A synopsis of the *Salmonella* Incidence Response Plan

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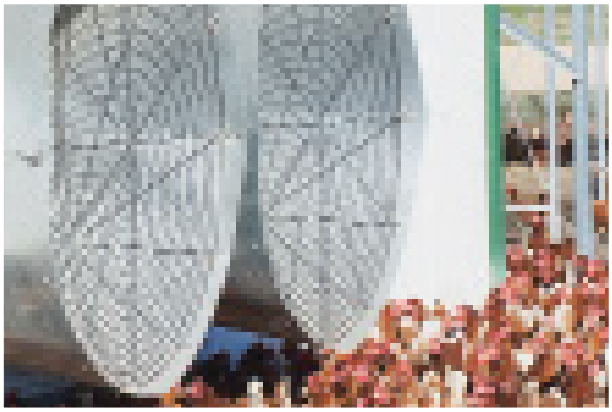
Introduction

1.1 Purpose of this synopsis

This synopsis is an adaptation of the *Salmonella* Incidence Response Plan, and is designed to provide a minimum standard for use by farmers if their farm is implicated in a foodborne outbreak.



2.0

Image 1 – Cracked and dirty eggs

Image 2 – Worker removing cracked and dirty eggs before washing

Fundamental stages of a *Salmonella* response

2.1 The farmer

Once a link between a food poisoning event and a farm has been established, the farm will be restricted from selling eggs until the health department and or food authority have confidence that the sale of eggs to the consumer will not pose a food safety risk.

The farmer may experience:

- the need to dispose of eggs that can no longer be sold
- reduced income
- media attention
- contacts from customers seeking clarification of the current status of egg availability
- contacts from suppliers enquiring about the status of the business
- competitive attention from other egg suppliers
- loss of contacts, visitations and various requests on compliance by regulators or customers litigation and financier enquiries.

2.2 Seek permission

If the sale of eggs is prohibited:

- seek permission from the relevant authority to allow the eggs currently in store and/or already produced to be pulped and pasteurised, and
- move the eggs securely, and have this process monitored by the relevant authority.

2.3 Seek technical advice

Seek technical advice (e.g. from your veterinarian) to establish a *Salmonella* Control and Monitoring program.

2.4 Monitor for cracked and dirty eggs

Start grading and monitoring eggs for cracks and dirt before washing and sanitation (refer section 2.7). Remove all B Grade eggs for pulping. If eggs are not normally washed, remove all cracked and dirty eggs, and do not wash dirty eggs by hand. Other procedures include:

- increasing the frequency of egg collection and the quality of grading at the farm level, to help remove cracked and dirty eggs (see image 1)
- employing extra staff on the grading floor to remove cracked and dirty eggs from the anaconda or input trolleys prior to washing (see image 2)
- holding a staff explanatory meeting and undertaking retraining
- reducing egg recovery to reduce the risk of another outbreak
- sampling cracked and dirty eggs (Appendix 2 – SOP 4).

Image 3 – Collecting floor eggs

Image 5 – Yolk from broken eggs

Image 4 – Dirty nest boxes

Image 6 – Anaconda not covered up, potential for eggs to get wet

2.5 Identify and mitigate factors leading to cracked and dirty eggs

Possible factors why customers are receiving cracked and dirty eggs include:

- selling ungraded, cracked and dirty eggs
- 100% recovery of all eggs
- not sending floor eggs for pulping (should be discarded if pulping is unavailable)
- old flocks with poor quality eggs.

High incidence of dirty eggs, due to:

- high number of floor eggs (see image 3)
- dirty nest boxes and/or worn nest pads (see image 4)
- dirty and wet egg belts and anacondas
- high incidence of broken eggs contaminating eggs with yolk (see image 5)
- vermin and insects contaminating eggs
- eggs getting wet because of rain or fogging (see image 6)
- faeces are reaching the floor of the cages, and can be accessed by the birds (see image 7)
- eggs are not rolling away from the birds promptly
- poor hygienic practices
- recycling dirty packaging and cardboard trays.

High incidence of cracked eggs, due to:

- aged flock with poor shell quality
- general poor eggshell quality across all ages
- poor husbandry, nutrition and egg size control
- worn nest pads (see image 8)
- non-operational egg saver wires
- crowding of eggs on the egg belt (see image 9)
- damage at transfer points during handling, packing and transport
- bird behaviour, and egg pecking and eating. (see image 10)

2.6 Equipment cleaning and sanitation

Review or establish Standard Operating Procedures (SOPs) for equipment cleaning and sanitation.

Image 7 – Faeces reaching floor of cage

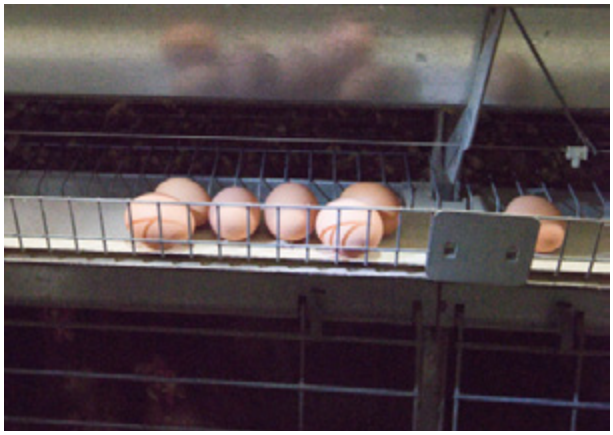


Image 9 – Crowding of eggs on belt

Image 8 – Nest pad

Image 10 – Egg pecking

Ensure that:

- a cleaning and sanitising program is in place and effective
- equipment is constructed and installed for effective cleaning and sanitising
- rooms are adequately constructed and maintained to prevent contamination
- potable water, hot and cold is present for cleaning.

2.7 Egg washing, sanitation and drying

Review or establish Standard Operating Procedures (SOPs) for egg washing, sanitation and drying. Consider:

- monitoring egg washing and sanitation parameters (e.g. pH, temperature and chemical concentration) at a minimum of every 30 minutes throughout the wash period
- whether the chemicals used are approved for contact with food products
- adequate drying of eggs after washing.

2.8 Feed additives

Hens are more likely to shed and be colonised by *Salmonella* from the environment when approaching peak production. Introduce feed additives such as:

- organic acids or more prescriptively Short Chain Fatty Acids (SCFA) such as formic, propionic, butyric, sorbic, acetic, fumarate, benzoic, lactic and fumaric
- phytogenics, phosphorylated yeast products or probiotics.

2.9 *Salmonella* vaccination program

Vaccination can be considered as a tool to help manage *Salmonella*. This could either be:

- a registered live *Salmonella* vaccine (e.g. Vaxsafe ST®), or
- a combination of the live *Salmonella* vaccine and an autogenous vaccine.

Consult your veterinarian for advice.

New pullet flocks can be vaccinated at the hatchery if requested, followed by vaccination through drinking water at 4 weeks of age and by intramuscular injection around 10-12 weeks of age. Vaxsafe ST® vaccine is normally administered in conjunction with other inactivated adjuvanted vaccines (Appendix 2 – SOP 9). If an autogenous vaccine were introduced, this would be undertaken by intramuscular injection at around 8-12 weeks of age.

An autogenous vaccine would only be available after the *Salmonella* has been recovered from the farm and correlated with the food safety incident. Autogenous vaccine is specific only to the particular *Salmonella* strain on a farm. The time frame to produce the vaccine is around 3 months.

2.10 Swabs

Conduct environmental swabbing of all sheds monthly (Appendix 2 – SOP 1 and 2). This can be reduced when the farm status is better understood.

Other types of swab include:

- environmental swabs of Day Old Chickens (DOC) (Appendix 2 – SOP 3) and Point Of Lay (POL) pullets
- surface swabbing of a representative sample of eggs pre and post washing (Appendix 2 – SOP 5)
- environmental swabs of the grading and packing floor (Appendix 2 – SOP 6).

Where pullets are reared externally, a *Salmonella* environmental test should be mandated as a condition of supply.

2.11 Feed and feed mills

Feed is often considered as a source of food safety pathogens, but the chance of detecting *Salmonella* Typhimurium is low. Other salmonellae can be found in vegetable protein meals and this can change the *Salmonella* status of the layer flock from negative to positive. Although feed that is free from *Salmonella* contamination is an important part of the *Salmonella* control program, the emphasis on feed should not be disproportionate.

Image 11 – Heat-treated mash feed

The ingredients used for final mixing are to be protected from contamination. Testing of finished feed for *Salmonella* can be conducted:

- on site for in house mills
- on major contact points within the mill
- by requesting your mill to do a routine SCFA flushing
- request finished feed testing from commercial mills when possible. Generally, heat-treated pelleted feed or heat-treated mash feeds are considered free of *Salmonella* but post manufacture contamination can occur. (see image 11)

As day old chickens have yet to establish a normal intestinal microbiota, it is more likely to be colonised by *Salmonella* from feed (and the environment). For the rearing of pullets, it is preferable to use pelleted or a heat-treated mash as a starter ration.

