicates that contamination events are perhaps rare, in part reflecting the intermittent nature of *S. Dublin* excretion.

Q.5. Characteristics of *Salmonella* Dublin

Q.5.1. *Salmonella* Dublin belongs to serogroup D₁ that also includes *Salmonella* Enteritidis and *Salmonella* Gallinarum. The reported heat resistance of *S. Dublin* at 60°C under otherwise ideal conditions is similar (D 0.5 - 0.6 min) to *S. Enteritidis* (D 0.7 - 0.8 min) and within the range for strains of *S. Typhimurium* (D 0.2-0.9) (ICMSF 1996). The organism has been reported to survive in the environment in dried faeces for over a year and in faeces on various stall surfaces for almost 6 years (Plym-Forshell and Ekesbo 1996). Survival in urine was up to 5 days.

Q.6. *Salmonella* Dublin isolates in the egg survey

Q.6.1. Table Q1 summarises the distribution of *S. Dublin* positive samples based on the where and when the eggs were sampled and tested. Although the 5 egg samples were tested at the laboratory on only 2 dates (one on 7th and 4 on 14th April 2003); the samples came from 4 different shops, had 5 different best before dates and were packed at 4 different packing stations. The 5 egg samples were purchased from shops in different parts of the country (Guildford, Clacton-on-Sea, Littlehampton and Ilkeston) and involved

4 different samplers. In addition the eggs represented 3 different production types (caged, free range, barn) and all isolates were from the shell.

Table Q.1: Distribution of the 5 S. Dublin positive egg samples according to where and when they were sampled and tested and the number of different packing stations codes on the packaging. For example the 5 S. Dublin positive samples were linked to 3 different retailers.

Retailers	Shops	Samplers	Dates Purchased	Best before Dates	Brand Names	Egg packing Stations	Dates laboratory received	Dates laboratory tested
3	4	4	3	5	3	4	2	2

Q.6.2. NMR who undertook the sampling in England and Wales purchased the 5 egg samples over two weekends (6th April; 12-13th April). Four different samplers were involved. Procedures were put in place with disinfection occurring before and after entry to a farm. Samples were sent to the laboratory on the Sunday (6th April, 13th April) and testing began on the Monday morning. The NMR samplers do undertake sampling on farms for milk recording. However, since the foot and mouth outbreak in 2001 all samplers are aware of strict disinfection and hygiene measures.

Q.6.3. For the 5 egg samples concerned it had been at least 3 days since the sampler had been on a farm. At the point of purchase each sampler established the type of egg and removed packets from the back of the shelf wherever possible (less likelihood other consumers may have contaminated them). The box was opened to check for faecal contamination, feathers and dirt, without touching the eggs. Four of the boxes were purchased from same packing station/type. The boxes of eggs were kept apart and checkout staff asked not to touch the eggs. The eggs were only opened for quick inspection after which they were placed in individual bags and packed in bubblewrap and containers sealed with tape, Paperwork was completed at the point of sale and the samples were not in contact with the samplers' car seat . Between 5 and 8 stores were visited on a sampling day.

Q.6.4. All of the S. Dublin positive samples came from eggs sampled in England and tested at Direct Laboratories, Wolverhampton. The first S. Dublin positive sample was tested at 11.48 on Monday 7th April. The 4 S. Dublin positive samples on Monday 14th April testing were begun at 11.05, 12.50, 13.41 and 13.47. Over 100 samples were processed that day with many between each of the S. Dublin positive samples. There was full traceability with dedicated reference numbers for each of the samples. Six staff were involved in processing the 5 egg samples between sampling and streaking enriched cultures onto XLD and BGA. One member of staff subcultured suspect colonies, carried out biochemical tests on the isolates and sent cultures to the HPA's Laboratory of Enteric Pathogens.

Q.6.5. Testing of eggs up to the enrichment stage was undertaken in a dedicated (containment level 2) facility. Nothing went into the laboratory except staff, sterile media and egg samples. No stock cultures were used. The laboratory made sure all staff were trained and supervisors checked standard operating procedures. Staff wore gloves and changed them between samples. The laboratory was sterilised each day and swabbed after each cleaning to ensure sterile conditions. Full details of the measures taken by Direct Laboratories and a plan of the laboratory layout can be found in Annex O.

Q.6.6. The laboratory took steps to minimise the stages where the tests on egg samples and isolates shared facilities with routine testing work which included testing of foods, dairy products, pasteurised milk, animal feeds, water and abattoir swabs was isolated. It was only after the enrichment of samples that staff other than the dedicated egg team had worked on the samples.

Q.7. Follow-up of *Salmonella* Dublin positive samples

Q.7.1. Following notification to the retailers of the 5 *S. Dublin* positive samples there followed extensive investigation at the farm level. The 5 positive samples came from 2 packing companies, 4 packing centres, 10 farms, 15 flocks, 4 breeds of laying hen, 4 hatcheries, 12 rearing farms and 7 feed mills (Table 2). These operations were located in different parts of the country. These findings indicate that the 5 egg samples were from a diverse range of farms and related supply chains.

