ANNEX

Testing scheme necessary to ascertain the achievement of the Union target for the reduction of the relevant *Salmonella* serotypes in adult breeding flocks of *Gallus gallus*

1. SAMPLING FRAME

The sampling framework to detect the presence of *Salmonella enteritidis, Salmonella infantis, Salmonella hadar, Salmonella typhimurium* and *Salmonella virchow* (the relevant salmonella serotypes) shall cover all adult breeding flocks of domestic fowl (*Gallus gallus*) comprising at least 250 birds (breeding flocks). It shall be without prejudice to the provisions in Regulation (EC) No 2160/2003 and Directive 2003/99/EC as regards the monitoring requirements in other animal populations or other serotypes.

2. MONITORING IN BREEDING FLOCKS

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

Breeding flocks shall be sampled at the initiative of the food business operator and as part of official controls.

2.1.1. *Sampling at the initiative of the food business 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 all layer breeding flocks. However, sampling of breeding flocks laying hatching eggs intended for the trade within the Union must take place on the holding.

A procedure shall be set up to guarantee that the detection of the presence of the relevant *Salmonella* serotypes during sampling at the initiative of the food business operator is notified without delay to the competent authority by the laboratory performing the analyses. Timely notification of the detection of the presence of any of the relevant *Salmonella* serotypes shall remain the responsibility of the food business operator and the laboratory performing the analyses.

By way of derogation from the first paragraph of this point, if the Union target has been achieved for at least two consecutive calendar years in the whole Member State, sampling at the holding may be extended to take place every three weeks, at the discretion of the competent authority. However, the competent authority may decide to keep or revert to a two-week testing interval in the case of detection of the presence of the relevant *Salmonella* serotypes in a breeding flock on the holding and/or in any other case deemed appropriate by the competent authority.

2.1.2. *Sampling as part of official controls*

Sampling as part of official controls shall consist of:

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

(a) routine sampling every 16 weeks at the hatchery;

(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 taking place 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 the detection of the presence of the relevant *Salmonella* serotypes from sampling at the hatchery.

2.1.2.2. If sampling at the initiative of the food business 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) at any time during the production cycle which is sufficiently distant in time from the sampling referred to in points (a) and (b).

2.1.2.3. By way of derogation from points 2.1.2.1 and 2.1.2.2, and if the Union target has been achieved for at least two consecutive calendar years in the whole Member State, the competent authority may replace the routine samplings by sampling:

(a) at the holding on one occasion at any time during the production cycle and once a year at the hatchery; or

(b) at the holding on two occasions at any times which are sufficiently distant in time from each other during the production cycle.

However, the competent authority may decide to keep or revert to the sampling laid down in point 2.1.2.1 or 2.1.2.2 in the case of detection of the presence of the relevant *Salmonella* serotypes in a breeding flock on the holding and/or in any other case deemed appropriate by the competent authority.

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 must be arranged on a hatch day when samples from all breeding flocks are available. If not possible, it has to be guaranteed that samples are collected from every flock at least at the frequency laid down in point 2.1.

All material from all hatchers from which hatched chicks are removed on the sampling day shall contribute to the set of samples in a proportionate way.

If there are more than 50 000 eggs of one breeding 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 obtain a total sampling surface of at least 1 m^2; if the hatching eggs from a breeding flock occupy more than

one hatcher, then such a composite sample shall be taken from each hatcher up to a maximum of five; or

(b) one sample taken with one or several moistened fabric swab(s) of at least 900 cm^2 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 each hatcher up to a maximum of five 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 of broken eggshells taken from a total of 25 separate hatcher baskets, namely 250 g in the initial sample, in up to five hatchers with hatched eggs from the flock, crushed, mixed and sub-sampled to form a 25 g subsample for testing.

