

ANNEX

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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 domestic fowl (*Gallus gallus*) comprising at least 250 birds (breeding flocks).

2. MONITORING IN BREEDING FLOCKS

2.1. Location, frequency and status of sampling

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 may decide to implement one of the options referred to in points (a) or (b) to the whole testing scheme for all broiler breeding flocks and one of those options for layer breeding flocks. Sampling on holdings, mainly exporting or trading hatching eggs to other Member States, shall in any case take place on the holding. It 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 laboratory performing the analyses. Timely notification of the detection of *Salmonella*, including the serotype, shall remain the responsibility of the operator and the laboratory performing the analyses.

By way of derogation, if the Community target has been reached for at least two consecutive calendar years, sampling at the holding may be extended to take place every three weeks, at the discretion of the competent authority. The competent authority may decide to revert to a two week testing interval in the case of detection of a positive flock on the holding and/or in any other case deemed appropriate by the competent authority.

2.1.2. Official control sampling

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

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, and;
- (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;

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- (c) confirmatory sampling at the holding, following the 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;
 - (c) during the production, at any time sufficiently distant from the samples referred to in points (a) and (b).
- 2.1.2.3. By way of derogation of paragraphs 2.1.2.1 and 2.1.2.2 and if the Community target has been achieved for at least two consecutive calendar years, the competent authority may replace the routine samplings by sampling:
- (a) at the holding at one occasion anytime during the production cycle and once a year at the hatchery; or
 - (b) at the holding at two occasions anytime sufficiently distant from each other during the production cycle.

A sampling carried out by the competent authority may replace a sampling at the initiative of the food business operator.

2.2. Sampling protocol

2.2.1. Sampling at the hatchery

At least one sample shall be taken per breeding flock on each sampling occasion. Sampling should be arranged on a hatch day when samples from all breeding flocks will be available and all material from all hatchers from which hatched chicks are removed on the sampling day should contribute to the set of samples in a proportionate way. If there are more than 50 000 eggs of one flock in the hatchers a second sample shall be collected from that flock.

The sample shall consist of at least:

- (a) 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 sampling surface of at least 1 m²; however, if the hatching eggs from a breeding flock occupy more than one hatcher, then such a composite sample shall be taken from all up to five hatchers; or
- (b) one sample taken with one or several moistened fabric swab(s) of at least 900 cm² surface area in total, taken immediately after the removal of the chickens from the whole surface area of the bottom of at least a total of five hatcher baskets, or from fluff from five places, including on the floor, in all up to five hatchers with hatched eggs from the flock, ensuring that at least one sample per flock from which eggs are derived, is taken; or
- (c) 10 g broken eggshells taken from a total of 25 separate hatcher baskets (i.e. 250 g initial sample) in up to five hatchers with hatched eggs from the flock, crushed, mixed and subsampled to form a 25 g subsample for testing.

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The procedure set out in points (a), (b) and (c) shall be followed for sampling at the initiative of the operator as well as for official sampling. It is not mandatory to include a hatcher with eggs from different flocks if at least 80 % of the eggs are in other sampled hatchers.

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 a 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 house in which the flock is kept, or where the flock has free access to more than one house on a particular holding, from each group of houses on the holding in which the flock is 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:

Number of birds kept in the flock	Number of faeces samples to be taken in the flock
250-349	200
350-449	220
450-799	250
800-999	260
1 000 or more	300

- (b) Boot swabs and/or dust samples:

Boot swabs used shall be sufficiently absorptive to soak up moisture. Tubegauze “socks” shall also be acceptable for that purpose.

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

The samples shall be taken while walking through the house using a route that will produce representative samples for all parts of the house or the respective sector. This shall include littered and slatted areas provided that 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 samples shall consist of:

- (i) five pairs of boot swabs, representing each about 20 % of the area of the house; the swabs may be pooled for analysis into a minimum of two pools; or
- (ii) at least one pair of boot swabs representing the whole area of the house and an additional dust sample collected from multiple places throughout the house from surfaces with visible presence of dust. One or several moistened

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fabric swab(s) of at least 900 cm² surface area in total shall be used to collect this dust sample.

- (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 in the following third to sixth subparagraphs:

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 that have 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 throughout 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 performed as described in point 2.2.2.1.
- (b) Confirmatory sampling following the detection of relevant *Salmonella* from sampling at the hatchery shall be performed as described in point 2.2.2.1. Additional samples can be collected for the possible testing of antimicrobials or bacterial growth inhibitors as follows: birds shall be taken at random from within each house of birds on the farm, normally up to five birds per house, unless the authority deems it necessary to sample a higher number of birds. If the source of infection is not confirmed, antimicrobial testing shall be carried out or new bacteriological testing for *Salmonella* shall be carried out on the flock or their progeny before trade restrictions are lifted. If antimicrobials or bacterial growth inhibitors are detected, the *Salmonella* infection shall be considered as confirmed.
- (c) Suspect cases

In exceptional cases where the competent authority has reasons to call the result into question (false positive or false negative results), it may decide to repeat the testing in accordance with point (b).

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 the culture of the sample by using the detection method described in 3.2.

3.1.2. Boot swabs and dust samples:

- (a) The pair(s) of boot/sock swabs and dust sample (fabric swab) shall be carefully unpacked to avoid dislodging adherent faecal material or loose dust material and placed in 225 ml of BPW which has been prewarmed to room temperature. The boot/socks and fabric swab shall be fully submersed in BPW to provide sufficient free liquid around the sample for migration of *Salmonella* away from the sample and therefore more BPW may be added if necessary. Separate preparations will be made of the boot swabs and the fabric swab.
- (b) Where five pairs of boot/sock swabs are pooled into two samples, place each pooled sample in of 225 ml BPW, or more if necessary, to fully submerge and provide sufficient free liquid around the sample for migration of *Salmonella* away from the sample.
- (c) Swirl to fully saturate the sample and continue the culture by using the detection method described in 3.2.

3.1.3. Other faecal material samples:

- (a) The faeces samples shall be pooled and thoroughly mixed and a 25 g subsample shall be collected for culture.
- (b) The 25 g subsample shall be added to 225 ml of BPW which has been prewarmed to room temperature.
- (c) The culture of the sample shall be continued by using the detection method described in 3.2.

If ISO standards on the preparation of relevant samples for the detection of *Salmonella* are agreed on they shall be applied and replace the above provisions on sampling preparation.

3.2. Detection method

The detection of *Salmonella* spp. shall be carried out according to Amendment 1 of EN/ISO 6579-2002/Amd1:2007. "Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. – Amendment 1: Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage".

As regards the boot swabs samples, dust 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.

Do not shake, swirl or otherwise agitate the samples in BPW after incubation as this releases inhibitory particulates and reduces subsequent isolation in MSRV.

3.3. Serotyping

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

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4. RESULTS AND REPORTING

A breeding flock shall be considered infected for the purpose of verifying the achievement of the Community target, when the presence of relevant *Salmonella* (other than vaccine strains) was detected in one or more 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, even if *Salmonella* is only detected in the dust sample. 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.

For statistical purpose, an infected flock shall be counted once only regardless of how often *Salmonella* have been detected in this flock during the production period.

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 of the results, in particular concerning exceptional cases.

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