



# ***Salmonella* Incidence Response Plan**

**A National Approach to *Salmonella* Outbreaks**

**Developed on behalf of the Australian Egg  
Corporation Limited**

by Dr Peter C. Scott

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# Table of Contents

1. Introduction .....	4
2. Background.....	5
3. Regulatory Response Plan .....	8
3.1 Procedural outline in the event of an egg related salmonellosis incident .....	10
3.1.1 Contingencies for Egg Farmers from <i>Salmonella</i> Notification by a Retailer, Consumer or Regulatory Authority .....	10
3.1.2 Actions Undertaken at the Farm Visit by the Responsible Authority .....	11
3.1.2.1 Outcomes of the visitation of the responsible authority .....	13
3.1.3 On Farm <i>Salmonella</i> Control Program .....	14
4. Key Risk Areas Associated with <i>Salmonella</i> Contamination of Eggs.....	16
5. The Fundamental Stages of the <i>Salmonella</i> Response.....	19
6. Supplementary Activities to Mitigate the Risk of <i>Salmonella</i> .....	27
7. Standard Long-term <i>Salmonella</i> Control Program.....	29
<b>Appendix 1. <i>Salmonella</i> testing.....</b>	<b>30</b>
List of laboratories that can test for <i>Salmonella</i> in chickens .....	30
<b>Appendix 2 Standard Operating Procedures.....</b>	<b>32</b>
Standard Operating Procedure 1: <i>Salmonella</i> Litter/Manure Sampling Procedure Using Gauze Swabs for Deep Litter Sheds.....	32
Standard Operating Procedure 2: <i>Salmonella</i> Litter/Manure Sampling Procedure Using Gauze Swabs for Fully Slatted Sheds .....	36
Standard Operating Procedure 3: <i>Salmonella</i> Litter/Manure Sampling Procedure Using Gauze Swabs for Deep Litter + Slatted Sheds .....	40
Standard Operating Procedure 4: <i>Salmonella</i> Litter/Manure Sampling Procedure Using Gauze Swabs for Conventional Multi-tier Cages with Manure Belt .....	43
Standard Operating Procedure 5: <i>Salmonella</i> Litter/Manure Sampling Procedure Using Gauze Swabs for Conventional Multi-tier Cages with Manure Pit (Without Manure Belt) .	46
Standard Operating Procedure 6: <i>Salmonella</i> Litter/Manure Sampling Procedure Using Boot Swabs for Deep Litter and Slatted Sheds .....	49
Standard Operating Procedure 7: <i>Salmonella</i> –Day Old Chick Paper/Three Day Old Brooding Paper Sampling Procedure .....	54
Standard Operating Procedure 8: <i>Salmonella</i> –Cracked and Dirty Eggs Sampling Procedure.....	56
Standard Operating Procedure 9: <i>Salmonella</i> –Washed Eggs Sampling Procedure To Validate Egg Washing .....	58
Standard Operating Procedure 10: <i>Salmonella</i> –Environmental Swabs Of Egg Packing and Grading Floor .....	60
Standard Operating Procedure 11: Shed Cleanout Procedure Rearing and Production Sheds.....	62
Standard Operating Procedure 12: Rodent Control In Poultry Farms .....	65
Standard Operating Procedure 13: Off-label use of Vaxsafe ST <sup>®</sup> vaccine in combination with Avian Adjuvanted Vaccines	68

# 1. Introduction

Foodborne illness outbreaks in the human population are managed by the relevant government agencies within each state and territory jurisdiction. Currently, there are no formal nationally structured standard operating procedures (outside of government) for investigating on-farm practices in relation to an outbreak of human salmonellosis cases linked to the consumption of eggs or egg products.

A set of procedures, developed by industry, in conjunction with the relevant government agencies, will ensure that the process is accurate and robust and is based on industry knowledge on how to assess the on-farm risks related to *Salmonella*. This will enable appropriate actions, if required, to implement a through-chain approach towards minimising the risk of a future outbreak under the same circumstances.

A national approach to these investigations would ensure that relevant information and processes are able to be reliably updated, with scope for individual state / territory requirements to be added by each responsible jurisdictional agency as required. Such a uniform program will also allow the mentoring and training of people within industry and government to ensure not only a sustainable program but one that is objective and accepted by both industry and government.

Although *Salmonella* Enteritidis (SE) is not endemic in the Australian poultry industry, a risk assessment needs to be considered for the Australian egg industry. This *Salmonella* Incidence Response Plan provides the opportunity to include the development of an accepted national response to the identification of SE in Australian commercial egg layer flocks.

## 2. Background

In the *Salmonella* genus, there are more than 2,500 serovars which are widely distributed in both wild and domestic animals and cold blooded species. *Salmonella* is so ubiquitous that it is hard not to find it in both animate and contaminated inanimate sources although only a few serovars are considered as a risk to food safety.

*Salmonella*, as microorganisms, are relatively non-particular about their growth media requirements, which are simple and while *Salmonella* grows best at 37°C, they can grow at temperatures from 5 to 45°C and within a pH range of 4 to 9. They can survive in the environment for years and colonise invertebrates which can act as passive carriers. Overall, they are very well adapted microorganisms and thus it is hard to control exposure and colonisation / infection in domestic livestock.

*Salmonella* can be inactivated by temperatures over 60°C if heated for longer than 10 minutes and, at temperatures above 70°C, inactivation will occur in seconds although this time and temperature inactivation can be affected by the nature of the food product including, for example, salting. Thus, the normal cooking process will kill *Salmonella* and reduce the food safety risk, which is the normal outcome for chicken meat. The egg industry though has the issue of the consumption in some food preparations of raw eggs or lightly cooked eggs.

The Australian layer and chicken meat industry is free of the two-avian host specific *Salmonella* serovars, *Salmonella* Pullorum (Pullorum disease) and *Salmonella* Gallinarum (Fowl Typhoid) which are both considered to be exotic to the Australian poultry industry. Similarly, Australia is also considered to be free from the human specific *Salmonella* called *Salmonella* Typhi that causes typhoid disease in humans.

Non-typhoid *Salmonella* are the type of *Salmonella* with which we are most familiar and they are not host specific, essentially affecting all species including mammals, reptiles and insects. Thus, they are common in the environment in which our domestic poultry live and controlling contamination in our livestock under normal conditions is difficult. Strict implementation of biosecurity and hygiene procedures though will assist in significantly reducing the risk of *Salmonella* contamination. The isolation of *Salmonella* from poultry and the poultry environment such as litter is relatively common. In most cases the infection of layer hens is generally asymptomatic with no signs of illness seen in these carrier poultry.

A few serovars such as *Salmonella* Typhimurium (ST) type 9 and *Salmonella* Hessarek may cause clinical disease and mortalities in day old chicks and young layers under physiological stressors. Non-typhoid *Salmonella* in layers generally colonise the intestinal tract with various degrees of persistency and are shed in the faeces thus providing a potential mechanism of contamination of the surface of eggs and the egg handling environment. Internal egg contamination results from the *Salmonella* penetrating the outer shell as a result of the egg being contaminated with faecal matter or the integrity of the shell being compromised by cracks or poor egg shell quality. It is imperative that eggs are handled correctly and where egg washing is implemented that this is done with regular or real time monitoring of temperature and chemical concentration. The washing of high risk eggs, being those that are potentially already internally contaminated, to increase recovery is contraindicated.

One *Salmonella* of this group that has a slightly different pathogenesis and epidemiology is SE. *Salmonella* Enteritidis can actively colonise the ovary of the layer and thus be deposited internally into a clean egg free of faecal contamination. Australia and New Zealand are very fortunate as their commercial poultry industries are SE free. However, SE clinical disease in humans in Australia does occur, with the greater majority of these cases associated with overseas travel, particularly to Asia. The presence of SE is of great concern in overseas egg industries as this type of *Salmonella* is vertically transmitted, which means that the *Salmonella* is incorporated into the egg during formation of the egg inside the hen.

The *Salmonella* spp. that are currently present in the Australian egg industry, although of concern for human health, are not able to be vertically transmitted and therefore are present

on the outside of the egg only. This means there are considerably different interventions required in the case of an SE outbreak compared to other types of *Salmonella*. This should be kept in mind when reading international literature on *Salmonella* control.

*Salmonella* Enteritidis presents a biosecurity risk to the egg industry and is currently not listed under the Emergency Animal Diseases (EAD) Response Agreement (the Deed) and, there is currently no formal agreed response plan should there be a SE outbreak linked to Australian eggs or even the identification of SE in a layer flock. While SE is notifiable in all states because it is not currently categorised as an EAD under the Deed, and there is no agreement to cost share any response costs. The Deed does allow the National Management Group to cost share an eradication response with industry (fifty percent government and fifty per cent industry). As a notifiable disease, state legislation allows control actions including potentially ordering destruction of infected birds and eggs.

The development of a response plan to SE that aligns with the *Salmonella* Incidence Response Plan would ensure that there is a national response process available that stakeholders are aware of. This will enable an agreed process to be followed in the event of a SE outbreak. The SE contamination of clean eggs means that the refrigeration of eggs stored for extended periods is a critical control point and vaccination against SE tends to be more efficacious because of the systemic characteristics of its colonisation.

The *Salmonella* that are causally associated with table eggs related food poisoning outbreaks in Australia include a number of ST, including, but not exclusively, phage types 9, 44, 135a and 135, 170 (108). The other less common *Salmonella* serovars associated with food poisonings outbreaks are *S. Infantis*, *S. Virchow* 34, *S. Singapore* and *S. Saintpaul*. In recent times, ST 9, 44, 135 and 170 have been the predominant serovars in significant food poisoning outbreaks.

All these salmonellae have been and continue to be recovered from environmental sampling of poultry sheds. The status of poultry layer genetic stock, which is under regular monitoring programs and which are high standard preventative programs, is historically free of those salmonellae associated with food safety issues. The serovars that have been historically recovered from day old layer stock included *S. Infantis*, *S. Hessarek* and more recently, *S. Agona*, *S. Anatum*, *S. Senftenberg* and a number of other *Salmonella* that are untypable. These latter salmonellae are not normally associated with eggs and food safety outbreaks. Generally, either in-house or commercially reared pullets are *Salmonella* free at point of lay and the epidemiological field picture identifies most layer flocks becoming positive in lay.

The Australian poultry industry has a relatively high proportion of farms with a *Salmonella* positive status and essentially there is no regulatory control over this other than the responsibility of the producer to provide a food safe product. This is very different to other Organisation for Economic Co-operation and Development (OECD) countries, particularly European countries, where there is tight legislative control on *Salmonella* in the poultry industry. In-feed additive treatments and flock vaccination are a normal part of these regulated control programs. Depopulations may be enforced or necessitated. This outcome has been driven by significant food safety events and then followed by sectors of the industry wanting a product distinction for the consumer. It is to be recognised that SE is one of the drivers of this in the European Union (EU).

Australia, as indicated, has no formal or even voluntary code regarding *Salmonella* control and depends on industry best practice management to achieve a food safe product. The isolation of any *Salmonella* from clinically affected livestock is defined as a case of salmonellosis which is notifiable (some state variation depending on the *Salmonella* involved) and the mandatory reporting is required to the state or territory CVO and primary industries authorities. Under state legislation any identification of SE from the environment, poultry or poultry product is notifiable. While the majority of the Australian egg industry has no significant food safety events related to table eggs, there are ongoing egg related food poisoning outbreaks associated in the majority of cases with producers who do not follow acceptable



egg handling practices. In most cases, there is also the concurrent association of poor food preparation practices and / or the production of high risk foods such as raw egg based mayonnaise, fried ice cream, mousse, egg butter and salad dressings.

*It is important to appreciate that in any egg related food safety case there is the requirement for a two-step process:*

1. The presence of *Salmonella* on or in the egg shell or even possibly in the albumin.
2. The preparation of a ready-to-eat product that has not been adequately cooked or treated by an effective equivalent process such as lowering the pH in some raw egg foods.

Invariably if the Australian egg industry is to avoid having costly compliance imposed on it through regulation because of ongoing egg related food poisoning outbreaks, it will need to become more proactive and united in facilitating *Salmonella* control programs and improved egg handling practices throughout the industry. This is in conjunction with the various state food safety authorities ensuring through regulation and consumer education that food preparation is done in a manner that mitigates the risk of *Salmonella* food poisoning.