2.12 Training

Staff involved in farming, the grading floor and marketing must all be trained on the *Salmonella* control program. Senior management and owners themselves are on occasions complicit in bad practices, and thus need to change and support staff in best practice. Hence, there must be:

- a change in staff responsibilities or the introduction of new staff
- a change in attitude and aptitude for all contractors
- incorporation of staff and contractor responsibilities into the *Salmonella* control program.

The methods of training may involve:

- workshops undertaken by a qualified person/entity (e.g. Registered Training Organisation)
- workshops undertaken in house reviewing SOPs and work instructions
- utilising chemical supply companies to conduct training and to supply wall posters
- requesting chemical companies to establish procedural and monitoring guidelines for the use of their chemicals – this should include using both hardware and materials for monitoring purposes
- allowing supervisory and quality assurance staff to visit other establishments, to review their operational procedures and equipment
- certificate III training courses – for more information, check: www.australianeggs.org.au/for-farmers/tools-and-training/
- continual contact with qualified people for answering questions
- making staff aware of their responsibilities, especially if a food safety non-compliance is observed
- do a corrective action if necessary, especially during candling and egg washing
- using *Salmonella* posters and the *Salmonella* risk identification report: www.australianeggs.org.au/what-we-do/leading-research/through-chain-salmonella-risk-identification/

2.13 Communication

2.13.1 Regulatory authorities

Cooperation is important to allow quick resolution and the return to normal business. It is important that all parties have confidence in each other during food safety events. Farmers are advised if they do not have the "temperament" to deal with regulatory authorities, that they appoint a third party to undertake this role.

2.13.2 Customers

Keep customers fully informed of the events to avoid speculation and uncertainty. This can be done:

- formally for the large retailers
- via interaction with onsellers, caterers and over the counter customers

- via consumer phone enquiries.

Nominate one suitable person to liaise with customers.

2.13.3 Creditors

In the case of large recalls and restrictions being enforced on sales, cash flow can become a significant problem. It is important to:

- discuss the situation with all suppliers (e.g. stock, feed and feed materials, disposables, etc.) to ensure the continuity of the necessary business inputs
- involve the cooperation of other egg producers where they can assist in supplying products to customers
- establish a line of credit with the bank.

2.13.4 Media

Generally, there is not a lot of direct media contact but:

- contact Australian Eggs for help if needed, or
- nominate one company representative to deal with the media, and advise the staff of this.

2.13.5 Litigation

If a foodborne outbreak involves large numbers of people and hospitalisation, litigation will initially be against the primary establishment (restaurant, reception centre, conference, etc.) and down the supply chain (e.g. caterer, egg marketer, egg wholesaler, and finally the farmer). Often, lawyers and insurance companies will also become involved. Insurance companies will generally pay out compensation for the primary establishment but then seek to recover the costs of legal action further down the supply chain.

Food poisoning episodes associated with eggs require:

- eggs contaminated with *Salmonella*, and
- sub-optimal food handling practices that allow the *Salmonella* levels to grow to an infectious level.

This is also further complicated by susceptibility of the consumer (e.g. persons in aged care).

Farmers should ensure that their professional liability and indemnity insurances are up to date and have sufficient policy coverage. All of the contractors and onsellers they deal with should likewise be covered.

During litigation, the farmer should notify their lawyer, and discuss the way forward and the lines of communication. To avoid any negative impact on the business, it is recommended to leave all of the dealings to your lawyer and insurance company.

2.14 *Salmonella* source

The presence of *Salmonella* on the farm (and thus in a percentage of the birds) does not imply a direct link with contaminated eggs and food poisoning. It is when there is a failure in the correct egg handling procedures that the risk of egg contamination increases.

Image 12 – Clean shed ready to be repopulated by a new batch of hens

In most cases, *Salmonella* colonise the hens with no clinical signs. Thus, controlling the infection, colonisation and the spread of *Salmonella* within housed poultry is difficult.

The ability to maintain a *Salmonella* free environment across all production systems (including multi-age sites and multi-age sheds) has its limitations due to the diversity of different factors that may introduce and spread *Salmonella*.

In Australia, food safety authorities are primarily concerned about the status of eggs and egg products as opposed to the status of the bird. Thus, the focus on egg handling and the final *Salmonella* status of the egg or egg product is important. It is a common sense conclusion that having *Salmonella* free birds almost entirely mitigates the risk of *Salmonella* in eggs from those birds.

2.14.1 Environment

Cross-contamination from the environment to commercial layers is the most common cause of change of the *Salmonella* status in a layer flock. Examples include:

- placement of day old chickens in poorly disinfected houses or on reused *Salmonella* positive litter (see image 12)
- placing point of lay pullets in *Salmonella* positive sheds, particularly those that are multi-age.

Often, sheds are not optimally washed and disinfected between flocks because of costs, labour resources, concern about damage to the facilities, and in more recent times the practical and physical limitations with aviary type housing systems.

Image 13 – Water pooling and mud formation

Image 14 – Clutter around the shed

Multi-age cage layer sheds and free-range areas also have limitations in achieving any effectiveness in environmental *Salmonella* reduction. The movement of dust containing *Salmonella* is limited to a few metres and thus unlikely to be responsible for the ingress of *Salmonella* on to a site, but can be responsible for the change of *Salmonella* status of adjoining sheds.

2.14.2 Water

Water can be contaminated with salmonellae by vermin or insect vectors, although mains and bore water are generally free from *Salmonella*. Therefore:

- surface water must be sanitised before use
- storage tanks should be tested several times a year even when the source of water is from the mains
- water storage tanks should be sanitised twice a year.

2.14.3 Avian livestock

Positive day old chickens are generally low risk to *Salmonella* in relation to egg related food safety. They are normally negative for *Salmonella* Typhimurium, and most suppliers conduct monitoring for *Salmonella* Pullorum, *Salmonella* Gallinarum and *Salmonella* Enteritidis.

Purchased pullets should be supplied with a mandated point of lay *Salmonella* environmental swab test results to ascertain the status of the birds.

2.14.4 Vermin and non-avian livestock

Rats and mice can introduce *Salmonella* into a poultry establishment. There should be:

- an effective rodent control program (Appendix 2 – SOP 8)
- adequate drainage to prevent water pooling and mud formation (see image 13)
- control of feed sources that act as an attractant to rodents
- tidy and uncluttered immediate surroundings. (see image 14)

Grazing animals, guard animals and domestic pets can all carry *Salmonella*, and should either be tested or excluded from the site.

2.14.5 Equipment and disposables

All equipment, including that belonging to contractors, must be washed and disinfected before entering the farm.

2.14.6 Personnel

Staff can carry *Salmonella*, and there should be:

- procedures in place for staff returning from overseas countries with a history of enteric disease
- procedures when can staff return to work after symptoms have ceased
- a microbiological check of each staff member for *Salmonella* (preferred option).

3.0

Standard long-term *Salmonella* control program

Most of the components in the *Salmonella* Incidence Response Plan are similar to a *Salmonella* Control Program for all farmers. These include:

- purchase of day old chickens that are free of *Salmonella*
- rearing of quality pullets with a good frame size, skeletal development and free of *Salmonella*
- vaccination of pullets against *Salmonella*
- nutritional formulation and specifications that optimise egg quality
- provision of feed containing additives to help control the colonisation and shedding of *Salmonella*
- facilitation and husbandry systems that optimise the production of eggs that are clean and have minimal cracks
- removal of all dirty and cracked eggs and be diverted to pulping and pasteurisation (or disposed of, if pulping and pasteurisation is not available)
- no washing of dirty eggs
- a washing system that has real time monitoring, or a manual monitoring system that requires testing every 30 to 60 minutes
- monitoring for *Salmonella*:
  - in day old chickens
  - in point of lay pullets
  - in layers in production
  - on the surface of washed eggs
  - on the grading floor environment
  - in the feed and feed mill
- careful selection of markets that do not undertake unsafe food practices
- a corrective action plan for the identification of high risk *Salmonella*.