Table Q.2.: Potential sources of the 5 *S. Dublin* positive egg samples in terms of producers, packers and feed suppliers.

Packing companies	Packing stations	Farms	Flocks	Breeds	Hatcheries	Rearing farms	Feed mills
2	4	10	15	4	4	12	7

Q.7.2. An extensive program of testing was conducted on birds and their environment (rearing and laying) for the flocks on farms linked to the 5 *S. Dublin* samples. Because layers have a life of about 72 weeks not all of the flocks tested would have included the birds linked to the *S. Dublin* positive eggs and in some cases tests were conducted on replacement flocks.

Q.7.3. Testing of layers comprised 62 pooled samples of 20 eggs, 22 pools of 30 environmental swabs, 2 pools of 12 environmental swabs from graders, 17 pools of 60 cloacal swabs and 10 dust samples. Tests in rearing comprised 91 pools of 30 environmental swabs, 1 fly test, 91 pools of 60 cloacal swabs, 1 mouse swab, 48 dust samples, 5 cow samples and 175 composite samples 8 post cleaning and 135 chick papers.

Q.7.4. The above tests were undertaken by 4 independent laboratories and represented over 12500 individual samples, all of which were negative for *S. Dublin* including on one farm where cows were kept. In addition one of the farms involved in the traceback was part of a VLA study and no *S. Dublin* was found during this work.

Q.7.5. The BEIC have reported that *S. Dublin* has never been found in their egg tests in the 2 retailer surveys conducted in 2003 no *S. Dublin* was found.

Q.8. Summary

Q.8.1. *Salmonella* Dublin is frequently reported from cattle, occasionally in other animals but rarely from poultry. It is a rare serotype in humans although when infections occur, they are often invasive and there is a high mortality rate.

Q.8.2. *Salmonella* Dublin belongs to the same serogroup (D₁) as *S. Enteritidis*. It would appear to be a cattle-adapted serotype whereas *S. Enteritidis* exhibits a strong association with poultry. Comparison of the 5 isolates by plasmid profiling and PFGE showed that they all had a 50 MDa plasmid and the same PFGE profile. Comparison of the PFGE profiles for the egg isolates with those from cattle and sheep in a VLA study undertaken in 2002 (Liebana *et al.* 2002) indicated that the egg isolates were very similar. The PFGE profiles for the egg isolates differed from the profiles for isolates of *S. Dublin* from human infections in 2003. They also differed slightly from the type strain of *S. Dublin* (John Threlfall, personal communication).

Q.8.3. *Salmonella* Dublin has not been isolated in previous surveys of UK-produced or non-UK eggs including recent surveys by the BEIC, 2 retailers and the HPA. We are not aware of any literature reports of *S. Dublin* being isolated from eggs in the UK or elsewhere. We are also not aware of any outbreaks of *S. Dublin* infection that have been attributed to shell eggs or egg products. Whilst unusual *Salmonella* serotypes are occasionally found in foodstuffs to find *S. Dublin* in 36% (5/14) of the isolates is most unusual.

Q.8.4. Contamination of the outside of an egg with *S. Dublin* is plausible if layers were to come into contact with faeces from cattle carrying *S. Dublin*. However, cattle were only present on one of the farms linked to a *S. Dublin* positive sample and this would not explain the contamination on the other 4 samples.

Q.8.5. There is no clear pathway which links the 5 *S. Dublin* isolates to a common source at the farm, packing station, retail shop or sampler level. The points of least variation between the 5 samples would appear to be the dates of receipt and dates of testing (2 each) at the laboratory.

Q.8.6. The samplers and the laboratory appear to have taken all reasonable precautions to guard against cross-contamination in their handling and testing of samples (Annex O) and no problems were found in the external quality assurance tests (see Annex M). The laboratory took steps to minimise the stages where the tests on egg samples and isolates shared facilities with routine testing work which included testing of foods, water and abattoir swabs.

Q.8.7. It has not been possible to identify a specific point in sample handling and testing regime where *S. Dublin* contamination might have occurred. Although this must remain a possibility in any laboratory handling and processing a large and diverse range of food and environmental samples, and where some facilities are shared. Given the variation between the samples at the retail, packing station and farm and farm supply level where there was no evidence of *S. Dublin* we conclude that the most plausible explanation is that cross-contamination occurred at some point in the handling and/on testing process. However, it is acknowledged that there is uncertainty in how this might have occurred.

Q.9. Conclusion

Q.9.1. The *S. Dublin* positive samples were unexpected and have been thoroughly investigated in an attempt to identify the most likely source(s) of contamination. It has not been possible to identify a definitive explanation for the presence of *S. Dublin* on eggs but on balance the most likely explanation is cross contamination during handling and/on testing.

