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Method for Identifying the Production system from egg characteristics

A report for the Australian Egg Corporation Limited

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Foreword

The overall aim in this project was to develop a non-destructive method for identifying the accuracy of the label used for batches of barn and free range eggs. The system would allow remote checking of the authenticity of product labelling.

Consumers need reassuring that product description matches product reality. There is concern that packs of free range and barn eggs may contain standard eggs from cage production systems. The benefits of a remote, non-destructive test for label authenticity include:

- Greater consumer confidence in the labelling standards used by the egg industry
- Improved image for the egg industry in terms of marketing standards
- Resolution of disputes about label accuracy
- Responding to customer and regulatory authority enquiries in a professional manner.

The method identified the dust pattern that develops on the shell when an egg rolls down the floor of a wire cage. The dust pattern was examined under ultraviolet light in a dark room, and a cage floor pattern was identified from parallel fluorescent dust lines. There is no single fluorescent pattern that identifies all barn or free range systems. In other words, the test is best used for identifying eggs that are laid on wire, rather than eggs laid on some other type of material.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Executive Summary

The overall aim in this project was to develop a non-destructive method for identifying the accuracy of the label used for batches of barn and free range eggs. The system would allow remote checking of the authenticity of product labelling.

Consumers need reassuring that product description matches product reality. There is concern that packs of free range and barn eggs may contain standard eggs from cage production systems. The benefits of a remote, non-destructive test for label authenticity include:

- Greater consumer confidence in the labelling standards used by the egg industry
- Improved image for the egg industry in terms of marketing standards
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- Responding to customer and regulatory authority enquiries in a professional manner.

The method identified the dust pattern that develops on the shell when an egg rolls down the floor of a wire cage. The dust pattern was examined under ultraviolet light in a dark room, and a cage floor pattern was identified from parallel fluorescent dust lines. The dust is mainly due to fluorescent particles present in the hens' feed. On average, 27% of cage laid eggs had parallel lines, and 0.4% of barn or free range eggs had similar marks.

Washing the eggs removed some of the parallel lines making diagnosis more difficult, so the test is best applied in unwashed eggs. Condensation on unwashed eggs did not mask the lines, and egg size had little effect on the proportion of washed eggs with these marks.

Eggs pick up fluorescent dust whilst they are still moist after being laid by the hen. If a moist egg rolls on the wire below the feed trough it is likely to show parallel lines. Regular dusting of this part of the cage floor is likely to result in fewer eggs with parallel lines, but occasional dusting is not likely to influence the outcome of the test.

Barn and free range egg producers use a wide variety of nestbox materials including sawdust, shavings, hay, straw, synthetic rubber mats, plastic mats, shell and grit. There is no single fluorescent pattern that identifies all barn or free range systems. In other words, the test is best used to identify eggs that are laid on wire, rather than eggs that are laid on some other type of material. The test is not a reliable way of checking whether barn or free range eggs are being sold under a standard (cage-laid) label.

For routine testing, it is recommended that 90 eggs should be sampled, and if 4 or more of those eggs have parallel fluorescent lines it can be concluded that some of the eggs in that batch were probably laid on wire. It is, however, advisable to make enquiries with the egg producer to check whether there is a legitimate reason for the fluorescent double lines, before raising a complaint.

The equipment needed for the test can be bought from an Ultraviolet Equipment Supplier. The cheapest 6 watt ultraviolet lamps are about $150, but there is better discrimination with more powerful lamps that emit less visible (blue) light, and those lamps are more expensive.

1. Introduction

In Australia, food traders are not allowed to falsely represent the items that they sell (e.g. Fair Trading Act 1987 [SA] and the Trade Practices Act 1974 [Commonwealth]). In the case of eggs, traders must not sell eggs that were laid by hens in cages as either 'barn' or 'free range' eggs. Modifying the label in this way would misrepresent the production system. Enforcement of this legal requirement has not been easy. The main aim in this study was to develop a simple method for checking the authenticity of labelling used for barn or free range eggs. The method was first proposed by Lob (1992). It is based on the facts that materials in the layer shed environment fluoresce when exposed to ultraviolet light, and, when an egg is laid by a hen it picks up some of that fluorescent material. The fluorescent patterns on the egg reflect the pattern of the surface onto which the egg was laid. In this study the effects of five variables on surface fluorescent patterns were examined; egg production system, egg washing and oiling, egg size, condensation on the eggs, and dusting of the cages. In addition a series of trials evaluated the likely source of the fluorescent material.

2. Materials and methods

2.1 Production system

11,520 eggs were examined at either their farm of origin in Victoria and South Australia or at a central egg packing plant. The type of floor material onto which the egg was laid was known for each farm from either the egg packing station auditor's comments or from direct inspection of the egg laying unit. 360 eggs from each of 20 cage, 7 barn and 5 free range units were examined for fluorescent marks under a uv lamp. All the eggs had brown shells except for two batches from white-shelled caged layer farms.

The eggs were examined under a 125W uv lamp (Philips HPW blacklight) in a dark room and classified into categories according to the pattern of white, off-white, bluish white or yellowish white fluorescent marks. From preliminary trials it was found that 365 nm uv wavelength produced a stronger background fluorescent red colour than 254 nm, and this produced better contrast for identifying the white fluorescent lines. For this and safety reasons, a blacklight uv_a lamp was the preferred source of uv light. Eggs were classified into five categories as follows.

- *double lines*. Two distinct parallel lines at 2.2 to 2.5 cm spacing, which corresponds to the distance between adjacent wires in a cage floor
- *indefinite*. Two parallel lines which were not 2.2 to 2.5 cm apart, or one clear line with another mark or line that was at an angle to the first line
- *single line*. One distinct line
- *scuffed*. Lines within a mark that had the appearance of a scuff, scratch or rubbing
- *nothing*. No obvious lines.

The double lines had an etched appearance, from the contrast provided by the background fluorescent red colour. The indefinite category included some eggs that could equivocally have been classed as double lines, but because there was an element of doubt they were called indefinite. White fluorescent marks that were specific to barn or free range units were noted, and the prevalence of those marks recorded for two farms.

2.2 Effects of egg washing, egg size, condensation, and cage dusting

The effect of automatic washing and grading on the prevalence of double lines was examined at an egg packing plant, using a total of 7,200 eggs from 5 battery cage layer farms. Trays of eggs were received at the plant on pallets, wrapped in clear cling film. They were de-stacked and put through an automatic nylon brush scrubber, spray washer and egg-oiling machine. The egg washer collected and re-used the washing solution, which included Dairy Chlor, Liquatec Low Foam Detergent, and Kemsol antifoam. The Dairy Chlor contained sodium hypochlorite to sanitise the washing solution, and the antifoaming agent was used to reduce frothing produced by broken eggs mixing with the washing solution. Oiling was with a drop of canola oil applied to the blunt end of the egg after washing. 360 eggs were examined before washing, and 360 eggs of each of the following weight grades were examined after washing, oiling and packing; 50-53g, 53-57g, and 59-62g. The post-washing samples were also used for assessing the effect of egg size on the prevalence of double lines. In addition, 360 eggs were examined under uv light before and after washing at a cage layer farm which was known to have a very low prevalence of double lines (<10%). This additional test was included to help determine the likely lower limit of double lines in washed eggs. The washing procedure used at this farm was the same as that for the egg packing plant except that the egg oiler was not operating at the time of the visit. The effect of the individual components included in the washing solution on white line fluorescence was examined in selected eggs that had double lines.

The effect of condensation on the appearance of the fluorescent white lines was assessed in 25 eggs, which were previously identified as having fluorescent double lines. The eggs were held in an atmosphere which had 98% relative humidity for 5 and 80 min. The prevalence of double fluorescent lines was assessed at the end of these two periods using a 125 W uv_a lamp whilst the eggs were wet, and after the moisture had been allowed evaporate.

Three trials evaluated the role of dust in causing the fluorescent double lines on the eggs. Firstly, at a caged layer farm, the effect of the blowers that normally blow air over the manure belt and across the wire floor below the feed trough was assessed in terms of the prevalence of double fluorescent lines. The blowers were switched off for 7 days and 360 eggs were sampled. The blowers were then switched on and run continuously for 30 days, and then a second 360 egg sample was taken. Secondly, the wire floor of the egg collection area in front of three cages was cleaned with a scouring pad and painted with a non-fluorescent spray paint (Holt's Dupli-Color™) to cover any zinc or zinc salts in the galvanised wire. The paint was allowed to dry and harden for 8 days and then the cages were stocked with hens. Dust was allowed to accumulate on the egg collection area, and after 30 days the prevalence of fluorescent double lines was assessed from eggs collected over a 3.5 day period. Thirdly, the effect of dusting cage floors under and around the feed troughs with a soft-bristle banister brush was examined at a caged layer farm. 360 eggs were examined that had been laid before and 360 eggs laid within 20 h of manual dusting.

2.3 Source of the fluorescence

In addition to the dusting trials described above, the following trials were conducted to examine the likely source of the double fluorescent lines.

The source of the fluorescent material was traced by searching for fluorescent materials in the caged layer shed environment, in the feed ingredients used in preparing layers mash, and by staining the double white fluorescent lines on cage laid eggs for starch using a potassium iodide/iodine stain (120/39 mmol/L/mmol/L), for free amino groups in protein using a 0.2% ninhydrin spray in ethanol, and for sulphydryl groups using ammonium 4-chloro-7-sulphobencofurazan (0.4 mmol/L in borate buffer plus 1 mmol/L EDTA) (Andrews *et al* 1982). Twelve eggs bearing distinct double lines were used in each test.

It was thought that eggs acquire the white fluorescent lines whilst they are still wet with lacquer produced by the hen. Trials were conducted on the uptake of different materials in the shed environment by wet eggs, on subsequent fluorescence of the egg surface. Eggs were palpated from the oviducts of barn hens that were about to lay their eggs. The rate of drying of the eggs was recorded for 10 eggs at an ambient shed temperature of 21 °C. In addition, eggs that were freshly palpated from hens' oviducts were immediately scraped over a cage wire floor surface before the egg lacquer dried. Three types of cage wire floor were tested using four eggs in each treatment: wire that had previously been cleaned with a scouring pad (cleaned wire); wire regions where the galvanising had corroded to produce a white surface layer (corroded wire); and wire that was encased in congealed dirt (dirty wire). In addition, one egg bearing wet lacquer was rubbed against wire that was coated in brown iron rust (rusty wire), and one egg bearing wet lacquer was rolled in shed dust that had collected on the roof of a nestbox, and was at least 3 m distance from a feed trough (shed dust). When the lacquer had dried, the eggs were viewed under a 125 W uv_a lamp, to determine the fluorescent colour of the marks.

2.4 Effect of exposure to sunlight and uv light

During the course of work at the central egg packing plant, it was noted that some eggs had an unusual background fluorescent colour. These eggs were at the top and sides of the pallets of eggs delivered to the plant. This lead us to examine the effect of exposure to sunlight on the background fluorescent red colour of the egg shell, by leaving 16 eggs in bright sunshine. In addition, the effects of exposure to sunlight on egg shell fluorescent colour viewed under a 6 W uv lamp and egg albumen height, were examined in 40 eggs. Colour of the fluorescence was scored using Methuen Colour Charts (Kornerup and Wanscher 1978), and albumen height was measured in Haugh Units.

