

## ANNEX

Testing scheme necessary to verify the achievement of the Community target for the reduction of *Salmonella enteritidis*, *Salmonella hadar*, *Salmonella infantis*, *Salmonella typhimurium* and *Salmonella virchow* in adult breeding flocks of *Gallus gallus*

### 1. Sampling frame

The sampling frame shall cover all adult breeding flocks of *Gallus gallus* comprising at least 250 birds ('breeding flocks').

### 2. Monitoring in breeding flocks

#### 2.1. Location, frequency and status of sampling

For the purpose of this Regulation, breeding flocks shall be sampled at the initiative of the operator and as part of official controls.

##### 2.1.1. Sampling at the initiative of the operator

Sampling shall take place every two weeks at the place designated by the competent authority from the following two possible options:

- (a) at the hatchery; or
- (b) at the holding.

The competent authority shall implement one of the above options to the whole testing scheme, and shall set up a procedure so that the detection of salmonella serotypes referred to in Article 1(1) ('relevant salmonella') during the sampling at the initiative of the operator is notified without delay to the competent authority by the operator, the sampler or the laboratory performing the analyses.

##### 2.1.2. Official control sampling

Without prejudice to Annex II, Part C.2 of Regulation (EC) No 2160/2003, official sampling shall consist in:

###### 2.1.2.1. If sampling at the initiative of the operator takes place at the hatchery:

- (a) routine sampling every 16 weeks at the hatchery, which shall on that occasion replace the corresponding sampling at the initiative of the operator;
- (b) routine sampling at the holding on two occasions during the production cycle, the first one being within four weeks following moving to laying phase or laying unit and the second one being towards the end of the laying phase, not earlier than eight weeks before the end of the production cycle;
- (c) confirmatory sampling at the holding, following detection of relevant salmonella from sampling at the hatchery.

###### 2.1.2.2. If sampling at the initiative of the operator takes place at the holding, routine sampling shall be carried out on three occasions during the production cycle:

- (a) within four weeks following moving to laying phase or laying unit;
- (b) towards the end of the laying phase, not earlier than eight weeks before the end of the production cycle;

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- (c) during the production, at any time sufficiently distant from the samples referred to in points (a) and (b).

## 2.2. Sampling protocol

### 2.2.1. Sampling at the hatchery

For each breeding flock, the sample shall consist of a minimum of one composite sample of visibly soiled hatcher basket liners taken at random from five separate hatcher baskets or locations in the hatcher, to reach a total of at least 1 m<sup>2</sup>. If the hatching eggs from a breeding flock occupy more than one incubator, then one such composite sample shall be taken from each incubator.

In cases where hatcher basket liners are not used, 10 g broken eggshells shall be taken from 25 separate hatcher baskets, crushed, mixed and a 25 g sub sample taken.

That procedure shall be followed for sampling at the initiative of the operator as well as for official sampling.

### 2.2.2. Sampling at the holding

#### 2.2.2.1. Routine sampling at the initiative of the operator

Sampling shall primarily consist of faecal samples and shall aim to detect a 1 % within flock prevalence, with 95 % confidence limit. To that effect, the samples shall comprise one of the following:

- (a) Pooled faeces made up of separate samples of fresh faeces each weighing not less than 1 g taken at random from a number of sites in the building in which the birds are kept, or where the birds have free access to more than one building on a particular holding, from each group of buildings on the holding in which the birds are kept. Faeces may be pooled for analysis up to a minimum of two pools.

The number of sites from which separate faeces samples are to be taken in order to make a pooled sample shall be as follows:

<b>Number of birds kept in a building</b>	<b>Number of faeces samples to be taken in the building or group of buildings on the holding</b>
250-349	200
350-449	220
450-799	250
800-999	260
1 000 or more	300

- (b) Five pairs of boot swabs:

Boot swabs used shall be sufficiently absorptive to soak up moisture. Tubegauze 'socks' are also acceptable.

The surface of the boot swab shall be moistened using appropriate diluent (such as 0,8 % sodium chloride, 0,1 % peptone in sterile deionised water, or sterile water).

Walking around shall be done in a manner which will sample representatively all parts of the sector, including littered and slatted areas when slats are safe to walk on. All separate pens within a house shall be included in the sampling. On completion of sampling in the chosen sector, boot swabs must be removed carefully so as not to dislodge adherent material.

The boot swabs may be pooled for analysis into a minimum of two pools.

- (c) In cage breeding flocks, sampling may consist of naturally mixed faeces from dropping belts, scrapers or deep pits, depending on the type of house. Two samples of at least 150 g shall be collected to be tested individually:
- (i) droppings belts beneath each tier of cages which are run regularly and discharged into an auger or conveyor system;
  - (ii) droppings pit system in which deflectors beneath the cages are scraped into a deep pit beneath the house;
  - (iii) droppings pit system in a step cage house when cages are offset and faeces fall directly into the pit.