Appendix 1 – List of laboratories that can test for *Salmonella* in chickens

Note: Contact the laboratory prior to collecting samples – phone numbers provided

Animal Consulting Enterprises Pty Ltd, East Bendigo, VIC – ACE Laboratory Services		(03) 5443 9665
Baiada Poultry Pty Limited, Bringelly, NSW - Birling Avian Laboratories		(02) 4778 6100
Biosecurity Queensland Veterinary Laboratories, Coopers Plains, QLD – Department of Agriculture and Fisheries – Biosecurity Sciences Laboratory		(07) 3276 6062
CSIRO, Geelong, VIC – Australian Animal Health Laboratory		(03) 5227 5000
Department of Primary Industries and Regional Development Diagnostic Laboratory Services, South Perth, WA – Animal Health Laboratories		(08) 9368 3333
Department of Primary Industry and Resources, Berrimah, NT- Berrimah Veterinary Laboratories		(08) 8999 2249
Department of Economic Development, Jobs, Transport & Resources, Bundoora, VIC – AgriBio, Vet Diagnostic Laboratory		(03) 9032 7515
Department of Primary Industries, Parks, Water and Environment, Launceston, TAS – Animal Health Laboratory		(03) 6777 2111
Gribbles Veterinary Pathology, Clayton, VIC		1300 307 190
Gribbles Veterinary Pathology Pty Ltd, Glenside, SA – VETLAB		1300 307 190
IDEXX Laboratories Pty Ltd	Mount Waverley, VIC – Melbourne Laboratory Rydalmere, NSW – Sydney Laboratory Unley, SA – Adelaide Laboratory East Brisbane, QLD – Brisbane Laboratory	1300 443 399
Microbiological Diagnostic Unit, Public Health Laboratory, Melbourne, VIC – Peter Doherty Institute for Infection and Immunity – The University of Melbourne		(03) 8344 5701
NSW Department of Primary Industries, Menangle, NSW – Department of Industry, Skills and Regional Development – Elizabeth Macarthur Agricultural Institute		(02) 4640 6333
Vetnostics, North Ryde, NSW – North Ryde Laboratory		1800 425 116
Vetpath Laboratory Services, Ascot, WA – Vetpath Laboratory Services		(08) 9259 3600

Appendix 2 – Standard Operating Procedures (SOP)

SOP 1:
***Salmonella* sampling in litter or manure procedure using swabs**

1.0 Introduction

Regular monitoring for *Salmonella* spp. in litter from egg laying birds or replacement pullet is completed to:

- detect any exotic, notifiable or atypical *Salmonella* spp. incursions into a flock, and
- monitor flocks for endemic *Salmonella* spp.

If the laboratory has a provisional culture of *Salmonella* it will send it to a central reference diagnostic laboratory for speciation and typing.

The laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation. Samples should always be recorded on accession sheets as being obtained from environmental litter samples.

2.0 Preparation for sample collection

2.1 Contact the laboratory

- Obtain a submission form from the laboratory (samples obtained through this SOP should always be recorded on accession sheets as "ENVIRONMENTAL LITTER SAMPLES")
- If applicable, and/or available, ask if the laboratory can supply the swabs
- Notify the laboratory 24 hours in advance of receiving samples to allow adequate time for preparation.

2.2 Calculate the number of swabs required

Type of sheds	Number of swabs
Sheds without pens or partitions	Three (3) swabs
Sheds with two or more pens	Two (2) swabs for each pen For example: » Shed with 2 pens – 4 swabs required » Shed with 3 pens – 6 swabs required
Single level shed or shed without pens or partitions	Two (2) swabs
Multi-level shed or shed with pens	Two (2) swabs for each pen or level
Deep litter + Slatted sheds	Four (4) swabs for each shed
Conventional multi-tier cages with manure belt	Two (2) swabs for each cage row
Conventional multi-tier cages with manure pit (without manure belt)	Three (3) swabs for each shed

2.3 Material

- Small plastic screw top jars (e.g. yellow top plastic jars) or small zips lock bags
- Cotton gauze swabs approximately 10cm square*
- Ball of cotton string
- Disposable latex gloves
- Sample transport media (peptone water)
- Whirl-Pak® bags or screw top plastic jar
- Scissors
- Marker pen
- Laboratory accession form
- Plastic post satchel for swab transport to laboratory

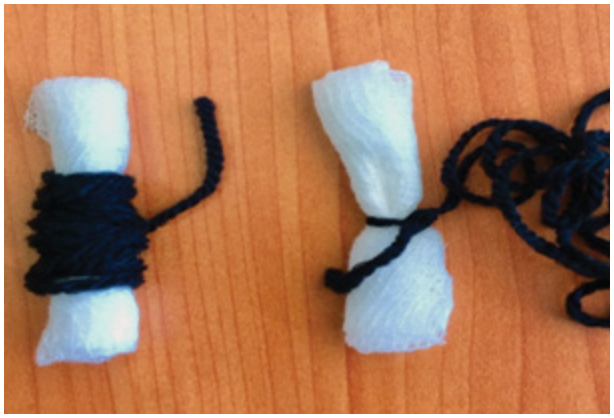


Figure A1 – Gauze swabs with string attached

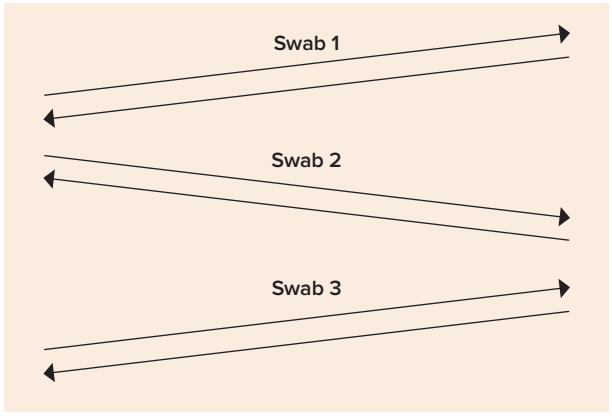


Figure A2 – Swabbing procedure for deep litter sheds without pens or partitions

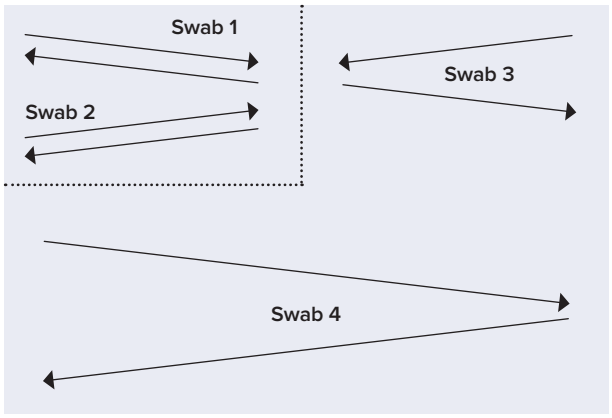


Figure A3 – Swabbing procedure for deep litter sheds with two pens

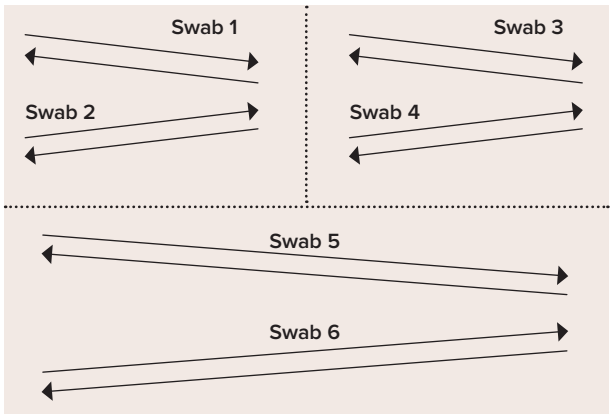


Figure A4 – Swabbing procedure for deep litter sheds with three pens

** Foot boot swabs can be used for shed floors and slats. Commercial 3M swabs can be used for environmental surfaces, like the grading floor and egg surfaces.*

*2.4 Preparation of swabs**

- Make multiple little bows using cotton gauze swabs with approximately 1m of string attached **(Figure A1)**.
- Wrap string around bow and place the required number of swabs for each shed (depending on shed type) into each plastic container or zip lock bag. Make up 50 or so sample containers.
- Store in a dry secure place for future use.

** Kits may be supplied by the diagnostic laboratory.*

3.0 Swabbing procedure

3.1 Shed without pens or partitions

- Wash hands and put on a pair of disposable latex gloves.
- Moisten the required number of swabs with water from the drinkers or solution provided by the laboratory.
- Each swab is to be held by the string and dragged twice the full length of the shed over different areas of litter in the patterns described in **Figure A2**.
- When the end of the slats is reached for the second time, retrieve the swab, cut the string with a pair of scissors and place swab in the Whirl-Pak® bag or screw top plastic jar.

3.2 Shed with pens or partitions

- Wash hands and put on a pair of disposable latex gloves.
- Moisten the required number of swabs with water from the drinkers or solution provided by the laboratory.
- Each swab is to be held by the string and dragged twice the full length of each pen over different areas of litter in the patterns described in **Figure A3** (two pens) and **Figure A4** (three pens).
- When the end of the slats is reached for the second time, retrieve the swab, cut the string with a pair of scissors and place swab in the Whirl-Pak® bag or screw top plastic jar.

