

NORMATIVE INSTRUCTION No.44, OF AUGUST 23rd, 2001

THE SECRETARY OF ANIMAL AND PLANT HEALTH AND INSPECTION OF THE MINISTRY OF LIVESTOCK AND FOOD SUPPLY, using the powers invested in him in article 83, clause IV, of the Internal Regulation of the Secretariat, approved by Ministerial Ordinance No.574, of December 8th, 1998, in consideration and in light of the provisions of the Ministerial Ordinance No.193, of September 19th, 1994, and in File MA 21000.005233/2001-68, decide:

Article I To approve the Technical Rules for the Control and the Certification of Aviary Centers and Establishment for the Aviary Mycoplasmosis (*Mycoplasma gallisepticum*, *synoviae* and *melleagridis*), according to the ATTACHMENT of this Normative Instructio.

Article II Revoke the SDA Normative Instruction No.13, of June 29th, 1999.

Article III This Normative Instruction will be in effect on the date of its publication.

LUIZ CARLOS DE OLIVEIRA

ATTACHMENT

TECHNICAL RULES FOR THE CONTROL AND CERTIFICATION OF AVIARY CENTERS AND ESTABLISHMENTS FOR AVIARY MYCOPLASMOSIS (*Mycoplasma gallisepticum*, *synoviae* and *melleagridis*).

CHAPTER I

INTRODUCTION

1. These rules define the measures of monitoring salmonellosis in aviary establishments permanently and eventually controlled (except commercial position, chicken for slaughter and ratites), that perform the commercial, or domestic and international transference of derived products, intended for breeding and for the production of poultry and hatching eggs, having to perform monitoring of their plants, obeying the directions of the National Avian Health Surveillance Program (PNSA).
2. To operate in the international market, the aviary establishment must be certified as free for aviary mycoplasmosis (*Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Mycoplasma melleagridis*), as established in Chapter IV of this ATTACHMENT.
3. The importing businesses or purchasers of genetic material of pure breeds, great grandparents and grandparents, must present the guarantee or certification as to free of the constant salmonellas in these rules.

CHAPTER II

DEFINITIONS

1. For the purpose of these rules, it is understood:
 - 1.1. Batch: group of poultry for the same purpose, origin and age, lodged in one or many barns.
 - 1.2. Boxes: are physical sections divided inside a barn.
 - 1.3. Barn: is the aviary production unit, characterized as the unit of a center, which lodges a group of breeders, poultry for slaughter or commercial laying hens, of the same age (with exception of pure breeds, genetically selected), and of the same breed.
 - 1.4. Commercial poultry: generation of poultry meant for slaughter and/or egg production for consumption.
 - 1.5. Center of reproduction: is the unit with an area adequately isolated, of common handling, constituted of one or more barns.
 - 1.6. Aviary establishment: is the location where poultry is kept for any purpose, which may be constituted of one or more centers.
 - 1.6.1. Aviary Establishment Permanently Controlled: they are the farms, genetically selected, of primary breeders (pure breeds), great grandparent farms, grandparent farms, head office farms, farms of breeding poultry free of specific pathogens (SPF), and the incubators of these establishments.
 - 1.6.2. Aviary establishments controlled eventually: they are the aviary establishments producing commercial eggs, poultry for slaughter, exploring other wild birds, or ornamental, and/or exotic, and the incubators of these establishments.
 - 1.7. Official service: Federal, State, and Municipal Animal Health Protection Service.
 - 1.8. Official laboratories: are the laboratories of the Ministry of Agriculture, Livestock and Food Supply (MAPA).
 - 1.9. Certified laboratories: federal, state, municipal or private laboratory units, qualified and recognized by the MA, to perform laboratory diagnosis of disease agents to which these rules refer to.
 - 1.10. Federal Inspector or Official Veterinarian: professional veterinarian of the Federal Public Service, who performs activities of Animal Health Protection.
 - 1.11. Official Veterinarian: Federal Inspector or Veterinarian of the Federal Public Service.
 - 1.12. Official Veterinarian for Certification: Federal Inspector or Official Veterinarian of the Animal Health Protection Service.
 - 1.13. Official Certified Veterinarian: Veterinarian of the state, district, private sector or independent professional, with authorization of jurisdiction of the federal official service, to issue the Animal Transit Guide – GTA.
 - 1.14. Responsible Technician: Veterinarian responsible for the hygienic-health control of the flocks of the aviary centers or premises.