Q.9.2. Investigations at the farm level found no evidence of contamination with this serotype and the farms linked to the positive samples were geographically widespread, as were the packing stations, the shops where samples were purchased and the samplers involved.

Q.9.3. On the basis of currently available evidence we consider that there is sufficient doubt concerning the robustness of the *S. Dublin* test results in this survey. There is no current evidence that *S. Dublin*, a cattle adapted serotype, can become established in layer flocks and contaminate eggs and therefore the 5 positive samples have been excluded from the main analysis in the report (see Chapter 6). However, for completeness Annex R provides a statistical analysis of the egg survey results with the *S. Dublin* positive samples included.

ANNEX R: STATISTICAL ANALYSIS WITH THE *SALMONELLA* DUBLIN RESULTS INCLUDED

R.1. Statistical analysis of survey results

R.1.1. This Annex presents the results from statistical analysis of the data with the *S. Dublin* results included. The statistical analysis with *S. Dublin* results excluded is in the main body of the report (Chapter 6).

R.1.2. Statistical analysis of the contamination rate of *Salmonella* in a box of 6 eggs showed that the overall prevalence of *Salmonella* in a box of six eggs was 0.51% for the UK as a whole, i.e. around 1 box in every 200 boxes.

R.2. Prevalence of *Salmonella* per box of 6 eggs

R.2.1. Table 1 shows the prevalence of *Salmonella* in a box of 6 eggs and the 95% confidence intervals (CI) (the lower and upper values) for the UK, England, Wales, Scotland and Northern Ireland. The lower and upper values represent the boundaries of the 95% confidence interval assuming that the observed prevalence followed a binomial distribution.

R.2.2. There were no statistically significant differences in *Salmonella* prevalence between England, Wales, Scotland and Northern Ireland (all $p > 0.10$). The UK figure of 0.51% (95% CI: 0.29% - 0.83%) is equivalent to 1 in 200 boxes (95% CI: 1 in 590 boxes to 1 in 160 boxes).

R.2.3. It should be noted that Wales had the highest *Salmonella* prevalence (1.53%) and the smallest sample size of all the countries (179 samples). The consequences of these are that:

- The confidence interval for *Salmonella* prevalence for eggs sampled in Wales is wider than the other countries.
- To identify statistically significant difference between Wales and the other countries, the *Salmonella* prevalence for eggs sampled would have to be at least 2%.

Table R.1.: The prevalence of *Salmonella* per box of 6 eggs in the UK and in England, Wales, Scotland and Northern Ireland. Figures shown include 95% confidence intervals.

Country (sample size)	Lower 95% CI	Prevalence	Upper 95% CI
UK (4753)	0.29%	0.51%	0.83%
England (2981)	0.27%	0.52%	0.91%
N. Ireland (782)	0.00%	0.00%	0.61%
Scotland (811)	0.00%	0.00%	0.58%
Wales (179)	0.21%	1.53%	5.25%

R.3. *Salmonella* prevalence and egg production types

R.3.1. Table 2 shows the prevalence of *Salmonella* contamination per box of 6 eggs for the four production types. Although *Salmonella* contamination was only found on eggs from caged production no statistically significant differences in *Salmonella* contamination of eggs was found between the four production types (all $p > 0.20$). The *Salmonella* prevalence of caged eggs would have to be at least 1% to show statistically significant differences from other production types.

Table R.2. : The prevalence of *Salmonella* per box of 6 eggs from caged, free range, barn and organic production systems. Figures shown include 95% confidence intervals.

Production type (sample size)	Lower 95% CI	Prevalence	Upper 95% CI
Barn (785)	0.01%	0.17%	0.87%
Caged (2376)	0.35%	0.66%	1.13%
Free Range (805)	0.01%	0.17%	0.87%
Organic (787)	0.00%	0.00%	0.56%

R.4. Prevalence of *Salmonella* per egg

R.4.1. The UK figure for individual egg prevalence was 0.08% for *Salmonella* (95% CI: 0.05% - 0.14%). The estimated prevalence of *Salmonella* per egg was based on the assumption that the rate of cross-contamination between eggs within the box was equivalent to the contamination rate between boxes. Given the low prevalence of *Salmonella* within boxes, we can assume that the source of contamination within a box of 6 eggs is from one egg only. In performing this calculation the assumption is although eggs are packed in a quasi-random order, the presence of more than one contaminated egg in a box is unlikely to occur frequently when the incidence of contamination is as

low as in this study. In reality this may not be the case and the overall figure may be somewhat higher than indicated. This assumption might underestimate the true *Salmonella* prevalence at single egg level.

R.5. *Salmonella* prevalence for other factors of interest

R.5.1. The following factors of interest were considered to evaluate whether there were differences in presence/absence of *Salmonella* on eggs:

- Lion Code (Yes, No)
- Size of retail outlet (Large, Medium, Small) (as categorised in 5.4.1)
- Storage temperature (Ambient, Chilled);
- Price per egg;
- Date of purchase;
- Remaining shelf-life (best before date - date of purchase).