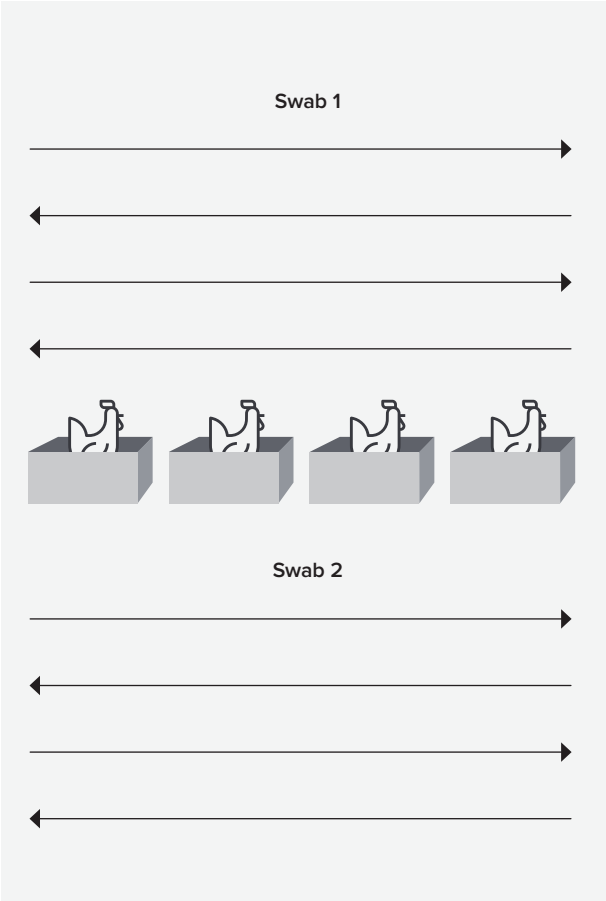


Figure A5 – Swabbing procedure for slatted shed

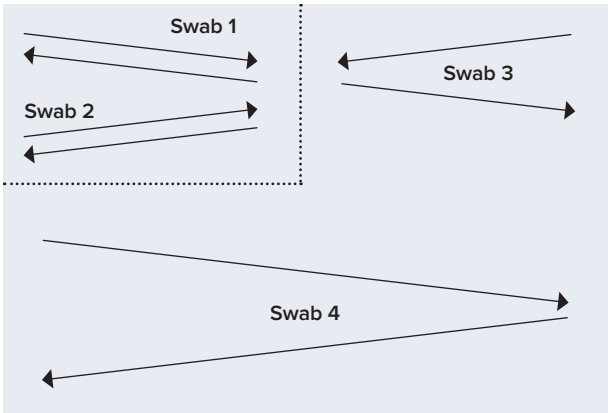


Figure A6 – Swabbing procedure for fully slatted sheds with two pens

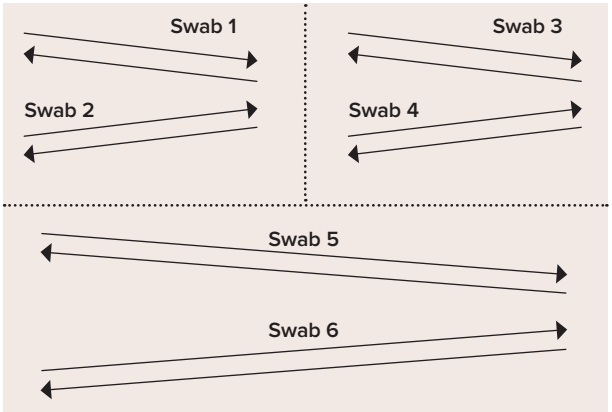


Figure A7 – Swabbing procedure for fully slatted sheds with three pens

3.3 Single level shed with 100% slats

For a slatted floor, swabbing of the slats has equivalent sensitivity for the detection of *Salmonella* as doing the manure itself underneath the slats.

- Wash hands and put on a pair of disposable latex gloves.
- Moisten the required number of swabs with water from the drinkers or solution provided by the laboratory.
- Walk down one side full length of the shed, dragging the swab over the top of the slats in the manner shown in **Figure A5** by the arrow "Swab 1". Repeat this up and down 3 more times.
- When the end of the slats is reached for the fourth time, retrieve "Swab 1", cut the string with a pair of scissors and place swab in the Whirl-Pak® bag or screw top jar.
- Walk down the other side of the shed, dragging the swab over the top of the slats in the manner shown in **Figure A5** indicated by the arrow "Swab 2". Repeat this up and down 3 more times.
- When the end of the slats is reached for the fourth time, retrieve "Swab 2", cut the string with a pair of scissors and place swab in the Whirl-Pak® bag or screw top jar.

3.4 Slatted sheds with pens

- Wash hands and put on a pair of disposable latex gloves.
- Moisten the required number of swabs with water from the drinkers or solution provided by the laboratory.
- Each swab is to be held by the string and dragged twice the full length of each pen over different areas of slats in the patterns described in **Figure A6** (two pens) and **Figure A7** (three pens).

3.5 Deep litter and slatted sheds

- Wash hands and put on a pair of disposable latex gloves.
- Moisten the required number of swabs with water from the drinkers or solution provided by the laboratory.
- Hold "Swab 1" by the string and drag it twice the full length of one side of the litter area in the pattern described in **Figure A8**.
- When the end of the litter area is reached for the second time retrieve "Swab 1", cut the string with a pair of scissors and place swab in the Whirl-Pak® bag or screw top jar.

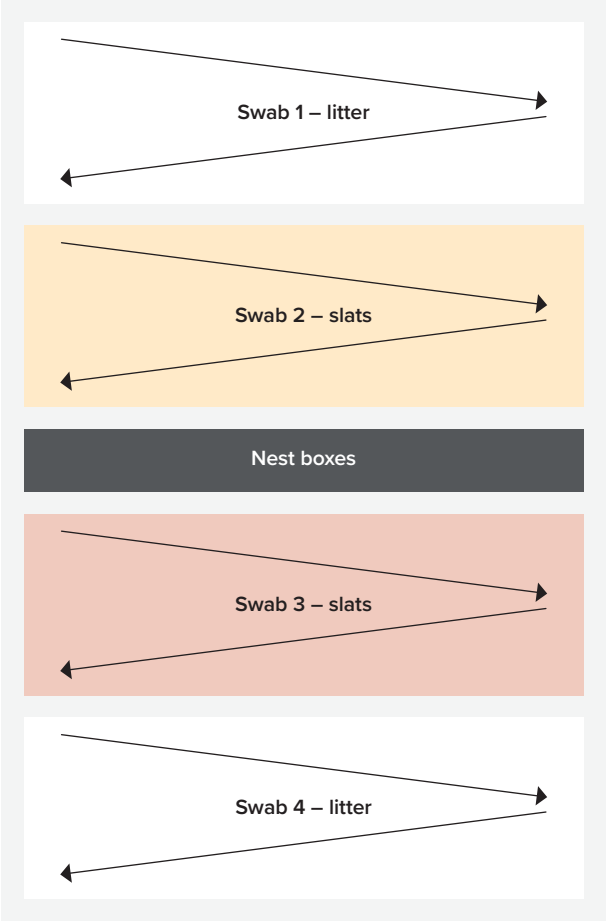


Figure A8 – Sampling pattern

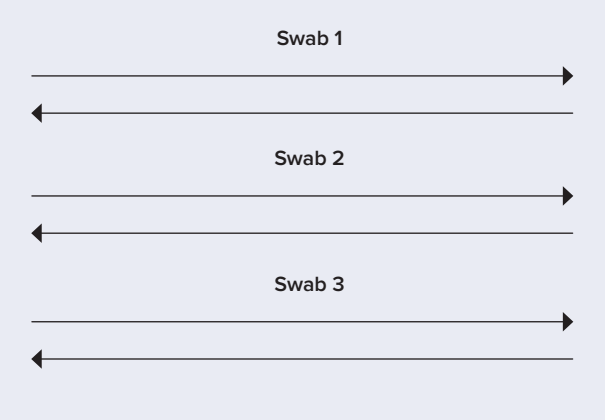


Figure A9 – Swabbing method for a single story conventional layer shed

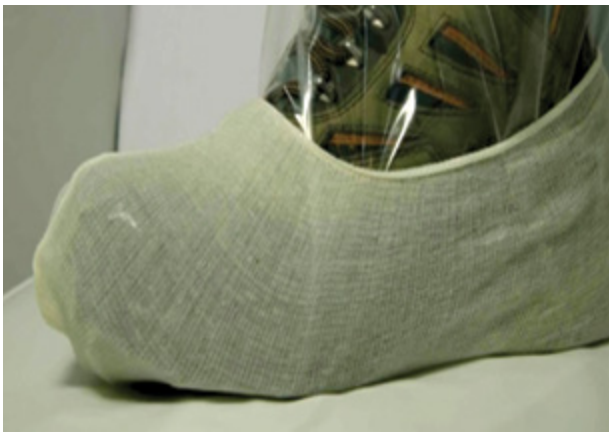


Figure A10 – Commercially available boot swab (Courtesy – Solar Biological Inc.)