The effect of 23 h exposure to uv_a light on the appearance of fluorescent double white lines was examined by holding 6 eggs, which had double lines, at 35 cm below a uv_a lamp (6 W Spectroline® BLF-6A, Spectronics Corporation, New York) for 23 h.

2.5 Survey of egg laying floor materials

A total of 50 egg producers in Queensland and South Australia took part in a telephone survey of the floor materials on which their hens laid eggs. Some producers operated more than one type of egg production system, and so there were 62 egg production units represented in the survey.

Since it was suspected that dust from the feed was at least partly responsible for the white fluorescent lines, 19 cage egg producers in Queensland were asked whether they periodically dusted or cleaned the egg collection area at the front of the cages.

2.6 Statistics

The proportions of barn-laid or free range eggs and cage-laid eggs with fluorescent double lines were estimated using logistic regression (generalised linear model with binomial distribution and logit link).

Point estimates of the two proportions (p_f in barn or free range populations and p_c in caged populations) were obtained, together with 95% confidence intervals, allowing for over-dispersion where the data exhibited greater variability than expected from binomial data, namely point estimate ±1.96 times the standard error using dispersion parameters estimated from the data, calculated on the logit scale then transformed to proportions.

Given caged eggs showed a substantially higher proportion of fluorescent double lines, a batch claimed to be barn or free range would be questioned for authenticity if the proportion of eggs with fluorescent double lines in a sample from that batch was greater than some critical value. The critical value was determined by a one-sided 0.1% level significance test of the null hypothesis H_0 that all eggs in the batch were barn or free range, against the alternative that the batch contained some cage eggs. For an assumed true proportion of eggs with fluorescent double lines in a barn or free range population (p_f, taken as either the upper 95% confidence bound $\tilde{p}_f$ for a conservative test, or the point estimate $\hat{p}_f$), the cumulative probability distribution of the number with fluorescent double lines from a given sample size was calculated based on a binomial distribution. The critical value was chosen as the number of eggs for which the probability of that number or more eggs with double fluorescent lines was less than 0.001 under H_0.

The sample size to be used during routine Label Authenticity Testing was based on power of the test, in other words the probability that a barn or free-range claim will be questioned, for various levels of contamination with cage eggs. It estimated the true proportion of fluorescent double line eggs in a caged population (p_c), using either the point estimate $\hat{p}_c$, or for more conservative power estimates, the lower 95% confidence bound $\tilde{p}_c$. For simplicity, these probabilities were estimated based on samples (rather than populations) assumed to contain the specified proportion of caged eggs. The significance level and hence critical number of fluorescent double line eggs would depend only on p_f and not on p_c. The value used for p_c would affect only the estimated power of the test.

3. Results

3.1 Production system

The free range and barn farms used nestboxes. None of the nestboxes had wire floors, or wire floor egg collection areas on which the eggs rolled as they passed from the nestboxes.

The prevalence of the different fluorescent marks for each type of production system is shown in Table 1. The prevalence of fluorescent double lines was a distinguishing feature for cage-laid eggs. On average 27% of the cage eggs showed this feature, whereas, on average 0.4% of the barn and free range eggs had double lines. The effect of sample size in determining the number of eggs with double lines at the point of rejecting the hypothesis that a batch of eggs was all barn or free range ($p<0.001$) is shown in Table 2. Table 2 shows critical numbers of fluorescent double line eggs and ranges of power for various sample sizes, using the conservative $\tilde{p}_f = 0.008$ to ensure a 0.001 significance level, and $\tilde{p}_c = 0.19$ for conservative estimates of power.

Table 1. Prevalence of the different types of white fluorescent marks on eggs according to egg production system % (range).

Fluorescent marks	Cage	Barn	Free Range
Number of farms	*20*	*7*	*5*
Double lines	27 (5-84)	0.4 (0-1.1)	0.4 (0- 1.4)
Indefinite	5 (1-13)	8 (0-16)	3 (0- 9)
Single lines	7 (0-14)	4 (1-14)	6 (2-14)
Scuffs	3 (0- 6)	7 (2-16)	9 (1-22)
Nothing	58 (10-82)	81 (54-96)	81 (53-96)

For example, using the conservative estimates $\tilde{p}_f$=0.008 and $\tilde{p}_c$=0.19, with a random sample of 90 eggs from a batch claimed to be barn or free range, the null hypothesis H_0 that the batch was all barn or free range eggs would be rejected at the 0.1% level if the sample contained 5 eggs with fluorescent double lines. The estimated probability of rejection was almost exactly 100% if the batch was entirely caged eggs, 96% if the batch was 50% caged eggs, and 52% if the batch was 25% caged eggs. The sample size would need to be much larger if a high power was required before questioning whether a batch was contaminated with a small number of cage eggs. For example, to have 99.9% chance of rejecting H_0 in a sample with 25% caged eggs, the sample size would need to be 430 with a critical value of 11 fluorescent double line eggs.

Table 2: Critical numbers of eggs with fluorescent double lines (using the conservative $\tilde{p}_f$ = 0.008) and ranges of power for various sample sizes. All power estimates are conservative, using $\tilde{p}_c$ = 0.19.

Sample size n	Critical number of eggs with fluorescent double lines	Power assuming different levels of dilution		
		100% caged	*50% caged*	*25% caged*
7 to 24	3	0.13 to 0.86	0.01 to 0.43	0.00 to 0.12
25 to 54	4	0.73 to 1.00	0.20 to 0.92	0.03 to 0.30
55 to 94	5	0.99 to 1.00	0.64 to 0.97	0.17 to 0.56
95 to 140	6	1.00	0.92 to 1.00	0.41 to 0.78
141 to 192	7	1.00	0.99 to 1.00	0.64 to 0.91
193 to 248	8	1.00	1.00	0.83 to 0.97
249 to 309	9	1.00	1.00	0.93 to 0.99
310 to 373	10	1.00	1.00	0.98 to 1.00

Retaining the conservative estimate $\tilde{p}_f$=0.008 so as to be confident the test was maintained at less than the 0.1% level, but using the less conservative estimate $\hat{p}_c$=0.27, the estimated power was 100%, 99.8%, or 82% for all, 50%, or 25% caged respectively, for a sample size equal to 90. To have 99.9% chance of rejecting H_0 in a sample with 25% caged eggs the sample size would need to be 262, with a critical number of 9 fluorescent double line eggs.

Using a 0.001 significance level would mean that we can be sure that at least 99.9% of the time when a batch is truly all barn or free range, it would not be questioned. This is regardless of sample size. However, in general, larger sample sizes will give greater power to reject batches containing some cage eggs, except when the increase in sample size crosses a boundary that increases the critical number of fluorescent double line eggs (the larger the sample, the more fluorescent double line eggs can be expected under H_0). Power is optimised at the largest sample size for a certain critical value, for which any increase will result in a jump in the critical value. The data for the individual farms are presented in Appendix 1, to allow estimation for any other chosen dilution rate of barn or free range eggs with cage-laid eggs.

At some of the cage and barn units the eggs had circular or part circular marks which corresponded to perforations on the conveyor belt onto which they rolled from the nestboxes. The prevalence of these marks was found to be 9% at one of the barn units. At another barn egg farm, which had square perforations in the conveyor belt, the prevalence of belt marks was 3%. Thirty three percent of those marks (1% of all eggs for that farm) resembled the double lines created by a wire floor cage. At two barn egg farms, the prevalence of stippling, which corresponded to the pattern of the rubber mat or the synthetic plastic turf mat in the nestboxes, was found to be 14 and 3% respectively.

3.2 Effects of egg washing, egg size, egg shell colour, condensation, and cage dusting

Washing cage-laid eggs caused a reduction in the prevalence of double lines from 20% to approximately 5% (Table 3; $p<0.001$). There was very little difference between the egg grades in the prevalence of double lines, but the 59-62g eggs had slightly more double lines than the 53-57g eggs ($p<0.05$).

Table 3. The effects of washing and egg size on the prevalence of fluorescent double lines on cage laid eggs.

Treatment	**Number of eggs examined**	**Number of eggs with double lines**	**Percent eggs with double lines**
Pre-wash ungraded	1800	366	20.3
Post-wash	5400	283	5.2
50-53g	1800	90	5.0
53-57g	1800	82	4.5
59-62g	1800	111	6.2

The four ingredients used for washing eggs in the egg packing plant were water, a chlorinated sanitising agent, a low foam detergent, and an anti-foaming agent. Wiping the egg with egg-washing detergent tended to disperse the white fluorescent lines, and applying drops of egg-washing chlorinated sanitising agent resulted in quenching of the fluorescence of both white lines and the background fluorescent redness. The anti-foaming agent had no obvious effect on the appearance or strength of the fluorescent lines.

White-shelled eggs had a similar fluorescent red background as brown-shelled eggs. There was no obvious difference in the nature of the fluorescent double lines in white and brown eggs, but the fluorescent white lines showed up more distinctly in white-shelled eggs. The prevalence of double lines at the two white egg farms was 58 and 73%.

When eggs were exposed to high humidity they developed beads of condensation on the surface. The condensation did not affect the prevalence of double white lines whilst the eggs were wet or after they had been allowed to dry by evaporation. All 25 eggs showed double white fluorescent lines before, during and after treatment with high humidity, although the lines were more distinct when the eggs were dry.

When the air blowers were switched off to allow dust to accumulate for 7 days the prevalence of double lines was 73%. When the blowers had been running for 30 days, the prevalence of double lines was similar (80%, $P > 0.05$). Painting the wire floor of the egg collection area and allowing the surface to accumulate dust over a 30 day period, resulted in 8 out of 17 eggs (47%) showing fluorescent double lines. At a separate caged layer farm, dusting the wire floor below the feed trough had no effect on the prevalence of fluorescent double lines. Before dusting, the prevalence of double lines was 21% and the day after dusting it was 21%.

3.3 Source of the fluorescence

When viewed under visible light, some batches of eggs at the egg packing plant and at the farms had obvious contamination with dust. In some cases the dust was present as lines, which corresponded to fluorescent double lines under uv light. However, inspection under visible lighting would not be sufficiently sensitive to reliably diagnose the production system.

A summary of the fluorescent materials found in the caged layer shed environment and feed is shown in Table 4. Cobwebs, wood shavings, fresh faeces, and fresh urine did not show any obvious or strong fluorescent colour. Material containing keratin, such as feather shafts and foot claws were strongly fluorescent producing a white or blue-white colour. Rubbing a bird's claw on a cage wire floor did not result in the wire becoming fluorescent, but instead tended to reduce the low-grade fluorescence present on the wire as it was rubbed off. In general dust on the cage floor and on the shed floor was not strongly fluorescent, except for the cage floor below the feed trough, which contained a large amount of white fluorescent material. Rolling an egg that had been wetted with water down a cage floor where birds had been standing did not produce any obvious fluorescent lines. This region of the cage usually has a polished appearance and is relatively dust-free because of

bird traffic. Whereas rolling a wetted egg on dusty wire that had accumulated below the feed trough, produced white fluorescent lines on the egg.