R.5.2. Since the survey was not designed to compare *Salmonella* prevalence among these factors all results should be taken with care. To enable the calculation of *Salmonella* prevalence and 95% confidence intervals in each factor one important assumption was made:

- the egg market shares for individual countries and production types (see Chapter 2) were considered to be constant in the different categories of the factors of interest. For example, in the storage temperature factor, chilled eggs were assumed to have the same distribution across the UK and across the different product types as the ambient eggs. In some cases this may be an unlikely assumption, so reported prevalence levels and confidence intervals should be interpreted with care.

R.5.3. Table 3 shows the prevalence of *Salmonella* in a box of 6 eggs and the 95% confidence intervals for Lion Code/Non-Lion Code eggs, ambient/chilled eggs and large/medium/small retailers. There were no statistically significant differences in *Salmonella* prevalence between Lion Code/Non-Lion Code eggs ($p > 0.5$) and between ambient/chilled eggs ($p > 0.50$). However, medium retail outlets had a statistically significant higher prevalence than large and small retail outlets ($p = 0.005$ and $p = 0.026$ respectively). There was no difference between large and small retail outlets ($p > 0.5$).

Table R.3.: The prevalence of *Salmonella* per box of 6 eggs from Lion Code/Non-Lion Code, ambient/chilled and large/medium/small eggs. Figures shown include 95% confidence intervals.

Factors of Interest (sample size)	Lower 95% CI	Prevalence	Upper 95% CI
Lion Coded (4753)			
Yes (4030)	0.26%	0.49%	0.83%
No (723)	0.09%	0.63%	2.13%
Storage Temperature (4753)			
Ambient (4515)	0.29%	0.52%	0.84%
Chilled (238)	0.00%	0.00%	5.87%
Retail Size (4753)			
Large (3582)	0.15%	0.34%	0.67%
Medium (593)	0.94%	2.42%	5.04%
Small (578)	0.00%	0.00%	1.11%

R.5.4. To calculate the prevalence and 95% confidence intervals for the continuous variables (price per box, date of purchase, remaining shelf-life), the data was divided into meaningful categories. Thus we have: [DN: we need some justification for this periods]

- Price per box was divided into two categories: boxes that cost less than £1 and boxes that cost more or equal than £1.
- Date of purchase was divided into 3 categories of 30 days each and a fourth category of 39 days.
- Remaining shelf-life was divided into 2 categories: less or equal than 14 days and more equal than 14 days.

R.5.5. Table 4 show the prevalence of *Salmonella* in a box of 6 eggs and the 95% confidence intervals for price per box, date of purchase, remaining shelf-life. There were no statistically significant differences in *Salmonella* prevalence in the price per box ($p > 0.90$), in the date of purchase (all $p > 0.09$) and in the remaining shelf-life ($p > 0.40$).

Table R.4.: The prevalence of *Salmonella* per box of 6 eggs. Figures shown include 95% confidence intervals.

Factors of Interest (sample size)	Lower 95% CI	Prevalence	Upper 95% CI
Price per box (4744)			
Less than £1 (3670)	0.26%	0.48%	0.82%
More or equal than £1(1074)	0.00%	0.21%	3.74%
Date of purchase (4753)			
First 30 days (1773)	0.11%	0.36%	0.90%
Second 30 days (1278)	0.28%	1.09%	2.86%
Third 30 days (1172)	0.00%	0.00%	0.58%
Fourth 39 days (530)*	0.00%	0.25%	2.04%
Remaining shelf-life (4591)			
Less or equal than 14 days (1745)	0.27%	0.64%	1.28%
More than 14 days (2846)	0.18%	0.42%	0.85%

*Includes the last nine days of the survey.

R.6. Comparison with the 1995/96 survey

R.6.1. The findings from the samples taken in England in the current survey were compared to those in the previous egg survey in England in 1995/96. Statistical analysis showed that the prevalence of *Salmonella* contamination of eggs in the 2003 survey was statistically significantly lower than the prevalence in 1995/96 ($p = 0.033$). Table 5 compares the *Salmonella* prevalence for the two surveys at the box level using England data from the 2003 survey. A statistical comparison with the findings of an earlier survey in England and Wales 1991 (de Louvois 1993) has not been undertaken as the earlier survey only sampled eggs from high street retail outlets.

Table R.5.: The prevalence of *Salmonella* per box of eggs in England in 2003 compared to the previous survey in 1995/96. Figures shown include lower and upper 95% confidence intervals.