- Walk down the slatted area, dragging the "Swab 2" over the top of the slats in the manner shown in **Figure A8** by the arrow "Swab 2".
- When the end of the slats is reached for the second time, retrieve "Swab 2", cut the string with a pair of scissors and place swab in the Whirl-Pak® bag or screw top plastic jar. Follow the same procedure for the other side of the shed, as shown in **Figure A8** by the arrows for "Swab 3" and "Swab 4".

3.6 Conventional multi-tier cages with manure belt

- Wash hands and put on a pair of disposable latex gloves.
- For each row of cages, two (2) drag swabs are to be moistened with water from the drinkers or solution provided by the laboratory.
- Each of the two (2) swabs is tied by the string over each of the holes where the manure falls from the belts, leaving the swabs dangling down.
- Once manure belts are started, manure from the top levels should fall directly onto the swabs.
- Once the manure belt run is completed, cut the string with a pair of scissors and place the swabs in a screw top plastic jar or Whirl-Pak® bag.

3.7 Conventional multi-tier cages with manure pit (without manure belt)

- Wash hands and put on a pair of disposable latex gloves.
- Moisten the required number of swabs with water from the drinkers or solution provided by the laboratory.
- For each shed use a total of three (3) drag swabs.
- Attach moistened swab by the string to a pole or length of a broom handle.
- Walk between the cages for the full length of the shed, dragging the swab over the top of the litter piles in the manner shown in **Figure A9** by the arrow "Swab 1".
- Repeat in the opposite direction, along a new row with "Swab 1" as indicated above.
- Retrieve "Swab 1", by cutting the string with a pair of scissors, and place the swab in a plastic screw top jar or Whirl-Pak® bag.
- Repeat along a new row with "Swab 2" as for "Swab 1" **(see Figure A9)**.
- Repeat with third swab.

4.0 Sample labelling

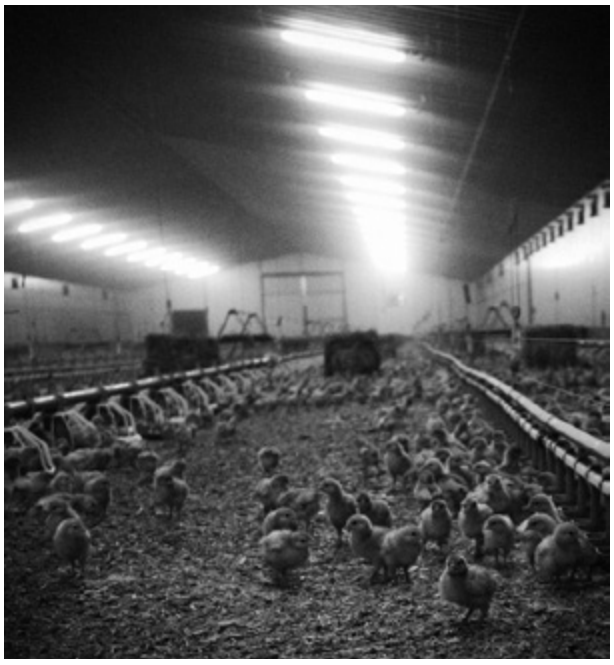
Swab containers must be labelled with a marker pen.

Farm	e.g. ABC Farm
Shed number	Shed S2
Date	15/07/15
Flock code and age	AA 22, 26 weeks
Collectors name	John Citizen
The words "environmental litter sample"	environmental litter sample

5.0 Packaging and transport to laboratory

- A completed laboratory accession form must be sent with each delivery.
- Swabs are to be packaged securely in a plastic lunch box and plastic post satchel, along with the completed accession form, and forwarded to the diagnostic laboratory.
- Ensure swabs are promptly express posted to the laboratory on the day of collection, so delays in culturing do not occur.
- Completed swabs must be protected from sunlight and stored in the fridge (between 4 and 8°C) until posted.

Swabs must not be frozen



SOP 2: *Salmonella* litter or manure sampling procedure using boot swabs for deep litter and slatted sheds

1.0 Introduction

Boot swab kits are available in different design and pack size to cover specific sampling applications, and currently most of the products comply with recommendations for sampling methods laid down by European and UK legislation and regulatory documents. Most of the commercially available products are presented either in two-pairs or five-pairs boot swab kits, with or without plastic boot covers or over boots.

2.0 Preparation for sample collection

2.1 Contact the laboratory

- Obtain an appropriate submission form from the laboratory (Samples obtained through this SOP should always be recorded on accession sheets as "ENVIRONMENTAL LITTER SAMPLES").
- If applicable, and/or available, ask if the laboratory can supply the swabs.
- Notify the laboratory 24 hours in advance of receiving samples to allow adequate time for preparation.

2.2 Calculate the pairs of swabs required

Type of shed	Number of boot swabs
Deep Litter System – sheds without pens or partitions	Two (2) pairs
Deep Litter System – sheds with two or more pens	One (1) pair for each pen Two (2) pens in shed – 2 pairs Three (3) pens in shed – 3 pairs
100% Slatted Shed – single level shed or shed without pens or partitions	Two (2) pairs required
100% Slatted Shed – multi-level shed or shed with pens	One (1) pair for each pen or level

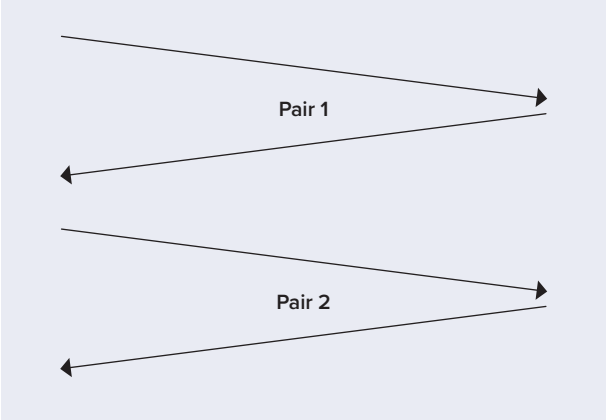


Figure A11 – Sampling procedure for deep litter shed with partition

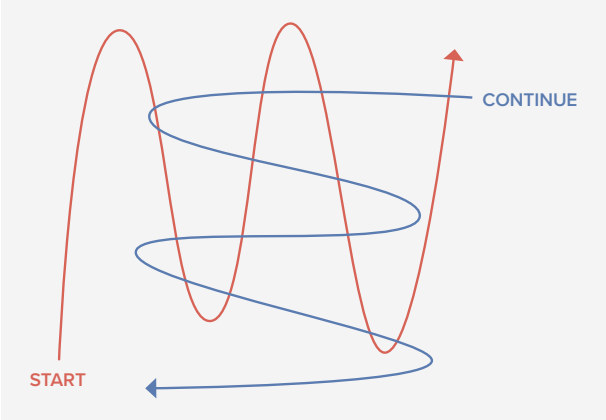


Figure A12 – Sampling procedure for deep litter sheds without pens or partitions

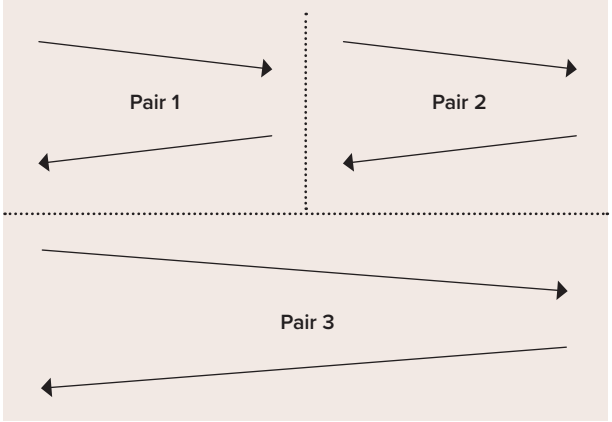


Figure A13 – Sampling procedure for deep litter sheds with three pens

*2.3 Material**

- Boot Swab Kit/Twirl-tie bag consists of a medical grade bag containing boot swab and other sampling consumables.
- Disposable latex gloves (may be included with the swab kit)
- Plastic Boot Cover or over boots (may be included with the swab kit)
- Marker pen
- Laboratory accession form
- Plastic post satchel for swab transport to laboratory

**Kits may be supplied by the diagnostic laboratory*

3.0 Swabbing procedure

3.1 Deep Litter sheds

- Wash hands prior to using the boot swab and wear disposable latex gloves.
- Immediately before sampling, slip a disposable plastic boot cover over a single shoe to protect personal clothing and to prevent cross-contamination of the samples from shoe sole carryover.

Don't use foot baths or any disinfectant/sanitizer prior to sample collection as it might kill the *Salmonella* in the sample, if there is any.