Importantly, producers need to appreciate that when dealing with human illness, and even in some cases death, that health departments will not be sympathetic to explanations of the previous historical performance of the property, economic considerations, practicality of farming operations, staff and resource difficulties and the moving of the responsibility of the outbreak solely to the food preparation. It is deemed the responsibility of the producer to produce a food safe product.

This document outlines a coordinated response by all stakeholders in the event of a human salmonellosis outbreak traced back to an egg farm. This document also includes a national response to a SE outbreak within the Australian egg industry, should this occur.

### 3. Regulatory Response Plan

Human salmonellosis would normally first present as a differential diagnosis to individual medical practitioners in either private or public practice as individual cases of gastrointestinal disease. On occasions, it may present as a point source outbreak with a concentrated spike of people attending hospitals or local medical clinics all with the history of consuming food at or from the same venue / outlet.

In the case of a point source outbreak, and before there is confirmation of the aetiological cause, the state health departments after being notified of the incident conducts preliminary visitations and investigations at the likely source of the contaminated food. Concurrent with this, ancillary laboratory tests are conducted to confirm the identity of the food pathogen involved.

Preliminary actions by the responsible authority regarding the source of the contaminated food will depend on the initial findings of the investigation and this may involve a closure of the premises particularly where significant breaches of best practice in food handling are observed. Continuing investigations will involve reviewing patient history, including the outcome of the laboratory findings, epidemiological investigations which will include microbiological sampling of prepared foods, raw material inputs and the food preparation environment.

Where a *Salmonella* is identified as the cause of the food poisoning incident then the knowledge of its strain (Multiple Locus Variable-number Tandem Repeat Analysis {MLVA}, phage type and/or Whole Genome Sequence {WGS}) can assist investigators to link an association with the probably source of raw material involved, such as eggs. Epidemiological studies at this stage may readily identify the primary source of the *Salmonella* isolate, such as finding the same strain of *Salmonella* in or on the surface of eggs and / or in raw egg product of ready-to-eat foods.

In some cases, the causal associations are difficult to identify and no conclusion as to the source of the outbreak can be identified. In many cases the definitive link is not identified to the cause of the outbreak, such as the inability to culture *Salmonella* from eggs, but the epidemiological links strongly indicate that eggs are involved. On occasions, regulatory authorities, even with limited epidemiological evidence linked to eggs, may formally consider eggs as the most probable causal association. The definitive link to an egg associated food safety incident is when the strain of *Salmonella* is linked back to the farm by the confirmatory identification of that particular *Salmonella* on the farm that was the supplier of the eggs. The utilisation of the gene technology tools, such as MLVA and WGS, allows the health departments / food safety regulators to establish a definitive link between the source of the eggs and the food safety outbreak that can be used not only for epidemiological investigations but also in a court of law.

In some cases, outbreaks do not occur as a defined point source but as a cluster of individual cases where the same *Salmonella* strain is involved which also results in health departments undertaking an epidemiological investigation to identify the common source of contamination. Ongoing suspicions lasting for long periods of time can suddenly all be linked the moment a trace back is achieved.

The Australian National Notifiable Disease Surveillance System collates all human salmonellosis laboratory diagnosed cases, which are notifiable conditions under state/territory public health legislation. Environmental isolates of *Salmonella* or incidental findings that are without any disease association do not require mandatory reporting, but by default when the typing of these *Salmonella* is undertaken at one of the two Australia *Salmonella* typing laboratories, these salmonellae go onto the record. The typing of these *Salmonella* firstly involves their serotyping (ST, *Salmonella* Infantis, *Salmonella* Agona, etc.), and then phage typing (ST 135, ST 44, etc.) and then MLVA typing (03-12-10-11-524, 03-12-10-10-524, etc.).



Information on *Salmonella* serotyping is monitored and utilised in outbreak investigations by the various health (Table 1), agriculture (Table 2) and food safety regulators (Table 3).

**Table 1: State health department contact details**

ACT	Phone: (02) 6205 0881 GPO Box 825 Canberra ACT 2601	SA	Phone: (08) 8226 2578. PO Box 287 Rundle Mall Adelaide SA 5000
NT	Phone: (08) 8999 2400 PO Box 40596, Casuarina NT 0810	TAS	Phone: 1300 135 513 GPO Box 125 HOBART TAS 7001
NSW	Phone: (02) 9391 9000 Locked mail Bag 961 North Sydney NSW 2059	VIC	Phone: 1300 651 160 50 Lonsdale Street Melbourne Vic 3000
QLD	Phone: (07) 3234 0111 GPO Box 48 Brisbane, Queensland 4001	WA	Phone: (08) 9222 4222 PO Box 8172 Perth Business Centre Perth WA 6849

**Table 2: State agriculture department contact details**

ACT	Department of Territory and Municipal Services Phone: 13 22 81 GPO Box 158, Canberra ACT 2601
NT	Department of Primary Industry and fisheries Phone: 1800 084 881 GPO Box 3000 Darwin NT 0801
NSW	Department of Primary Industries Phone: 1800 808 095 Locked Bag 21, Orange NSW 2800
QLD	Department of Agriculture and Fisheries Phone: 13 25 23 (QLD) or (07) 3404 6999 GPO Box 46, Brisbane QLD 4001
SA	Department of Primary Industries and Regions SA Phone: 1800 675 888 or (08) 8226 0995 GPO Box 1671, Adelaide, SA 5001
TAS	Department of Primary Industries, Water, Parks and Environment Phone: 1300 368 550 GPO Box 44, Hobart Tasmania 7001
VIC	Department of Economic Development, Jobs, Transport and Resources Phone: 136 186 GPO Box 4509, Melbourne VIC 3001
WA	Department of Agriculture and Food WA Phone: (08) 9368 3333 Locked Bag 4 Bentley Delivery Centre WA 6983

**Table 3: Food safety authorities for New South Wales and Queensland**

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NSW	Biosecurity and Food Safety Phone: 1300 552 406 or (02) 9741 4850 PO Box 6682, Silverwater NSW 1811 Australia
QLD	Safe Food Production Queensland Phone: 1800 300815 or (07) 3253 9800 PO Box 549, Stones Corner QLD 4120

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The primary concern of the food regulators is the presence of *Salmonella* in the internal contents of eggs or in finished ready-to-eat egg products. As a consequence of the endemic nature of *Salmonella* and its asymptomatic carrier status in domestic hens, the identification of *Salmonella* in the environment of layers is expected from time to time. The pivotal area of focus is required on the table eggs post laying, grading, handling and preparation for sale.

It is recognised by food regulators that the major cause of food poisoning cases related to eggs is the use of cracked and dirty or unwashed eggs with *Salmonella* on their shell surface in the production of raw or lightly cooked egg foods. The washing of eggs is not a mandatory procedure for egg producers. The statistical data on whether unwashed compared to washed eggs are associated with a higher frequency of food poisoning cases has not been undertaken. However, what is emphatically known from investigating field cases is that attempting to recover dirty eggs as clean A Grade eggs by washing has significant correlation with food poisoning episodes when these “recovered” eggs are used in the preparation of raw egg foods.

### **3.1 Procedural outline in the event of an egg related salmonellosis incident**

#### **3.1.1 Contingencies for Egg Farmers from *Salmonella* Notification by a Retailer, Consumer or Regulatory Authority**

It is important that producers have designated responsible personnel to handle enquiries about possible food safety events. In such situations, there is concern about the accuracy and integrity of the information supplied to the enquirer, particularly when dealing directly with the public. Information provided can potentially be used inappropriately and affect the wellbeing of the business. This can also apply to retailer and regulatory authorities where technical human resources can vary in experience and knowledge and again if information is interpreted incorrectly, this could result in outcomes that are not objective for all concerned. The designated people within an organisation ideally should include senior management, head of QA and a veterinarian or someone familiar with the operational aspects of farming including knowledge of *Salmonella* and commercial egg layers. The appointment of one designated person for all correspondence is desirable.

Initial enquires or information may not be complete and thus it is important to obtain as much information as possible regarding the food poisoning episode and the possible link to the supply of eggs. Information sought should include:

- i) the primary purchaser of the eggs, ii) pictures of the containers, iii) delivery dockets, iv) Julian dates v) egg stamp and vi) as much as possible that confidentiality considerations allow about the nature of the food safety episode and the reasons for the possible links with eggs from the property. Such information can quickly identify any issues with traceability, such as

noting that the egg buyer is not a customer, cartons are not of the supplier, or other discrepancies.

It is critically important to cooperate with the responsible authorities whose primary aim is implementing corrective actions as soon as practicably possible. Limited cooperation is implied as noncompliance and often leads to a lack of confidence from the regulatory authorities and the greater likelihood of the implementation of quarantine, stopping supply and stand down directives. The various authorities have such powers where they have reasonable evidence or even suspicions regarding the need to intervene because of public safety.

Commercial layer farms that already have evidence of a *Salmonella* mitigation program in place are considered as already demonstrating best practice and this puts the producer in a favourable position and consideration by the regulatory investigators. It is understood that commercial layer farms do not uncommonly have *Salmonella* positive layer flocks and that the consideration of an egg being a food capsule free of potential food safe related *Salmonella* is erroneous. Thus, while best practice on farms and sound egg handling protocols will significantly reduce the quantitative potential of egg contamination with *Salmonella* there is a responsibility of the food preparers to ensure that foods containing raw eggs are handled in a food safe manner.

Their responsibility in this aspect is clearly detailed in numerous food safe documentation, the one from NSW Food Authority being a good example.

Food Safety Guidelines for the Preparation of Raw Egg Products.  
[http://www.foodauthority.nsw.gov.au/Documents/retail/raw\\_egg\\_guidelines.pdf](http://www.foodauthority.nsw.gov.au/Documents/retail/raw_egg_guidelines.pdf)

The enumeration of *Salmonella* in an intact egg to a level that will cause food poisoning when consumed, even when eggs are stored at room temperature for several weeks, is highly unlikely (refer to Yolk Mean Time studies in the EIRDC report National Food Safety Risk Profile of Eggs and Egg Products, Project SAR-47, 2005).

<https://www.aecl.org/assets/www.aecl.org/outputs/SAR-47A-Final-Report.pdf>

Thus, invariably there is always the need for the multiplication step of *Salmonella* from the contaminated eggs to grow to an infectious dose in a raw egg dish when the *Salmonella* is exposed to the yolk and or other nutrients.

In summary, *Salmonella* food poisoning caused by eggs is a two-step process. Firstly, it involves the internal contamination of the eggs and then the multiplication of this *Salmonella* to an infectious dose in a raw egg food product. The combination of a high incidence of contaminated eggs and poor food handling practices, often results in a higher probability of an outbreak occurring.

### **3.1.2 Actions Undertaken at the Farm Visit by the Responsible Authority**

In most instances, it will be necessary to undertake an inspection of the farm suspected of supplying contaminated eggs. For the responsible authority, it will be important for the investigators to have some familiarity with the processes and activities carried out on a poultry farm. This is where cooperation between industry and government is vital to ensure that the necessary mentoring processes are in place to enable meaningful outcomes from farm visitations and also confidence of the producer in the due process.

Over recent years there has been an increase in the licensing of egg production farms in some states as well as the mandatory auditing by the food safety authorities. This is in conjunction with other third party audits undertaken - for example by Australian Egg Corporation Limited (AECL) through its Egg Standard of Australia (ESA) quality assured program and the increasing auditing by retailers. These third party audits cover all aspects of the production including food safety, biosecurity and welfare. This increase in auditing and compliance requirements is positive for the industry in mitigating *Salmonella* related problems.

In cases where the health department has strong epidemiological links with particular eggs, they can immediately request the producer to cease the sale of eggs and recall all the producer's eggs after a particular date. Where the traceability of these eggs readily identifies their location for easy access and retrieval a public recall notice may not be implemented but in the case where there are dispersed sales through a retailer or markets then a formal public recall will be implemented. This invariably means that the egg producers branded and related branded product will be named.

Here is a link to the Food Standards Australia and New Zealand (FSANZ) website in how to conduct a product recall.

<http://www.foodstandards.gov.au/industry/foodrecalls/firp/Pages/default.aspx>

The subsequent action will depend on the outcome of further investigation, the severity of the outbreaks, which can involve deaths in some cases, and the farm visitation.

