ANNEX II

MICROBIOLOGICAL CONTROL PROGRAMME IN HATCHERIES AND DISEASE SURVEILLANCE PROGRAMMES IN ESTABLISHMENTS KEEPING POULTRY AND IN HATCHERIES

PART 1

Microbiological control programme in hatcheries as referred to in Article 7

Microbiological control programme for purpose of hygienic controls shall include the following:

(a) environmental samples must be collected and undergo a bacteriological examination;

(b) samples must be taken at least every six weeks and each sampling must include 60 samples.

PART 2

Diseases surveillance programmes in hatcheries as referred to in Article 7 and in establishments keeping poultry as referred to in Article 8

1. Objective of the diseases surveillance programmes

Demonstration that flocks kept on approved establishments keeping poultry are free from the disease agents listed in points 2 and 3.

The disease surveillance programmes shall, as a minimum, comprise of the disease agents and the listed kept species referred to in point 2.

2. Disease surveillance for *Salmonella* serotypes of animal health relevance

2.1. Identification of infection with the agents:

(a) *Salmonella* Pullorum: covering *Salmonella enterica* subspecies *enterica* serovar Gallinarum biochemical variant (biovar) Pullorum;

(b) *Salmonella* Gallinarum: covering *Salmonella enterica* subspecies *enterica* serovar Gallinarum biochemical variant (biovar) Gallinarum;

(c) *Salmonella arizonae*: covering *Salmonella enterica* subspecies arizonae serogroup K (O18) arizonae.

2.2. Target poultry species:

(a) for *Salmonella* Pullorum and *Salmonella* Gallinarum: *Gallus gallus*, *Meleagris gallopavo, Numida meleagris, Coturnix coturnix, Phasianus colchicus, Perdix perdix, Anas* spp.;

(b) for *Salmonella arizonae*: *Meleagris gallopavo*.

2.3. Examinations:

Each flock must be clinically examined during each laying or productive period at the best time for detecting the disease in question.

2.4. Sampling matrix:

(a) samples must be taken from each flock in the establishment keeping poultry, as appropriate:

(i) for serological testing: blood;

(ii) for bacteriological testing:

— post mortem tissues, especially liver, spleen, ovary, oviduct and ileo-caecal junction,

— environmental samples,

— swabs from the cloaca of live birds, in particular from those that appear sick or that have been identified as highly sero-positive;

(b) samples to be taken in the hatchery for bacteriological testing:

(i) chicks that fail to hatch (namely embryos dead-in-shell);

(ii) second grade chicks;

(iii) meconium of chicks;

(iv) down or dust from hatchers and from the walls of the hatchery.

2.5. Sampling frame and frequency of sampling:

(a) in the establishment keeping poultry:

(i) sampling for *Salmonella* Pullorum and *Salmonella* Gallinarum:

Species	**Time of sampling**		**Number of birds to be sampled/ Number of environmental samples**
	Breeding poultry	**Productive poultry**	
Gallus gallus, Meleagris gallopavo, Numida meleagris, Coturnix coturnix, Phasianus colchicus, Perdix perdix and *Anas spp*	At the point of lay	During production at least once a year	60

(ii) sampling for *Salmonella arizonae:*

Species	**Time of sampling**		**Number of birds to be sampled/ Number of**
	Breeding poultry	**Productive poultry**	

			environmental samples
Meleagris gallopavo	At the point of lay	During production at least once a year	60

(iii) the number of birds to be sampled in accordance with points (i) and (ii) may be adapted by the competent authority to the known prevalence of infection in the specific Member State concerned and its past incidence in the establishment. In any case a statistically valid number of samples for serological/bacteriological testing to detect infection shall always be taken;

(b) in the hatchery, samples shall be collected and examined at least once every 6 weeks. The testing shall include at least:

(i) one pooled sample of down and meconium from chicks from each hatcher;

and

(ii) sample of:

— either 10 second grade chickens and 10 dead-in-shell chickens from every flock of origin present in a hatcher on the day of sample collection,

or

— 20 second grade chickens from every flock of origin present in a hatcher on the day of sample collection.

