Poultry Enterprise Environmental Sample Handling and Processing for Salmonella Spp. Detection

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Abstract
This protocol details the methodology for processing environmental samples for Salmonella spp. detection.

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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
- Mirella Research Sterile Bags (400 x 500 mm) AAF2 by Contributed by users

Protocol

Primary sample collection and handling

Step 1.

Primary Sample Collection

The sampling methodology is described in the protocol Poultry Enterprise Environmental Sampling Methodology 10.17504/protocols.io.n6mdhc6.

Primary Sample Handling

All samples are collected into a sterile plastic container or bag for processing (Whirl-pak)
All primary samples are to be labelled with the date of collection, sample type, sample ID, sample location, and flock ID
All wet primary samples (rinsate or swabs pre-moistened with buffered peptone water) are refrigerated as soon as practical from sample collection and during transportation

All dry collected primary samples (chick papers, hatch debris) are stored cool and out of the sun prior to and during transportation

Sample Processing
All samples were delivered to the laboratory and processed on the same day as collection.

Primary Sample Processing
To maintain sample integrity, or minimise cross contaimination during processing
All samples collected into a sterile plastic container for processing were kept in the originial collection container or bag for processing, unless described otherwise.

The primary sample is processed as described in the protocol below

Microbiological testing of the primary sample
Microbiological testing of the primary sample is detailed in the protocol Poultry Enterprise Environmental Microbiological Testing for Salmonella spp. 10.17504/protocols.io.n6sdhee

Primary Samples

Step 2.
The primary sample types collected from each location, material used for sample collection and the sample pool sizes for microbiological testing are summarised in the table below.

Location	Sample Type	Sample Material	Primary Sample Pool Size (No. Units or Volume)
Parent Egg Production	Dust	Gauze Swab	4
	Manure Belt	Gauze Swab	4
	Egg Belt	Gauze Swab	4
	Boot Swab	Boot Swab	1
Parent Rearing	Drag Swab	Swab Assembly	1
	Chick Papers	Paper or Cardboard	10
Hatchery	Chick Papers	Paper	10
	Hatch Debris	Hatch remains: shell and remnants	3 x 100 g (sub-sample of sample)
Broiler Farm	Boot Swab	Boot Swab	1
	Dust - Wall	Gauze Swab	4
	Dust - Fan	Gauze Swab	4-6
Processing	Carcass Rinse	Rinsate	10 mL (sub-sample of rinsate)
	Portion Rinse	Rinsate	10 mL (sub-sample of rinsate)

Buffered Peptone Water

Step 3.
Sterile buffered peptone water (BPW) is added as part of the primary sample processing
BPW is mixed accordance with the manufacturers instructions and sterilised by autoclaving

Chick Papers

Step 4.

Ten chick papers collected from day old chick deliveries or at the hatchery is pooled as a single sample

900mL of BPW is added to each sample of 10 chick papers
Leave the sample at room temperature to incubate for approximately 30 minutes.

The sample is then manually macerated by massaging the paper within the sterile bag.

Massage gently to ensure the paper is fully wet through and BPW covers the whole sample
Do not overmix

The whole sample is incubated

AMOUNT
900 ml Additional info:
Buffered Peptone Water

Hatch Debris

Step 5.
Each collected sample of hatch debris is crushed manually and mixed thoroughly by shaking/tipping.

Three 100g sub-samples are aliquoted from each hatch debris sample as the primary sample.
Each 100g sub-sample is processed as a separate sample

100mL of BPW is added to each 100g sample and gently mixed
Do not overmix

The whole sample is incubated

AMOUNT
100 ml Additional info:
Buffered Peptone Water
AMOUNT
100 g Additional info:
Hatch Debris

Carcass or portion rinse samples

Step 6.
Carcass rinse samples and portion rinse samples are collected in accordance with the Australian Standard AS4465.

Carcass Rinse Sample
A fresh chicken carcass is collected from the processing line or chiller into a sterile plastic bag.

500 mL of BPW is added to a single bagged chicken, and massaged gently for 2 - 3 minutes.
A sub-sample (10 mL) of the rinsate is collected as the primary sample

Portion rinse samples
A fresh chicken portion rom the processing line into a sterile plastic bag

200 mL of BPW is added to a single portion and massaged gently for 2 - 3 minutes.
A sub-sample (10 mL) of the rinsate is collected as the primary sample

The whole rinsate sample is incubated

AMOUNT

500 ml Additional info:
Carcass Rinse -Buffered
Peptone Water

AMOUNT

200 ml Additional info:
Portion Rinse - Buffered
Peptone Water

Boot Swabs

Step 7.
Each boot swab is processed as a single sample.

200mL of BPW is added to each boot swab

Massage sample gently to ensure the swabs are fully wet or covered with BPW
Do not overmix

The whole sample is incubated

AMOUNT

200 ml Additional info:
Buffered Peptone Water

Dust, Manure Belt, Egg Belt , and drag swab assemblies

Step 8.
Each pool of gauze swabs (4 or 6 swabs or a single drag swab assembly) is processed as a single sample

100 mL of BPW is added to each pool of gauze swabs

Massage gently to ensure the swabs are fully wet and covered by the BPW
Do not overmix

The whole sample is incubated

AMOUNT

100 ml Additional info:
Buffered Peptone Water