The following additional observations were made when searching for potential sources of the fluorescent material in the white fluorescent lines. The white fluorescent lines did not contain any measurable free-sulphydryl groups as determined with an ammonium 4-chloro-7-sulphobenzofurazan fluorescent probe and laboratory spectrofluorimeter. The fluorescent double lines produced distinct black lines when treated with iodine/potassium iodide stain and viewed under normal light, indicating that the lines had a high starch content. In addition they stained purple with ninhydrin, indicating that they were also rich in protein. The white corrosive layer that normally develops on galvanised cage wire floors produced a white fluorescence when viewed under a uv_a lamp, and scrapings of the white fluorescent lines on eggs contained about ten times more zinc compared with areas of egg shell that had no white fluorescent lines, as determined by atomic absorption spectrophotometry.

Table 4. Summary of results for materials obtained from a layer shed and a feed mill that were tested for fluorescence.

Material	**Colour of fluorescence**
Barley	Pale purple-blue endosperm + brown-orange husk
Wheat	Pale translucent blue endosperm
Triticale	Pale blue endosperm, brown husk
Crushed pea	Blue-white to white endosperm + pink husk
Soyabean meal	Pearl blue + green yellow
Rovabio	Blue-white with orange particles
Blood meal	Light brown
Meatmeal	Brown-yellow
Lupins	Brown
Lysine	Off-white to cream
Methionine	White
Layers' mash	Pink, blue, khaki, white and orange particles
Feather shafts	White
Chicken shank skin	White
Chicken claws	White

The interval between removing eggs from the hens' oviducts and drying of the lacquer on approximately 90% of the eggs' surface was 28 ± 3 sec ± se. When eggs bearing a wet lacquer were scraped against dirty cage wire floor, off-white or brown fluorescent marks were produced on the eggs. In the case of clean wire the fluorescent marks were off-white, and corroded wire produced blue fluorescent marks. Rusty wire produced black fluorescent lines and general shed dust resulted in khaki coloured fluorescent marks. Scraping or scratching an egg with a clean stainless steel knife created brown marks when viewed under uv. When a cage wire floor was viewed directly under uv light, the following fluorescent colours were noted, in decreasing order of prevalence: brown, yellow, black and off-white.

3.4 Effect of exposure to sunlight and uv light

A preliminary trial indicated that when eggs were exposed to sunlight over a 6 h period, the background fluorescent colour of the egg shell changed from ruby to greyish rose followed by a dull red colour. Those changes occurred when the average ambient sunlight intensity was 128 klux and average temperature in the shade was 33.1 °C.

In a subsequent trial on the effect of sunlight on egg shell fluorescence and egg albumen height, the average intensity of the sunlight was 115 ± 3 klux ± se, and temperature in the shade was 27.5 ± 0.7 °C ± se. The fluorescent colour of the shell changed from red (11-8-B) to violet brown (10-8-E) and then greyish ruby (12-5-D) with progressive exposure to sunlight. Albumen height was reduced by 2 h exposure to sunlight ($p<0.05$), by which time the fluorescent colour of the shell had changed to greyish ruby (Table 5). It should be noted that the exact fluorescent colour depends on the uv lamp that is used, because lamps differ in the amount of visible light they emit.

Table 5. Effect of duration of exposure to sunlight on egg shell fluorescent colour and mean egg albumen height ± se.

	Duration of sun exposure h			
	0	0.5	1.0	2.0
Egg weight g	52 ± 1	52 ± 1	52 ± 1	53 ± 1
Colour score †	11-8-B red	10-8-E violet brown	12-5-D greyish ruby	12-5-D greyish ruby
Albumen height	94 ± 2	91 ± 1	92 ± 2	82 ± 2

† according to the Methuen Handbook of Colour.

White double lines were still present after they had been exposed to a 6 W uv_a lamp for 23 h, but background colour faded to a greyish rose and the contrast provided for the white lines was less pronounced.

3.5 Survey of egg laying floor materials

Twenty two of the 50 farms in the survey were producing barn and/or free range eggs. None of those farms were using wire floor nestboxes, or allowed the eggs to roll from the nestboxes onto a wire collecting area. Instead, the most common nestbox bedding material was sawdust or shavings (49% prevalence), followed by hay or straw (19%), synthetic turf or rubber or plastic matting (19%) and shell or grit (13%). In 78 % of the barn and free range systems, the eggs were hand-collected from the nestboxes, and in the remainder the eggs rolled onto a belt conveyor. In all caged layer units the eggs were laid onto cage wire floor.

The cleaning frequency used in cage layer sheds varied considerably. One of the 19 cage egg producers in Queensland never cleaned the wire floor of the egg collection area in front of the cages, and one producer cleaned some of the cages once a month. At the other extreme, some units had daily automatic cleaning systems.

4. Discussion

The feature which distinguished eggs that had been laid on a wire floor in a cage was the presence of fine (up to 1.2 mm thick) parallel fluorescent lines. The spacing between the parallel lines was about 2.3 cm, and corresponded to the common spacing between adjacent wires on the floor of conventional cages. The fluorescent colour of the lines was usually white. In one instance parallel black lines were observed under the uv lamp, but those marks were traced to residual dirty water that had been picked up from a roller conveyor in the egg washer.

When this test is conducted on a routine basis for eggs claimed to be barn or free range, it is suggested that a random sample of 90 eggs is screened. If 4 or more eggs have fluorescent double lines, then that batch should be investigated, since 4 or more fluorescent double line eggs is extremely unlikely (less than one chance in 1000) if the batch was all barn or free range. It would, however, be advisable to check the laying conditions used at that farm to establish that there were no exceptional but acceptable circumstances that might explain the high prevalence of double lines.

A potential weakness of the uv test for production system is that some barn or free range egg farms may use wire floor nestboxes or have wire egg collection areas below the nestboxes. However, none of the farms that supplied eggs for this study, and none of the barn or free range farms in the survey, had those wire systems. In other words, wire floors are unusual in barn and free range units, but where fluorescent wire marks occur in barn or free range eggs, this feature needs to be checked to vindicate that particular farm.

Another potential source of confusion in this test is the design of the egg conveyor used at one farm. The conveyor produced by one equipment manufacturer had 2.2 cm x 2.2 cm square perforations in the belt. The size of the perforations corresponded closely to the distance between wires in the floor of cage systems. However, when eggs from a barn unit that had this type of conveyor were tested, the prevalence of double lines was 1%, suggesting that the marks created by the perforations would be low.

Egg washing reduced the value of the test. Chlorine in the washing solution caused quenching of the fluorescence and brushes in the washer probably removed some of the distinguishing marks. This has two implications. Firstly, the uv test for production system could only be applied successfully to washed eggs that would otherwise have a high prevalence of distinguishing wire marks. Secondly, the test is best applied to unwashed eggs. In Australia and North America this limits the test to eggs that have arrived at an egg packing plant, but in European countries where eggs are not washed, the test could also be used for eggs that are on display in retail outlets.

The range in the prevalence of double lines in the cage layer farms was large (5 to 84%). Two of the farms with a low prevalence (<10%) were operating systems that minimised dust accumulation. These systems included egg-saver wires and brushes attached to the feed hopper that brushed the cage floor below the feed trough whilst feed was being dispensed. Egg-saver wires would reduce the exposure of wet eggs to dust below the feed trough. Manure belt air blowers probably have limited effect on the prevalence of double lines.

Some of the fluorescent lines appeared to contain dust, when examined under normal lighting. In addition, the fluorescent lines were found to be rich in starch and protein, and the dust on the wire floor under the feed trough had a high content of fluorescent material. Taken together this indicated that the primary source of fluorescence was dust from the feed. Zinc salts formed during corrosion of the galvanising on the wire floor could also contribute some fluorescence.

The wide range of materials used as bedding in the barn and free range nestboxes made it difficult to select a fluorescence pattern that distinguished these types of production system. Eggs laid on rubber mats had a stippled fluorescence in about 14% of the eggs, and stippling was present in 3% for eggs laid on synthetic turf. The presence of stippling could be used to confirm that the eggs were laid under barn or free range conditions, but since about half the barn and free range farms in Australia use sawdust or shavings, this feature would not be a reliable indicator for all units. Belt marks were obvious in up to 6% eggs that were conveyed automatically from cages or nestboxes, but this feature was also not diagnostic for production system, because belts may be used in a wide range of production systems.

During the course of this work it was noted that eggs at the top and sides of a pallet often showed fading of the fluorescent red colour in the shell. Normally, the outer surface of an egg shell fluoresces with a vivid red colour, and this is due to porphyrins deposited by the shell gland in the hen (With 1974). It would be unusual for the red fluorescent colour to be absent, but in that situation it could either be that the eggs had been subjected to uv light or the hens had received the coccidiostat Nicarbazin in the feed (Schwartz *et al* 1975). Preliminary trials showed that exposing eggs directly to sunlight resulted in similar fading of the fluorescent redness in the shell. The fading was subsequently found to be related to albumen height. Eggs that faded from a red to a greyish ruby fluorescent colour were at risk of having a lower albumen height. A runny egg white with a low albumen height gives the egg consumer the impression that the egg is old.

5. Implications

5.1 Project Intellectual property

The test for label authenticity is not patentable because knowledge of this test is already in the public domain.

5.2 Benefits to the Australian Egg Industry

In Australia most eggs are washed. The tests would be best applied at central egg packing Plants that perform the washing and receive unwashed eggs from a large number of producers. Random samples taken from deliveries of barn and free range eggs could be screened to check that the deliveries do not contain cage-laid eggs. In addition, the fading test could be used to identify eggs within a batch of barn, free range or cage eggs, which should be screened for albumen height measurement.

Both the Label Authenticity and the Sun Exposure Tests will help ensure market access by maintaining label integrity and consumer satisfaction. As such it is unrealistic to put a dollar value on these benefits.

The cost to the industry would be small. The cost of equipment is given below. There would be an additional cost from time spent performing the tests, but this would not be onerous as the tests could be conducted by staff who normally monitor albumen height. The exact cost would depend on whether the tests are used on a random basis or on every batch of barn and free range eggs. The additional cost would probably be borne by firms that pack the eggs, as this is where the tests would be applied. When performed properly, the test for label authenticity would protect the egg packing companies from possible fines for selling-on eggs that have been mislabelled, but it would be hypothetical to put a financial value to this benefit.

5.3 Dissemination strategy and communication plan

The following can be incorporated in guidelines on performing label authenticity testing in eggs.

Label Authenticity Test

The aim of this test is to determine whether eggs despatched from a barn or free range unit have been contaminated with cage-laid eggs. Select 90 eggs from a delivery of unwashed free range or barn eggs. Transfer them to a dark room.