Box Level			
	Lower 95% CI	Prevalence	Upper 95% CI
2003 England	1/370 boxes	1/200 boxes	1/110 boxes
2003 England	0.27%	0.52%	0.91%
1995/96	1/120 boxes	1/100 boxes	1/90 boxes
1995/96	0.83%	0.99%	1.17%

ANNEX S : RAW DATA FOR THE SURVEY RESULTS

Table S.1.: The proportion of eggs from different outlets relative to the market share

Retail outlet size	Sampling plan	Survey sample
Large retailers	3800	3582
Medium retailers	585	592
Small retailers	339	579

Table S.2.: Number of samples in survey according to egg production types and retail outlets in Great Britain

Retail Outlets	Caged	Free Range	Barn	Organic	Total
Large	1820	550	578	634	3582
Medium	359	107	46	81	593
Small	197	148	161	72	578

Table S.3.: Number of sample in survey according to egg production types and retail outlets in England

Retail outlets	Caged	Free Range	Barn	Organic	Total
Large	1234	310	330	410	2284
Medium	119	53	40	30	242
Small	143	111	147	54	455

Table S.4.: Number of sample in survey according to egg production types and retail outlets in Wales

Retail Outlets	Caged	Free Range	Barn	Organic	Total
Large	72	20	21	25	138
Medium	11	4	2	3	20
Small	7	6	5	3	21

Table S.5.: Number of sample in survey according to egg production types and retail outlets in Scotland

Retail Outlets	Caged	Free Range	Barn	Organic	Total
Large	315	102	118	103	638
Medium	62	20	3	14	99
Small	24	26	9	15	74

Table S.6.: Number of sample in survey according to egg production types and retail outlets in Northern Ireland

Retail Outlets	Caged	Free Range	Barn	Organic	Total
Large	199	98	129	96	522
Medium	167	30	1	34	232
Small	23	5	0	0	28

ANNEX T: LETTER SENT TO RETAILERS DURING SAMPLING.

<Name and Address of retailer>

<Date>

Dear <Name of Retailer> ,

Sample purchase for UK-wide survey of *Salmonella* contamination of UK produced shell eggs on retail sale

The Food Standards Agency is conducting a survey of *Salmonella* contamination of UK produced shell eggs on retail sale. Sampling will begin on 3rd March 2003 and will run for at least 12 weeks.

Representatives of the Agency will be purchasing samples of shell eggs from a number of your stores. Samples will also be collected from a variety of retailers, including small independent shops as well as larger retail outlets.

At the end of the survey, the results and all information that has been collected about the samples will be published on the Agency's Web-site www.food.gov.uk.

We will notify you when the results of the survey are to be published on the web-site. Please note that the survey is not for enforcement purposes.

The primary objective of the survey is to determine whether the level of *Salmonella* contamination of UK produced eggs has changed since the previous survey in 1995/96 in the light of various interventions, the main one of which is vaccination. It should be noted that this survey focuses on UK produced eggs and is not intended to provide information on seasonality. It is however possible that a small number of non-UK eggs will be sampled, as they are generally not identifiable at retail. However, as far as possible, the aim will be to identify the source of all *Salmonella* positive eggs and the results of any non-UK eggs will be reported separately.

I would be grateful if you could pass this letter on to the relevant person(s) if you are not the contact point.

If you have any queries, please contact Mrs Florence Opehan, Microbiological Safety Division on the following telephone number: 020 7276 8960 or send an E-mail to florence.opehan@foodstandards.gsi.gov.uk

19/03/04

Yours sincerely

Dr Judith Hilton
Head, Microbiological Safety Division

ANNEX U: GLOSSARY OF TECHNICAL TERMS USED IN THE SURVEY

U.1. Confidence interval – A confidence interval is an interval, which determines a range of likely values for a population/regression parameter (e.g., the prevalence of *Salmonella*, a logistic regression coefficient). It gives an estimated range of values that has a specified probability of containing the parameter being estimated. The most commonly used is the 95% confidence interval that has a 0.95 probability of containing the parameter. The width of the confidence interval gives some indication about how uncertain we are about the unknown parameter.

U.2. Bonferroni Correction – The Bonferroni Correction is a multiple-comparison correction based on the division of the p-value by the number of comparisons. It is used when several categories (e.g., countries, production type) are being compared simultaneously, so spurious significant differences are avoided.

U.3. Caged eggs

U.3.1 Unenriched cage systems (Regulation 74/1999 (*Article 5*)). At least 550cm² per hen of cage area, measured in a horizontal plane, which may be used without restriction, in particular not including non-waste deflection plates liable to restrict the area available, must be provided for each laying hen. A feed trough that may be used without restriction must be provided. Its length must be at least 10cm multiplied by the number of hens in the cage.

U.3.2. Unless nipple drinkers or drinking cups are provided, each cage must have a continuous drinking channel of the same length as the feed trough as previously mentioned. Where drinking points are plumbed in, at least two nipple drinkers or two cups must be within reach of each cage. Cages must be at least 40cm high over at least 65% of the cage area and not less than 35cm at any point. Floors of cages must be constructed so as to support adequately each of the forward-facing claws of each foot. Floor slope must not exceed 14% or 8%. In the case of floors using other than rectangular wire mesh, Member States may permit steeper slopes and cages shall be fitted with suitable claw-shortening devices.