- Carefully remove the pre-moistened boot swab from the bag (Twirl-tie bag) and place it securely over the boot or shoe.
- Walk the entire length of the shed and return to the collection point as per **Figure A11** (shed with partition) and **Figure A12** (shed without pens or partition).
- For multiple pens/rows use fresh swab **(Figure A13)** and plastic boot cover **(Figure A14)** for each row/pen.
- Immediately after the sample collection carefully remove the boot swab and return it to its original Twirl-tie bag. For multiple swabs from same shed, return all the swabs in a single Twirl-tie bag to pool the swabs.
- Once all the swabs are collected from any particular shed, seal the bag and identify the samples.

3.2 Slatted shed (single level shed with 100% slats)

For a slatted floor, swabbing of the slats has equivalent sensitivity for the detection of *Salmonella* as doing the manure itself underneath the slats.

- Wash hands prior to using the boot swab and wear disposable latex gloves.
- Immediately before sampling, slip a disposable plastic boot cover over a single shoe to protect personal clothing and to prevent cross contamination of the samples from shoe sole carryover.

Figure A14 – Sampling procedure using boot swabs

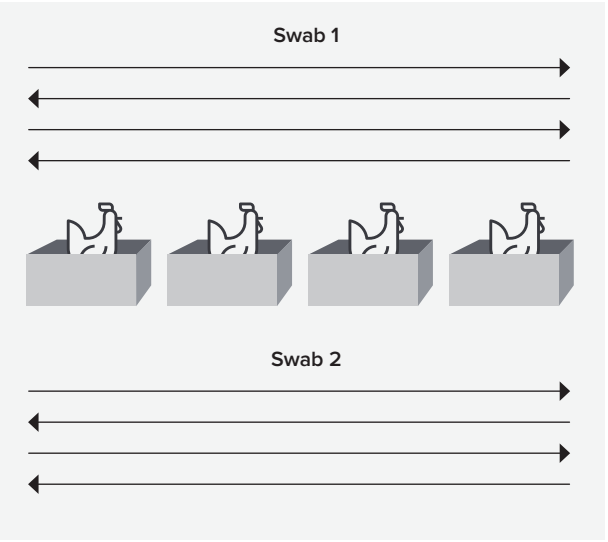


Figure A15 – Sampling procedure for a slatted shed

Don't use foot baths or any disinfectant/sanitizer prior to sample collection as it might kill the *Salmonella* in the sample, if there is any.

- Carefully remove the pre-moistened boot swab from the bag (Twirl-tie bag) and place it securely over the boot or shoe.
- Walk down the full length of one side of the shed and return to the collection point so that the boot swab should represent the maximum area to which birds have access, including the underneath of feeders and drinkers (if safe to do so).
- Immediately after the sample collection, carefully remove the boot swab and return it to its original Twirl-tie bag. For multiple swabs from the same shed, return all of the swabs in a single Twirl-tie bag to pool the swabs.
- Walk down the other side of the shed, in the manner shown by the arrow "Swab 2" in Figure A15.

4.0 Sample labelling

Swab containers must be labelled with a marker pen.

Farm	e.g. ABC Farm
Shed number	Shed S2
Date	15/07/15
Flock code and age	AA 22, 26 weeks
Collectors name	John Citizen
The words "environmental litter sample"	environmental litter sample

5.0 Packaging and transport to laboratory

- A completed laboratory accession form must be sent with each delivery.
- Swabs are to be packaged securely in a plastic lunch box and plastic post satchel, along with the completed accession form, and forwarded to the diagnostic laboratory.
- Ensure swabs are promptly express posted to the laboratory on the day of collection, so delays in culturing do not occur.
- Completed swabs must be protected from sunlight and stored in the fridge (between 4 and 8°C) until posted.

Swabs must not be frozen

SOP 3: Day old chick paper or three-day old brooding paper sampling procedure

1.0 Introduction

All day old chick deliveries should be tested for *Salmonella* spp. excretion by collecting and testing of chick box papers or where no chick papers are available by testing the brooding paper after 3 days. This gives an initial assessment of flock status and allows enhanced control and biosecurity measures to be put in place.

2.0 Preparation for sample collection

2.1 Contact the laboratory

- Obtain an appropriate submission form from the laboratory (Samples obtained through this SOP should always be recorded on accession sheets as "CHICK PAPER OR BROODING PAPER").
- If applicable, and/or available, ask if the laboratory can supply the swabs.
- Notify the laboratory 24 hours in advance of receiving samples to allow adequate time for preparation.

2.2 Material

- New white plastic garbage bags (so the bags can be written on)
- Marker pen
- Courier kit (can be obtained through the laboratory)

3.0 Sampling procedure

- Ten (10) chick papers (or sections of 3-day old brooding paper) are to be randomly collected from boxes (cages) immediately following placement of chicks.
- If multiple donor flocks are placed, and are identifiable, chick papers should be collected from EACH donor flock.
- Place each group of chick (brooding) papers into an individual bag.

4.0 Sample labelling

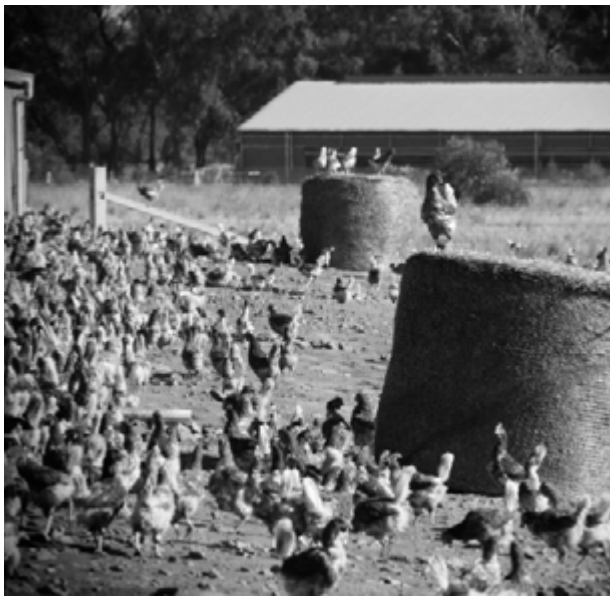
Swab containers must be labelled with a marker pen.

Farm	e.g. ABC Farm
Shed number	Shed S2
Date	15/07/15
Flock code and age	AA 22, 26 weeks
Sex	Male
Breed	Name of egg laying breed
Donor flock	If known
Collectors name	John Citizen
The words "Chick/ brooding Paper"	Chick papers

5.0 Packaging and transport to laboratory

- A completed laboratory accession form must be sent with each delivery.
- Samples are to be packaged securely in a plastic lunch box and plastic post satchel, along with the completed accession form, and forwarded to the diagnostic laboratory.
- Ensure samples are promptly express posted to the laboratory on the day of collection, so delays in culturing do not occur.
- Samples must be protected from sunlight and stored in the fridge (between 4 and 8°C) until posted.

Samples must not be frozen



SOP 4: Cracked and dirty eggs sampling procedure

1.0 Introduction

Cracked and dirty eggs are most likely to be contaminated with *Salmonella* when eggs are derived from donor flocks that are positive and shedding *Salmonella*. In such situations the *Salmonella* is associated with the faeces and/or has entered the egg through a shell defect. Intact clean eggs are significantly less likely to be contaminated with *Salmonella*.

2.0 Preparation for sample collection

2.1 Contact the laboratory

- Obtain an appropriate submission form from the laboratory (Samples obtained through this SOP should always be recorded on accession sheets as "CRACKED AND DIRTY EGGS").
- If applicable, and/or available, ask if the laboratory can supply the swabs.
- Notify the laboratory 24 hours in advance of receiving samples to allow adequate time for preparation.

2.2 Material

- Small plastic screw top jars (e.g. yellow top plastic jars)
- Plastic bags (e.g. Zip lock bag)
- Environmental/3M swabs (optional)
- Marker pen
- Laboratory accession form
- Plastic post satchel for swab transport to laboratory

3.0 Sampling procedure

- Collect multiple cracked and dirty eggs from each shed and identify them by shed or flock code.
- Place each group of eggs into a separate plastic bag and smash the eggs into pulp.
- Cut the corner of the bag and collect 25 ml in a screw top jar or wet an environmental/3M swab with the contents.
- Secure and seal the 25 ml of liquid in a plastic container or place the environmental/3M swab in the sealed plastic container, and label the container to identify the contents and identify the donor flock.

4.0 Sample labelling

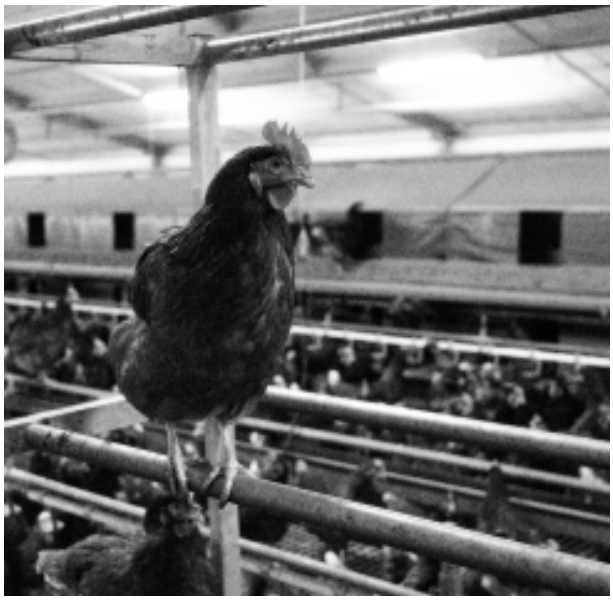
Swab containers must be labelled with a marker pen.

