

ANNEX

Testing scheme necessary to verify the achievement of the Community target for the reduction of *Salmonella enteritidis* and *Salmonella typhimurium* in adult laying hens of *Gallus gallus*, as referred to in Article 1(2)

1. SAMPLING FRAME

The sampling frame shall cover all flocks of adult laying hens of *Gallus gallus* (laying flocks) referred to in Article 1 of Regulation (EC) No 2160/2003.

2. MONITORING IN LAYING FLOCKS

2.1. Frequency and status of sampling

Laying flocks shall be sampled at the initiative of the food business operator (operator) and by the competent authority.

Sampling at the initiative of the operator shall take place at least every fifteen weeks. The first sampling shall take place at the age of 24 ± 2 weeks.

Sampling by the competent authority shall take place at least:

- (a) in one flock per year per holding comprising at least 1 000 birds;
- (b) at the age of 24 ± 2 weeks in laying flocks housed in buildings where salmonella was detected in the preceding flock;
- (c) in any case of suspicion of *Salmonella enteritidis* or *Salmonella typhimurium* infection, as a result of the epidemiological investigation of food-borne outbreaks in accordance with Article 8 of Directive 2003/99/EC of the European Parliament and of the Council⁽¹⁾;
- (d) in all other laying flocks on the holding in case *Salmonella enteritidis* or *Salmonella typhimurium* are detected in one laying flock on the holding;
- (e) in cases where the competent authority considers it appropriate.

A sampling carried out by the competent authority may replace one sampling at the initiative of the operator.

2.2. Sampling protocol

In order to maximise sensitivity of sampling, both faecal material and the environment shall be sampled at least as provided for in (a) and (b):

- (a) In cage flocks, 2×150 grams of naturally pooled faeces shall be taken from all belts or scrapers in the house after running the manure removal system; however, in the case of step cage houses without scrapers or belts 2×150 grams of mixed fresh faeces must be collected from 60 different places beneath the cages in the dropping pits.
- (b) In barn or free-range houses, two pairs of boot swabs or socks be taken, without changing overboots between boot swabs.

In the case of sampling by the competent authority, 250 ml containing at least 100 gram of dust shall be collected from prolific sources of dust throughout the house. If there is not sufficient dust, an additional sample of 150 grams naturally pooled faeces or an additional pair of boot swabs or socks shall be taken.

In the case of sampling referred to in point 2.1(b), (c) and (d), the competent authority shall satisfy itself by conduction further tests as appropriate that the results of examinations for salmonella in birds are not affected by the use of antimicrobials in the flocks.

Where the presence of *Salmonella enteritidis* and *Salmonella typhimurium* is not detected but antimicrobials or bacterial growth inhibitory effect are it shall be accounted for as an infected laying flock for the purpose of the Community target referred to in Article 1(2).

3. EXAMINATION OF THE SAMPLES

3.1. Transport and preparation of the samples

Samples shall be sent by express mail or courier to the laboratories referred to in Article 11 of Regulation (EC) No 2160/2003, on the day of collection. At the laboratory, samples shall be kept refrigerated until examination, which shall be carried out within 48 hours following receipt.

3.1.1. Boot swab samples

- (a) The two pairs of boot swabs ('or socks') shall be carefully unpacked to avoid dislodging adherent faecal material, pooled and placed in 225 ml Buffered Peptone Water (BPW) which has been pre-warmed to room temperature;
- (b) The sample shall be swirled to fully saturate it and culture shall be continued by using the detection method in 3.2.

3.1.2. Other faecal material and dust samples

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

If ISO standards on the preparation of faeces 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 method recommended by the Community Reference Laboratory (CRL) for Salmonella in Bilthoven, the Netherlands, for detection shall be used. This method is described in the current version of draft Annex D of ISO 6579 (2002): 'Detection of *Salmonella* spp. in animal faeces and in samples of the primary production stage'. In this method, a semi-solid medium (modified semi-solid Rappaport-Vassiladis medium, MSR_V) is used as the single selective enrichment medium.

3.3. Serotyping

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

3.4. Alternative methods

With regard to samples taken at the initiative of the operator, the methods of analysis provided for in Article 11 of Regulation (EC) No 882/2004⁽²⁾, may be used instead of the methods for the preparation of samples, detection methods and serotyping provided for in point 3 of this Annex, if validated in accordance with EN/ISO 16140/2003.

3.5. Storage of strains

At least the strains isolated from samples collected by the competent authority, shall be stored for future phagetyping or anti-microbial susceptibility testing, using the normal methods for culture collection, which must ensure integrity of the strains for a minimum of two years.

4. RESULTS AND REPORTING

A laying flock shall be considered positive for the purpose of verifying the achievement of the Community target, where the presence of *Salmonella enteritidis* and *Salmonella typhimurium* (other than vaccine strains) was detected in one or more samples in the laying flock. Positive laying flocks shall be counted only once, irrespective of the number of sampling and testing operations and only be reported in the first year of detection.

Reporting shall include:

- (a) the total number of flocks of laying hens tested and the number of laying flocks tested for each status of sampling referred to in point 2.1;
- (b) the total number of infected flocks and the results of the testing for each status of sampling referred to in point 2.1;
- (c) explanations on the results, in particular concerning exceptional cases.

The results referred to in this point and any additional relevant information shall be reported as part of the report on trends and sources provided for in Article 9(1) of Directive 2003/99/EC.

Status: This is the original version (as it was originally adopted).

- (1) OJ L 325, 12.12.2003, p. 31.
- (2) OJ L 191, 28.5.2004, p. 1.