- 1.15. Monitoring of Flocks: is the health supervision of the serological tests and of other biological exams, as well as the epidemiological analysis related to the health conditions of the birds lodged at an aviary center or premises.
- 1.16. MA : Ministry of Agriculture, Livestock and Food Supply
- 1.17. SDA : Secretariat of Animal and Plant Health and Inspection
- 1.18. DDA : Animal Defense Department
- 1.19. CLA : Animal Laboratory Coordinating Unit
- 1.20. PNSA : National Plan of Poultry Health
- 1.21. DIPOA : Animal Origin Products Inspection Department.
- 1.22. DFA : Federal Agricultural Delegacy
- 1.23. SSA : Animal Health Service
- 1.24. SIF : Federal Inspection Service

CHAPTER III

DEMANDS

1. To attend to the PNSA, the aviary establishments of permanent and eventual controls must:
 - 1.1. Acquire registry and licensing at the DFA of the jurisdiction.
 - 1.2. Be under the surveillance and control of the local Animal Health Service of the DFA or of the State Secretariat of Agriculture.
 - 1.3. Be supervised by the TECHNICIAN in charge, registered at the Federal Agriculture Delegacy of the State.
2. The aviary establishment with participation of the PNSA will not be able to use:
 - 2.1. any vaccine for aviary mycoplasmosis at permanently controlled premises.
 - 2.2. any vaccine prepared with oily adjuvant during the four weeks prior to the laboratory tests;
 - 2.3. any drug scientifically proven to interfere in the results of serological tests, and/or interfere with the isolation of the salmonellas in a period of three weeks prior to the tests;
 - 2.4. the exceptional cases must be evaluated by the DDA/SDA, as long as it is presented and approved by a specific technical-scientific project.
3. Only antigen, control serums and “kits” approved by the MAPA, can be used, as per the expiration date.
4. Laboratory proof will only be able to be used when properly approved by the PNSA.
5. The aviary establishment must provide monthly a calendar of collections, which will be forwarded to the DFA of the State where it is located, along with the schedules of birth, importation and dates of routine sample collection performed by the TECHNICIAN in charge, focusing on the inspection and the supervision of the official service.

CHAPTER IV

CERTIFICATION

1. Certification for the pure breed aviary centers or premises, great grandparent and grandparent hatcheries.
 - 1.1. Free of chicken *Mycoplasma gallisepticum* and *Mycoplasma synoviae*.
 - 1.2. Free of turkey *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Mycoplasma melleagridis*.
2. Certification of the centers (aviary establishment of parent hatcheries).
 - 2.1. Free for chicken *Mycoplasma gallisepticum*.
 - 2.2. Free of turkey *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Mycoplasma melleagridis*.
 - 2.3. Under supervision and monitoring for chicken *Mycoplasma synoviae*.

CHAPTER V

LABORATORY EXAMS

1. The exams used for laboratory monitoring and diagnostic in the different stages of the process are:
 - 1.1. Immunological diagnosis:
 - 1.1.1. Fast plate agglutination with serum or yolk from embryonated eggs.
 - 1.1.2. Slow agglutination with serum (SAL), or yolk from embryonated eggs.
 - 1.1.3. Inhibition of the Hemagglutination, (IH).
 - 1.1.4. Enzyme linked immuno sorbent assay (ELISA)
 - 1.2. Mycoplasmalogic diagnosis:
 - 1.2.1. Isolation in culture medium.
 - 1.2.2. Chain reaction of polymerase (PCR).
 - 1.3. Identification of culture:
 - 1.3.1. Indirect Immunofluorescence (IIF).
 - 1.3.2. Direct immunofluorescence (DIF).
 - 1.3.3. Inhibition of metabolism (IM).
 - 1.3.4. Inhibition of Growth (IG)
 - 1.3.5. Chain reaction of polymerase (PCR).
2. The completion and interpretation of the tests mentioned above, will obey the criterions established in specific technical rules and regulations of the MA.
3. The laboratory exams will only be accepted when performed at an official laboratory, and/or certified by the MAPA, identifying the antigen, the starting number, and the amount used.
4. Other laboratory exams may be used as long as they are approved by the DDA/SDA/MA.

