

ANNEX

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

1. Frequency and status of sampling
 - (a) The sampling frame shall cover all flocks of fattening and breeding turkeys covered by the scope of Regulation (EC) No 2160/2003.
 - (b) Flocks of turkeys shall be sampled on the initiative of the food business operator and by the competent authority.
 - (i) Sampling of flocks of fattening and breeding turkeys on the initiative of the food business operator shall take place in accordance with Article 5(3) of Regulation (EC) No 2160/2003 within three weeks before the birds are moved to the slaughterhouse. The results remain only valid until maximum six weeks after sampling and therefore repeated sampling of the same flock might be required.
 - (ii) Additionally, sampling of flocks of breeding turkeys on the initiative of the food business operator shall take place:
 - in rearing flocks: at day-old, at four weeks of age and two weeks before moving to the laying phase or laying unit,
 - in adult flocks: at least every third week during the laying period at the holding or at the hatchery.
 - (iii) Sampling by the competent authority shall include at least:
 - once a year, all flocks on 10 % of holdings with at least 250 adult breeding turkeys between 30 and 45 weeks of age but including in any case all holdings where *Salmonella enteritidis* or *Salmonella typhimurium* was detected during the previous 12 months and all holdings with elite, great grand parents and grand parent breeding turkeys; this sampling may also take place at the hatchery,
 - all flocks on holdings in case of detection of *Salmonella enteritidis* or *Salmonella typhimurium* from samples taken at the hatchery by food business operators or within the frame of official controls, to investigate the origin of infection,
 - once a year, all flocks on 10 % of the holdings with at least 500 fattening turkeys, but in any case:
 - all flocks on the holding when one flock tested positive for *Salmonella enteritidis* or *Salmonella typhimurium* in samples taken by the food business operator, unless the meat of the turkeys in the flocks is destined for industrial heat treatment or another treatment to eliminate salmonella, and
 - all flocks on the holding when one flock tested positive for *Salmonella enteritidis* or *Salmonella typhimurium* during the previous round in samples taken by the food business operator, and
 - each time the competent authority considers it necessary.

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

2. Sampling protocol

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

2.1. Sampling at the hatchery

Sampling shall occur at the hatchery in accordance with the provisions laid down in point 2.2.1 of the Annex to Regulation (EC) No 1003/2005⁽¹⁾.

2.2. Sampling at the holding

2.2.1. Breeding turkeys

Samples shall be taken in accordance with the provisions laid down in point 2.2.2 of the Annex to Regulation (EC) No 1003/2005.

2.2.2. Fattening turkeys

At least two pairs of boot/sock swabs shall be taken. For free range flocks of turkeys, samples shall only be collected in the area inside the house. All boot/sock swabs must be pooled into one sample.

In flocks with less than 100 turkeys, where it is not possible to use boot/sock swabs as access to the houses is not possible, they may be replaced by hand drag swabs, where the boot swabs or socks are worn over gloved hands and rubbed over surfaces contaminated with fresh faeces, or if not feasible, by other sampling techniques for faeces fit for the intended purpose.

Before putting on the boot/sock swabs, their surface shall be moistened with maximum recovery diluents (MRD: 0,8 % sodium chloride, 0,1 % peptone in sterile deionised water), or sterile water or any other diluent approved by the national reference laboratory referred to in Article 11 of Regulation (EC) No 2160/2003. The use of farm water containing antimicrobials or additional disinfectants shall be prohibited. The recommended way to moisten boot swabs shall be to pour the liquid inside before putting them on. Alternatively, boot swabs or socks may be autoclaved with diluents within autoclave bags or jars before use. Diluents may also be applied after boots are put on using a spray or wash bottle.

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

Alternatively, the competent authority may decide that one pair of boot swabs shall be taken, covering 100 % of the area of the house if combined with a dust sample, collected from multiple places throughout the house from surfaces with visible presence of dust.

