Poultry Enterprise Environmental Sampling Methodology for Salmonella spp. Detection Version 3

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Abstract

Details of envrionmental sampling methodology of different production locations within a broiler production enterprise.

Specific details regarding the sampling methodology and samples collected are provided.

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Guidelines

Sampling Protocol

All sampling procedures in this protocol have been designed to be conducted in commercial production facilities while birds are housed.

No sampling at height is required to obtain sufficient samples representative of the house or the flock to be sampled.

Animal Welfare

This prtocol is designed to minimise adverse interference with birds while they are housed.

Sampling is conducted at routine times when birds would be normally inspected by farm staff.

No birds are directly handled during the sampling procedure, however they will be walked through during sampling within sheds.

Walking pace should be maintained, and it is recommended that sampling be conducted when birds are being routinely checked to minimise flock disturbance.

Samples can be obtained by collection of materials that are routinely used or disposed of during production, such as hatch debris or chick papers.

Repeated Sampling

Where sampling is to be conducted repeatedly in the same environment, it is recommended that sampling be conducted at the same time of the day on each sampling ocassion to eliminate sampling or operator bias.

To ensure repeatability between sampling events, select the same sites to be sampled on each sampling ocassion.

To ensure this, identify simple to access sampling locations prior to sampling.

Top ensure adequate sample is collected during sampling samples should be collected from sites dispersed evenly along the length of the shed or within the environment to be sampled.

To ensure repeatability within the shed, it is easier if the sampling sites are collected from the same site, the length of the equipment or at an easily identifiable site such as the end of a cage frame.

Avoid sampling at height if possible.

Before start

Sampling Equipment

All sampling equiement should be assembled and sterilised in single units for sampling prior to sampling.
Pre-label all sample bags with shed ID, sample ID and sample location

Boots and Boot swabs

Clean dry sanitised boots should be used on entry to all sheds prior to sampling if using boot swabs to sample sheds.
To ensure cross contamination is minimised between sheds new boots must be worn prior to entry.

Personal protective equipment

Appropriate personal protective equipment should be worn when sampling sheds, this should include coveralls, gloves for collecting samples, face mask (P2), and hairnet.

Materials

- Buffered Peptone Water CM0509 by Oxoid Microbiology Products - Thermo Fischer
- Nasco Whirl Pak B00736 by Contributed by users
- Nasco Whirl-Pak B01297 by Contributed by users
- Cotton Gauze Swabs (10 x 10cm x 8 ply) G5201 Emh40/0404 by Contributed by users
- Mirella Research Sterile Bags (400 x 500 mm) AAF2 by Contributed by users
- Carnivore Hair Nets (polypropylene with double elastics) C933 by Contributed by users

Protocol

Sample Equipment Preparation

Step 1.

Drag Swab Assemblies

- 2 to 4 cotton gauze swabs (10 x 10 cm)
- 2 m cotton string

Lay all cotton gauze swabs flat
Firmly tie the cotton string around the middle of all cotton gauze swabs
Fan the cotton gauze to make a 'butterfly'

Sterilize each drag swab assembly separately by autoclaving

Prior to use pre-moisten each drag swab assembly by dipping into 10mL sterile Buffered Peptone Water (BPW)
Squeeze out excessive moisture before use

Boot Swabs

Boot swabs are constructed from polypropylene hair nets used to protect hair from dust
Two hair nets (pair of boot swabs) are sterilized by autoclaving.

Prior to use pre-moisten each boot swab by dipping into 10mL of sterile BPW

Surface Swabs

Cotton gauze swabs (10cm x 10cm)
Sterilised as sampling units of 4 or 6 by autoclaving

Prior to use pre-moisten each gauze swab unit by dipping into 5mL of sterile BPW

Sampling Methodology

Step 2.

Using a clean pair of gloves for each sample and sample type

Surface Sampling (Dust)

Each swab should be used to sample approximately a quarter of the length to be sampled
Hold a single cotton gauze swab and lightly wipe the surface to be sampled while walking at a steady pace
Place the used swab into a labelled sterile plastic bag or container
Repeat the procedure until the length of the unit has been sampled
Results appear to be better if a minimal amount of surface material is obtained.

Surface sampling (Fan dust)

Each swab should be used to sample approximately one quarter of the guard covering the fan unit within reach
Hold a single cotton gauze swab and lightly wipe the surface to be sampled

Place the used swab into a labelled sterile plastic bag or container
Repeat the procedure until the unit has been sampled
One fan is equivalent to one sample of four to six swabs (more swabs may be required if the fan units are large)
Results appear to be better if a minimal amount of surface material is obtained.

Manure Belt Sampling
Frequntly the manure belt in caged sheds (cage scraper style) cannot be accessed under the cages but may be accessible at the end of the cage frame
Each swab should be used to sample a quarter of the exposed surface of the manure belt.
Holding a single cotton gauze swab, wipe the exposed surface of each of the manure belts
Place the used swab into a labelled sterile plastic bag or container
Repeat the procedure until the exposed surface of the unit within reach has been sampled
Results appear to be better if a minimal amount of surface material is obtained, avoid large quantities of manure

Boot Swabs
Boot swabs are typically used on littered floor, in poultry flocks housed on the floor. They have been proved to be useful on concrete or slatted floors as well.
Clean dry boots must be worn on entry to the shed.
Once inside the shed (just inside the entrance)
Put a plastic overshoe over the boot
Using clean gloves
Place a pre-moistened bootswab over the plastic overshoe
Walk the length or width of the shed as normal while collecting all other samples.
At the sampling half way point or the middle of the shed exchange the bootswab and plastic overshoe for a new set.
Remove each bootswab and place into a labelled sterile plastic bag or container
Remove the plastic overshoe and dispose of
With a new pair of clean gloves replace the plastic overshoe and then a new bootswab
Continue collecting boot swab samples until the shed has been sampled.