The procedure set out in points (a), (b) and (c) shall be followed for sampling at the initiative of the food business operator and as part of official controls. However, it shall not be mandatory to include a hatcher with eggs from different flocks if at least 80 % from 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 food business 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 poultry house in which the breeding flock is kept, or where the breeding flock has free access to more than one poultry house on a particular holding, from each group of houses on the holding in which the breeding 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 breeding flock	**Number of faeces samples to be taken in the breeding 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 produces representative samples for all parts of the poultry house or the respective sector. It shall include littered and slatted areas provided that slats are safe to walk on. All separate pens within a poultry house shall be included in the sampling. On completion of the 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 poultry 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 poultry house and an additional dust sample collected from multiple places throughout the poultry house from surfaces with visible presence of dust; one or several moistened fabric swab(s) of at least 900 cm^2 surface area in total must be used to collect the 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 scrape into a deep pit beneath the house;

(iii) droppings pit system in a step-cage poultry 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 breeding 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 poultry house 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.

(d) **[**[F1]In cage houses where a sufficient amount of faeces does not accumulate on scrapers or belt cleaners at the discharge end of belts, four or more moistened fabric swabs of at least 900 cm^2 per swab, 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, shall be used to swab as large a surface area as possible at the discharge end of all accessible belts after they have been run, ensuring

each swab is coated on both sides with faecal material from the belts and scrapers or belt cleaners.

(e) In multi-tier barn or free range houses in which most of the faecal material is removed from the house by dropping belts, one pair of boot swabs shall be taken by walking around in littered areas in accordance with point (b) and at least 2 moistened fabric swabs shall be taken as hand-held swabs from all accessible dropping belts, as in point (d).**]**

Textual Amendments

F1 Inserted 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).

2.2.2.2. *Sampling as part of official controls*

(a) Routine sampling shall be performed as described in point 2.2.2.1.

(b) Confirmatory sampling following the detection of the relevant *Salmonella* serotypes 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 poultry house of birds on the holding, normally up to five birds per house, unless the competent 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 the presence of the relevant *Salmonella* serotypes shall be carried out on the breeding 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) Suspicion of false results

In exceptional cases where the competent authority has reason to question the results of the testing (such as 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. **Transport and preparation of the samples**

3.1.1. *Transport*

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. Transportation can be at ambient temperature as long as excessive heat (over 25 °C) and exposure to sunlight are avoided. 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.

3.1.2. *Hatcher basket liners:*

(a) Place the sample in 1 litre of buffered peptone water (BPW) which has been pre-warmed at room temperature and mix gently.

(b) Continue the culture of the sample by using the detection method described in point 3.2.

3.1.3. *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 pre-warmed to room temperature.

(b) 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 must be made of the boot swabs and the fabric swab.

(c) Where five pairs of boot/sock swabs are pooled into two samples, each pooled sample must be placed in of 225 ml of BPW, or more if necessary, to fully submerge the sample and provide sufficient free liquid around the sample for migration of *Salmonella* away from the sample.

(d) Swirl to fully saturate the sample and continue the culture by using the detection method described in point 3.2.

3.1.4. *Other faecal material samples:*

(a) The faeces samples shall be pooled and thoroughly mixed and a 25 g sub-sample shall be collected for culture.

(b) The 25 g sub-sample shall be added to 225 ml of BPW which has been pre-warmed to room temperature.

(c) The culture of the sample shall be continued by using the detection method described in point 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 those referred to in points 3.1.2, 3.1.3 and 3.1.4 on sampling preparation.

[[F1]3.1.5.

In case of collection of fabric swabs in accordance with point 2.2.2.1(d) or one pair of boot swabs and 2 moistened fabric swabs in accordance with 2.2.2.1(e), pooling shall occur in accordance with point 3.1.3(b).**]**

3.2. **Detection method**

[[F2]The detection of *Salmonella* spp. shall be carried out according to EN ISO 6579-1.**]**

Textual Amendments

F2 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).

As regards the boot swabs samples, dust samples and other faecal material samples referred to in point 3.1, the incubated BPW enrichment broth for future culture may be pooled. To do so, incubate both samples in BPW as referred to in point 3.1.3. Take 1 ml of incubated broth from

each sample and mix thoroughly, then take 0,1 ml of the mixture and inoculate the modified semi-solid Rappaport-Vassiliadis (MSRV) plates.