2.6. Processing of samples and testing methods:

(a) samples collected must be subject to:

(i) serological testing[(1)];

(ii) bacteriological testing either as an alternative or in addition to serological testing referred to in point (i); however, samples for bacteriological testing must not be taken from poultry or eggs that have been treated with antimicrobial medicinal products during the two to three weeks prior to testing;

(b) samples collected must be processed as follows:

(i) direct enrichment in Selenite-cysteine broth for faecal/meconium and intestinal samples or other appropriate media suitable for samples where competing flora is expected;

(ii) non-selective pre-enrichment followed by selective enrichment in soya based Rappaport-Vassiliadis (RVS) broth or Müller-Kauffmann Tetrathionate-Novobiocin broth (MKTTn) for samples (such as embryos dead-in-shell) where competing flora is expected to be minimal;

(iii) direct plating of aseptically collected tissues on to a minimally selective agar, such as MacConkey agar;

(iv) *Salmonella* Pullorum and *Salmonella* Gallinarum do not readily grow in the modified semi-solid Rappaport Vassiliadis (MSRV) medium that is used for monitoring of zoonotic *Salmonella* spp. in the Union, but it is suitable for *Salmonella arizonae*;

(v) detection techniques must be capable of differentiating serological responses to *Salmonella* Pullorum and *Salmonella* Gallinarum infection from serological responses due to the use of *Salmonella* Enteritidis vaccine, where this vaccine is used[(2)]. Such vaccination must therefore not be used if serological monitoring is to be used. If vaccination has been used, bacteriological testing must be used, but the confirmation method used must be capable of differentiating live vaccinal strains from field strains.

2.7. Results:

A flock is considered positive when, following the positive results of the testing performed in accordance with points 2.3 to 2.6, a second test of an appropriate type confirms the infection by the disease agents.

3. Diseases surveillance for *Mycoplasma* spp. of relevance for poultry:

3.1. Identification of infection with the following agents:

(a) *Mycoplasma gallisepticum*;

(b) *Mycoplasma meleagridis.*

3.2. Target species:

(a) *Mycoplasma gallisepticum: Gallus gallus, Meleagris gallopavo;*

(b) *Mycoplasma meleagridis: Meleagris gallopavo.*

3.3. Examinations:

Each flock must be clinically examined during each laying or productive period at the best time for detecting the disease in question.

3.4. Sampling matrix:

Samples to be taken from each flock in the establishment keeping poultry, as appropriate:

(a) blood;

(b) sperm;

(c) swabs taken from the trachea, the choanae or the cloaca;

(d) post mortem tissues, especially air sacs from day-old chicks with lesions;

(e) in particular for the detection *of Mycoplasma meleagridis*, oviduct and penis of turkeys.

3.5. Sampling frame and frequency of sampling:

(a) sampling for *Mycoplasma gallisepticum:*

Species	**Time of sampling**		**Number of birds to be sampled**
	Breeding poultry	**Productive poultry**	
Gallus gallus	• at 16 weeks of age • at the point of lay • and then every 90 days	During production every 90 days	• 60 • 60 • 60
Meleagris gallopavo	• at 20 weeks of age • at the point of lay • and then every 90 days	During production every 90 days	• 60 • 60 • 60

(b) sampling for *Mycoplasma meleagridis:*

Species	**Time of sampling**		**Number of birds to be sampled**
	Breeding poultry	**Productive poultry**	
Meleagris gallopavo	• at 20 weeks of age • at the point of lay • and then every 90 days	During production every 90 days	• 60 • 60 • 60

(c) the number of birds to be sampled according to points (a) and (b) may be adapted by the competent authority to the known prevalence of infection in the specific Member State concerned and its past incidence in the establishment. In any case a statistically valid numbers of samples for serological/bacteriological testing shall always be taken.

3.6. Examinations, sampling and testing methods:

Testing for the presence of infection by serological, bacteriological and molecular tests must be carried out by validated methods recognised by the competent authority.

3.7. Results:

A flock is considered positive when, following the positive results of the testing performed in accordance with points 3.3 to 3.6, a second test of an appropriate type confirms the infection by the disease agents.

PART 3

Additional information on diagnostic techniques

Laboratories that have been designated by the competent authority to carry out the testing as required in Parts 1 and 2 of this Annex may consult the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Organisation for Animal Health (OIE), Edition 2018 for further detailed description of the diagnostic techniques.

(**1**) Occasionally in avian species other than *Galliformes* serological testing results in an unacceptable proportion of false-positive reactions.

(**2**) There is currently no test that can differentiate between the response to *Salmonella* Pullorum and *Salmonella* Gallinarum infection and vaccination for this serotype.

Changes to legislation:
There are currently no known outstanding effects for the Commission Delegated Regulation (EU) 2019/2035, ANNEX II.