U.3.3. Enriched cage systems (Regulation 74/1999(*Article 6*)).

At least 750cm² of cage area per hen, 600cm² of which shall be usable; the height of the cage other than that above the usable area shall be at least 20cm at every point and no cage shall have a total area that is less than 2000cm the cage must contain a nest. Litter such that pecking and scratching are possible and appropriate perches allowing at least 15cm per hen. A feed trough which may be used without restriction must be provided. Its length must be at least 12cm multiplied by the number of hens in the cage. Each cage must have a drinking system appropriate to the size of the group; where

nipple drinkers are provided, at least two nipple drinkers or two cups must be within the reach of each hen. To facilitate inspection, installation and depopulation of hens there must be a minimum aisle width of 90cm between tiers of cages and a space of at least 35cm must be allowed between the floor of the building and the bottom tier of cages. Cages must be fitted with suitable claw-shortening devices.

U.4. Free-range eggs (Regulation 2295/2003) must be produced in establishments which satisfy at least the conditions specified in Article 4 of Council Directive 1999/74/EC with effect from the dates referred to in that Article, and in which:

—hens have continuous daytime access to open-air runs, except in the case of temporary restrictions imposed by veterinary authorities.

—the open-air runs to which hens have access are mainly covered with vegetation and not used for other purposes except for orchards, woodland and livestock grazing if the latter is authorised by the competent authorities.

—the open-air runs must satisfy at least the conditions specified in Article 4(1)(3)b)(ii) of Council Directive 1999/74/EC whereby the maximum stocking density is not greater than 2 500 hens per hectare of ground available to the hens or one hen per 4m² at all times; however, where at least 10m² per hen is available and where rotation is practised and hens are given even access to the whole area over the flock's life, each paddock used must at any time assure at least 2.5m² per hen.

—the open-air runs do not extend beyond a radius of 150m from the nearest pophole of the building. However an extension of up to 350m from the nearest pophole of the building is permissible provided that a sufficient number of shelters and drinking troughs within the meaning of that provision are evenly distributed throughout the whole open-air run with at least four shelters per hectare.

U.5. Barn eggs (Regulation 74/1999 (*Article 4*)). Hens must have:

(a) either linear feeders providing at least 10cm per bird or circular feeders providing at least cm per bird;

(b) either continuous drinking troughs providing 2.5cm per hen or circular drinking troughs providing 1cm per hen. In addition, where nipple drinkers or cups are used, there shall be at least one nipple drinker or cup for every 10 hens. Where drinking points are plumbed in, at least two cups or two nipple drinkers shall be within reach of each hen;

(c) at least one nest for every seven hens. If group nests are used, there must be at least 1m² of nest space for a maximum of 120 hens;

(d) adequate perches, without sharp edges and providing at least 15cm per hen. Perches must not be mounted above the litter and the horizontal distance between perches must be at least 30cm and the horizontal distance between the perch and the wall must be at least 20cm;

(e) at least 250cm² of littered area per hen, the litter occupying at least one third of the ground surface. The floors of installations must be constructed so as to support adequately each of the forward-facing claws of each foot. In

addition to the provisions laid down there shall be no more than four levels where headroom between the levels must be at least 45cm. The drinking and feeding facilities must be distributed in such a way as to provide equal access for all hens. The levels must be so arranged as to prevent droppings falling on the levels below. If laying hens have access to open runs, there must be several popholes giving direct access to the outer area, at least 35cm high and extending along the entire length of the building; in any case, a total opening of 2m must be available per group of 1000 hens. Open runs must be appropriate to the stocking density and to the nature of the ground, in order to prevent any contamination. Equipped with shelter from inclement weather and predators and, if necessary, appropriate drinking troughs.

U.5.1. The stocking density must not exceed nine laying hens per m² usable area. However, where the usable area corresponds to the available ground surface, Member States may, until 31 December 2011, authorise a stocking density of 12 hens per m² of available area for those establishments applying this system on 3 August 1999.

U.6. Organic hens/ eggs – The organic production type must comply with EU Regulation 2092/91. There are several guidelines that relate to the production of organic eggs, United Kingdom Register of Organic Food Standards (UKROFS) and the Soil Association Standards. UKROFS state the following for poultry and poultry products.

U.6.1. The poultry house at least one third shall be solid, that is, not of slatted or of grid construction, and covered with a litter material such as straw, wood shavings, sand or turf. Laying hens should have sufficiently large part of the floor area available to the hens must be available for the collection of bird droppings. They must have perches of a size and number commensurate with the size of the group of the birds. They must have exits /entry pop-holes of a size adequate for the birds, and these pop-holes must have a combined length if at least 4m per 100m² area of the house available to the birds. Each poultry house must not contain more than 3000 laying hens. Natural light may be supplemented by artificial means to provide a maximum of 16 hours of light per days with a continuous nocturnal rest period without artificial light of at least eight hours. Poultry must have access to an open-air run whenever possible and have such access for at least one third of their life.