The samples in BPW must not be shaken, swirled or otherwise agitated 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.

[[F2]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**

It shall be guaranteed that at least one isolated strain of the relevant *Salmonella* serotypes from sampling as part of official controls per house and per year are stored for possible future phagetyping or antimicrobial susceptibility testing, using the normal methods for culture collection, which must ensure integrity of the strains for a minimum period of two years. If the competent authority decides so, isolates from sampling by food business operators shall also be stored for these purposes.

4. RESULTS AND REPORTING

A breeding flock shall be considered positive for the purpose of ascertaining the achievement of the Union target:

— when the presence of the relevant *Salmonella* serotypes (other than vaccine strains) has been detected in one or more samples taken in the flock, even if the relevant *Salmonella* serotypes is only detected in the dust sample, or

— when the confirmatory sampling as part of official controls in accordance with point 2.2.2.2(b) does not confirm the detection of relevant *Salmonella* serotypes but antimicrobials or bacterial growth inhibitors have been detected in the flock.

This rule shall not apply in exceptional cases described in point 2.2.2.2(c) where the initial *Salmonella* positive result from sampling at the initiative of the food business operator has not been confirmed by the sampling as part of official controls.

A positive breeding flock shall only be counted once regardless of how often the relevant *Salmonella* serotypes has been detected in this flock during the production period or whether the sampling was carried out at the initiative of the food business operator or by the competent authority. However, if sampling during the production period is spread over two calendar years, the result of each year shall be reported separately.

Reporting shall include:

(a) a detailed description of the options implemented for the sampling scheme and the type of samples taken, as appropriate;

(b) the total number of adult breeding flocks comprising at least 250 birds which were tested at least once during the year of reporting;

(c) the results of the testing including:

(i) the total number of breeding flocks positive with any *Salmonella* in the Member State;

(ii) the number of breeding flocks positive with at least one of the relevant *Salmonella* serotypes;

(iii) the number of positive breeding flocks for each *Salmonella* serotype or for *Salmonella* unspecified (isolates that are untypable or not serotyped);

(d) the number of cases where the initial *Salmonella* positive sample from sampling at the initiative of the food business operator was not confirmed by the sampling as part of official controls;

(e) 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.

Changes to legislation:
There are outstanding changes not yet made to Commission Regulation (EU) No 200/2010. Any changes that have already been made to the legislation appear in the content and are referenced with annotations.
View outstanding changes

Changes and effects yet to be applied to :

- Annex heading word omitted by S.I. 2019/740 reg. 7(7)
- Annex point 2.1.1 word omitted by S.I. 2019/740 reg. 7(9)(b)(i)
- Annex point 2.1.2.3 word omitted by S.I. 2019/740 reg. 7(10)(a)
- Annex point 4 word omitted by S.I. 2019/740 reg. 7(13)(a)
- Annex point 1 words omitted by S.I. 2019/740 reg. 7(8)
- Annex point 4 words omitted by S.I. 2019/740 reg. 7(13)(b)
- Annex point 2.1.1 words substituted by S.I. 2019/740 reg. 7(9)(a)
- Annex point 2.1.1 words substituted by S.I. 2019/740 reg. 7(9)(b)(ii)
- Annex point 2.1.2.3 words substituted by S.I. 2019/740 reg. 7(10)(b)
- Annex point 3.2 words substituted by S.I. 2019/740 reg. 7(11)
- Annex point 3.4 words substituted by S.I. 2019/740 reg. 7(12)
- Annex point 3.4 words substituted by S.I. 2023/793 reg. 5(2)
- Annex point 2.1.1 words substituted in earlier amending provision S.I. 2019/740, reg. 7(9)(a) by S.I. 2020/1463 reg. 7(4)(b)

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

- Signature words omitted by S.I. 2019/740 reg. 7(5)
- Art. 1A inserted by S.I. 2019/740 reg. 7(3)
- Art. 1A words substituted in earlier amending provision S.I. 2019/740, reg. 7(3) by S.I. 2020/1463 reg. 7(4)(a)