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ANNEX

Testing scheme necessary to verify the achievement of the Union target as referred to in Article 1(2)

1. SAMPLING FRAME

The sampling frame shall comprise all flocks of broilers of *Gallus gallus* ('broilers') within the framework of the national control programmes provided for in Article 5 of Regulation (EC) No 2160/2003.

2. MONITORING IN BROILERS

2.1. Frequency of sampling

- (a) Food business operators shall sample all flocks of broilers within three weeks before slaughter.

By way of derogation from the sampling obligation set out in the first subparagraph, the competent authority may provide that food business operators shall sample at least one flock of broilers per round on holdings with more than one flock where:

- (i) an all in / all out system is used in all flocks of the holding;
- (ii) the same management applies to all flocks;
- (iii) feed and water supply is common to all flocks;
- (iv) during at least the last six rounds, tests for *Salmonella* spp. according to the sampling scheme set out in the first subparagraph in all flocks on the holding and samples of all flocks of at least one round were carried out by the competent authority;
- (v) all results from the testing according to the first subparagraph and point (b) for *Salmonella enteritidis* or *Salmonella typhimurium* were negative.

By way of derogation from the sampling obligations set out in this point the competent authority may authorise sampling in the last six weeks prior to the date of slaughter in case the broilers are either kept more than 81 days or fall under organic broiler production according to Commission Regulation (EC) No 889/2008⁽¹⁾.

- (b) The competent authority shall each year sample at least one flock of broilers on 10 % of the holdings with more than 5 000 birds. That sampling may be done on a risk basis and each time the competent authority considers it necessary.

A sampling carried out by the competent authority may replace the sampling by the food business operator as required by point (a).

2.2. Sampling protocol

2.2.1. General instructions for sampling

The competent authority or the food business operator shall ensure that samples are taken by persons trained for that purpose.

At least two pairs of boot swabs shall be taken for sampling. Boot swabs are put on the boots and the sample is taken by walking around in the poultry house. Swabs from one flock of broilers may be pooled into one sample.

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Before putting on the boot swabs, their surface shall be moistened by:

- (a) the application of maximum recovery diluents (MRD: 0,8 % sodium chloride, 0,1 % peptone in sterile deionised water);
- (b) the application of sterile water;
- (c) the application of any other diluents approved by the national reference laboratory referred to in Article 11 (3) of Regulation (EC) No 2160/2003; or
- (d) being autoclaved in a container together with diluents.

The way to moisten boot swabs shall be to pour the liquid inside before putting them on or to shake them in a container of diluent.

It shall be ensured that all sections in a house are represented in the sampling in a proportionate way. Each pair of boot swabs must cover about 50 % of the area of the house.

On completion of sampling, the swabs shall be carefully removed from the boots so as not to dislodge adherent material. Boot swabs may be inverted to retain material. They shall be placed in a bag or pot and labelled.

The competent authority may decide to increase the minimum number of samples in order to ensure representative sampling on a case-by-case evaluation of epidemiological parameters, such as biosecurity conditions, the distribution or size of the flock.

If the competent authority approves one pair of boot swabs may be replaced by a dust sample of 100g collected from multiple places throughout the house from surfaces with visible presence of dust. As an alternative, one or several moistened fabric swab(s) of a combined surface of at least 900 cm² may be used to gather dust from multiple surfaces throughout the house. Each swab shall be well coated with dust on both sides.

2.2.2. *Specific instructions for certain types of holdings*

- (a) For free range flocks of broilers, samples shall only be collected inside the house.
- (b) Where access to the houses is not possible due to limited space in flocks with less than 100 broilers, and it is therefore not possible to use boot swabs when walking around, they may be replaced by the same kind of hand fabric swabs that are used for dust, where the swabs are rubbed over surfaces contaminated with fresh faeces, or if this is not feasible, by other sampling techniques for faeces fit for the intended purpose.

2.2.3. *Sampling by the competent authority*

The competent authority shall satisfy itself by conducting further tests and/or documentary checks as appropriate to verify that results are not altered through the presence of antimicrobials or other substances inhibiting the growth of bacteria.

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

2.2.4. *Transport*

Samples shall be sent without undue delay either by via express mail or courier, to the laboratories referred to in Articles 11 and 12 of Regulation (EC) No 2160/2003. During transport they shall be protected from heat over 25°C and exposure to sunlight.

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Where it is not possible to send the samples within 24 hours from the time of sampling they shall be stored refrigerated.

3. LABORATORY ANALYSIS

3.1. Preparation of the samples

At the laboratory samples shall be kept refrigerated until examination. Examination shall start within 48 hours following the time of receipt of the samples and within four days from the date of sampling.

Dust samples shall be analysed separately. However, the competent authority may decide to pool them with the pair of boot swabs for analysis.

The sample shall be swirled to fully saturate it and culturing shall be continued by using the detection method set out in point 3.2.

The two pairs of boot swabs shall be carefully unpacked to avoid dislodging adherent faecal material, pooled and placed in 225 ml of buffered peptone water (BPW) pre-warmed to room temperature, or 225 ml of diluent is added directly to the two pairs of boot swabs in their container as received in the laboratory.

The boot swabs 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.

If EN/ISO standards on the preparation of faeces for the detection of *Salmonella* are agreed on, they shall replace the provisions on the preparation of samples set out in this point as appropriate.

[^{F1}3.2. Detection method

The detection of *Salmonella* spp. shall be carried out according to EN ISO 6579-1.]