There are normally several stacks of cages within a house. Pooled faeces from each stack shall be represented in the overall pooled sample. Two pooled samples shall be taken from each flock as described below.

In systems where there are belts or scrapers, these shall be run on the day of the sampling before sampling is carried out.

In systems where there are deflectors beneath cages and scrapers, pooled faeces which has lodged on the scraper after it has been run, shall be collected.

In step-cage systems where there is no belt or scraper system it is necessary to collect pooled faeces from the deep pit.

Droppings belt systems: pooled faecal material from the discharge ends of the belts shall be collected.

#### 2.2.2.2. Official sampling

- (a) Routine sampling shall be as described in point 2.2.2.1.
- (b) Confirmatory sampling following detection of relevant salmonella from sampling at the hatchery shall be carried out as follows.

In addition to the sampling as described in point 2.2.2.1, the sampling may include a sample of birds taken at random from within each house of birds on the farm, normally up to five birds per house, unless the authority deems necessary to sample a higher number of birds. The examination shall consist in a test for research of anti-microbials or of bacterial growth inhibitory effect in samples. A test is considered failed if a positive is found in any of the birds.

In case the presence of relevant salmonella is not detected but anti-microbials or bacterial growth inhibitory effect are, sampling of the flock for relevant salmonella and bacterial growth inhibitory effect shall be repeated until no bacterial growth inhibitory effect is detected, or the breeding flock is destroyed. In the latter case, the breeding flock shall be accounted for as an infected breeding flock for the purpose of the Community target.

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(c) Suspect cases

In exceptional cases where the competent authority has reasons to suspect false negative results at the first official sampling at the holding, a secondary official confirmatory sampling may be performed, composed of faeces or birds (for the detection of salmonella in organs).

In exceptional cases where the competent authority has reasons to suspect false positive sampling performed at the initiative of the operator at the holding, follow-up official sampling may be performed.

3. Examination of the samples

3.1. Preparation of the samples

3.1.1. Hatcher basket liners

- (a) place in 1 litre Buffered Peptone Water (BPW) which has been prewarmed at room temperature and mix gently;
- (b) continue culture of the sample by using the detection method in 3.2.

3.1.2. Boot swabs samples

- (a) carefully unpack the pair of boot swabs (or 'socks') to avoid dislodging adherent faecal material and place in 225 ml BPW which has been prewarmed to room temperature;
- (b) where five pairs of boot swabs are pooled into two samples, place five individual samples into a minimum of 225 ml BPW and ensure that all the samples are totally immersed in the BPW;
- (c) swirl to fully saturate the sample and continue culture by using the detection method in 3.2.

3.1.3. Other faecal material samples

- (a) at the laboratory place each sample (or pooled sample as appropriate) into an equal weight of Buffered Peptone Water and mix gently;
- (b) allow the sample to soften for 10-15 minutes then mix gently;
- (c) immediately after mixing remove 50 g of the mixture and add to 200 ml of Buffered Peptone Water which has been pre-warmed to room temperature;
- (d) continue culture of the sample by using the detection method in 3.2.

3.2. Detection method

The method recommended by the Community Reference Laboratory for Salmonella in Bilthoven, Netherlands, shall be used: the method is a modification of ISO 6579 (2002), where a semi solid medium (MSRV) is used as the single selective enrichment medium. The semi-solid medium should be incubated at  $41,5 \pm 1$  °C for  $2 \times (24 \pm 3)$  hours.

As regards the boot swabs samples and other faecal material samples referred to in paragraph 3.1., it is possible to pool incubated BPW enrichment broth for future culture. To do that, incubate both samples in BPW as normal. Take 1 ml of incubated broth from each sample and mix thoroughly then take 0,1 ml of the mixture and inoculate the MSRV plates in the usual way.

3.3. Serotyping

At least one isolate from each positive sample shall be typed, following the Kaufmann-White scheme.

#### 4. Results and reporting

A breeding flock shall be considered positive for the purpose of verifying the achievement of the Community target, when presence of relevant salmonella (other than vaccine strains) was detected in one or more faecal samples (or if there is a secondary official confirmation in the Member State, in the relevant faecal samples or birds organ samples), taken at the holding. This shall not apply in exceptional cases of suspect breeding flocks where salmonella detection at the holding at the initiative of the operator was not confirmed by official sampling.

The cumulative results from sampling and testing in breeding flocks at holding level shall be accounted for, i.e. each breeding flock shall be counted only once irrespective of the number of sampling and testing operations. Positive breeding flocks shall be counted only once, irrespective of the number of sampling and testing operations.

Reporting shall include:

- (a) detailed description of the options implemented for the sampling scheme and the type of samples taken, as appropriate;
- (b) number of existing breeding flocks and those tested;
- (c) results of the testing;
- (d) explanations on the results, in particular concerning exceptional cases.