It will be the requirement at this stage of the investigation for the responsible authority to provide the testing material and disposables needed and undertake the testing at an approved National Association Of Testing Authorities, Australia (NATA) accredited laboratory.

The potential scenarios why the farm may be visited by the responsible authorities are:

1. The causal association of eggs with a food poisoning outbreak and the strain of *Salmonella* from the human cases being identical to that recovered from the eggs sourced from the supplier.
2. Strong epidemiological links with eggs being the cause of the outbreak despite no recovered *Salmonella* from eggs or egg products.
3. A number of small outbreaks or individual cases where the same *Salmonella* strain is involved and in most of the cases there is a strong epidemiological link with the consumption of eggs from a particular supplier.
4. The identification of a strain of *Salmonella* with MLVA typing/WGS that has historical links to an egg supplier.

The major purpose of the initial visit will be to:

- Collect samples for *Salmonella* testing to see if there is any link to the outbreak strain.
  - Environmental sampling of shed floors and slats, nest boxes, egg belts, collection tables, anacondas and rat bait stations.
  - Sampling of grading floor surfaces, surface of eggs pre-and post-washing if washing and egg sanitation is practiced.
  - Ancillary testing including feed, transport vehicles, used egg fillers, drinking and cooling water, etc.
- Review the current operational and husbandry procedures in place which could include:
  - Feed additives
  - On farm hygiene practices
  - Vermin control programs
  - Identify the types of systems in place including free range, barn and cage production.
  - Egg handling and grading procedures.
  - Previous or existing on farm *Salmonella* monitoring results

- Egg washing and if effective and actively monitored.
- Quality control program in place.
- Staff training
- Traceability
- External egg inputs from contractors, other suppliers, etc.
- Handling of cracked and dirty egg
- Compliance with egg standards including QA programs (with internal and external audits)

### 3.1.2.1 Outcomes of the visitation of the responsible authority

Each state responsible authority for eggs has specific requirements that egg producers are to meet and despite some variation in detail, the foundation document is the FSANZ, Primary Production & Processing Standard for Eggs and Egg Products. It is to be noted that even in the absence of any related *Salmonella* food poisoning incident, a supplier who sells cracked and or dirty eggs is noncompliant and this alone can result in the enforced withdrawal of eggs from sale.

Outcomes that will result in further actions on the supplier by the responsible authority include:

1. Identification from environmental swabs the identical strain of *Salmonella* as verified by the MLVA typing or WGS.
2. Identification of a very similar strain of *Salmonella* as indicated by MLVA typing and serotyping or WGS. It is to be noted that MLVA types do drift and while an identical match confirms the linkage and association a partial match does not discount the linkage.
3. Identification of no *Salmonella* linked with the outbreaks but evidence of noncompliance with the requirements of the FSANZ Eggs Standards such as the packaging of cracked and or dirty eggs or other aspects like inadequate monitoring of the egg washing parameters, inadequate traceability records or inaccurate stamping of eggs.

With the finding of any of the above the responsible authority can enforce the following:

1. Require a food recall.
2. Discontinue the sale of eggs with all eggs to be destroyed or sent for pulping and pasteurisation until further notice.
3. Designate certain eggs that can leave the site for washing and sanitation at another approved site provided strict traceability is maintained.
4. Enforce the implementation of a *Salmonella* control program to the satisfaction of the responsible authority. At this stage, the health departments are dependent on the Departments of Agriculture or the Food Safety authority in the state to implement and oversee the program. This may involve the use of a third-party consultant with knowledge, technical expertise and integrity in this area.

The duration of the “biosecurity restriction” period depends on several factors which include the severity of the outbreak, the history of the producer regarding previous outbreaks and the general compliance of the producer in regard to implementation of the *Salmonella* control program. In some cases, this may require capital. Producers who are seen to be overtly challenging to the responsible authority invariably can create a lack of confidence and the



entrenchment of imposed restrictions will occur until the responsible authority is satisfied that a suitable *Salmonella* control program has been implemented.

In most major food poisoning cases, there is always the chance that there will be some legal action taken against the caterer, restaurant or whomever the supplier of the contaminated food product was. The settlement of these claims invariably will involve a claim against the egg supplier. It is for this reason that there is sometimes noted caution and formality of information and results being provided by the responsible authorities to the egg producer.

### 3.1.3 On Farm *Salmonella* Control Program

This section of the *Salmonella* Incidence Response plan document outlines what constitutes a *Salmonella* Control and Monitoring Program. For regulators, this will be a series of documents, Standard Operating Procedures (SOP) and Work Instructions (WI). Having documentation alone is not adequate and there is a requirement to provide physical evidence of the implementation of the program that is auditable.

Producers who feel as if they are victims in such enforcement activity, particularly when the outbreak involves poor food handling practices, need to appreciate that the sole responsibility of the food authorities and the health departments is to ensure food safety and human health. When an outbreak occurs, it is these agencies that are held accountable for the failure in protecting the public. While not incurring a financial loss as a producer can, it is still in the interest of the responsible authorities to get the matter addressed as quickly as possible with minimal impact. The food authority which is generally more closely associated with the producer is very understanding of this, while the health department is more focused on the primary human health impacts.

The primary document *Salmonella* Control and Monitoring Program outlines the following:

- Background
- Preventative and Monitoring Program for Rearing flocks
- Preventative and Monitoring Program for Layer Flocks
- Monitoring Procedures for Egg Handling and Grading
- Shed Wash-down and Disinfection

The specifics of the program include:

- The use of in feed additives as an aid in *Salmonella* control.
- The incorporation of a *Salmonella* vaccination program.
  - This initially may involve the use of a registered live *Salmonella* vaccine but may lead to a final program that incorporates the live *Salmonella* vaccine with an autogenous killed bacterin that includes the isolates responsible for the *Salmonella* outbreak.
- Vermin control program.
- Multiple SOPs that detail the following:
  - Sampling of day old chickens.
  - Environmental swabbing of dirt floors, slats, manure belts, egg belts, egg collection equipment and bait stations.
  - Environmental swabbing of the grading floor surfaces.
  - Surface swabbing of unwashed and washed eggs (where applicable).



- Sampling of cracked and dirty eggs.
- Grading floor procedures
  - The monitoring and removal of cracked and dirty eggs prior to entry into the washer.
  - What constitutes reject eggs for disposal?
  - Criteria of what constitutes a B grade egg destined for pulping?
  - Candling procedures.
  - Manual or active monitoring of the wash parameters including temperature, detergent and sanitiser levels.
  - Egg handling, packaging and storage.

The monitoring of the environment is a far more sensitive indicator of the flock status than monitoring individual birds. This is because in layer flocks there are generally only a low level of birds that are positive for *Salmonella* and are actively shedding.

Similarly, the number of clean eggs that are internally positive for *Salmonella* is extremely low under most circumstances.

The bulk surface swabbing or rinse of unwashed eggs provides another indication of the amount of shedding going on in the flock and the surface swabbing of washed eggs provides an indication of the effectiveness of the egg washing and sanitation process.

In a flock that is positive on drag swabs for a high-risk *Salmonella* such as ST then the culture of a 25-ml sample of a tray of pulped cracked and dirty eggs is a very sensitive indicator of level of *Salmonella* shedding occurring in the flocks and the level of *Salmonella* mitigation enhancement required.

Appendix 2 lists the SOPs that are used by the egg industry.

## 4. Key Risk Areas Associated with *Salmonella* Contamination of Eggs.

### ***Cracked and/or Dirty Eggs***

While all producers can have incidental food safety cases or outbreaks, the historical outbreaks are a more common causal association with medium sized producers that have a mixed customer base. Importantly most of these producers do not have access to a pulping and pasteurisation plant and thus these eggs tend to find alternative markets in an attempt to recover some value from these eggs.

In fact, because of the increasing value of egg product, the egg producers that operate pulping or pasteurisation plants or have access to them, put all their B grade eggs through the plant and even go beyond this by increasing the sensitivity of their crack and dirt detector and may even put all eggs from an aged flock with poor egg shell quality straight to pulp to increase production.

The level and incidence of *Salmonella* with all Australian producers is similar and this is the case between cage, barn and free range systems.

*The number one risk factor is the sale of cracked and/or dirty eggs by default into the catering market.*

The common and erroneous belief amongst egg producers is that the washing of dirty eggs reduces the risk of *Salmonella*.

The egg as a safe food capsule and its natural properties:

The egg has evolved to accommodate for the problem of environmental contamination as can be seen of birds laying eggs in chronically dirty nests, on rock outcrops, buried in dirt and on the ground. Without innate mechanisms to protect an egg from internal contamination the viability of the concept of an egg would not endure in nature. The reasons for this are multiple:

- External shell membrane.
- The shell.
- Internal shell membrane.
- Inhibitors including enzymes in the egg white.
- The vitelline membrane separating the white from the yolk.

Various studies included in the references demonstrate how the intact egg with membrane and shell integrity is resistant to the penetration of bacterial and even when bacterium enter into the egg white their replication is impeded for several weeks.

The integrity of the membranes and albumen inside the egg is affected by time and temperature. Once the integrity has been compromised, *Salmonella* (if present in the egg contents) can gain access to the yolk, where it can grow if temperatures are greater than 7°C.

When that shell membrane loses its integrity through being damp with contaminated faeces or the shell structure itself is compromised because of micro cracks or poor shell quality then these protective mechanisms are compromised. Bacteria can then enter the egg – critically 90% of this ingress occurs within the first 2 hours after lay.

Thus, the attempt to recover A Grade eggs from B Grade dirty eggs by washing and sanitation is contraindicated.

Repeated investigations in Australia identify the majority of egg related *Salmonella* food safety outbreaks are related to the attempted recovery of dirty eggs by washing and / or the sale of cracked eggs to caterers.

The number one activity to reduce the risk of further *Salmonella* outbreaks by an egg producer having experienced a *Salmonella* outbreak is to identify the cracked and dirty eggs prior to entry into the washer. This is 90% of the solution and all other aspects of the *Salmonella* control program are 1% of the components.

The unfortunate aspect of previously experiencing no problems under these husbandry conditions is the development of the 3 corners of a triangle.

1. A food pathogen *Salmonella* entering a flock.
2. The washing of dirty and cracked eggs
3. The sale of these eggs to a caterer making raw egg dishes.

A producer with the orientation of these 3 corners can go for years of incident free production to an episode that could seriously compromise their business.

### **Eggs with *Salmonella* Contamination of the Shell Surface**

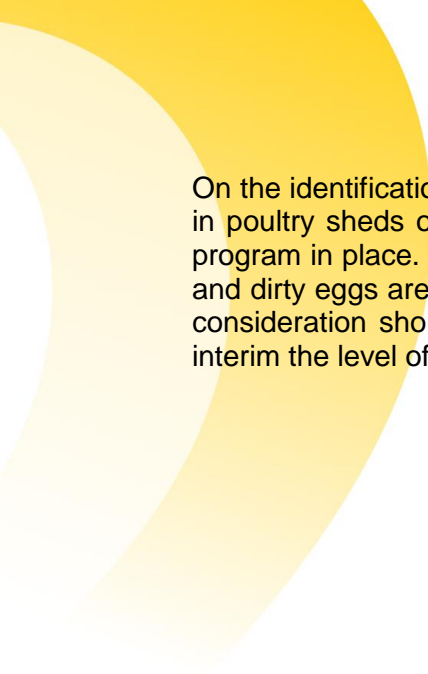
*Salmonella* positive layers can intermittently shed *Salmonella* and this can result in the contamination of the shell surface of the egg. Provided the shell integrity is normal, the external shell membrane is intact and is not affected by moisture, then the *Salmonella* is impeded from entering into the internals of the egg.

Despite this, during food preparation and the breaking of the shell, *Salmonella* may enter the food being prepared and survive and maybe even grow if this a raw egg dish and hence there is the potential for a food poisoning episode. An effective washing of eggs reduces this risk assessment.