CHAPTER VI

COLLECTION OF SAMPLES

1. The collections for official monitoring will only be accepted when performed by a federal inspector, an official veterinarian, or under the supervision of one of them.
2. To receive certification, the samples will be analyzed by the SSA/DFA of the State in which the establishment is located, the samplings will be forwarded by the company TECHNICIAN in charge before the MA, and/or the random collection performed by the official service.
3. All material forwarded for laboratory examining must officially be forwarded with the standard DDA/SDA collection form, filled out correctly and signed by the TECHNICIAN in charge before the MA, by the federal inspector, or by the official veterinarian.
4. The official sampling must be performed randomly among the barns in the same center for, serological tests, biological exams on SPF poultry, on embryonated eggs, or on mycoplasmaologic exams.
5. With the focus on monitoring the health status, certification maintenance and of regular collections at pure breed premises, great grandparent and grandparent hatcheries, this stage must be performed directly by the federal inspector, or by the official veterinarian, by random double sample collections, at least once a year, being later forwarded for analysis at official or certified laboratories.
6. DFA's criteria as to the Animal Health Service, and/or of the State Secretariat of Agriculture, of the State in which the aviary establishment is located in, at any time and in the presence of the federal inspector, or of an official veterinarian, random double samplings may be collected to be submitted for laboratory exams, respecting the criterions and rules of biosafety, at official or MAPA certified laboratories.
7. The official monitored material for this matter could be sent to any MAPA certified laboratory for this matter, chosen by the federal inspector, or by the official veterinarian responsible for the collection. .
8. The MA will establish a system for the random selection of samples and of official and certified laboratories, which will be supervised by a federal inspector or by an official veterinarian responsible for the collection.
9. The costs for the official collections and for the shipping of this material to be analyzed by certified laboratories will be at the expense of the requesting company.
10. Al the material officially collected must be sealed and sent along with a standard DDA/SDA form.
11. Random samplings performed by the official service may or not meet companies' schedules, of which a federal inspector, or an official veterinarian are responsible for, and also responsible for collecting or supervising the process, and for securing the material, being of responsibility of the company to provide the necessary means and materials for this operation.
12. The same criterions used for parent hatcheries will be applied for ornamental and wild poultry.

CHAPTER VII

PERFORMING LABORATORY EXAMS

1. The procedure of laboratory exams per batch for the Certification of aviary centers or establishment free for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* for

chickens, and *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Mycoplasma melleagridis* for turkeys, in light of the provisions in Chapter IV, will be composed of:

1.1. Breeding stock or hatching eggs and commercial production for restocking imported aviary flocks:

1.1.1. The sample collection will be performed on arrival, and the laboratory tests performed as set forth to the specific rules for poultry and hatching egg importation and exportation, intended for restocking aviary flocks.

1.1.1.1. When dealing with live or dead poultry, serological and/or mycoplasma techniques will be used depending on the case.

1.1.1.2. When dealing with eggs, yolk agglutination from embryonated eggs and mycoplasma exams may be used,

1.1.2. The poultry produced from pure breeds, and great grandparent hatcheries born in Brazil, will follow the same procedure mentioned in item 1.1.1., having its first collection performed in the incubatory at the moment of birth, by the SSA/DFA in the State where it is located, and sent to the official laboratory.

1.2. Health monitoring of aviary flocks

1.2.1. On 12 (twelve) week old breeding stocks:

1.2.1.1. On chickens and turkeys: SAR of at least three hundred samples for *Mycoplasma gallisepticum* and one hundred samples for *Mycoplasma synoviae*, selected randomly, with the presentation of each barn, and/or box per each supplemented center, when concerning reagents with HI or ELISA.

1.2.2. On breeding stocks at the beginning of production, with posture of about 5%:

1.2.2.1. SAR on one hundred and fifty samples per center, for *Mycoplasma gallisepticum* and one hundred for *Mycoplasma synoviae* for chicken.

1.2.2.2. SAR on one hundred and fifty samples per center, for *Mycoplasma gallisepticum* and *Mycoplasma melleagridis* and one hundred for *Mycoplasma synoviae* for turkeys.

1.2.2.3. When tested positive for HI or ELISA, collect tracheal swabs from twenty birds for confirmation per cultivation and/or PCR at certified or official laboratory, chosen by the official animal health protection service.