If a large number of units have to be tested during the course of a day, and the operator is sensitive to ultraviolet light, apply sunscreen to the face, use gloves that protect the hands whilst providing a good grip on the eggs, and put on an eyeshield or safety spectacles that prevents the transmission of ultraviolet light. Consult your safety equipment supplier for the appropriate brands of safety equipment, and inform them that staff will be working with ultraviolet 'a' radiation. Examine the surface colour of each egg when it is held under a 6 W ultraviolet lamp (e.g. a Spectroline® BLF-6 hand lamp). Brown- and white-shelled eggs normally have a deep rich red colour when held under this light. On average, about one quarter of the eggs laid on a wire floor also have fluorescent white double lines on the shell. Some of these lines may be quite short (about 3 mm in length), whilst others will pass almost completely around the circumference of the shell. The lines often have an etched appearance. The distance between the double lines corresponds to the distance between the wires of a cage floor. If more than 4 of the 90 eggs have white double lines, there is a strong (more than 999 in a thousand) chance that the batch has been contaminated with cage-laid eggs. In this situation, the best advice is to enquire from the truck driver and the free range or barn egg producer whether there is a legitimate explanation for the presence of apparent wire marks on the eggs.

5.4 Equipment specification

The label authenticity test does not warrant an in-line ultraviolet testing booth at Australian egg packing plants. The test would only be applied to barn and free range eggs, and in the present egg market the booth would not be justified because of the small contribution barn and free range eggs make to overall throughput. In addition, at the egg packing plant where much of the work in this project was performed, there was insufficient space at the loading point in the conveyor to install and evaluate the feasibility of an ultraviolet testing booth. This limitation may not apply to all packing lines in Australia, but it could limit their use. It would not be realistic to install an ultraviolet lamp in the normal egg candling booth, and switching the lights from the visible spectrum to ultraviolet 'a' when a Label Authenticity Test needs to be applied. In modern systems, candling booths are situated after egg washing, and the Label Authenticity Test is best applied in unwashed eggs.

Instead, it is recommended that the test is used in a sample of eggs that are taken to a test room or laboratory. The room should be darkened so that light does not interfere with the fluorescence. The ultraviolet lamp should emit uv light in the 'a' band (315-400 nm). This wavelength range is relatively innocuous compared with uv light in the 'b' band, but some individuals are more sensitive than others to the effects of ultraviolet 'a' radiation. Sensitive people will experience a prickling feeling similar to that from exposure to sunlight. In this case, it is advisable to use sunscreen blocker on the face, to protect the hands with gloves, and to wear an eye shield or safety spectacles that do not allow transmission of uv_a light. Skin irritation amongst sensitive individuals can also be minimised by using an ultraviolet lamp with a low power rating.

When purchasing an ultraviolet lamp the first thing to consider is whether the lamp requires a ballast, or whether it is self-ballasted. A separate ballast will increase the cost above that of the lamp alone. In addition, handheld ultraviolet lamps will need a stand, which will introduce an extra cost. Seek advice from a specialist dealer in ultraviolet equipment. Ultraviolet equipment dealers can be identified from the local Yellow Pages.

The following lamps were available from a dealer in Adelaide at the time of writing, and would be suitable for this application:

Model	*Cost ($)*
Spectroline® BLF-6 or BLF-6A hand lamps †	140
Spectroline® SB-100P lamps †	1200

† does not include a stand

The Spectroline® BLF-6 or BLF-6A hand lamps have a low power rating and emit more visible light, producing a bluish hue. However, they can be used for both the label authenticity and the fading tests. The Spectroline® SB-100P lamp is more powerful and emits less visible light, giving potentially greater discrimination in the Label Authenticity Test. When purchasing equipment, it might be advisable to take a tray of cage-laid eggs to the ultraviolet equipment supplier to help choose the most appropriate equipment.

6. References

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With, T. K. (1974). Porphyrins in egg shells. *Biochemical Journal* **137,** 597-598.

Appendix 1. Prevalence of fluorescent double lines at each farm in the study

Farm number	**Number of eggs examined**	**Number of eggs with double lines**	**% eggs with double lines**
CAGED			
1	360	58	16
2	360	177	49
3	360	73	20
4	360	95	26
5	360	82	23
6	360	78	22
7	360	92	25
8	360	99	28
9	360	18	5
10	360	41	11
11	360	56	15
12	360	40	11
13	360	102	28
14	360	53	15
15	360	304	84
16	360	45	13
17	360	209	58
18	360	263	73
19	360	26	7
20	360	60	17
BARN or FREE RANGE			
1	360	0	0
2	360	1	0.3
3	360	5	1.4
4	360	2	0.5
5	360	3	0.8
6	360	1	0.3
7	360	0	0
8	360	0	0
9	360	1	0.3
10	360	1	0.3
11	360	0	0
12	360	4	1.1

Appendix 2. Statistics report

The purpose of this Appendix is to describe the statistical approach used in assessing whether cage-laid eggs are present in a batch of barn and/or free-range eggs.

The probability of getting k or more double lines given that the eggs were barn/free-range was calculated and shown in Table 1. The cut-off at ≤ 0.01 for a one-tail test at the 1% significance level was determined for different sample sizes. The test was based on an estimate of the proportion of double lined eggs from a sample of barn/free-range eggs. A conservative estimate at the upper bound of the 95% C.I. was used, namely $\tilde{p}_f$ = 0.0080. The estimate for the proportion of double lined eggs from a caged sample, using the lower bound of the 95% C.I. was $\tilde{p}_c$ = 0.190. The probabilities using the point estimates of p_f and p_c, namely $\hat{p}_f$ = 0.0042 and $\hat{p}_c$ = 0.274, were shown in Table 2. Power of rejecting the null hypothesis test that all eggs are barn/free-range was also calculated in these tables as well as the probabilities for the null hypothesis that all eggs are caged.

Taking into consideration dilution at 25%, 50% and 75% of caged eggs in the population, the power of rejecting the null hypothesis test that all eggs are barn/free-range was calculated in Table 3 and 4 (for the different estimated values of p_f and p_c). The power determined the appropriate sample size, which is recommended to be 90 eggs.

The sampling method recommended for the 90 eggs was to totally randomly sample across the whole population. However, if this is not practical then the recommendation is to randomly select 15 trays from the total number of trays, and from each of the 15 trays chosen, randomly select 6 eggs. This was described as sampling 15 sets of 6 eggs. This will perform relatively well across the different possible population scenarios.

Therefore, in a sample of 90 eggs, and using the conservative value 0.008 for p_f, the null hypothesis that all eggs are barn/free-range would be rejected (at the 1% level) if the number of double lined eggs is four or more. Then the sample is presumed to contain at least some caged eggs, not necessarily that all were caged eggs.

If the sample did consist of all caged, we would not expect the number with double lines to be less than 15 (the null hypothesis that all eggs are caged would be rejected at the 1% level if 14 or fewer were double lined, using the point estimate 0.274 for p_c). Thus, if between 4 and 14 eggs have double lines, both scenarios, all barn/free-range and all caged, would be rejected, and further investigation is warranted, with a mixture being a feasible explanation. An estimate for the proportion of caged eggs in a mixture is given by d=(number of eggs with double lines - nx $\hat{p}_f$)/(nx $\hat{p}_c$ - nx $\hat{p}_f$), which is taken as 1 if the number of double lined eggs is greater than nx $\hat{p}_c$ (and equal to 0 if the number of double lines is less than nx $\hat{p}_f$) where n is the sample size.

Note that even with more than 14 double lined eggs, we cannot confidently say the sample contains all caged eggs, only that we cannot reject the null hypothesis that all are caged. A mixture is still possible. A sample with more than 14 double lined eggs is consistent with, but does not necessarily imply, a sample consisting solely of caged eggs. Certainly if the sample consisted of anything less than 100% caged eggs, then it would be very unlikely (<5%) to have 33 or more double lined eggs – we would reject the null hypothesis that less than 100% were caged. However, this is a very conservative test, not likely to be rejected often – even with 100% caged eggs, we have a less than 5% chance of 33 or more double lined eggs.

On average, in a 100% caged sample of 90 eggs, we would expect to get 25 double-lined eggs. But getting 25 double-lined eggs does not necessarily mean the sample was all caged – it could have been only 90% or 80% or 70% caged for example. If the sample is 80% caged eggs, it has 72 caged and 18

barn/free-range eggs, and the probability of getting 25 or more double-lined eggs is greater than 10.5% (from the 72 caged eggs alone). So one cannot confidently say, even with 25 double-lined eggs, that the sample is all caged eggs. (And with 24 or 23 or so double-lined eggs it is still quite possible the sample could have been all caged.) A full Bayesian analysis would show that with 25 or more double-lined eggs, the sample is more likely than not to consist of 100% caged eggs, but there may not be much in it. In other words, one should not be unduly confident that the sample is 100% caged.

Out of 360 eggs per farm, the numbers of eggs which displayed double lines from caged farms ranged between 18 and 304 eggs, whereas, the eggs that displayed double lines for barn/free-range numbered between 0 and 5 eggs. The variation between the two types of farms is vastly different and it is evident that the distributions based on these samples are not overlapping. To show the distributions of barn/free-range and caged, Figures 1 to 4 have been presented. It is evident by Figures 1 to 4 that the larger the difference between pf and pc, the less overlapping between distributions, and the larger n, the less overlapping of distributions.

Figure 1: Probability distributions of barn/free-range and caged for n = 90 using $\tilde{p}_f$ = 0.008045 and $\tilde{p}_c$ = 0.189817.

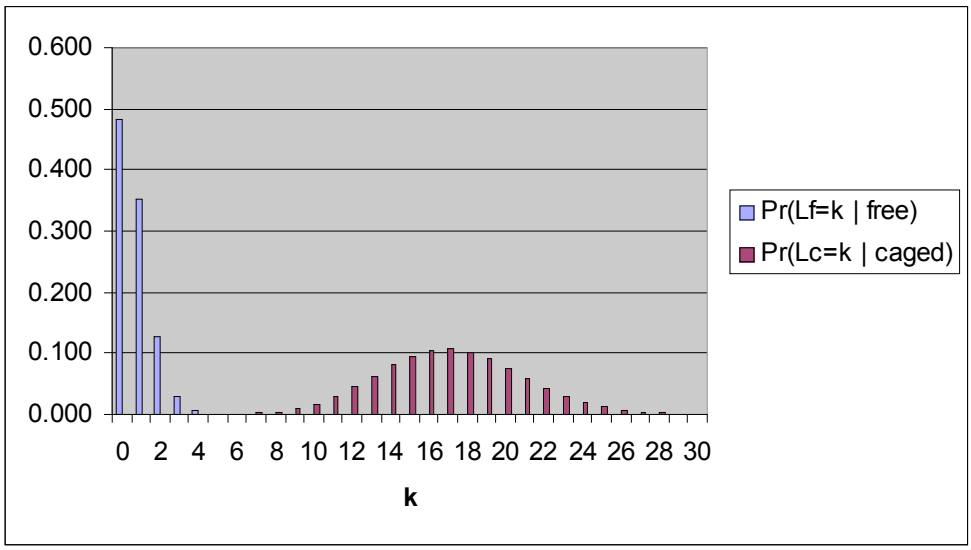


Figure 2: Probability distributions of barn/free-range and caged for n = 90 using $\hat{p}_f$ = 0.004167 and $\hat{p}_c$ = 0.2738.