### **High Risk *Salmonella* Causally Associated with Egg Related Food Safety Outbreaks**

The *Salmonella* isolates most commonly associated with notable food safety episodes include the following:

- ST Phage types 9, 44, 135 A, 135 and 170 (108).
- *Salmonella* Infantis
- *Salmonella* Virchow
- *Salmonella* Chester
- *Salmonella* Hessarek



On the identification of these *Salmonella* in particular when conducting environmental swabs in poultry sheds or the grading floor there is a need to reinforce the *Salmonella* mitigation program in place. This means ensuring that the overall egg hygiene is re-examined, cracked and dirty eggs are sent for pulp and if the donor flock is aged and has poor egg shell quality consideration should be put to diverting the eggs to pulp or depopulating the flock. In the interim the level of in feed additives should be increased.

## 5. The Fundamental Stages of the *Salmonella* Response

At this stage the linkage with the *Salmonella* responsible for the food poisoning event has been verified as having come from the farm. The farm has been placed under an order restricting the eggs from sale until the health department and food authority have confidence that the sale of the recommencement of eggs to the consumer will not pose a food safety risk.

The producer at this point may be experiencing the following:

- The need to dispose of eggs that can no longer be sold.
- Reduced income because of the loss of sales.
- Media attention.
- Contacts from clients, consumers, and other 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.
- Multiplicity of contacts, visitations and various requests on compliance.
- Litigation enquiries.
- Financier enquiries.

All the above establishes a response by producers that can be a mixture of stress, anger, frustration and isolation. It is important at this stage to establish relationships with those within the responsible authority, egg industry colleagues and technical support with the aim of normalising the business as quickly as possible.

Critically there is a need to remain objective and establish compliance with the requirements of the responsible authority to gain their confidence.

The following sequential steps should be implemented:

1. Where the sale of eggs is prohibited, permission should be sought to allow the eggs currently in store and being produced to go for pulping and pasteurisation. This process will eliminate any food safety risk but enable some income still to be received.
  - i. It is important that the industry cooperates in this regard.
  - ii. Eggs will need to be moved securely and the process monitored by the responsible authority.
2. Seek technical assistance to establish a *Salmonella* Control and Monitoring program which is the first step towards satisfying the responsible authority.
3. Immediately commence the monitoring and grading of eggs for cracks and dirt prior to washing (refer to egg washing section below) and sanitation and remove all B Grade eggs for pulping. Where eggs are not routinely washed, the same principle applies in removing all cracked and dirty eggs (the practice of manually washing dirty eggs is to cease immediately).
  - i. Increase the frequency of egg collection and quality of grading at the farm level to assist in the removal of cracked and dirty eggs.

- ii. This will involve extra staff in the grading floor to identify the cracked and dirty eggs for removal from the anaconda or input trolleys prior to washing.
  - iii. At this stage, a staff explanatory meeting should be undertaken with some retraining
  - iv. Egg recovery is expected to be initially significantly reduced but this is necessary to reduce the risk of another outbreak that would more seriously compromise the business.
4. Investigate as to why cracked and dirty eggs were reaching the consumer.
- i. STOP selling ungraded cracked and dirty eggs to caterers at discount prices.
  - ii. STOP attempting to achieve near 100% recovery of all eggs for sale.
  - iii. Send all floor eggs for pulping (or discard if pulping is unavailable).
  - iv. Depopulate old flocks with poor quality eggs or divert all their eggs for pulping.
  - v. Identify why there is a high incidence of dirty eggs.
    - a) High level of floor eggs.
    - b) Dirty nest boxes and / or worn nest pads.
    - c) Dirty egg belts and anacondas.
    - d) Faulty transfer points causing increased egg breakage.
    - e) High incidence of broken eggs contaminating eggs with yolk.
    - f) Vermin contamination of eggs.
    - g) Eggs getting wet because of rain or fogging.
  - vi. Identify why there is a high incidence of cracked eggs
    - a) Aged flock with poor shell quality.
    - b) General poor egg shell quality across all ages.
      - a. Review husbandry
      - b. Nutrition
      - c. Egg size control
    - c) Worn nest pads.
    - d) Nonoperational egg saver wires.
    - e) Crowding of eggs on the egg belt.
    - f) Damage at transfer points.
    - g) Handling, packing and transport.
    - h) Bird behaviour and egg picking and eating.
5. Review or establish SOPs for egg washing and sanitation
- i. Monitoring of this process and measuring parameters such as pH, temperature and chemical concentration should be done every 30 minutes throughout the wash period (or more often if required).
  - ii. Real time testing is preferred with automatic monitoring systems in place.
  - iii. Ensure the chemicals used are approved for contact with food products.




6. Introduce a feed additive to aid in the control of *Salmonella* colonisation and shedding.
    - i. Generally, this involves the use of organic acids or more prescriptively Short Chain Fatty Acids (SCFA).
    - ii. Organic acids which are recognised to be helpful in the control of enterobacteria like *Salmonella*. These include formic, propionic, butyric, sorbic, acetic, fumarate, benzoic, lactic, fumaric, etc. These acids are only effective in their associated form and thus the pKa (a measure of the strength of an acid) is important for the acid to achieve activity against the *Salmonella* in the intestine. Some products are also formulated with natural earth complexes or essential oils to act as protectants and carriers to assist the organic acid in achieving activity within the intestinal tract. Organic acids are a tool in reducing *Salmonella* colonisation and levels in layers. In Europe, their use of organic acids has become mandatory in some milling situations. The inclusion rates and use in different age groups will be tailored by the farm veterinarian in response to the result of ongoing monitoring.
    - iii. Flocks approaching peak production are more likely to shed *Salmonella* and are more likely to be colonised by *Salmonella* from the environment so this time will be a focus for organic acid inclusion in feed.
    - iv. Consider the inclusion of other products such as phytogenics, phosphorylated yeast products, probiotics; collectively called synbiotics.
  
  7. Incorporation of a *Salmonella* vaccination program.
  
  8. Vaccination should be considered as one element in the program. This could involve the use of registered live *Salmonella* vaccine (Vaxsafe ST<sup>®</sup> is currently the only registered live *Salmonella* vaccine in Australia) or a combination of the live *Salmonella* vaccine and an autogenous vaccine. In the first instance an autogenous vaccine would not be available until the isolate has been recovered from the farm and had been correlated with the food safety episode before it is cloned, characterised and made into an inactivated autogenous bacterin vaccine. The time frame here would be around 3 months. Initially new pullet flocks would be primed using Vaxsafe ST<sup>®</sup> by a coarse aerosol spray at the hatchery, followed by through drinking water at 4 weeks of age and finally by intramuscular injection of the pullets around 10-12 weeks of age. The Vaxsafe ST<sup>®</sup> is normally administered in conjunction with other inactivated adjuvanted vaccines. If there was the introduction of the autogenous vaccine, this would be undertaken by intramuscular injection at around 8 and 12 weeks of age. Vaccination strategies for production flocks would require consultation with the farm veterinarian. Commence *Salmonella* monitoring to establish a sound knowledge of the level of contamination
    - i. Undertake environmental swabbing of all sheds monthly
      - a) This can subsequently be reduced as a better understanding of the farm status is achieved.
      - b) The future frequency of environmental monitoring is determined by the test result findings.
    - ii. Commence surface swabbing of a representative sample of eggs prior to and after washing.
    - iii. Environmental swabs of Day Old Chickens (DOC) and Point Of Lay (POL) pullets.
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- a) Where pullets are reared externally a *Salmonella* test should be mandated as a condition of supply.
- iv. Testing of finished feed for *Salmonella*.
  - a) This can be done on site for in house mills.
    1. Major contact points within the mill should also be tested.
    2. Introduce an SCFA flushing program into the mill.
  - b) Request finished feed testing from commercial mills.
  - c) While feed is often considered by many including regulatory authorities of being a source of high risk food safety pathogens, epidemiological studies have shown that this is incorrect, and particularly for ST. There are several salmonellae not uncommonly found in vegetable protein meals such as *S. Agona*, *S. Anatum* and *S. Singapore*, that can change the status of the layer flock. Thus, while feed free from *Salmonella* contamination is an important part of the *Salmonella* control program, the emphasis on feed should not be disproportionate.
  - d) The ability of most in-feed additives to effectively and completely sanitise the feed free of *Salmonella* is limited because of their inability to make adequate contact with the *Salmonella* and for the required duration of time. Noting that the feed is manufactured and consumption commenced within several days. Formaldehyde 30% is more effective but its use is limited by feed mills unless the appropriate Workplace Health and Safety (WHS) procedures are in place. Heat treated pelleted feed or heat treated mash feeds are generally considered free of *Salmonella* but post manufacture contamination is recognised.
  - e) As the DOC has not established a normal intestinal microbiota, it is significantly more susceptible to the colonisation of *Salmonella* from feed (and the environment). For the rearing of pullets, it is preferably to use pelleted or a heat-treated mash as a starter ration.

## 9. Training

- i. Having written procedures in place are of limited value unless they are implemented correctly and with some degree of first principle understanding. Staff involved in farming, the grading floor and marketing all must be formally trained on the *Salmonella* control program.
- ii. This may require a change in attitude as to what is an acceptable practice required for the future compared to the past.
  1. Senior management and owners themselves are on occasions complicit in bad practices and thus need to change and support staff in best practice. This can prove on occasions difficult because the absence of previous food safety issues historically formulates a belief that past and existing practices are satisfactory.
  2. A change in staff responsibilities or the introduction of new staff maybe required.
  3. This change in attitude and aptitude will be required for all contractors also and thus their responsibility must be incorporated in the *Salmonella* control program.

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- iii. The methods of training may involve.
    1. Workshops undertaken by a qualified person.
    2. Workshops undertaken in house reviewing SOPs and WI.
    3. Utilisation of chemical supply companies to undertake certificate training and supply wall posters.
    4. Requesting chemical companies to establish procedural and monitoring guidelines for the use of their chemicals. This should incorporate the introduction of hardware and materials for monitoring purposes.
    5. Allowing supervisory and quality assurance staff to visit other establishments and review their operational procedures and equipment.
    6. Certificate III training courses.
    7. Continual contact with qualified people for answering questions.
  - iv. All staff must be made aware of their responsibilities and most importantly, if there is a non-compliance observed that creates a STOP in procedures, seeking a resolution to the problem and corrective action is necessary. This is pivotally important in processes such as candling and egg washing.

## 10. Communications

- i. Regulatory Authorities: Cooperation is the key here to an expedient resolution and returning to normal business. It is important that all parties have confidence in each other during food safety events. While the producer is focused on primary commercial pressures, the authorities are faced with a combination of public health, media, political issues and seeking a satisfactory outcome. It is to be appreciated that when a food safety event occurs, it is the food safety authority that is viewed by the community to have failed in its responsibility. Producers are advised if they do not have the “temperament” to deal with regulatory authorities, that they appoint a third part to undertake this role. When a product recall is involved there are people available to assist you in this process.
  - ii. Customers: It is important that customers are kept fully informed of the events to avoid speculation and general uncertainty. The way this will be done will vary considerably from the formality of the large retailers, interaction with on sellers, caterers, over the counter customers and consumer phone enquiries. It is important to nominate one suitable person to liaise with customers.
  - iii. Creditors: In the case of large recalls and the restrictions being enforced on sales, cash flow can become a significant problem. It is important to discuss the situation with all suppliers to ensure the continuity of the necessary business inputs. This may also involve the cooperation of other egg producers where they can assist in supplying products to customers. The restriction of inputs like replacement stock, feed and feed materials, disposables etc. can further seriously compromise cash flow and beyond that associated with the loss of egg sales. Establishing a line of credit with the bank may be required in some cases.
  - iv. Media: Generally, there is not a lot of direct media contact but the observation of print media reports can be another pressure on the
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business both physically and psychologically. It is important to nominate one company representative to deal with the media and advise the staff of this.


- v. Litigation: Where there are significant outbreaks involving large numbers of people and hospitalisation, it is not uncommon for litigation to occur. This will invariably initially be against the establishment (restaurant, reception centre, conference, etc.) and lawyers and insurance companies will become involved. The parties involved in the chain may be complex being the establishment, caterer, egg marketer, egg wholesaler and finally the producer. On some occasions, there may be several egg suppliers involved. Insurance companies will generally pay out compensation for the primary establishment but then seek legal action monies further down the chain suppliers. This is where the debate starts about the level of responsibility noting that a food poisoning episode associated with eggs requires the two components:
1. Eggs contaminated with *Salmonella*.
  2. Suboptimal food handling practices that allow the *Salmonella* levels to enumerate to an infectious dose.
    - a. Further complicated by susceptibility of the consumer (e.g. aged care)

Producers should always ensure that their professional liability and professional indemnity insurances are up to date and have sufficient policy coverage. Also, all the contractors and on sellers they deal with are likewise covered.