U.6.2. Open air runs must be covered with vegetation be provided with protective facilities and permit hens to have easy access to adequate numbers of drinking and feeding troughs. For health reasons building must be emptied of livestock between each batch of poultry reared. The building and fittings are to be cleaned and disinfected during this time. In addition, when rearing of each batch of hens has completed, runs must be left empty for at least two months to allow vegetation to grow back and for health reasons. These requirements shall not apply to small numbers of hens which are not kept in runs and which are free to roam throughout the day (cited from UKROFS Standard 2000).

U.6.4. The Soil Association Standards allow up to 500 laying birds are permitted in any one housing unit. Occasionally permission to allow up to 2000 birds is permitted, but a 100m ranging distance must be supplied and birds are not allowed to be housed more than 6 per m². A special derogation must first be granted from Soil Association Certification Limited. Birds must be fed with a minimum 80% of their feed grown to SA organic standards. The permitted allowance of non-organic ingredients (20%) must be from sources guaranteed free of genetically modified organisms. This allowance will decrease in future years until 100% organic feed must be given.

U.6.5 Beak trimming is prohibited under Soil Association Standards. It is used to prevent birds harming each other by pecking, which arises when the stocking rates are too high and the birds' environment is not stimulating enough, leading to high levels of stress and unnatural behaviour. Antibiotics routine, preventative use of antibiotics, administered in the feed, is prohibited in organic systems (cited from the Soil Association website <http://www.soilassociation.org/web/sa/saweb.nsf/0/80256c840055c30580256ca600594c96?OpenDocument>).

U.7. Laid in Britain Scheme- The "Laid in Britain Quality Assurance" scheme is aimed specifically at independent egg producers, and is run by the UK Egg Producers Association (UKEP) Ltd. This scheme uses, competitive exclusion has been chosen as the method of disease control because of its proven record against all salmonellae, coliforms, pasturella and other potential food safety hazards.

U.8. Lion code- The mark has featured prominently on egg boxes since 1998 following the launch of the British Egg Industry Council's (BEIC) Code of Practice. From 1 January 2000 the red Lion Quality mark has also been stamped on all individual eggs produced under the Code. The code covers all aspects of production from farm to retail including welfare and hygiene. New measures in the Lion Quality Code of Practice include:

- Vaccination of laying hens against *Salmonella enteritidis*
- Registration and traceability of laying hens and eggs
- Controls on storage time and temperature including a 21 day best before date on the shell and packaging.

(cited from the British Egg Information Service (BEIS) website <http://www.britegg.co.uk/beis/beis2nf.htm>).

ANNEX V COMMENTS FROM RETAILERS ON SALMONELLA POSITIVE RESULTS

V.1 Safeway – Paul Raynor and Jill Morris – Comments on *Salmonella* positives

V.1.1. We were surprised and disappointed to learn that the FSA found *Salmonella* in two samples of eggs purchased from Safeway stores.

V.1.2. We only sell eggs from vaccinated flocks and all of our own test results have been clear for *Salmonella* since vaccination began in 1998.

V.1.3. Although we are confident through the vaccination programme and our negative results, we do continue to advise customers that expectant mothers, infants, the elderly and those susceptible to infections should avoid eating dishes containing raw or lightly cooked eggs.

V.2 The Co-operative Group –Cathryn Higgs – Comments on *Salmonella* positives

V.2.1. We were naturally concerned by the Agency's findings and in conjunction with our supplier initiated an immediate and thorough investigation of the laying flocks, hatcheries and feed mills concerned. It is however unfortunate that there was a delay of 8 months in notification of the results which inevitably hindered the efficacy of our investigations.

V.2.2. All Co-op Brand Eggs are from flocks accredited to the Lion Code. The Code, which is designed to enhance standards of food safety in the industry, includes a requirement to vaccinate flocks against *Salmonella* and also to test eggs (shell and content separately) for the presence of *Salmonella*. The current level of testing, which includes the farms of concern, amounts to some 23,000 eggs a year and with the exception of the results notified by the Agency there have been no positive findings. In addition independent testing by BEIC, where again no positive results were found, has been backed up. Despite the reassurance that this large scale testing provides we remain concerned by the Agency's findings and are continuing to investigate the matter in conjunction with the maintenance of existing preventative controls and monitoring as an outgoing safeguard.

November 2003

ANNEX W : REFERENCES

ACMSF. *Report on Salmonella in eggs*. Advisory Committee on the Microbiological Safety of Food. HMSO, London, 1993.

ACMSF. *Second report on Salmonella in eggs*. Advisory Committee on the Microbiological Safety of Food. HMSO, London, 2001.