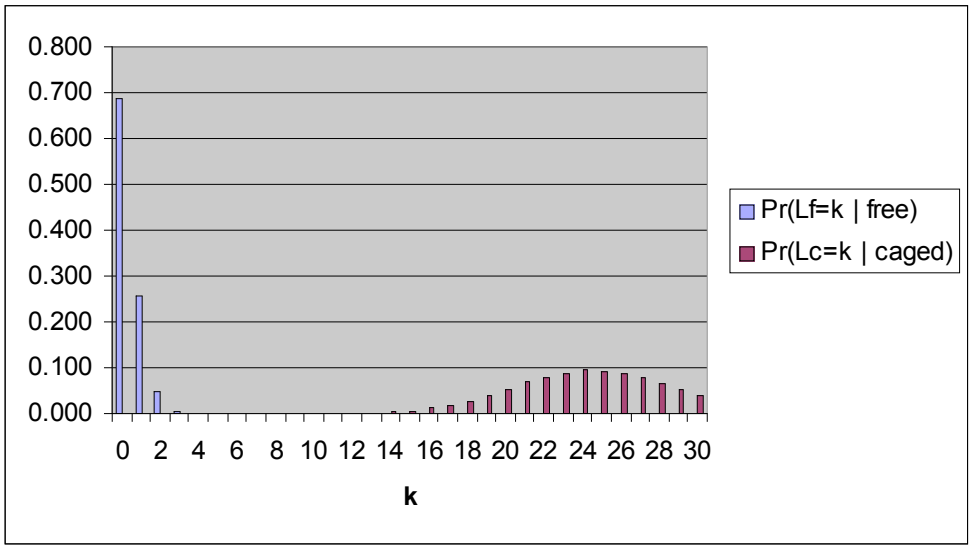


Figure 3: Probability distributions of barn/free-range and caged for n = 40 using $\tilde{p}_f$ = 0.008045 and $\tilde{p}_c$ = 0.189817.

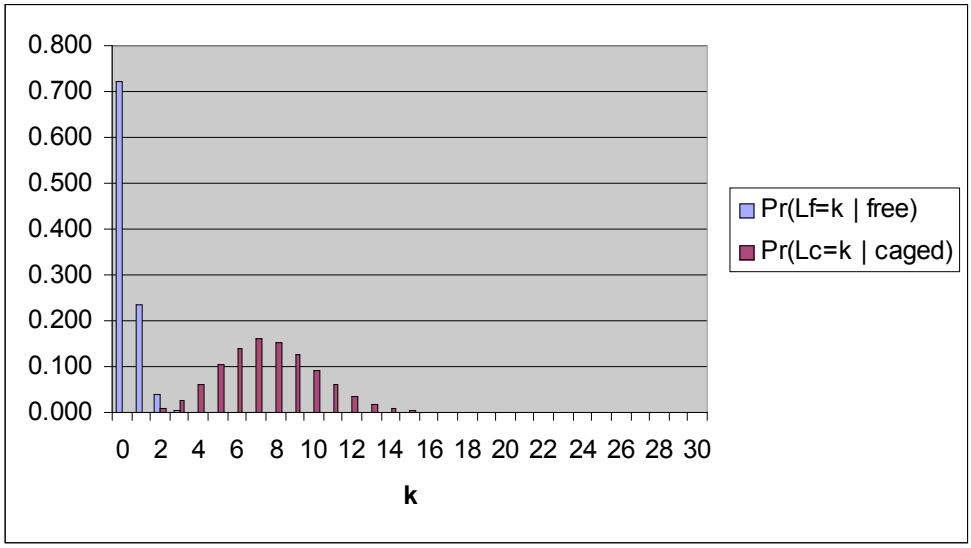
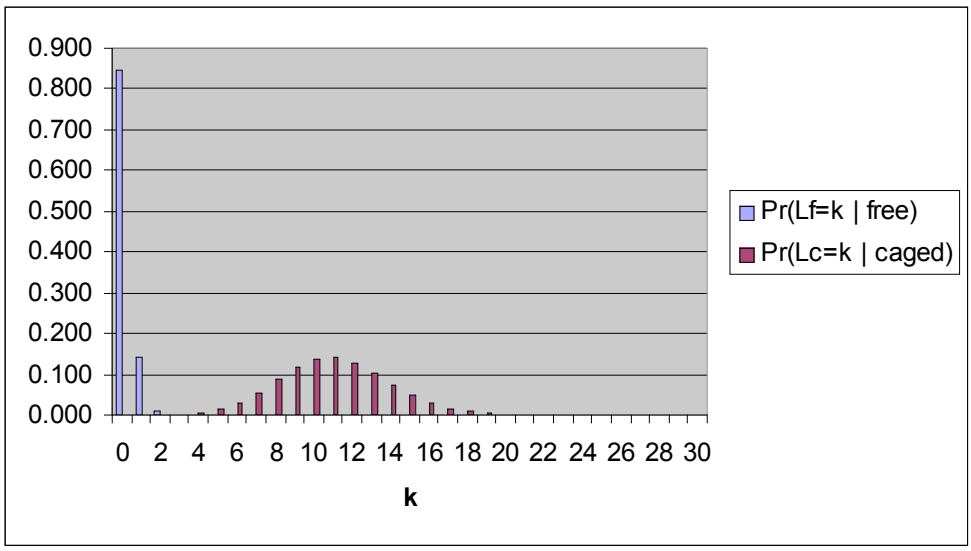


Figure 4: Probability distributions of barn/free-range and caged for n = 40 using $\hat{p}_f$ = 0.004167 and $\hat{p}_c$ = 0.2738.



A number of questions are of interest including:

1) What is the cut-off point (% of double-lines) which is a diagnostic to indicate the presence of some cage-laid eggs?
2) What sample size should be selected for the test?
3) What is the impact of dilution of a batch of barn eggs with some cage-laid eggs?

Question 1

To answer question 1, a null hypothesis has to be determined. In future the intention is to sample from a batch of eggs claimed to be 'barn/free-range' eggs and to test the eggs under UV light to diagnose whether they are indeed from a barn and free-range population. Therefore, the null hypothesis under investigation is

H_{0F}: all the eggs in the batch are barn/free-range

H_{AF}: eggs are not barn/free-range (i.e. may be a mixture "diluted", or all caged)

If the above null hypothesis is rejected then it may be of interest to identify whether the number of double lines detected indicate that all the eggs are from a caged population. Therefore, a secondary hypothesis may be tested:
H_{0C}: all the eggs in the batch are caged
H_{AC}:eggs are not all caged (i.e. may be a mixture "diluted", or all barn/free-range).

To test the first hypothesis, H_{0F}, that all the eggs are barn/free-range, the data collected from the barn/free-range farms is used to estimate the mean proportion of eggs with double lines and the standard error.

Assuming that the proportion of double lines from the barn/free-range population follows a binomial distribution $B(N,p_f)$, the expected or mean proportion is
$E[P_f] = p_f$,
and the standard error (s.e.) is $\sqrt{\frac{p_f(1-p_f)}{N_f}}$

where P_f is the true sample proportion of double lines for free-range, p_f is the expected or mean proportion of double lines for barn/free-range and N_f is the total number of eggs sampled from barn/free-range farms.

If the distribution is over-dispersed i.e. more variable than the binomial assumption would suggest, then the variance and hence the standard error is adjusted. The adjustment is the multiplication of the variance by the residual mean deviance, which is estimated by a Generalised Linear Model (GLM). Both the mean and standard error were estimated in GenStat 6th edition using a Generalised Linear Model with a logit link function. The model was:

$$log(\frac{p_f}{1-p_f}) = \mu + error$$

where μ is the proportion of double lines on the logistic scale and the error is the unexplained variation in the model.

To estimate the proportion of double lines from a caged population (p_c) and the associated standard error, the same theory applies by substituting p_f for p_c .

Since p_f and p_c are not known exactly we have only estimates $\hat{p}_f$ and $\hat{p}_c$, which are imprecise, to be cautious in differentiating between samples of all barn/free-range eggs and samples of all caged eggs, we construct the 95% confidence intervals of "believable" values for p_f and p_c, and use the upper confidence limit, $\tilde{p}_f$, for p_f and the lower limit, $\tilde{p}_c$, for p_c.

Using $\tilde{p}_f$ for p_f, a suitable cut-off point to indicate presence of caged eggs in a sample can be chosen for any specified sample size n, based on the number k for which there is a less than 1% chance of getting k or more double lined eggs (under H_{0F}: all barn/free-range, a 1% level one-sided significance test).

For any given sample size n, and number k of double lined eggs, we can calculate the probability of k or fewer double-lined eggs in a sample of size n under H_{0C}: all caged, using either $\hat{p}_c$ or $\tilde{p}_c$. The probabilities for each of these hypotheses are calculated in a Microsoft Excel spreadsheet, which will be provided to the client.

If the distribution is over-dispersed, the 95% C.I. constructed will be based on the GLM $\hat{\mu}$ parameter and the standard error for $\hat{\mu}$ on the logit scale assuming approximate normality on the logit scale. Once this 95% C.I. is constructed, it will be back-transformed onto the original scale which will cause the 95% C.I. to be skewed. The skewness is appropriate when dealing with probabilities which are necessarily bounded between 0 and 1.

Question 2

To decide on an appropriate sample size, the power of the test has to be taken into consideration.

Question 3

To consider the impact of barn/free-range eggs "diluted" with some caged eggs, both the expected number of lines and also the power of rejecting the null hypothesis that all eggs are barn/free-range can be calculated. By considering the power of the hypothesis test in the presence of dilution, an appropriate sample size for both Question 2 and Question 3 can be recommended.

An additional equation is presented to estimate the expected percentage of dilution for a given number of double lines detected. This may be useful, when it is unlikely that all eggs sampled are from a barn/free-range population.

Sampling methods are also discussed for different scenarios of how caged eggs are distributed through the batch and which sampling method is most recommended.

Results & Conclusions

Questions 1, 2 and 3

The three questions cannot be answered independently, therefore will be discussed as a whole. Firstly, the GLM results for barn/free-range eggs estimated the residual mean deviance to equal 2.004, which is slightly over-dispersed (no over-dispersion corresponds to the residual mean deviance = 1). The GLM estimated $\hat{\mu}$ = -5.476 and the s.e. = 0.334. Considering there was over-dispersion, the 95% C.I. was constructed on the logistic scale. The C.I. is –5.476 ± 1.98 x 0.334, which is (-6.13732, -4.81468) where 1.98 corresponds to an approximate t-value at 2.5% significance for degrees of freedom approximately equal to 100. Back-transforming to the original scale, the 95% C.I. is (0.002156, 0.008045) and the point estimate proportion $\hat{p}_f = 0.004167$.

The GLM results for caged eggs estimated the residual mean deviance to equal 82.52 which is hugely over-dispersed for a binomial distribution. Therefore, the 95% C.I. was constructed on the logit scale. The GLM estimated $\hat{\mu}$ = -0.976 and the s.e. = 0.240. The 95% C.I. is –0.976 ± 1.98 x 0.240, which is (-1.4512, -0.5008). Back-transformed, the 95% C.I. is (0.189817, 0.377353) and the point estimate proportion $\hat{p}_c = 0.2738$.