Where there is the likelihood of litigation, the producer should notify his lawyer and discuss the way forward and the lines of communication. Litigation can be complex and drawn out and can impose further stressors on the business. To avoid this negatively impacting on the business because of diversionary activity, it is recommended to leave all the dealings to your lawyer and insurance company.

## 11. *Salmonella* Source


- i. Producers who have not previously had a monitoring program may / will question where this food safety *Salmonella* came from. Invariably it has been on the farm for some time. The presence of *Salmonella* on the farm (and thus in a % of the birds) does not imply the 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.
- ii. *Salmonella* of the non-typhoid type are very successful organisms and there are over 2,500 strains which are ubiquitous within the environment and in many different species of animals including birds, mammals and reptiles. In most cases, they colonise the host with no clinical signs, such as the case in commercial layers. Thus, limiting the infection, colonisation and spread of *Salmonella* within housed poultry is difficult. It is only the most stringent elite breeder companies that can with their extremely rigorous biosecurity procedures obtain a *Salmonella* free status: but even these sites have breaks and may require depopulation. In the normal commercial situations, including multiple age sites, in some cases multiple age sheds, cage, barn and free range and the relatively porous horizontal contacts makes the



ability to maintain a *Salmonella* free status impracticable or commercially feasible. There is caution in the statement because in some countries freedom from particular strains of *Salmonella* such as ST and SE is mandated. In Australia, other than for SE which is notifiable, the food safety authorities and as documented in FSANZ, are primarily concerned about the status of eggs and egg product and not 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 through having *Salmonella* free layers, it almost entirely mitigates the risk of *Salmonella* in eggs from those birds.

iii. Sources of *Salmonella*

1. Environment: *Salmonella* do normally need an animal host to replicate, but they have a good ability to survive in the environment for long periods and under a variety of conditions. They can also replicate at the right temperatures in inanimate nutrient substrates. Cross contamination from the environment to commercial layers is the most common cause of change of status of a layer flock. The placement of DOC in poorly disinfected houses or on reused positive litter is an example. As is placing point of lay pullets in positive layer houses and particularly those that are multi-age. Overall the standard of house hygiene prior to repopulation is not best practice in Australia, with houses not being effectively washed and disinfected between flocks because of the significant costs, labour resources, concern about damage to the facilities and in more recent times the practical and physical limitations with aviary type housing systems. Multi-age cage layer sheds and free range areas also have limitations to 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* onto a site, but can be responsible for the change of status of adjoining sheds.
  2. Feed: Has been discussed above and its relevance needs to be kept in perspective. *Salmonella* positive feed can change the status of a flock, particularly in young birds. Mash feed of course will have the status of the raw materials that make up the feed formulation. Attempts should be made to minimise the positive *Salmonella* status of feed as an aid to the *Salmonella* control program.
  3. Water: Mains water and bore water are generally considered free of *Salmonella*. Surface water though can contain *Salmonella* and thus all surface water should be sanitised to eliminate *Salmonella* and other avian pathogens like avian influenza. Storage tanks should be also tested several times a year, as within the industry, the finding of *Salmonella* in a water storage tank is common, even when the source of water is the mains. Contamination is probably from vermin or possibly insect vectors. The biannual bolus sanitising of water storage tanks is recommended.
  4. Vermin and Non-Avian Livestock: Rats and mice can introduce *Salmonella* into a poultry establishment and thus a rodent
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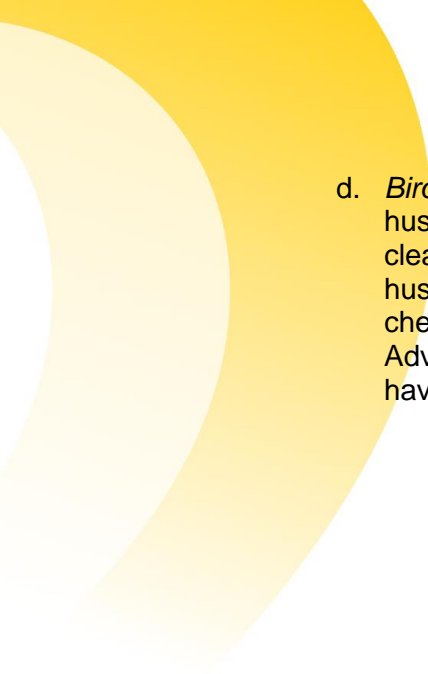
control program should be in place. The inclusion of bait stations in the *Salmonella* monitoring program often reveals positive results. Grazing animals, guard animals and domestic pets can all carry *Salmonella* and should either be tested or excluded from the site.

5. Avian Livestock: Positive DOC are recognised within the Australian industry but generally considered low risk *Salmonella* in relation to egg related food safety. They are normally negative for ST and most suppliers conduct monitoring for *S. Pullorum*, *S. Gallinarum* and SE. While the commercial Australian egg industry is recognised as negative for these salmonellae, testing is normally done to meet export requirements. It is important to test the DOC either by culturing chick box papers, using 3M swabs on chick boxes or 3-day old brooding paper to determine the background status of your birds at arrival. A change in status then would confirm an in-house rearing contamination. Purchased pullets should also be supplied with a mandated POL *Salmonella* environmental swab test results again, to ascertain the status of your layer.
6. Equipment and Disposables: All material prior to entering the farm should be washed and disinfected. This includes in house and that belonging to contractors.
7. Personnel: Staff should also be considered a potential source of *Salmonella* contamination of layers. While monitoring of staff is not a normal feature of the commercial egg laying industry, it is a requirement of several international poultry breeding operations which handle very valuable livestock. Procedures should be in place for any staff returning from overseas countries with a history of enteric disease not to be able to return to work for several days after symptoms have ceased and the consideration of them taking a probiotic. A microbiological check for *Salmonella* of the staff member would be the preferred option for the company.



## 6. Supplementary Activities to Mitigate the Risk of *Salmonella*

1. **Washing, Sanitising and Disinfecting Procedures:** These are particularly important for the placement of DOC in the pullet rearing facilities which need to be completely cleaned and disinfected. The young chicken is at its most sensitive stage in life for colonisation by *Salmonella* due to the absence of a properly developed gut flora. The clean out procedure involves:
  - a. A wet detergent wash down.
  - b. The application of an efficacious disinfectant (refer SOP on shed wash down).
  - c. Insecticide treatment of the shed.
  - d. Flushing and sanitising of the drinker lines.
  - e. Pre-placement microbiological assessment of the wash down procedure.
  - f. Vermin control program.
  - g. To avoid the ongoing and cyclic build-up of *Salmonella* in layer production sheds, there is a need to implement a policy of single age sheds and an effective shed sanitation program after depopulation. Where sheds are heavily soiled, this will involve a wet detergent wash down. Where organic loads are light, a dry clean is to be undertaken. This is followed by a total surface disinfection where there is an application that adequately “wets” all surfaces.
  - h. Using disinfectants such as glutaraldehyde or blended double chain (four) quaternary ammonium products, should be able to accommodate moderate organic loads.
  - i. Insecticides for the control of mites, litter beetles and flies are to be used at each cleanout.
  
2. **Egg shell quality:** The egg shell has intrinsic properties that impeded the entry of bacteria into the egg white. This includes the cuticle, the shell, the outer membrane and inner membrane. If these are compromised then the risk of *Salmonella* entering the egg is higher. The factors that can influence egg shell quality include:
  - a. *The size of the egg:* The amount of total calcium laid down on the egg does not effectively increase after around 37 weeks of age in the layer and thus as the egg increases in size after this age the shell becomes thinner. It is thus important by the rearing of a pullet with a good skeletal frame, weight at the commencement of lay, quality nutrition, adjustment of net nutrient intake and the macromineral balance optimise the egg shell thickness by mid lay. Then for the terminal part of lay control egg size through nutritional management.
  - b. *Nutrition:* Important to ensure that the rations formulated with the correct specifications for the stage of lay and that a quality premix is used incorporating organic minerals.
  - c. *Bird Health:* Diseases like Infectious Bronchitis (IB) can affect egg shell quality. Pullets are to be completely vaccinated in lay with the two IB groups recognised in Australia and then for security vaccinated throughout lay every 8 weeks with IB. General ill health with any disease like fowl cholera will also result in poor egg shell quality.

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- d. *Bird Behaviour and Husbandry*: it is well recognised that bird behaviour and husbandry can affect shell quality. The activity of free ranging for reasons not clearly understood itself affects the terminal quality of the egg shell. Disruptive husbandry will cause changes in egg shell quality including target eggs, body checks and generally thin eggs because of variations in net nutrient intake. Advanced feather loss typically produces “stressed” appearance eggs that have pale poor egg shell quality.

## 7. Standard Long-term *Salmonella* Control Program

Most of the components in the *Salmonella* Incidence Response Plan are consistent with what is required in a *Salmonella* Control Program for all producers. These include:

- Purchase of quality DOC 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.
- Feed containing additives as an aid to control the colonisation and shedding of *Salmonella*.
- Facilitation and husbandry systems that optimise the production of eggs that are clean and have minimal cracks.
- The removal of all dirty and cracked eggs from A Grade eggs and divert to pulping and pasteurisation.
- No washing of dirty eggs.
- Washing system that has real time monitoring or a manual monitoring system that requires testing every 30 to 60 minutes.
- The monitoring for *Salmonella* of:
  - Day old chicks
  - Point of lay pullets.
  - Layers in production.
  - Surface of washed eggs
  - Grading floor environment
  - Feed and feed mill
- Careful selection of markets that do not undertake unsafe food practices.
- Corrective action plan for the identification of high risk *Salmonella*.

# Appendix 1. *Salmonella* testing

There are various methods of examining potential *Salmonella* from diagnostic samples. Basic microbiological techniques include growing the bacteria in selective broths or media under controlled conditions and undertaking various standard procedures (microscopic examination, biochemical tests, antimicrobial susceptibility testing) to identify the bacteria as a *Salmonella* species. For ST, further testing can involve phage typing, pulsed-field gel electrophoresis (PFGE), multi-locus variable-number tandem repeat analysis and whole genome sequencing.

Multiple-locus variable-number tandem repeat analysis (MLVA) is used as an epidemiological tool for examining human outbreaks of bacterial disease. It is basically a method of examining the bacterial genes, in relation to a number of standardised strains where the genome has been sequenced. The results of the MLVA testing comprise a “genetic fingerprint” of the *Salmonella* and are compared to known databases to enable an accurate identification. This allows the tracking of organisms throughout an outbreak and recognition of different strains if there is more than one involved.

Phage typing is commonly used to describe strains of ST and use viruses of bacteria called bacteriophages. Bacteriophages (phages) are often specific to particular bacteria and even to particular strains of bacteria. The use of multiple known phages on a bacterial culture allows typing, as certain phages kill the bacteria and others do not. On a bacterial culture plate this will leave areas where the bacteria do not grow (having been killed by the phage) and others where it does grow (where it is not killed by other phages). Therefore, when a standardised set of phages are applied to a bacterial culture plate in a standardised pattern the resulting pattern can be used to define the particular strain of the bacteria.

The Australian *Salmonella* Reference Centre historically published the results of laboratory investigations into human cases as well as of animal monitoring in Australia on a quarterly basis. This allows us to identify which phage types are involved in human cases and respond accordingly when those phage types are detected from on-farm monitoring. Thus, phage typing is particularly relevant for the poultry industry. MLVA testing is used in human epidemiological investigations and due to privacy restrictions on the release of MLVA results this testing is not as helpful in the farm setting.