Textual Amendments

F1 Substituted by Commission Regulation (EU) 2019/268 of 15 February 2019 amending Regulations (EU) No 200/2010, (EU) No 517/2011, (EU) No 200/2012 and (EU) No 1190/2012 as regards certain methods for *Salmonella* testing and sampling in poultry (Text with EEA relevance).

3.3. Serotyping

At least one isolate from each positive sample taken by the competent authority shall be serotyped, following the current White-Kauffmann-Le Minor scheme.

Food business operators shall ensure that for all isolates, it is at least excluded that they do not belong to the serotypes *Salmonella enteritidis* and *Salmonella typhimurium*.

[^{F1}3.4. Alternative methods

Alternative methods may be used instead of the methods for detection and serotyping provided for in points 3.1, 3.2 and 3.3 of this Annex, if validated in accordance with EN ISO 16140-2 (for alternative detection methods).]

3.5. Storage of strains

The competent authority shall ensure that at least one isolated strain of *Salmonella* serotypes from sampling as part of official controls per house and per year is stored for future phagetyping

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or antimicrobial susceptibility testing, using established methods for culture collection, which must ensure integrity of the strains for a minimum period of two years from the date of the analysis.

The competent authority may decide that isolates from sampling by food business operators shall also be stored for future phagetyping or antimicrobial susceptibility testing to provide for isolates to be tested in accordance with Article 2 of Commission Decision 2007/407/EC⁽²⁾.

4. RESULTS AND REPORTING

4.1. Calculation of prevalence for the verification of the Union target

A flock of broilers shall be considered positive for the purpose of verifying the achievement of the Union target, where the presence of *Salmonella enteritidis* and/or *Salmonella typhimurium* (other than vaccine strains) was detected in the flock.

Positive flocks of broilers shall be counted only once per round, irrespective of the number of sampling and testing operations and only be reported in the year of the first positive sampling.

4.2. Reporting

Reporting shall include:

- (a) the total number of flocks of broilers which were tested at least once during the year of reporting;
- (b) the total number flocks positive with any *Salmonella* serotype in the Member State;
- (c) the number of broiler flocks positive at least once for *Salmonella enteritidis* and *Salmonella typhimurium* including monophasic strains with the antigenic formula [^{X1}1,4,[5],12:i:-];
- (d) the number of positive broiler flocks for each *Salmonella* serotype or for *Salmonella* unspecified (isolates that are untypable or not serotyped).

Editorial Information

- X1** Substituted by [Corrigendum to Commission Regulation \(EU\) No 200/2012 of 8 March 2012 concerning a Union target for the reduction of *Salmonella enteritidis* and *Salmonella typhimurium* in flocks of broilers, as provided for in Regulation \(EC\) No 2160/2003 of the European Parliament and of the Council \(Official Journal of the European Union L 71 of 9 March 2012\)](#).

The information shall be provided separately for the sampling within the overall national *Salmonella* control programme as provided for in point 2.1. (a) and (b), the food business operators sampling as provided for in point 2.1. (a) and the competent authorities sampling as provided for in point 2.1. (b).

The results of the tests shall be considered relevant food chain information as provided for in Section III of Annex II to Regulation 853/2004 of the European Parliament and of the Council⁽³⁾.

At least the following information shall be made available to the competent authority from each flock of broilers tested:

- (a) holding reference, remaining unique in time;
- (b) house reference, remaining unique in time;

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(c) month of sampling.

The results 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⁽⁴⁾.

The food business operator shall notify the competent authority of the confirmed detection of *Salmonella enteritidis* and *Salmonella typhimurium* without undue delay. The food business operator shall instruct the analysing laboratory to act accordingly.

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- (1) [OJ L 250, 18.9.2008, p. 1.](#)
- (2) [OJ L 153, 14.6.2007, p. 26.](#)
- (3) [OJ L 226, 25.6.2004, p. 22.](#)
- (4) [OJ L 325, 12.12.2003, p. 31.](#)

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Changes and effects yet to be applied to :

- Annex heading word omitted by [S.I. 2019/740 reg. 9\(5\)\(a\)](#)
- Annex point 1 word omitted by [S.I. 2019/740 reg. 9\(5\)\(b\)](#)
- Annex point 2.2.1(c) word omitted by [S.I. 2019/740 reg. 9\(5\)\(c\)](#)
- Annex point 2.2.3 word omitted by [S.I. 2019/740 reg. 9\(5\)\(d\)](#)
- Annex point 4.1 word omitted by [S.I. 2019/740 reg. 9\(5\)\(i\)](#)
- Annex point 3.1 word substituted by [S.I. 2019/740 reg. 9\(5\)\(e\)](#)
- Annex point 4.2 words omitted by [S.I. 2019/740 reg. 9\(5\)\(j\)](#)
- Annex point 3.2 words substituted by [S.I. 2019/740 reg. 9\(5\)\(f\)](#)
- Annex point 3.4 words substituted by [S.I. 2019/740 reg. 9\(5\)\(g\)](#)
- Annex point 3.5 words substituted by [S.I. 2019/740 reg. 9\(5\)\(h\)](#)
- Annex point 3.4 words substituted by [S.I. 2023/793 reg. 4\(2\)](#)

Changes and effects yet to be applied to the whole legislation item and associated provisions

- Art. 1A inserted by [S.I. 2019/740 reg. 9\(3\)](#)
- Art. 1A words substituted in earlier amending provision [S.I. 2019/740, reg. 9\(3\)](#) by [S.I. 2020/1463 reg. 7\(6\)](#)