To test the first null hypothesis, H_{0F}, that the eggs are all barn/free-range, an estimate of the proportion of double lines that are barn/free-range has to be used. The conservative estimate at the upper bound of the 95% C.I. is 0.008045. Using $\tilde{p}_f$ = 0.008045, the probability of getting k number of double lined eggs given that all the eggs are barn/free-range can be calculated [Pr(L>=k | free)]. The cumulative probability was calculated so a one-tailed test (upper tail) could be performed at the 1% level. Therefore, a cut-off at k double lined eggs can be determined where the probability is ≤ 0.01. If k or more lines are detected for a given sample size n, then we reject the null hypothesis that the eggs are all barn/free-range. These probabilities have been calculated in an Excel spreadsheet where k is equal to 0 to 20, however k equal to 0 to 10 for different sample sizes n have been presented in Table 1.

In Table 1 (a), probabilities have been calculated for each k double lined eggs for a given n. The Bold values indicate the cut-off where you would reject the null hypothesis for the given sample size. It is evident, that the smaller the sample size the less the k double lined eggs likely to be detected. By rejecting the null hypothesis at k equal to 4 for a sample size 90, it can be said that the eggs could possibly be from a mixture or diluted 'batch' (population) or they may be all caged. There could be other explanations for why the detection of double lines was more than expected for all barn/free-range batches, therefore further investigation is required.

Assuming that the only alternative hypothesis to the eggs being all barn/free-range was all caged (no dilution or other explanations), the power of the above test can be calculated. To calculate power more conservatively, the lower bound from the 95% C.I. for the estimated proportion of double lined eggs based on the caged dataset $\tilde{p}_c = 0.189817$ was used. The results of power are provided by the Bold and Italic values in Table 1 (b). The Pr(L>=k | caged), is the probability of rejecting the null hypothesis that all eggs are barn/free-range given that all the eggs are truly from a caged population or in other words, the probability of correctly rejecting the null hypothesis H_{0F}. By looking at the Bold and Italic and Bold values, the power of the test is very good for all sample sizes except for sample size of 20.

If the null hypothesis that the eggs are all barn/free-range was rejected, it may be of interest to test that all the eggs are from a caged population. To do this, the Pr (L<=k | caged) is calculated and presented in Table 1 (c). The Bold and Underline values show the cut-off where the null hypothesis would be rejected (Pr ≤ 0.01) that all the eggs in the batch are caged. For sample sizes 20 – 40, no cell was Bold and Under line values because at the cut-off for caged you would not have rejected the previous null hypothesis that all eggs are barn/free-range. It does not make sense to only test the null hypothesis that all eggs are caged. It is only after rejecting the null hypothesis that all eggs are barn/free-range that you would consider testing the null hypothesis that all eggs are caged.

At sample size 90, the null hypothesis H_{0F} is rejected at 4 or above. Although any p_c-value within the 95% C.I. 0.190 to 0.377 for p_c is consistent with the cage-laid sample data provided, our best estimate of p_c is the point estimate 0.274, and it is recommended that this be used for tests of H_{0C} i.e. Table 2 (c). Using the lower bound 0.190 could result in false claims that the eggs were all caged (e.g. in sample = 90 where 10 lined eggs were detected, H_{0C} would be retained using $\tilde{p}_f = 0.190$ which could be incorrect). By testing the null that all eggs are caged, you could not reject the null at 14 double lined eggs or above (not shown on Table 2(c), need to refer to Excel spreadsheet for n=90). Therefore, what could be said about the double lined eggs detected between 4 and 14? One possibility is that the batch is diluted, however nothing can be confidently concluded when 4 to 14 double lined eggs are detected in a sample size of 90. This may require further investigation into the farm from where the batch came.

Table 2 is similar to Table 1, but has used the point estimates of proportions lined for barn/free-range and caged eggs rather than the upper bound for barn/free-range and the lower bound for caged. A similar interpretation to Table 1 applies. For the test of H_{0C}: all caged, Table 2 (c) is to be preferred (giving 14 or fewer lined eggs to reject H_{0C}) so as not to be overly willing to believe all the eggs were caged.

In Table 2, by changing p_f and p_c, the cut-off (Bold, Bold and Italic and Bold and Underlined values) for the null hypothesis, H_{0F}, that all eggs are barn/free-range has shifted down and the cut-off has shifted up for the null hypothesis, H_{0C}, that all eggs are caged. It is also evident that the cut-off for rejecting the null hypothesis, H_{0C}, that all eggs are caged in Table 2 is greater than 10 for large sample sizes (no Bold, Bold and Italic and Bold and Underlined values, because greater than 10). To determine these cut-offs, the Excel spreadsheet can be used by setting n, p_f, and p_c appropriately to find k such that the Pr(L<=k | caged) ≤ 0.01.

Table 1: For different sample sizes n and assuming p_f = 0.00804 and p_c = 0.18982, (a) Probability of rejecting the null hypothesis that all eggs are barn/ free-range,(b) the power of rejecting the null when all eggs are caged, and(c) the probability of rejecting that all eggs are caged, L = number of double lined eggs.

	Pf = 0.00804					Pc = 0.18982						
n	k =	0	1	2	3	4	5	6	7	8	9	10
(a)												
100	Pr(L>=k | free)	1.000	0.554	0.193	0.047	**0.009**	0.001	0.000	0.000	0.000	0.000	0.000
90	Pr(L>=k | free)	1.000	0.517	0.164	0.036	**0.006**	0.001	0.000	0.000	0.000	0.000	0.000
80	Pr(L>=k | free)	1.000	0.476	0.136	0.027	**0.004**	0.000	0.000	0.000	0.000	0.000	0.000
70	Pr(L>=k | free)	1.000	0.432	0.109	0.019	**0.003**	0.000	0.000	0.000	0.000	0.000	0.000
60	Pr(L>=k | free)	1.000	0.384	0.084	0.013	**0.001**	0.000	0.000	0.000	0.000	0.000	0.000
50	Pr(L>=k | free)	1.000	0.332	0.061	**0.008**	0.001	0.000	0.000	0.000	0.000	0.000	0.000
40	Pr(L>=k | free)	1.000	0.276	0.041	**0.004**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
30	Pr(L>=k | free)	1.000	0.215	0.024	**0.002**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20	Pr(L>=k | free)	1.000	0.149	0.011	**0.001**	0.000	0.000	0.000	0.000	0.000	0.000	0.000
(b)												
100	Pr(L>=k | caged)	1.000	1.000	1.000	1.000	***1.000***	1.000	1.000	1.000	0.999	0.998	0.995
90	Pr(L>=k | caged)	1.000	1.000	1.000	1.000	***1.000***	1.000	1.000	0.999	0.997	0.993	0.984
80	Pr(L>=k | caged)	1.000	1.000	1.000	1.000	***1.000***	1.000	0.999	0.996	0.990	0.978	0.954
70	Pr(L>=k | caged)	1.000	1.000	1.000	1.000	***1.000***	0.999	0.995	0.987	0.968	0.934	0.879
60	Pr(L>=k | caged)	1.000	1.000	1.000	1.000	***0.998***	0.993	0.981	0.954	0.905	0.829	0.726
50	Pr(L>=k | caged)	1.000	1.000	1.000	***0.998***	0.991	0.972	0.932	0.862	0.758	0.627	0.484
40	Pr(L>=k | caged)	1.000	1.000	0.998	***0.988***	0.960	0.900	0.797	0.657	0.498	0.344	0.216
30	Pr(L>=k | caged)	1.000	0.998	0.985	***0.942***	0.848	0.698	0.516	0.339	0.196	0.100	0.045
20	Pr(L>=k | caged)	1.000	0.985	0.916	***0.761***	0.543	0.326	0.164	0.069	0.024	0.007	0.002
(c)												
100	Pr(L<=k | caged)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.002	**0.005**	0.011
90	Pr(L<=k |	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	**0.00**	0.01	0.033

	caged)									**<u>7</u>**	6	
	Pr(L<=k									0.02	0.04	
80	caged)	0.000	0.000	0.000	0.000	0.000	0.001	0.004	**<u>0.010</u>**	2	6	0.086
	Pr(L<=k									0.06	0.12	
70	caged)	0.000	0.000	0.000	0.000	0.001	**<u>0.005</u>**	0.013	0.032	6	1	0.200
	Pr(L<=k									0.17	0.27	
60	caged)	0.000	0.000	0.000	0.002	**<u>0.007</u>**	0.019	0.046	0.095	1	4	0.397
	Pr(L<=k									0.37	0.51	
50	caged)	0.000	0.000	0.002	***0.009***	0.028	0.068	0.138	0.242	3	6	0.654
	Pr(L<=k									0.65	0.78	
40	caged)	0.000	0.002	0.012	0.040	0.100	0.203	0.343	0.502	6	4	0.877
	Pr(L<=k									0.90	0.95	
30	caged)	0.002	0.015	0.058	0.152	0.302	0.484	0.661	0.804	0	5	0.982
	Pr(L<=k									0.99	0.99	
20	caged)	0.015	0.084	0.239	0.457	0.674	0.836	0.931	0.976	3	8	1.000

Bold	~ cut-off --> reject H_{0f}: all free (more conservative than the test in Table 2 (a))
Bold and Italic	~ at cut-off, power of rejecting Ho when eggs truly are from a caged population
Bold and Underline	~ if we reject H_{0F}: all free, cut-off at lower tail for test of H_{0C}: all caged

Table 2: For different sample sizes n and assuming p_f = 0.00417 and p_c = 0.2738, (a)Probability of rejecting the null hypothesis that all eggs are barn/free-range,(b) the power of rejecting the null when all eggs are caged, (c) the probability of rejecting that all eggs are caged, L = number of double lined eggs.