## List of laboratories that can test for *Salmonella* in chickens

**Note: Contact the laboratory prior to collecting samples**

Animal Consulting Enterprises Pty Ltd, EAST BENDIGO, VIC - ACE Laboratory Services ACCREDITATION NO: 15636	Phone: (03) 5443 9665
Baiada Poultry Pty Limited, BRINGELLY, NSW - Birling Avian Laboratories ACCREDITATION NO: 14235	Phone: (02) 4778 6100
Biosecurity Queensland Veterinary Laboratories, COOPERS PLAINS, QLD - Department of Agriculture and Fisheries - Biosecurity Sciences Laboratory ACCREDITATION NO: 13389	Phone: (07) 3276 6062
CSIRO, GEELONG, VIC- Australian Animal Health Laboratory ACCREDITATION NO: 13546	Phone: (03) 5227 5000
Department of Agriculture and Food Western Australia, SOUTH PERTH, WA - Animal Health Laboratories ACCREDITATION NO: 13724	Phone: (08) 9368 3333

Department of Primary Industry and Fisheries, BERRIMAH, NT- Berrimah Veterinary Laboratories ACCREDITATION NO: 13626	Phone: (08) 8999 2249
Dept. of Economic Development, Jobs, Transport & Resources, BUNDOORA, VIC- Biosciences Research Division - AgriBio, The Centre for Agribioscience - Bioprotection Platform ACCREDITATION NO: 14477	Phone: (03) 9032 7515
Dept. of Primary Industries, Parks, Water and Environment, LAUNCESTON, TAS- Animal Health Laboratory ACCREDITATION NO: 384	Phone: (03) 6777 2111
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# Appendix 2 Standard Operating Procedures

## **Standard Operating Procedure 1: Salmonella Litter/Manure Sampling Procedure Using Gauze Swabs for Deep Litter Sheds**

### **Introduction**

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

1. detect any exotic, notifiable or atypical *Salmonella* spp. incursions into a flock and
2. 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.

To ensure quick and accurate results, 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.

### **Preparation for sample collection**

#### **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.
- To ensure quick and accurate results, the laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation.

#### **2. Calculate the number of swabs required**

- Sheds without pens or partitions- 3 swabs required
- Sheds with two or more pens- 2 swabs for each pen. For example,
  - » Shed with 2 pens– 4 swabs required
  - » Shed with 3 pens– 6 swabs required

#### **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

#### 4. Preparation of swabs\*

- Make multiple little bows using cotton gauze swab with approximately 1 metre 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.



Figure A1- Gauze swabs with string attached

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

#### 5. Swabbing procedure

**Shed without pens or partitions** • Wash hand 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. Contamination with other bacteria is unimportant as the culture media is selective for *Salmonella* spp.
- 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 for the 2<sup>nd</sup> time are reached, retrieve the swab, removing it from its string by cutting with scissors and place swab in the whirl-pak bag or screw top plastic jar.

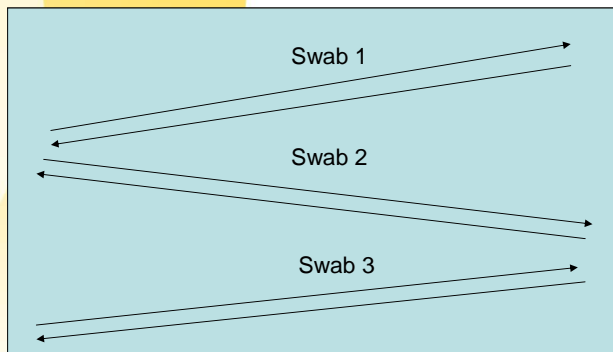


Figure A2. Swabbing procedure for deep litter sheds without pens or partitions

### Shed with pens or partition

- Wash hand 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. Contamination with other bacteria is unimportant as the culture media is selective for *Salmonella* spp.
- 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 and Figure A4.
- When the end of the slats for the 2<sup>nd</sup> time are reached, retrieve the swab, removing it from its string by cutting with scissors and place swab in the whirly-pak bag or screw top plastic jar.

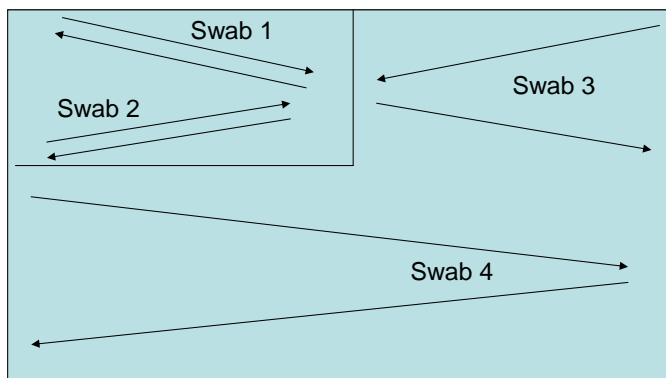


Figure A3. Swabbing procedure for deep litter sheds with **two** pens

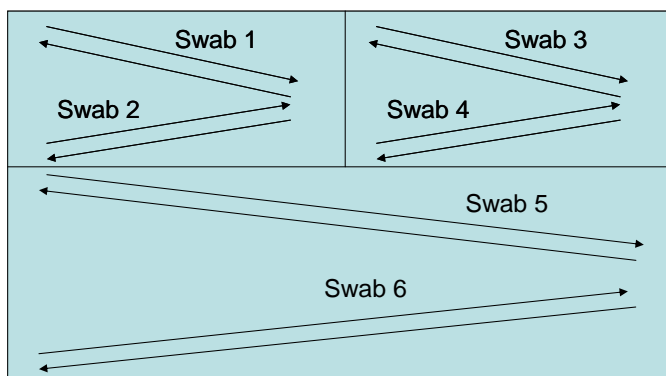


Figure A4. Swabbing procedure for deep litter sheds with **three** pens

## 6. 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

## 7. 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 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.**

## **Standard Operating Procedure 2: Salmonella Litter/Manure Sampling Procedure Using Gauze Swabs for Fully Slatted Sheds**

### **Introduction**

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

1. detect any exotic, notifiable or atypical *Salmonella* spp. incursions into a flock and
2. 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.

To ensure quick and accurate results, 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.

### **Preparation for sample collection**

#### **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.
- To ensure quick and accurate results, the laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation.

#### **2. Calculate the number of swabs required**

- Single level Shed or shed without pens or partitions - 2 swabs required
- Multi-level shed or shed with pens- - 2 swabs for each pen or level

#### **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

#### 4. Preparation of swabs\*

- Make multiple little bows using cotton gauze swab with approximately 1 metre of string attached.
- 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.



Figure A5- Gauze swabs with string attached \*Kits may be supplied by the diagnostic laboratory

#### 5. Swabbing procedure

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

##### Single Level Shed with 100% slats

- Wash hand 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. Contamination with other bacteria is unimportant as the culture media is selective for *Salmonella* spp.
- Walk down one side full length of the shed, dragging the swab over the top of the slats in the manner shown in Figure 1 by the arrow “Swab 1”. Repeat this up and down 3 more times.
- When the end of the slats for the 4<sup>th</sup> time are reached, retrieve “Swab 1”, removing it from its string by cutting with 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 A6 by the arrow “Swab 2”. Repeat this up and down 3 more times

- When the end of the slats for the 4<sup>th</sup> time are reached, retrieve “Swab 2”, removing it from its string by cutting with scissors and place swab in the Whirl-Pak<sup>®</sup> bag.

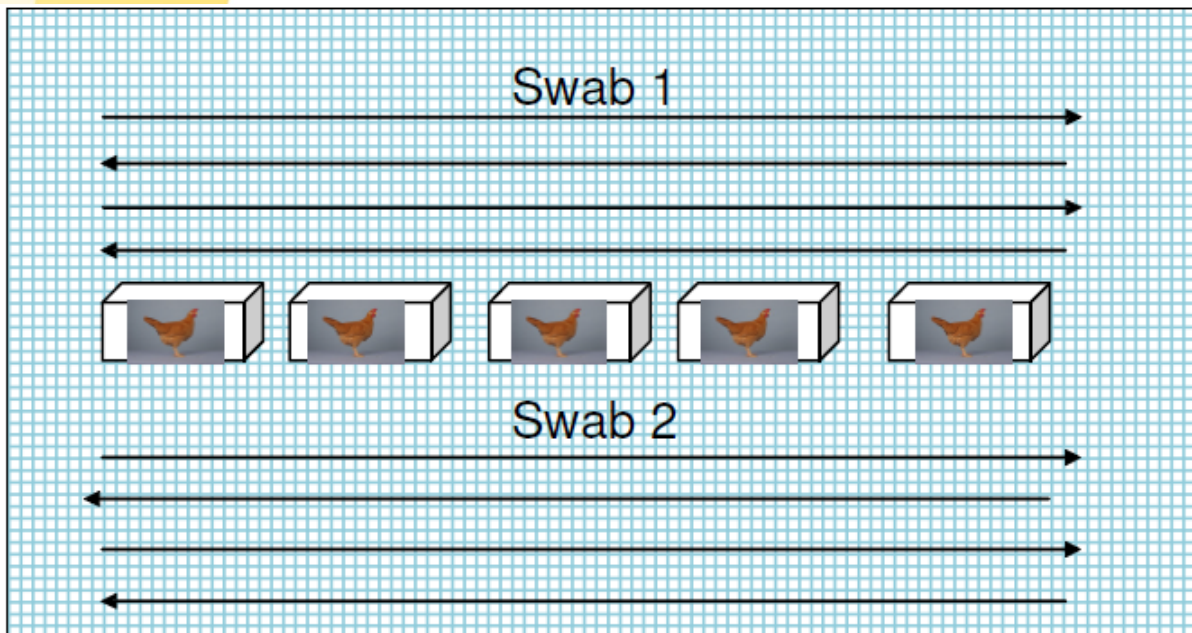


Figure A6. Swabbing procedure for slatted shed

#### Slatted Sheds with Pens • 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. Contamination with other bacteria is unimportant as the culture media is selective for *Salmonella* spp.
- 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 A7 and Figure A8.

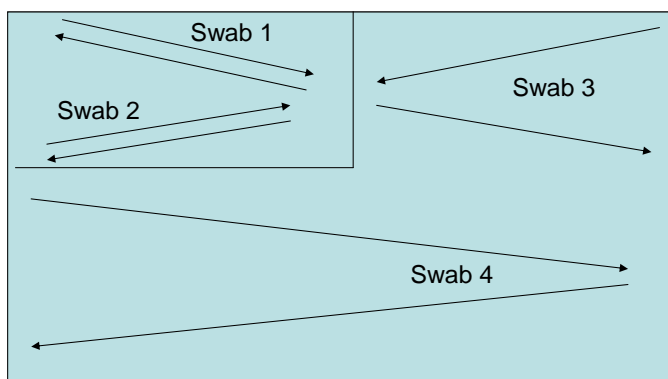


Figure A7. Swabbing procedure for fully slatted sheds with **two** pens



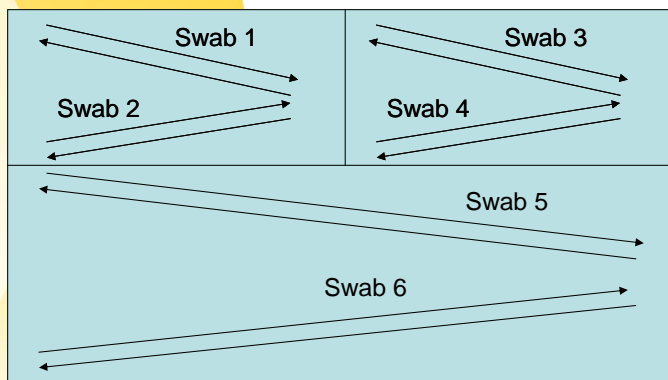


Figure A8. Swabbing procedure for fully slatted sheds with **three** pens

## 6. 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

## 7. 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 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.**

## **Standard Operating Procedure 3: Salmonella Litter/Manure Sampling Procedure Using Gauze Swabs for Deep Litter + Slatted Sheds**

### **Introduction**

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

1. detect any exotic, notifiable or atypical *Salmonella* spp. incursions into a flock and
2. 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.

To ensure quick and accurate results, 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.

### **Preparation for sample collection**

#### **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.
- To ensure quick and accurate results, the laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation.

#### **2. Calculate the number of swabs required**

Total 4 (four) swabs required for each shed.

#### **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

#### 4. Preparation of swabs\*

- Make multiple little bows using cotton gauze swab with approximately 1 metre of string attached.
- 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.