Hatchery Sampling
Hatch Debris - egg shell and adhering material remaining in a chick hatching basket after chicks have been removed post hatch
Chick Papers - paper or cardboard used to line the inside of a chick transport basket

Parent Rearing Farms - Day old chick delivery sampling

Step 3.
Randomly collect no less than ten chick papers from chick transport boxes on the day of delivery.
All chick papers are pooled into a single sterile plastic bag.
Label each pool of papers with the flock ID, Shed ID and the date of collection

Parent Rearing Farms - Environmental sampling during rearing

Step 4.

Four drag swab assemblies are dragged across the surface of the litter within the shed.
The entire surface of the shed is sampled, with each swab used to sample approximately one quarter of the shed each
All drag swab assemblies are pooled into a single sterile plastic bag

Parent Egg Production Farms - Cage Shed Sampling

Step 5.

Four pre-moistened cotton gauze swabs are used to collect each egg belt, manure belt and dust sample by wiping each respective surface.

Egg belt samples are collected from the bottom of the egg belt surface (height: 1.00 - 1.20 m).
Dust samples are collected from the uppermost surface of the nest box or front of cage (height: 1.20 - 1.40 m)
Dust and egg belt samples are collected from each row of cages within the shed, along the horizontal length of each frame.

Manure belt samples are collected from all exposed manure belt surfaces within reach (height: 0.50 - 1.80 m).
Manure belts are sampled at the end of each frame of cages.

While collecting all other samples within the shed, two pairs of boot swabs are worn.
Boot swabs are worn over clean dry boots, and new plastic boot covers.
The first pair of boot swabs is exchanged, after half the shed is walked, for a new pair of boot covers and boot swabs.

Each sample (four gauze swabs) is pooled separately into a sterile plastic bag, and labelled by frame and cage row.
Boot swabs are bagged separately into a sterile plastic bag and labelled by boot swab number (1 to 4).

Parent Egg Production Farms - Barn Shed Sampling

Step 6.

Four pre-moistened cotton gauze swabs are used to collect each environmental sample by wiping the respective surface as detailed below.

Dust samples are collected from the nest box lid, along the horizontal length of the nest box assembly.
At least two samples are collected from each side of the shed.

Dust samples are collected from each of the fan guards along the horizontal length of each shed.
A minimum of 2 fan guard assemblies are swabbed per side of shed, or at the end of the shed if fans arranged in a tunnel ventilation

Egg belt samples are collected at the exposed end of the egg belt, either within the shed at the end of the

nest boxes if available, or in the egg packing room.
A minimum of 2 egg belt samples are collected while the egg belt is running, for a period of no less than 2 minutes.

While collecting all other samples within the shed, two pairs of boot swabs are worn.
Boot swabs are worn over clean dry boots, and new plastic boot covers.
The first pair of boot swabs is exchanged, after half the shed is walked, for a new pair of boot covers and boot swabs.

Each sample (four gauze swabs) is pooled separately into a sterile plastic bag, and labelled by sample type and location collected.
Boot swabs are bagged separately into a sterile plastic bag and labelled by boot swab number (1 to 4).

Hatchery Samples

Step 7.
On each hatch day, hatch debris or chick papers are collected for each parent breeder flock of interest.

Hatch debris is randomly collected from at least 10 chick hatching baskets from each trolley for the hatch, until the equivalent of 100 eggs is obtained.
Hatch debris are pooled into a sterile plastic bag, labelled by donor flock and date of hatch.

Chick papers are randomly collected from the bottom of at least 10 transport baskets after chicks had been standing for no less than 1 hour.
Chick papers are pooled into a single sterile plastic bag, labelled by donor flock and date of hatch.

Broiler Production Farms

Step 8.
Six pre-moistened cotton gauze swabs are used to collect each environmental sample by wiping the respective surface as detailed below.

Wall dust samples are collected by wiping the surface of the wall the length of the shed above the concrete short wall (height: 1.00 - 1.20 m).
At least two samples are collected from each side of the shed.

Fan dust samples are collected from each of the fan guards along the horizontal length of each shed.
A minimum of 2 fan guard assemblies are swabbed per side of shed, or at the end of the shed if fans arranged in a tunnel ventilation

While collecting all other samples within the shed, two pairs of boot swabs are worn.
Boot swabs are worn over clean dry boots, and new plastic boot covers.
The first pair of boot swabs is exchanged, after half the shed is walked, for a new pair of boot covers and boot swabs.

Each sample (six gauze swabs) is pooled separately into a sterile plastic bag, and labelled by Shed ID, sampling location and sample type
Boot swabs are bagged separately into a sterile plastic bag and labelled by boot swab number (1 to 4).

Primary Processing - Carcase rinse or portion rinse sampling

Step 9.

Carcase rinse and portion rinse samples are collected daily from each production shift as part of routine primary processing quality assurance testing in accordance with the Australian Standard AS4465

Whole chicken carcases or carcase portions are selected randomly from each production line.
The sample is collected into a sterile plastic bag and identified by production line and date.

Warnings

Safety Procedures

Sampling in commecial production facilities requires an awareness of all safety procedures.

Working at height

Safety procedures must be observed when sampling at height. Avoid at all costs unless absolutely necessary

Working in enclosed spaces

Depending on the shed design, manure may be collected underneath slatted floors or in pits. Avoid sampling in these locations.
Alternative samples can be collected by sampling the ends of conveyors or collecting floor samples

Moving parts and equipment

Be aware that there are many conveyors and moving parts within commercial operations.
Do not sample in locations while equipment is moving if it is not guarded and safe to do so