				0.0041		0.273						
			Pf	7	Pc	8						
n	k =	0	1	2	3	4	5	6	7	8	9	10
(a)												
10	Pr(L>=k |									0.00		0.00
0	free)	1.000	0.341	0.066	**0.009**	0.001	0.000	0.000	0.000	0	0.000	0
	Pr(L>=k |									0.00		0.00
90	free)	1.000	0.313	0.055	**0.006**	0.001	0.000	0.000	0.000	0	0.000	0
	Pr(L>=k |									0.00		0.00
80	free)	1.000	0.284	0.044	**0.005**	0.000	0.000	0.000	0.000	0	0.000	0
	Pr(L>=k |									0.00		0.00
70	free)	1.000	0.253	0.035	**0.003**	0.000	0.000	0.000	0.000	0	0.000	0
	Pr(L>=k |									0.00		0.00
60	free)	1.000	0.222	0.026	**0.002**	0.000	0.000	0.000	0.000	0	0.000	0
	Pr(L>=k |									0.00		0.00
50	free)	1.000	0.188	0.019	**0.001**	0.000	0.000	0.000	0.000	0	0.000	0
	Pr(L>=k |									0.00		0.00
40	free)	1.000	0.154	0.012	**0.001**	0.000	0.000	0.000	0.000	0	0.000	0
	Pr(L>=k |									0.00		0.00
30	free)	1.000	0.118	**0.007**	0.000	0.000	0.000	0.000	0.000	0	0.000	0
	Pr(L>=k |									0.00		0.00
20	free)	1.000	0.080	**0.003**	0.000	0.000	0.000	0.000	0.000	0	0.000	0
(b)												
10	Pr(L>=k |									1.00		1.00
0	caged)	1.000	1.000	1.000	***1.000***	1.000	1.000	1.000	1.000	0	1.000	0
	Pr(L>=k |									1.00		1.00
90	caged)	1.000	1.000	1.000	***1.000***	1.000	1.000	1.000	1.000	0	1.000	0
	Pr(L>=k |									1.00		1.00
80	caged)	1.000	1.000	1.000	***1.000***	1.000	1.000	1.000	1.000	0	1.000	0
	Pr(L>=k |									1.00		0.99
70	caged)	1.000	1.000	1.000	***1.000***	1.000	1.000	1.000	1.000	0	0.999	7
	Pr(L>=k |									0.99		0.98
60	caged)	1.000	1.000	1.000	***1.000***	1.000	1.000	1.000	0.999	7	0.992	2
	Pr(L>=k |									0.98		0.91
50	caged)	1.000	1.000	1.000	***1.000***	1.000	0.999	0.998	0.992	0	0.955	2
	Pr(L>=k |									0.89		0.68
40	caged)	1.000	1.000	1.000	***1.000***	0.998	0.993	0.979	0.949	3	0.806	9
	Pr(L>=k |									0.60		0.29
30	caged)	1.000	1.000	***0.999***	0.995	0.980	0.943	0.869	0.753	4	0.441	2
	Pr(L>=k |									0.15		0.02
20	caged)	1.000	0.998	***0.986***	0.941	0.839	0.676	0.480	0.294	5	0.069	6
(c)												
10	Pr(L<=k |									0.00		0.00
0	caged)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0	0.000	0
90	Pr(L<=k |	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.00	0.000	0.00

	caged)									0		0
	Pr(L<=k |									0.00		0.00
80	caged)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0	0.000	1
	Pr(L<=k |									0.00		**0.00**
70	caged)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1	0.003	**7**
	Pr(L<=k |									**0.00**		0.03
60	caged)	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	**8**	0.018	8
	Pr(L<=k |									0.04		0.15
50	caged)	0.000	0.000	0.000	0.000	0.001	0.002	**0.008**	0.020	5	0.088	6
	Pr(L<=k |									0.19		0.44
40	caged)	0.000	0.000	0.000	0.002	**0.007**	0.021	0.051	0.107	4	0.311	7
	Pr(L<=k |									0.55		0.82
30	caged)	0.000	0.001	**0.005**	0.020	0.057	0.131	0.247	0.396	9	0.708	7
	Pr(L<=k |									0.93		0.99
20	caged)	0.002	0.014	0.059	0.161	0.324	0.520	0.706	0.845	1	0.974	2

Bold	~ cut-off --> reject Ho: all free
Bold and Italic	~ at cut-off, power of rejecting Ho when eggs truly are from a caged population
Bold and Underline	~ if we reject H_{0F}: all free, cut-off at point estimate for test of H_{0C}: all caged (more conservative than the test in Table 1(c)).

To answer what is the appropriate sample size, the power of the test of the null hypothesis, H_{0F}, that all eggs are barn/free-range should be considered (as in Table 1 & 2 (b)) and also the impact of dilution. Therefore, the results and discussion of Question 3 will be first presented before determining an appropriate sample size.

To consider the impact of dilution, both the power to reject the null hypothesis H_{0F} that all eggs are barn/free-range, and the expected number of double lined eggs, can be calculated for different degrees of dilution (25%, 50%, 75%).
Firstly the power of the hypothesis at the point of rejection (cut-off = m), was calculated when the batch has 25% dilution of caged eggs, 50% dilution and 75% dilution for different ranges of n. This was calculated based on $\tilde{p}_f$ = 0.00804 and $\tilde{p}_c$ = 0.18982 (the upper bound for the C.I. around the point estimate of barn/free-range and the lower bound around the point estimate for caged) as shown in Table 3, and also for the point estimates for barn/free-range and caged $\hat{p}_f$ = 0.00417 and $\hat{p}_c$ = 0.2738 shown in Table 4. Table 3 gives more conservative estimates of power, based on a smaller difference between proportions of lined eggs in barn/free-range samples and caged samples.

It is evident in Table 3 that the power is very poor for dilution of 25% or below for sample sizes of below 90, and the power is poor for dilutions of 50% or 75% when the sample sizes are 30 or below. In Table 4, the power appears much better compared to Table 3. This is because the estimated p_f and p_c used to calculate power were less conservative (greater difference between them), therefore making the power of the test appear much better.

Table 3: Power of rejecting the null hypothesis that all eggs are barn/ free-range given that there is dilution, for p_f = 0.00804 and p_c = 0.18982

	Pf = 0.008045 & Pc = 0.189817	
n	Pr(Total lines>=cut-off at 99% C.I. | dilution)	Power
100	Pr(Total lines>=4* | 25% caged & 75% free)	0.8066
100	Pr(Total lines>=4 | 50% caged & 50% free)	0.9934
100	Pr(Total lines>=4 | 75% caged & 25% free)	0.9999
90	Pr(Total lines>=4 | 25% caged & 75% free)	0.7188
90	Pr(Total lines>=4 | 50% caged & 50% free)	0.9854
90	Pr(Total lines>=4 | 75% caged & 25% free)	0.9995
80	Pr(Total lines>=4 | 25% caged & 75% free)	0.6406
80	Pr(Total lines>=4 | 50% caged & 50% free)	0.9684
80	Pr(Total lines>=4 | 75% caged & 25% free)	0.9984
70	Pr(Total lines>=4 | 25% caged & 75% free)	0.5085
70	Pr(Total lines>=4 | 50% caged & 50% free)	0.9342
70	Pr(Total lines>=4 | 75% caged & 25% free)	0.9942
60	Pr(Total lines>=4 | 25% caged & 75% free)	0.4032
60	Pr(Total lines>=4 | 50% caged & 50% free)	0.8692
60	Pr(Total lines>=4 | 75% caged & 25% free)	0.9825
50	Pr(Total lines>=3 | 25% caged & 75% free)	0.4907
50	Pr(Total lines>=3 | 50% caged & 50% free)	0.8952
50	Pr(Total lines>=3 | 75% caged & 25% free)	0.9824
40	Pr(Total lines>=3 | 25% caged & 75% free)	0.3636
40	Pr(Total lines>=3 | 50% caged & 50% free)	0.7846
40	Pr(Total lines>=3 | 75% caged & 25% free)	0.9457
30	Pr(Total lines>=3 | 25% caged & 75% free)	0.1814
30	Pr(Total lines>=3 | 50% caged & 50% free)	0.5918
30	Pr(Total lines>=3 | 75% caged & 25% free)	0.8243
20	Pr(Total lines>=3 | 25% caged & 75% free)	0.0749
20	Pr(Total lines>=3 | 50% caged & 50% free)	0.3158
20	Pr(Total lines>=3 | 75% caged & 25% free)	0.5726

* refers to m=4, at cut-off (Bold, Bold and Italic and Bold and Underlined values) shown in Table 1.

Table 4: Power of rejecting the null hypothesis that all eggs are barn/ free-range given that there is dilution, for p_f = 0.00417 and p_c = 0.2738

	Pf = 0.004167 & Pc = 0.2738	
N	Pr(Total lines>=cut-off at 99% C.I. | dilution)	Power
100	Pr(Total lines>=3* | 25% caged & 75% free)	0.9861
100	Pr(Total lines>=3 | 50% caged & 50% free)	1.0000
100	Pr(Total lines>=3 | 75% caged & 25% free)	1.0000
90	Pr(Total lines>=3 | 25% caged & 75% free)	0.9704
90	Pr(Total lines>=3 | 50% caged & 50% free)	0.9999
90	Pr(Total lines>=3 | 75% caged & 25% free)	1.0000
80	Pr(Total lines>=3 | 25% caged & 75% free)	0.9511
80	Pr(Total lines>=3 | 50% caged & 50% free)	0.9997
80	Pr(Total lines>=3 | 75% caged & 25% free)	1.0000
70	Pr(Total lines>=3 | 25% caged & 75% free)	0.9011
70	Pr(Total lines>=3 | 50% caged & 50% free)	0.9988
70	Pr(Total lines>=3 | 75% caged & 25% free)	1.0000
60	Pr(Total lines>=3 | 25% caged & 75% free)	0.8440
60	Pr(Total lines>=3 | 50% caged & 50% free)	0.9955
60	Pr(Total lines>=3 | 75% caged & 25% free)	0.9999
50	Pr(Total lines>=3 | 25% caged & 75% free)	0.7100
50	Pr(Total lines>=3 | 50% caged & 50% free)	0.9836
50	Pr(Total lines>=3 | 75% caged & 25% free)	0.9992
40	Pr(Total lines>=3 | 25% caged & 75% free)	0.5763
40	Pr(Total lines>=3 | 50% caged & 50% free)	0.9445
40	Pr(Total lines>=3 | 75% caged & 25% free)	0.9951
30	Pr(Total lines>=2 | 25% caged & 75% free)	0.6386
30	Pr(Total lines>=2 | 50% caged & 50% free)	0.9480
30	Pr(Total lines>=2 | 75% caged & 25% free)	0.9921
20	Pr(Total lines>=2 | 25% caged & 75% free)	0.4408
20	Pr(Total lines>=2 | 50% caged & 50% free)	0.8117
20	Pr(Total lines>=2 | 75% caged & 25% free)	0.9461

* refers to m=3, at cut-off (Bold, Bold and Italic and Bold and Underlined values) shown in Table 2.

Therefore, the sample size based on the power of the hypothesis test that all eggs in the batch are barn/free-range when dilution is present is recommended to be 90. This will allow better detection of double lines when there is only 25% dilution of caged eggs.

Based on a recommended sample size of 90, the expected number of double lined eggs can be calculated.

For example, if the sample size n = 100 and assuming 25% dilution then:

- For 25 caged eggs, the number of lined ~ B (25, p_c) and
- For 75 barn/free-range eggs, the number of lined ~ B (75, p_f)

Therefore, the expected number of lined eggs from the caged distribution is 25 x p_c , and the expected from the barn/free-range distribution is 75 x p_f . Adding the expected number of lined eggs from the two distributions will give the total number of lined eggs expected from the diluted sample. Using our two sets of p_f and p_c that have been used throughout this report, Table 5 and Table 6 are presented for the different dilution rates.

For example to calculate the expected number of lined eggs for 25% dilution in a sample size of 90 as in Table 5, and assuming $p_f = 0.0080$ and $p_c = 0.1898$ are close to the true proportion, it is: 67.5 x 0.0080 + 22.5 x 0.1898 = 4.8.

Table 5 and 6 provide a guide to the number of lined eggs that may be expected when sampling in the presence of dilution. Table 6 is preferred as it uses our best (point) estimates of p_f and p_c.