Adak GK, Long SM, O'Brien SJ. Trends in indigenous foodborne disease and deaths, England and Wales: 1992-2000. *Gut* 2002; **51(6)**: 832-841.

BEIC. BEIC calls for ban on imported eggs. Press release 25/10/02. British Egg Industry Council 2002. www.britegg.co.uk/advertsection/press24.html

Clayton D, Hills M. *Statistical Models in Epidemiology*. Oxford Science Publications, 1993. pp. 362.

Cogan TA, Humphrey TJ. The rise and fall of *Salmonella* Enteritidis in the UK. *J Appl Microbiol* 2003; **94 Suppl**: 114 -119.

Counter DE, Gibson EA. *Salmonella dublin* infection in self-contained dairy herds in East Anglia: excretion at calving. *Vet Rec* 1980; **107(9)**: 191-193.

Davies, R, Breslin M. Effect of vaccination and other preventive methods for *Salmonella* Enteritidis on commercial laying chicken farms. *Vet Rec* 2003; **153**: 673-677.

Davison AC, Hinkley DV. *Bootstrap Methods and their Application*. Cambridge University Press, 1997. pp. 582.

Defra Eggs Statistic Notices
<http://statistics.Defra.gov.uk/egg/statnot/eggnotice.pdf>

de Louvois J. *Salmonella* contamination of eggs; a potential source of human salmonellosis. *PHLS Microbiology Digest* 1993; **10 (3)**: 158-162.

Farrington CP. Estimating prevalence by group testing using generalised linear models *Statistics in Medicine* 1992; **11**: 1591-1597.

ICMSF. *Microorganisms in foods 5. Characteristics of microbial pathogens*. The International Commission on Microbiological Specifications for Foods of the International Union of Biological Societies, Blackie Academic and Professional, London. 1996. pp.513.

Kado CL, Liu S-T. Rapid procedure for detection and isolation of large and small plasmids. *J Bacteriol* 1981; **145**: 1365-1373.

Liebana E, Garcia-Migura L, Clouting C, Cassar CA, Clifton-Hadley FA, Lindsay EA, Threlfall EJ, Chappell SA, Davies RH. Investigation of the genetic diversity among isolates of *Salmonella enterica* serovar Dublin from animals and humans in England, Wales and Ireland. *J Appl Microbiol* 2002; **93**: 732-744.

Maguire H, Cowden J, Jacob M, Rowe B, Roberts D, Bruce J, Mitchell E. An outbreak of *Salmonella dublin* infection in England and Wales associated with soft unpasteurized cows' milk cheese. *Epidemiol Infect* 1992; **109**: 389-396.

Mintel. Eggs, Market Intelligence report – June 2002, Mintel International Group Ltd, London.

National Food Survey 2000.

<http://statistics.defra.gov.uk/egg/publications/nfs/2000/default.asp>

Plym-Forsshell L, Ekesbo I. Survival of salmonellas in urine and dry faeces from cattle- an experimental study. *Act Vet Scand* 1996; **37(2)**: 127-131.

Powell NG, Threlfall EJ, Chart H, Rowe B. Subdivision of *Salmonella enteritidis* PT4 by pulsed-field gel electrophoresis: potential for epidemiological surveillance. *FEMS Microbiol Lett* 1994; **119**: 193-198.

Public Health Laboratory Service (PHLS). Public Health Investigation of *Salmonella* Enteritidis in raw shell eggs. *CDR Weekly* 2003; **13(2)**: 1-3.

Richardson A. The transmission of *Salmonella dublin* to calves from adult carrier cows. *Vet Rec* 1973; **92(5)**: 112-115.

Roberts JA, Sockett PN. The socio-economic impact of human *Salmonella enteritidis* infection. *Int J Food Microbiol* 1994; **21(1-2)**: 117-129.

Roberts JA, Cumberland P, Sockett PN, Wheeler J, Rodrigues LC, Sethi D, Roderick PJ; Infectious Intestinal Disease Study Executive. The study of infectious intestinal disease in England: socio-economic impact. *Epidemiol Infect* 2003; **130(1)**: 1-11.

Small RG, Sharp JMC. A milk-borne outbreak of *Salmonella dublin*. *J Hyg Camb* 1979; **82**: 95

Soil Association

<http://www.soilassociation.org/web/sa/saweb.nsf/0/80256c840055c30580256ca600594c96?OpenDocument>

Threlfall EJ, Fisher IS, Ward LR, Tschape H, 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.

United Kingdom Register of Organic Food Standards (UKROFS) – UKROFS Standard for Organic Food Production: Standard for organic livestock and organic livestock products, August 2000 Edition.

Veterinary Laboratories Agency. *Salmonella* in livestock production in GB, 2002. VLA Weybridge.

Wilson IG, Heaney JC, Powell, GG. *Salmonella* in raw shell eggs in Northern Ireland: 1996-7. *Commun Dis Public Health* 1998; **1(3)**: 156-160.