On completion of sampling the boot/sock swabs shall be carefully removed 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 shall supervise education of the food business operators to guarantee the correct application of the sampling protocol.

In the case of sampling by the competent authority because of suspicion salmonella infection in a flock on that holding and in any other case considered appropriate, the competent authority shall satisfy itself by conducting further tests as appropriate so that the results of examinations for salmonella in flocks of turkeys are not affected by the use of antimicrobials in those flocks.

Where the presence of *Salmonella enteritidis* and *Salmonella typhimurium* is not detected but antimicrobials or bacterial growth inhibitory effect are detected it shall be considered as an infected flock of turkeys 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 preferably be sent by express mail or courier to the laboratories referred to in Articles 11 and 12 of Regulation (EC) No 2160/2003, within 24 hours after collection. If not sent within 24 hours, they shall be stored refrigerated. At the laboratory samples shall be kept refrigerated until examination, which shall be started within 48 hours following receipt and within 96 hours after sampling.

The pair(s) of boot/sock swabs 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. The boot/sock swabs shall be fully immersed in BPW and therefore more BPW may be added if necessary.

The dust sample shall preferably be analysed separately. However, the competent authority may decide to pool it with the pair of boot/sock swabs for analysis.

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

Other samples (e.g. from hatcheries) shall be prepared in accordance with the provisions laid down in point 2.2.2 of the Annex to Regulation (EC) No 1003/2005.

If ISO standards on the preparation of faeces for the detection of salmonella are agreed on, they shall be applied and replace the provisions on the preparation of samples set out in this point.

3.2. Detection method

The detection method recommended by the Community reference laboratory (CRL) for salmonella in Bilthoven, the Netherlands, shall be used.

That method is described in the Annex D of ISO 6579 (2002): 'Detection of salmonella spp. in animal faeces and in samples of the primary production stage'. The latest version of Annex D shall be used.

In that detection method, a semi-solid medium (modified semi-solid Rappaport-Vassiladis medium, MSRV) 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 on the initiative of the food business 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 points 3.1, 3.2 and 3.3 of this Annex, if validated in accordance with EN/ISO 16140/2003.

3.5. Storage of strains

Laboratories shall guarantee that at least one isolated strain of salmonella spp. per house and per year can be collected by the competent authority and stored for possible future phage typing 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

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

4.1. Detection of *Salmonella enteritidis* and/or *Salmonella typhimurium*

The laboratory shall immediately report each detection of *Salmonella enteritidis* and/or *Salmonella typhimurium* to the competent authority and give holding and flock references.

4.2. Calculation of prevalence for the verification of the Community target

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

Positive flocks of turkeys 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.

The prevalence shall be calculated separately for flocks of fattening turkeys and flocks of adult breeding turkeys.

4.3. Annual reporting

The annual reporting shall include:

- (a) the total number of flocks of fattening and adult breeding turkeys sampled by the competent authority or by the food business operator;
- (b) the total number of flocks infected with *Salmonella enteritidis* or *Salmonella typhimurium*, of fattening and adult breeding turkeys;
- (c) all serotypes of salmonella isolated (including other than *Salmonella enteritidis* and *Salmonella typhimurium*) and the number of flocks infected per serotype;
- (d) explanations of the results, in particular concerning exceptional cases.

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⁽³⁾.

4.4. Additional information

At least the following information shall be made available from each flock of turkeys tested for analysis at national level or by the European Food Safety Authority at its request:

- (a) sample taken by the competent authority or by the food business operator;
- (b) holding reference, remaining unique in time;
- (c) house reference, remaining unique in time;
- (d) month of sampling.

- (1) [OJ L 170, 1.7.2005, p. 12.](#)
- (2) [OJ L 165, 30.4.2004, p. 1, corrected version OJ L 191, 28.5.2004, p. 1.](#)
- (3) [OJ L 325, 12.12.2003, p. 31.](#)