Table 5: Expected number of lined eggs in diluted batches for samples size = 90 using upper and lower bound estimates of p_f and p_c respectively

Pf = 0.008045 & Pc = 0.189817	
Dilution	E[# lined eggs]
25%	4.8
50%	8.9
75%	13.0

Table 6: Expected number of lined eggs in diluted batches for samples size = 90 using point estimates of p_f and p_c

Pf = 0.004167 & Pc = 0.2738	
Dilution	E[# lined eggs]
25%	6.4
50%	12.5
75%	18.6

The equation used in the above method to calculate the expected number of lined eggs, could be rearranged to estimate the dilution rate, d, the proportion of caged eggs in the sample, for a given number of lined eggs, by replacing the expected number of double lined eggs with the observed number, L.

i.e. $L = n \times d \times p_c + n \times (1 - d) \times p_f = n \times d \times p_c + n \times p_f - n \times d \times p_f = d(n \times p_c - n \times p_f) + n \times p_f$

$$\rightarrow d = \frac{L - np_f}{n(p_c - p_f)}$$

where $0 \leq d \leq 1$, as long as $L \leq n \times p_c$, otherwise d may exceed 1 which does not make intuitive sense. If $L > n \times p_c$, d estimate to be 1 or 100% i.e. all the sample eggs are caged.

The recommended sample size is 90, and it is recommended to use Table 1 to do the hypothesis test for H_{0F}. Therefore at n = 90, double lined eggs detected greater or equal to 4, it would be concluded that not all the eggs are barn/free-range. If the double lined eggs were greater or equal to 14, it can be concluded that the result is consistent with the batch being all caged based on the Pr(L<=k | caged) in Table 2. Therefore for a sample of size 90, if all eggs are barn/free-range it is unlikely to get ≥ 4 double lined eggs, while if all eggs are caged it is unlikely to get ≤ 14 double lined eggs. If the number of double lined eggs detected is between 4 and 14, then neither scenario is likely. However, if the number of double lined eggs is greater than 14, while this is consistent with H_{0C}: all caged, some dilution scenarios may be more likely. For instance, with 75% caged eggs, the expected number of double lined eggs in a sample of 90 is 18.6 (Table 6). For 100% caged eggs, the expected number of double lined eggs in a sample of 90 is 90 x 0.2738 = 24.6, so if 25 or more lined eggs are found the most likely (but by no means the only possible) scenario is that all the eggs were caged.

Sampling

To sample the eggs that claim to be barn/free-range, it is assumed that the officer has a batch or population to choose from. A batch consists of a number of trays and each tray holds 30 eggs.

The hypothesis tests in the previous sections are all based on assuming random sampling. Therefore, sampling individual eggs totally at random would be ideal to best cover all population possibilities in lieu of any other information. The sampling strategy with the greatest power to detect caged eggs depends on the distribution of caged eggs in the population. Some population possibilities or scenarios with 20% dilution could be the following:

a) All caged eggs are together in one bunch i.e. in a batch/population of 100 trays, 20 trays located together would all be caged
b) 20 trays of all caged eggs are randomly scattered amongst the 100 trays
c) All the 600 caged eggs are randomly distributed amongst the 100 trays
d) Caged eggs are uniformly distributed i.e. 6 caged eggs are placed in each of the 100 trays.

With no information about this, random sampling is the ideal. However in practice, true random sampling is almost impossible. Therefore, a compromise is by sampling in sets of eggs e.g. if the total sample is 90 as recommended, 15 sets of 6 eggs could be sampled. A 'set' could mean that the 6 eggs could be randomly selected from the same tray and 15 trays could be randomly chosen from the population. In terms of the above possible population scenarios, this may be the best compromise which is closest to being totally random.

If the scenario was actually either a) or b), then larger sets of eggs sampled across the population of eggs could actually have more power to detect eggs that are not barn/free-range. However, selecting larger sets of eggs i.e. greater than 6 eggs per tray can do much worse at detecting eggs that are not barn/free-range when the caged eggs are distributed in the scenarios of c) and d).

Random sampling is best performed using a table of random numbers or a random number generator such as in Microsoft Excel.

Therefore if totally random sampling is not possible, it is recommended to sample 15 sets of 6 eggs randomly from the batch, as already discussed above.

Which p_f and p_c to use?

We don't know p_f and p_c precisely. We have obtained ranges of believable values for them in the form of 95% C.I.'s. We may choose to use different values of p_f and p_c in different situations so as to err on the side of caution.

- We do not want to be too hasty to say a sample is not all barn/free-range because our estimate of p_f was actually too small, hence we base the test of H_{0F} on $\tilde{p}_f$, the upper limit of the 95% C.I. (Table 1(a)).
- We do not want to claim higher power than is reasonable because the true difference between proportion lined for barn/free-range and caged was smaller than here said, hence we use the upper bound $\tilde{p}_f$ and the lower bound $\tilde{p}_c$ here (Table 1 (b) and Table 3).
- When testing H_{0C}: all caged, we do not want to be too hasty to say a sample is all caged (retain the null hypothesis H_{0C}), therefore use the larger value $\hat{p}_c$ rather than the lower bound $\tilde{p}_c$ (Table 2(c)).
- When estimating the expected numbers of lined eggs under different dilution scenarios and when estimating the dilution proportion, use the single best estimates $\hat{p}_f$ and $\hat{p}_c$ (Table 6 and below).

Recommendations

In summary it is recommended to sample 90 eggs, and if totally random sampling is not possible, sample 15 sets of 6 eggs randomly from the population. Based on this sample, use Table 1(a) to test the null hypothesis that all the eggs are barn/free-range. This is more conservative than Table 2, as it allows for uncertainty in the value of p_f, the proportion of barn/free-range eggs expected to display double lines. Using these recommendations, reject the null hypothesis, H_{0F}, that all eggs are barn/free-range if 4 or more double lined eggs are observed in a sample of 90.

If H_{0F} is rejected, one may wish to test the null hypothesis, H_{0C}, that all eggs are caged. Using Table 2 (c)(extended to 14), reject H_{0C} if 14 or fewer double lined eggs are observed. If both H_{0F} and H_{0C} are rejected (there are between 4 and 14 double lined eggs) neither scenario, all barn/free-range or all caged, is likely. Further investigation is warranted. A mixture is a likely explanation.

While 15 or more double lined eggs are consistent with all caged eggs, this is not necessarily the only nor the most likely scenario. However, if 25 or more double lined eggs are found in a sample of 90, it is more likely than not that all the eggs were caged.

Appendix 3 – Raw data for the production systems study

farm	type	line_no	total	proportion (p)
1	caged	58	360	0.1611
2	caged	177	360	0.4917
3	caged	73	360	0.2028
4	caged	95	360	0.2639
5	caged	82	360	0.2278
6	caged	78	360	0.2167
7	caged	92	360	0.2556
8	caged	99	360	0.2750
9	caged	18	360	0.0500
10	caged	41	360	0.1139
11	caged	56	360	0.1556
12	caged	40	360	0.1111
13	caged	102	360	0.2833
14	caged	53	360	0.1472
15	caged	304	360	0.8444
16	caged	45	360	0.1250
17	caged	209	360	0.5806
18	caged	263	360	0.7306
19	caged	26	360	0.0722
20	caged	60	360	0.1667
21	barn_freerange barn_freerange	0	360	0.0000
22	barn_freerange	1	360	0.0028
23	barn_freerange	5	360	0.0139
24	barn_freerange	2	360	0.0056
25	barn_freerange	3	360	0.0083
26	barn_freerange	1	360	0.0028
27	barn_freerange	0	360	0.0000
28	barn_freerange	0	360	0.0000
29	barn_freerange	1	360	0.0028
30	barn_freerange	1	360	0.0028
31	barn_freerange	0	360	0.0000
32	barn_freerange	4	360	0.0111

Appendix 4 – Computer output for production systems study

Generalised linear models

CAGED DATA

```
  67  "Modelling of binomial proportions. (e.g. by logits)."
  68       MODEL   [DISTRIBUTION=binomial;   LINK=logit;   DISPERSION=*]   line_no;
NBINOMIAL=total
  69   FIT [PRINT=model,summary,estimates; CONSTANT=estimate; FPROB=yes; TPROB=yes;
FACT=9]\
  70

70.....................................................................



***** Regression Analysis *****

 Response variate: line_no
  Binomial totals: total
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant


*** Summary of analysis ***

                                      mean  deviance approx
             d.f.     deviance     deviance     ratio  F pr.
Regression      0           0.            *
Residual       19        1568.        82.52
Total          19        1568.        82.52

Dispersion parameter is estimated to be 82.5 from the residual deviance
* MESSAGE: The following units have large standardized residuals:
         Unit     Response    Residual
           15       304.00        2.56
           18       263.00        2.03


*** Estimates of parameters ***

                                                      antilog of
                 estimate         s.e.     t(19)  t pr.   estimate
Constant           -0.976        0.240     -4.06  <.001     0.3769
* MESSAGE: s.e.s are based on the residual deviance

  71        PREDICT    [PRINT=description,predictions,se;    COMBINATIONS=estimable;
BACKTRANSFORM=link;\
  72   ADJUST=marginal]

72.....................................................................
```

```
*** Predictions from regression model ***

These predictions are estimated mean proportions, formed on the scale of the
response variable, corresponding to one binomial trial.

The standard errors are appropriate for interpretation of the predictions as
summaries of the data rather than as forecasts of new observations.

 Response variate: line_no

  Prediction        s.e.
      0.2738      0.0477
 * MESSAGE: s.e's, variances and lsd's are approximate, since model is not
linear.
* MESSAGE: s.e's are based on the residual deviance.
```

FREE-RANGE DATA

```
  36  "Modelling of binomial proportions. (e.g. by logits)."
  37       MODEL   [DISTRIBUTION=binomial;   LINK=logit;   DISPERSION=*]   line_no;
NBINOMIAL=total
  38   FIT [PRINT=model,summary,estimates; CONSTANT=estimate; FPROB=yes; TPROB=yes;
FACT=9]\
  39

39.....................................................................


***** Regression Analysis *****

 Response variate: line_no
  Binomial totals: total
     Distribution: Binomial
    Link function: Logit
     Fitted terms: Constant


*** Summary of analysis ***

                                        mean  deviance approx
             d.f.     deviance     deviance     ratio  F pr.
Regression      0         0.00            *
Residual       11        22.04        2.004
Total          11        22.04        2.004

Dispersion parameter is estimated to be 2.00 from the residual deviance


*** Estimates of parameters ***

                                                        antilog of
                 estimate         s.e.     t(11)  t pr.   estimate
Constant           -5.476        0.334    -16.38  <.001   0.004184
* MESSAGE: s.e.s are based on the residual deviance

  40        PREDICT    [PRINT=description,predictions,se;    COMBINATIONS=estimable;
BACKTRANSFORM=link;\
  41   ADJUST=marginal]

41.....................................................................
```

*** Predictions from regression model ***

These predictions are estimated mean proportions, formed on the scale of the response variable, corresponding to one binomial trial.

The standard errors are appropriate for interpretation of the predictions as summaries of the data rather than as forecasts of new observations.

Response variate: line_no

Prediction	s.e.
0.00417	0.00139

* MESSAGE: s.e's, variances and lsd's are approximate, since model is not linear.
* MESSAGE: s.e's are based on the residual deviance.