Farm	e.g. ABC Farm
Shed number	Shed S2
Date	15/07/15
Flock code and age	AA 22, 26 weeks
Sex	Male
Breed	Name of egg laying breed
Collectors name	John Citizen
The words "Cracked and dirty eggs"	Cracked and dirty eggs

5.0 Packaging and transport to laboratory

- A completed laboratory accession form must be sent with each delivery.
- Samples are to be packaged securely in a plastic lunch box and plastic post satchel, along with the completed accession form, and forwarded to the diagnostic laboratory.
- Ensure samples are promptly express posted to the laboratory on the day of collection, so delays in culturing do not occur.
- Samples must be protected from sunlight and stored in the fridge (between 4 and 8°C) until posted.

Samples must not be frozen



SOP 5: Washed eggs sampling procedure to validate egg washing

1.0 Introduction

Eggshells are washed and sanitised to eliminate the potential for surface contamination with food safety pathogens such as *Salmonella*. *Salmonella* that is present on the eggshell may potentially penetrate the shell and enter the internal substrate of the egg, or physically be displaced into a consumable food during the process of breaking the eggshell.

2.0 Preparation for sample collection

2.1 Contact the laboratory

- Obtain an appropriate submission form from the laboratory (Samples obtained through this SOP should always be recorded on accession sheets as "WASHED EGGS").
- If applicable, and/or available, ask if the laboratory can supply the swabs.
- Notify the laboratory 24 hours in advance of receiving samples to allow adequate time for preparation.

2.2 Material

- Marker pen
- 3M sponge swab
- Whirl-Pak® bags
- Laboratory accession form
- Courier pack
- Sterile water

3.0 Sampling procedure

- Wash hands and put on a pair of disposable latex gloves.
- Randomly select a minimum of 90 washed eggs and separate it into 3 different fillers (30 eggs in each filler) that have been processed at separate times throughout the day.
- Moisten the 3M sponge swab in sterile water (free of any sanitising agents).
- Firmly wipe the 3M sponge swab across the surface of the washed eggs making contact with them all.
- Return the sponge in original Whirl-Pak® bag and seal using the wire twist.

4.0 Sample labelling

Each plastic pouch to be labelled with a marker pen.

Farm	e.g. ABC Farm
Date	15/07/15
Collectors name	John Citizen
The words "Washed eggs"	Washed eggs

5.0 Packaging and transport to laboratory

- A completed laboratory accession form must be sent with each delivery.
- Swabs are to be packaged securely in a plastic lunch box and plastic post satchel, along with the completed accession form, and forwarded to the diagnostic laboratory.
- Ensure swabs are promptly express posted to the laboratory on the day of collection, so delays in culturing do not occur.
- Completed swabs must be protected from sunlight and stored in the fridge (between 4 and 8°C) until posted.

Swabs must not be frozen

SOP 6: Environmental swabs of egg packing and grading floor

1.0 Introduction

The surfaces in the egg packing and grading floors are a potential source of *Salmonella*. Contamination from cracked and dirty eggs infected with *Salmonella* can also contribute to cross-contamination of clean eggs prior to and after washing/sanitation.

Environmental sponges can be used to assess the process control and hygiene. The concept of environmental sampling as applied to an egg handling facility, as in any food handling and processing environment, is aimed at the discovery of the most probable sites that can harbour *Salmonella*. Detection of these sites will assist as to where to concentrate the efforts with regards to sanitation programs.

Sampling sites should include, but not be limited to, the following:

- graded and ungraded cooler floor/door/walls
- packing head (transfer point) and packing table
- anaconda surface
- all other surfaces where broken egg residues are evident

2.0 Preparation for sample collection

2.1. Contact the laboratory

- Obtain an appropriate submission form from the laboratory (Samples obtained through this SOP should always be recorded on accession sheets exactly where the sample is collected, for example "ANACONDA SURFACE").
- If applicable, and/or available, ask if the laboratory can supply the swabs.
- Notify the laboratory 24 hours in advance of receiving samples to allow adequate time for preparation.

2.2 Material

- 3M sponge stick
- Marker pen
- Laboratory requisitions
- Plastic post satchel for swab transport to laboratory

3.0 Sampling procedure

- A number of high load surface egg contact areas in the packing/grading floor are to be tested using 3M sponge sticks.
- Wash and sanitise hands before beginning.
- Put on disposable gloves and remove the sponge from the bag.
- Drag sponge across the defined area to be tested in a back and forth grid to completely cover the area.
- Return the sponge in the original sterile bag and seal using the wire twist.

4.0 Sample labelling

Each plastic pouch to be labelled with a marker pen.

Farm	e.g. ABC Farm
Date	15/07/15
Collectors name	John Citizen
Area sampled	Anaconda surface or egg packer surface

5.0 Packaging and transport to laboratory

- A completed laboratory accession form must be sent with each delivery.
- Swabs are to be packaged securely in a plastic lunch box and plastic post satchel, along with the completed accession form, and forwarded to the diagnostic laboratory.
- Ensure swabs are promptly express posted to the laboratory on the day of collection, so delays in culturing do not occur.
- Completed swabs must be protected from sunlight and stored in the fridge (between 4 and 8°C) until posted.

Swabs must not be frozen

SOP 7: Shed cleanout procedure – rearing and production sheds

1.0 Introduction

The aim of a wash down and disinfection of a poultry shed is to reduce significantly/eliminate pathogen load. The effectiveness of shed disinfection is particularly influenced by the organic load in the shed and the presence of contaminating sources. Thus, it is preferable for the shed to be depopulated of all livestock, for disposable equipment to be removed, and the organic load substantially reduced before the terminal disinfection. This is usually followed up by the washing and disinfection of ancillary equipment such as feeders, drinker lines, fan cowlings, and other facilities.

The inclusion of an insecticide spray is aimed at reducing or eliminating some of the vectors and passive carriers of poultry pathogens.

The choice of chemicals is influenced by the nature of the surfaces to be cleaned, user-friendly nature and suitability for equipment, OH&S issues, and importantly the type of pathogen or food safety organism being addressed.

In summary:

- Detergents vary in type, efficacy and safety and should be rinsed off before the application of the disinfectant.
- Disinfectants: most common ones used in the poultry industry are glutaraldehyde and quaternary ammonia compounds (only third generation and above QUATS should be used because of their enhanced efficiency), and Virkon®.

Note: cleaning and disinfection protocols are guidelines but, in all cases, the first principles remain the same.

2.0 Pullet rearing sheds

- Depopulate the shed of all livestock.
- Remove disposable materials and the remaining feed from the trough and augers. Drain drinker lines, and empty water from end of line water storage vessels.
- Run manure belts and empty all manure from the shed, and undertake a dry clean of all equipment and facilities including fans and cowlings, cages, floors and infrastructure.
- Apply an approved animal house detergent using high-pressure low volume application equipment. The application of the detergent as a foam is preferred.
- Rinse detergent off with a high-pressure low volume spray and allow to partially dry.
- Apply disinfectant (glutaraldehyde buffered or double chain QUAT) with a low volume spray application adequate to just lightly wet all surfaces including the cooling pads (if present). Where Virkon® is used, the surfaces particularly need to be free of organic load.
- Flush drinker lines out then treat in situ with a peracetic acid type product or other commercial product. Allow these products to stay in the drinker lines for several hours before flushing, then repeat the treatment and flushing for a second time.
- Apply an insecticide/beetle control product to the shed infrastructure, equipment and cooling pads.
- Check the inside of silos and clean where required, and repair any defects.
- If the shed comes with a cooling pad, drain the cooling pad system and lightly sanitise the pad. In winter, leave the system empty of water and in summer recharge the system with water and add a bromide capsule (Actrol® Mini Dose Cartridge). This provides a slow release sanitiser into the cooling water.
- Check and replace rodent bait stations.
- Ensure all amenity rooms and equipment being brought back into the shed are washed and disinfected similarly.
- Before re-population, the major surfaces of the shed should be swabbed for *Salmonella*.

3.0 Production or layer sheds

Procedure as for the rearing facilities and similar principles. The nature of the various shed designs and facilities though make it more difficult to achieve an optimal wash down and disinfection.