Figure A9- Gauze swabs with string attached

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

#### 5. Swabbing procedure

- Wash hand 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. Contamination with other bacteria is unimportant as the culture media is selective for *Salmonella* spp.
- Hold Swab 1 by string and drag it twice the full length of one side of the litter area in the pattern described in Figure A10.
- When the end of the litter area is reached second time retrieve “Swab 1”, removing it from its string by cutting with scissors and place swab in the Whirly-Pak<sup>®</sup> bag.
- Now walk down the slatted area, dragging the Swab2 over the top of the slats in the manner shown in Figure A10 by the arrow “Swab 2”.
- When the end of the slats for the second time are reached, retrieve “Swab 2”, removing it from its string by cutting with scissors and place swab in the Whirly-Pak<sup>®</sup> bag or screw top plastic jar.

- Follow the same procedure for the other side of the shed as shown in Figure 1 by the arrows “Swab 3’ and ‘Swab 4’.

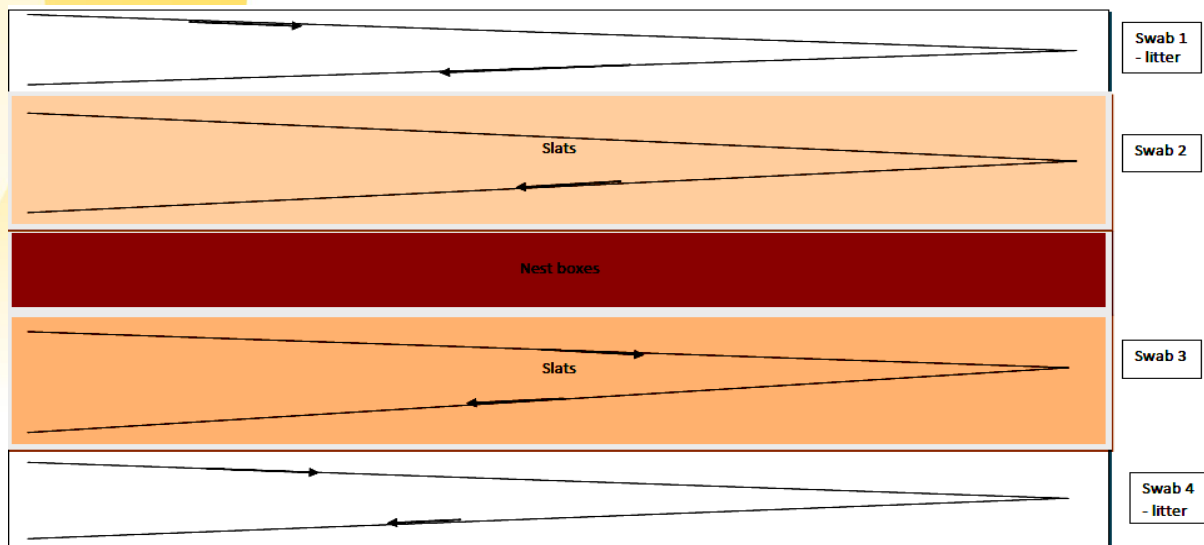


Figure -A10: Sampling pattern

## 6. 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

## 7. 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 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.***

## **Standard Operating Procedure 4: Salmonella Litter/Manure Sampling Procedure Using Gauze Swabs for Conventional Multi-tier Cages with Manure Belt**

### **Introduction**

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

1. detect any exotic, notifiable or atypical *Salmonella* spp. incursions into a flock and
2. 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.

To ensure quick and accurate results, 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.

### **Preparation for sample collection**

#### **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.
- To ensure quick and accurate results, the laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation.

#### **2. Calculate the number of swabs required**

- Two (2) swabs for each cage row

#### **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

#### 4. Preparation of swabs\*

- Make multiple little bows using cotton gauze swab with approximately 1 metre of string attached.
- 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.



Figure A11- Gauze swabs with string attached

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

#### 5. Swabbing procedure

- For each row of cages, two (2) drag swabs are to be moistened with water (or peptone broth if supplied) from the drinkers in the shed. Contamination with other bacteria is unimportant as the culture media is selective for *Salmonella* spp.
- 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 be falling directly onto the swab.
- Once the manure belt run is completed, swabs are to be removed from the string by cutting and placing in the screw top plastic jar or Whirl Pak bag

#### 6. 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

## 7. 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 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.***

## **Standard Operating Procedure 5: Salmonella Litter/Manure Sampling Procedure Using Gauze Swabs for Conventional Multi-tier Cages with Manure Pit (Without Manure Belt)**

### **Introduction**

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

1. detect any exotic, notifiable or atypical *Salmonella* spp. incursions into a flock and
2. 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.

To ensure quick and accurate results, 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.

### **Preparation for sample collection**

#### **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.
- To ensure quick and accurate results, the laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation.

#### **2. Calculate the number of swabs required**

- Three (3) swabs for each shed

#### **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
- Scissors

- Marker pen
- Laboratory accession form
- Plastic post satchel for swab transport to laboratory

#### 4. Preparation of swabs\*

- Make multiple little bows using cotton gauze swab with approximately 1 metre of string attached.
- 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.



Figure A12- Gauze swabs with string attached

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

#### 5. Swabbing procedure

- For each shed use a total of three (3) drag swabs.
- 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. Contamination with other bacteria is unimportant as the culture media is selective for *Salmonella* spp.
- 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 1 by the arrow “Swab 1”.
- Repeat in the opposite direction, along a new row with “Swab 1” as indicated above.
- Retrieve “Swab 1”, removing it from its string by cutting with scissors and place swab in the plastic screw top jar or Whirl-Pak sample bag.

- Repeat along a new row with “Swab 2” as for “Swab 1” (see Figure A13)
- Repeat with the 3<sup>rd</sup> swab.

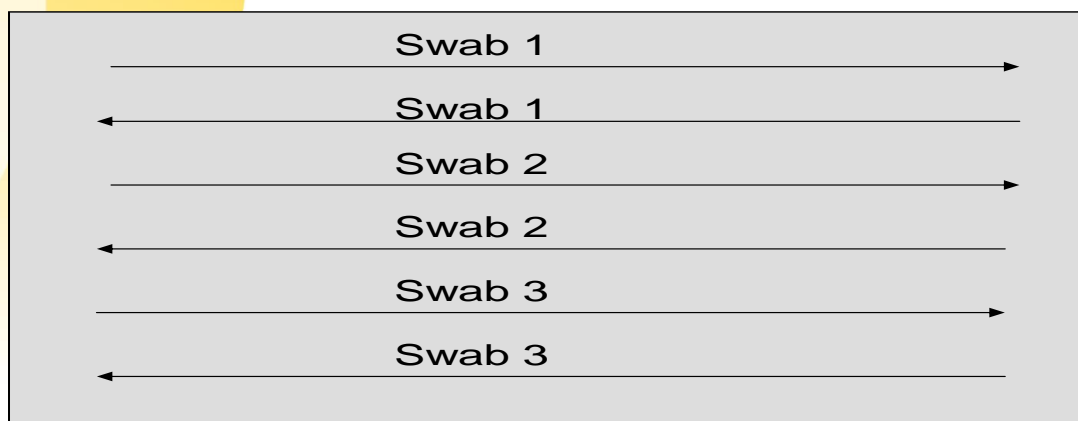


Figure A13- Swabbing method for single story conventional layer shed

## 6. 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

## 7. 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 posted to the laboratory by 1.00 p.m. 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.***

## **Standard Operating Procedure 6: *Salmonella* Litter/Manure Sampling Procedure Using Boot Swabs for Deep Litter and Slatted Sheds**

### **Introduction**

Commercially available sterile, pre-moistened fabric sock style boot cover is becoming increasingly popular to demonstrate a poultry flock *Salmonella* status. Boot swabs were proven to be as sensitive as conventional methods, such as hand collection of litter and drag swabs (Carrique-Mas et al., 2008).

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



Figure- A14: Commercially available boot swab, Courtesy- Solar Biological Inc.

### **Preparation for sample collection**

#### **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.
- To ensure quick and accurate results, the laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation.

## 2. Calculate the pairs of swabs required

### Deep Litter System

- Sheds without pens or partitions
  - o 2 pairs required
- Sheds with two or more pens
  - o 1 pair for each pen
  - o 2 pens in shed – 2 pairs required
- 3. o 3 pens in shed – 3 pairs required

### 100% Slatted Shed

- Single level shed or shed without pens or partitions
  - o 2 pairs required
- Multi-level shed or shed with pens
  - o 1 pair for each pen or level

### Material

- Boot Swab Kit (Twirl-tie bag?) - consists of a medical grade bag containing boot swab and other sampling consumable.
- Disposable latex gloves (may be included with the swab kit)
- Plastic Boot Cover/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*

## 5. Swabbing procedure

### Deep Litter sheds

- Wash hands prior to using boot swab and wear clean 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 bath or any disinfectant/sanitizer prior to sample collection as it might kill the Salmonella in the sample if there 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 the Figure A15 and A16 (for shed without pens or partition).

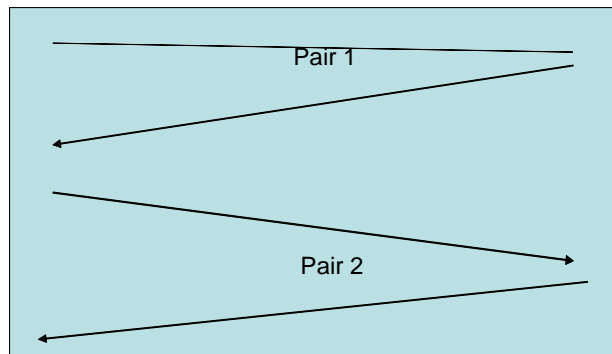


Figure A15- Sampling procedure for deep litter shed with partition

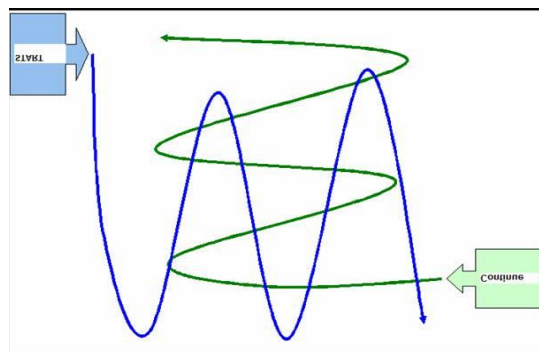


Figure A16- Sampling procedure for deep litter sheds without pens or partitions

- For multiple pens/rows use fresh plastic boot cover and swab for each row/pen (Figure A15)
- 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.

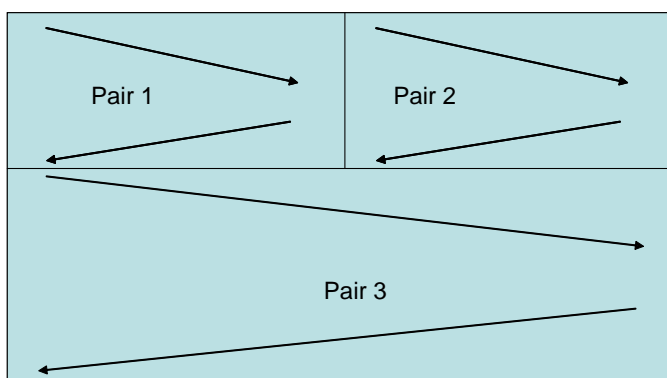


Figure A17. Sampling procedure for deep litter sheds with **three** pens



Figure A18: Sampling procedure using boot swab

### 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 boot swab and wear clean 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 bath or any disinfectant/sanitizer prior to sample collection as it might kill the Salmonella in the sample if there 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 one side full length of the shed and return to the collection point in a manner so that that 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 same shed, return all the swabs in a single twirl-tie bag to pool the swabs
- Walk down the other side of the shed, in the manner shown in Figure A19 by the arrow "Swab 2".
- Once all the swabs are collected from any particular shed, seal the bag and identify the samples.

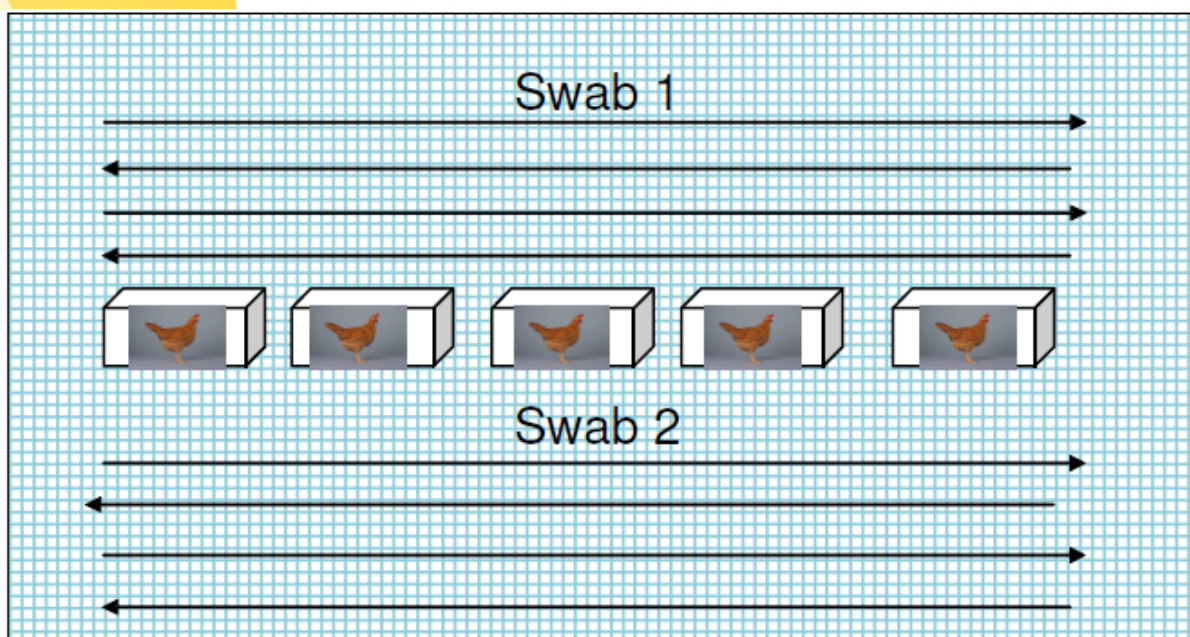


Figure A19. Sampling procedure for slatted shed

## 6. 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

## 7. 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 posted to the laboratory by 1.00 p.m. 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.**

## **Standard Operating Procedure 7: Salmonella –Day Old Chick Paper/Three Day Old Brooding Paper Sampling Procedure**

### **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.

### **Preparation for sample collection**

#### **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.
- To ensure quick and accurate results, the laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation.

#### **2. Material**

- New white plastic garbage bags (so the bags can be written on)
- Indelible black or blue marker
- Courier kit (can be obtained through laboratory)

#### **3. 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. 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, 3 days
- Sex - Male
- Breed - Hy-Line Brown
- Donor Flock - if known
- Collectors name - John Citizen
- The words "Chicks/brooding Paper" - Chicks Papers

#### 5. 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 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.***

## **Standard Operating Procedure 8: Salmonella –Cracked and Dirty Eggs Sampling Procedure**

### **Introduction**

Cracked and dirty eggs are of a high probability 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 the shell defect. Intact clean eggs are significantly less likely to be contaminated with *Salmonella*.

### **Preparation for sample collection**

#### **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.
- To ensure quick and accurate results, the laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation.

#### **2. Material**

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

#### **3. Sampling Procedure**

- Collect multiple cracked and dirty eggs from each shed and identify.
- Place each group of eggs into a separate plastic bag and then smash the eggs to 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. 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, 3 days
- Sex - Male
- Breed - Hy-Line Brown
- Donor Flock - if known
- Collectors name - John Citizen
- The words "Cracked & Dirty Eggs" - Cracked and Dirty Eggs

#### 5. 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 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.***

## **Standard Operating Procedure 9: Salmonella –Washed Eggs Sampling Procedure To Validate Egg Washing**

### **Introduction**

The shell of eggs are washed and sanitised to eliminate the potential for the surface contamination with food safety pathogens such as *Salmonella*. *Salmonella* that is present on the egg shell 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 egg shell.

### **Preparation for sample collection**

#### **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.
- To ensure quick and accurate results, the laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation.

#### **2. Material**

- Indelible black or blue marker
- 3M sponge swab
- Whirl-Pak® bags
- Laboratory accession form
- Courier pack
- Sterile water

#### **3. Sampling Procedure**

- Wash hand and put on a pair of disposable latex gloves.
- Identify a minimum of 90 filler packed eggs divided onto 3 separate fillers 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. Sample Labelling

Each plastic pouch to be labelled with a marker pen

- Farm or Grading Place e.g. - ABC Farm
- Date - 15/07/15
- Collectors name - John Citizen
- The words "Washed Eggs" - Washed Eggs

#### 5. 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 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.***

## **Standard Operating Procedure 10: Salmonella –Environmental Swabs Of Egg Packing and Grading Floor**

### **Introduction**

The surfaces in the egg packing and grading floors are a potential source of *Salmonella*. Contamination from cracked and dirty eggs affected with *Salmonella* can also contribute to cross contamination to 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 in the station environment will then provide with assistance as to where to concentrate the efforts with regards to sanitation programs.

Sampling site should include, but not limited to, 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

### **Preparation for sample collection**

#### **1. 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.
- To ensure quick and accurate results, the laboratory should be notified 24 hours in advance of receiving samples to allow adequate time for preparation.

#### **2. Material**

- 3M sponge stick
- Permanent felt marker
- Pen and laboratory requisitions
- Plastic post satchel for swab transport to laboratory



## **Standard Operating Procedure 11: Shed Cleanout Procedure Rearing and Production Sheds**

### **Introduction**

The principle aim of any wash down and disinfection of a poultry shed is to reduce significantly / eliminate pathogen load. The effectiveness of a shed disinfection is particularly influenced by the organic load in the shed and the presence of contaminating sources. Thus it is preferential for the shed to be depopulated of all livestock, disposable equipment 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 facilitation.

The inclusions of an insecticide spray are aimed at reducing and / 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 created, user friendly nature, 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 double QUATS / 4 QUATS should be used because of their enhanced efficiency) and Virkon®.

It is to be noted that cleaning and disinfection protocols are guidelines but in all cases the first principles remain the same.

### **Pullet Rearing Sheds.**

- Depopulate the shed of all livestock
- Remove disposable materials and remove 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 shed and undertaken 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 services 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 shed comes with cooling pad, drain the cooling pad system and lightly sanitise pad. In winter leave system empty of water and in summer recharge system with water and add a bromide capsule (Actrol® Mini Dose Cartridge). Provides a slow release sanitiser into the cooling water.
- Check and re charge 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*.

### **Production / Layer Sheds**

Procedure as for the rearing facilities and similar principles. The nature of the various shed designs and facilitation 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 a particular area that requires attention in regard to cleaning for *Salmonella* control.
- Aviary Systems provide the difficulties of alternate system sheds but with the additional third dimension.

### **Pre Placement of Point of Lay Pullets**

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 Disinfection Specific's to Production Houses

- Increased attention to the dry cleaning of all surfaces to remove the bulk of the accumulated organic load.
- Use of a low volume high pressure detergent wash-down to ensure the effective removal of all organic material from the surfaces. This may require a protease in the detergent.



- The removal or part removal of structures like slats, nest box pads, supplementary equipment for hand cleaning and disinfection.
- The cooling pads and tunnels must be cleaned and disinfected.
- Manure belt drying systems to be cleaned and disinfected as feasibly possible.
- The cleaning and disinfection of amenities rooms, egg collection rooms / bays and cool rooms.
- Where required, Red Mites (*Demanyssus gallinae*) specific insecticide treatment programs will need to be applied. Noting that Red Mites are not an obligate parasite and thus can live off the bird in the shed environment for many months.
- All water storage tanks should be drained and flushed to remove organic and mineral deposit build up and refilled with clean water and sanitised with 1 litre of 12.5% sodium hypochlorite per 25,000 litres of water.
- Before re population the major surfaces should be swabbed for *Salmonella*.

### **During Production**

Ongoing the production houses should have attention to the following:

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

## **Standard Operating Procedure 12: Rodent Control In Poultry Farms**

### **Introduction**

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

#### **Brown rat (*Rattus norvegicus*)**

Natural burrowers

Dig burrows close to food and water supply in long vegetation and clutter

Weigh between 250 and 500g

Tail shorter than head and body

Ears small and close set

#### **Black rat (*Rattus rattus*)**

Excellent climbers

Nest high up in roofs and trees

Weigh between 200 and 300g

Tail longer than head and body

Ears prominent

#### **House mouse (*Mus musculus*)**

Found inside and outside sheds

Gnawing causes extensive damage to wiring and facilities if not controlled

Weigh 15 to 25g.

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.

## **Control Strategy**

### **Food/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.

### **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 7.5mm.

## **Baiting Strategy**

### **Exterior Baiting**

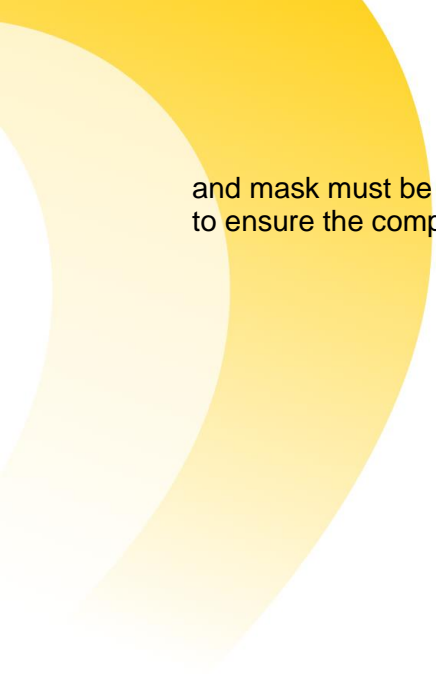
- If rat burrows are identified, place bait in burrows and kick in entrance
- Check daily to see if holes have been re-opened, if so repeat procedure
- Perimeter baits around sheds using one bait station every 15 meters. Baits must be laid both on the ground and in elevated positions to ensure baits 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.

### **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.

## **Risk Management Plan (Health and Safety Consideration)**

Baiting is a control method that uses rodenticides (chemicals which are poisonous to rodents), which are available in the form of fumigants, tracking powders, and acute and delayed toxicity baits. All rodenticides are poisonous at various levels for poultry, livestock, and humans. Therefore caution in the use of rodenticides is required, and manufacturer's label instructions should be strictly followed. Personal Protective Equipment such as gloves



and mask must be worn at all times while handling the rodenticide. Management also needs to ensure the compliance with the procedure and operators are trained properly.

## **Standard Operating Procedure 13: Off-label use of Vaxsafe ST<sup>®</sup> vaccine in combination with Avian Adjuvanted Vaccines**

### **Introduction**

Vaxsafe ST<sup>®</sup> is a commercial live *Salmonella* vaccine to vaccinate the birds against *Salmonella*. Currently this is the only available commercial vaccine in Australia which is registered for drinking water administration. But for long term protection in layers, it is recommended to vaccinate the flock with Vaxsafe ST<sup>®</sup> in combination with an adjuvanted vaccine through intramuscular injection following priming the flock with in-water administration. But it has to be noted that this is an “**off label use**” of the vaccine.

### **Material**

- Appropriate number of effective doses of Vaxsafe ST<sup>®</sup> vaccine. Note-Vaxsafe ST<sup>®</sup> 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.

### **Preparation of Vaccines**

#### **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<sup>®</sup> 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 500 ml adjuvanted vaccine bottle containing 1,000 doses. Gently invert the vaccine bottle several times to aid mixing.

Check the Vaxsafe ST<sup>®</sup> 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.

#### **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 bottle containing 1,000 doses each. Gently invert the vaccine bottle several times to aid mixing.

Check the Vaxsafe ST<sup>®</sup> vaccine vial carefully and if there is any evidence that Vaxsafe ST<sup>®</sup> 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.

### **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 bottle containing 1,000 doses each. Gently invert the vaccine bottle several times to aid mixing.

Check the Vaxsafe ST<sup>®</sup> vaccine vial carefully and if there is any evidence that Vaxsafe ST<sup>®</sup> 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.

### **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<sup>®</sup> 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<sup>®</sup>, Nobilis EDS<sup>®</sup>, Newcavac<sup>®</sup> etc. Any of these vaccines can be utilised as an adjuvanted vaccine for Vaxsafe ST<sup>®</sup> administration.