Specific considerations:

- Controlled Environment Cage Sheds by the nature of their surfaces, concrete floors, manure belts and wire cages allow easier removal of the organic load and terminal disinfection.
- Flat Deck Barn and Free Range Sheds invariably have more difficult surfaces to clean, and may involve the removal (partial removal) of slats, timber surfaces, dirt or only partial concrete floors, curtains and other infrastructure. Nest box systems and nest pads are particular areas that require attention in regard to cleaning for *Salmonella* control.
- Compared to cages, all alternative housing systems have additional challenges for husbandry and disease control. An aviary system, being a

three dimensional system as opposed to a flat deck configuration, has additional requirements relating to husbandry, feed and water access, manure removal, ventilation/cooling, and bird housing and depopulation, all of which add complexity to washing down and disinfection.

4.0 Pre placement of point of lay pullets (POL)

Ensure the *Salmonella* status of the POL of lay pullets is known by undertaking *Salmonella* testing of the pullets litter/manure ten (10) days prior to transfer.

Wash down and disinfect specifically to the production system by:

- dry cleaning of all surfaces and removing the bulk of the accumulated organic load.
- using a low volume high-pressure detergent wash-down to remove all organic material from the surfaces. This may require a protease in the detergent.
- removing or part removal of structures like slats, nest box pads, and supplementary equipment for hand cleaning and disinfection.
- cleaning and disinfecting the cooling pads and tunnels.
- cleaning and disinfecting as feasibly as possible the manure belt drying systems.
- cleaning and disinfection of amenities rooms, egg collection rooms/bays and cool rooms.
- using specific insecticide treatment where required for Red Mites (*Dermanyssus gallinae*). Red Mites are not an obligate parasite and thus can live off the bird in the shed environment for many months.
- draining and flushing all water storage tanks to remove organic and mineral deposit build up, and refilling them with clean water sanitised with 1 litre of 12.5% sodium hypochlorite per 25,000 litres of water.
- swabbing major surfaces for *Salmonella* before re-population.

5.0 During production

The production houses should have ongoing attention to the following:

- egg belts monitored for egg residue and cleaned.
- egg elevators regularly cleaned.
- anaconda cleaned down weekly.
- vermin control program maintained.
- fly bait and/or fly surface sprays (e.g. Agita®) used as required in summer.

SOP 8: Rodent control in poultry farms

1.0 Introduction

There are three main species of rodent that are present around poultry facilities.

1.1 Brown rat (Rattus norvegicus)

- Natural burrowers
- Dig burrows close to food and water supply in long vegetation and clutter
- Weigh between 250 and 500 g
- Tail shorter than head and body
- Ears small and close set

1.2 Black rat (Rattus rattus)

- Excellent climbers
- Nest high up in roofs and trees
- Weigh between 200 and 300 g
- Tail longer than head and body
- Ears prominent

1.3 House mouse (Mus musculus)

- Found inside and outside sheds
- Gnawing causes extensive damage to wiring and facilities if not controlled
- Weigh 15 to 25 g.

Rodents are almost blind and rely on touch and smell to guide them. They use the same paths, forming trails to find their way to food and water. Food is stored as a cache in the burrow or nest for pregnant females. Rodents are generally nocturnal, so identifying rodents during the day may indicate a severe rodent problem. To control rodents, we must take advantage of these aspects of rodent behaviour.

2.0 Control strategy

2.1 Food or water source removal

- A vegetation free perimeter of at least 1 meter is to be present around all sheds.
- All overhanging branches of trees are to be cut back.
- Areas of stagnant water are to be filled in or drained where appropriate.
- Feed spills outside sheds are to be cleaned up immediately.

2.2 Rodent proofing

- All holes in exterior walls are to be filled, particularly those around rooflines and where cross augers enter sheds.
- No gaps around doors are to exceed 7.5 mm.

2.3 Baiting strategy

2.3.1 Exterior baiting

- Place baits in burrows and block the entrance.
- Check daily to see if holes have been re-opened, if so repeat procedure.
- Place perimeter baits around sheds using one bait station every 15 meters. Baits must be laid both on the ground and in elevated positions to ensure baits are accessible to both species of rat.
- Check baits weekly to detect rodent activity, replacing baits when necessary.
- Baits should be used both when sheds are empty and when they are in use.

2.3.2 Interior baiting

- Place sachets of bait along inside walls and on other horizontal areas where rodents could move. Baits must be laid both on the ground and in elevated positions to ensure baits are accessible to both species of rat.
- Check baits weekly to detect rodent activity, replacing baits when necessary
- Baits should be used both when sheds are empty and when they are in use.

3.0 Risk management plan (health and safety consideration)

All rodenticides (chemicals which are poisonous to rodents) are poisonous at various levels for poultry, livestock, and humans. Therefore, caution in the use of rodenticides is required, and the manufacturer's label instructions should be strictly followed. Personal Protective Equipment such as gloves and masks must be worn at all times while handling the rodenticide. Management also needs to ensure compliance with the procedures and operators are trained properly.

SOP 9: Off-label use of Vaxsafe ST® vaccine in combination with avian adjuvanted vaccines

1.0 Introduction

Vaxsafe ST® is the only registered commercial live *Salmonella* vaccine in Australia and normally is administered via drinking water. For long term protection in layers, it is recommended after live priming the flock with Vaxsafe ST® to vaccinate the flock with Vaxsafe ST® in combination with an adjuvanted vaccine by intramuscular injection. **This is an "off-label use" of the vaccine but extensively adopted by the Australian poultry industry.**

2.0 Material

- Appropriate number of effective doses of Vaxsafe ST® vaccine. Note – Vaxsafe ST® comes in 1000, 2000 and 5000 dose vial sizes.
- Diluent – to dilute the ST vaccine in the vial before transfer and mixing with the adjuvanted killed vaccine. Commercial Marek's diluent is preferred but distilled water can also be used as a diluent.
- 5 ml syringe and a needle, preferably a large bore like an 18G.

3.0 Preparation of vaccines

3.1 Vaccine batch with 1000 dose vial size

Withdraw 3 ml of diluent (e.g. Marek's diluent or distilled water) from the diluent bottles to obtain a total of 3 ml in the syringe. Add this 3 ml of diluent to a vial of Vaxsafe ST® vaccine and gently invert the vial a number of times until all the freeze-dried vaccine is dissolved.

Draw up the total volume of the reconstituted vaccine into the same syringe. Determine the total volume in the syringe and dispense the total amount of this volume into a 500 ml adjuvanted vaccine bottle containing 1,000 doses. Gently invert the vaccine bottle several times to aid mixing.

Check the Vaxsafe ST® vaccine vial carefully and if there is any evidence that ST vaccine did not dissolve properly or part of the vaccine is left in the vial, repeat the procedure again. After transfer, shake the bottle of killed adjuvanted vaccine to mix the vaccines together to homogeneity.

3.2 Vaccine batch with 2000 dose vial size

Withdraw 3 ml of diluent from the diluent bottles to obtain a total of 3 ml in the syringe. Add this 3 ml of diluent to a vial of vaccine and shake gently until all the freeze-dried vaccine is dissolved.

Draw up the total volume of the reconstituted vaccine into the same syringe. Determine the total volume in the syringe and dispense exactly **half** amount of this volume into each of **two** 500 ml adjuvanted vaccine bottles containing 1,000 doses each. Gently invert the vaccine bottles several times to aid mixing.

Check the Vaxsafe ST® vaccine vial carefully and if there is any evidence that Vaxsafe ST® vaccine did not dissolve properly or part of the vaccine is left in the vial, repeat the procedure again. After transfer, shake the bottles of killed adjuvanted vaccine to mix the vaccines together to homogeneity.

3.3 Vaccine batch with 5000 dose vial size

Withdraw 3 ml of diluent from the diluent bottles to obtain a total of 3 ml in the syringe. Add this 3 ml of diluent to a vial of vaccine and shake gently until all the freeze-dried vaccine is dissolved.

Draw up the total volume of the reconstituted vaccine into the same syringe. Determine the total volume in the syringe and dispense exactly **1/5 amount** of this volume into each of **five** 500 ml adjuvanted vaccine bottles containing 1,000 doses each. Gently invert the vaccine bottles several times to aid mixing.

Check the Vaxsafe ST® vaccine vial carefully and if there is any evidence that Vaxsafe ST® vaccine did not dissolve properly or part of the vaccine is left in the vial, repeat the procedure again. After transfer, shake the bottles of killed adjuvanted vaccine to mix the vaccines together to homogeneity.

4.0 Administration of vaccine

Use the vaccine mix according to the instructions of the killed adjuvanted vaccine, which is intramuscular for most adjuvanted killed vaccine. Also use the same dose recommended for the killed vaccine.

NB:

1. The dilution of Vaxsafe ST® must be done with due diligence and requires careful observation and patience.
2. Commercially available avian adjuvanted vaccine commonly used in Australia includes: Nobilis EDS+NDV®, Nobilis EDS®, Newcavac® etc. Any of these vaccines can be utilised as an adjuvanted vaccine for Vaxsafe ST® administration.