1.2.3. Establishment permanently controlled (periodic control every three months).

1.2.3.1. SAR on one hundred and fifty samples per aviary center, randomly selected and with the presentation of each barn and/or box from the center, for *Mycoplasma gallisepticum* and *Mycoplasma melleagridis*, the last one exclusively for turkeys, and

one hundred samples for *Mycoplasma synoviae*, supplementary when as reagents, with HI and ELISA. The tests must be permanently until it is totally eliminated from the batch, allowing a variation of up to two weeks during the intervals, to comfortably adapt blood collecting to other handling procedures.

1.2.3.2. When tested positive for HI or ELISA, collect tracheal swabs and serum from twenty birds for confirmation per cultivation and/or PCR at certified or official laboratory, chosen by the official service.

1.2.4. Establishment eventually controlled, except commercial poultry, chicken for slaughter (periodic control every three months):

1.2.4.1. SAR per center of one hundred and fifty serum samples from poultry randomly selected and with presentation of each barn and/or box of the center for *Mycoplasma gallisepticum*, *Mycoplasma melleagridis* and *Mycoplasma iowa*, according to Chapter IV of this rule, and one hundred samples for *Mycoplasma synoviae*, supplemented when reagents with HI and ELISA, and repeated every three months until full elimination from the batch, allowing a variation of about two weeks to comfortably adapt the blood collection to other handling procedures.

1.2.4.2. On breeding stocks, where there is no possibility of using swabs, proceed to randomly collect from three birds within every thousand, as long as the minimum is ten and the maximum of twenty per center.

2. On other breeding stocks, the recommended laboratory exams are of mycoplasmas.
3. In cases of death during the first days of the batch, the aviary establishment must forward material from thirty rejected or agonizing birds to a official or MA certified laboratory for isolation of mycoplasmas or PCR.

CHAPTER VIII

INTEPRETATION OF THE RESULTS

1. On poultry or hatching eggs from pure breeds, imported great grandparent and grandparent hatchers born in Brazil:
 - 1.1. Positive for *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, sacrifice/slaughter of the center.
 - 1.2. Positive for *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma melleagridis*, exclusive for turkeys, according to Chapter IV of this rule, sacrifice/slaughter at the center.
2. Parent Hatcheries:
 - 2.1. If tested positive for *Mycoplasma gallisepticum* on chickens or *Mycoplasma galisepticum*, *Mycoplasma synoviae* or *Mycoplasma melleagridis* on turkeys, there will be a slaughter and slaughter at the center, and the extinguishing of all eggs, incubating or not, originated there, according to Chapter IV of his rule.

- 2.1.1. Until receiving the results from the tests above, all the batches or centers will be isolated, also prohibited for incubation.
- 2.2. Two evaluations should be used, considering the free centers or under surveillance, and monitoring for *Mycoplasma synoviae*.
 - 2.2.1. If tested positive for *Mycoplasma synoviae* on chickens, these centers may be treated with antibiotics and retested after the antibiotic residue is eliminated from the system.
 - 2.2.2. The centers considered under surveillance and inspected for *Mycoplasma synoviae* cannot be commercialized internationally, maintaining production and incubation of the center under surveillance and supervision until the final productive cycle.
 - 2.2.3. The establishment considered under surveillance and controlled must reinforce measures of biosafety such as:
 - 2.2.3.1. To be protected by security fences with one access only, with a wash and disinfecting system for vehicles.
 - 2.2.3.2. To have available criterions for the strict transit control, and of access to people (gates, doors, receptions, concrete walls and other).
 - 2.2.3.3. To have internal surfaces set up in a way that allows adequate cleaning and disinfection;
 - 2.2.3.4. To dispose of ways legally approved by the MA, and by the departments of environmental control, for the destination of production residues (dead poultry, manure, egg remains, packaging, etc.) and others;
 - 2.2.3.5. to isolate different age categories, by means of fencing and /or by means of non fruit-trees, with a restricted single access, controlled flow, measures of biosafety for the internal area, for vehicles, personnel and material;
 - 2.2.3.6. To allow the entrance of people, vehicles, equipment and material in the internal areas of the property, only when strict biosafety measures are complied;
 - 2.2.3.7. Must apply control measures of liquid effluents, by means of septic tanks, observing the distance of the water paths and groundwater, to avoid contaminations.
 - 2.2.3.8. Physical-chemical and microbiological water control, performed at a public laboratory.

CHAPTER IX FORWARDING RESULTS

1. The laboratory test results must be issued on a standard form, and communications according to the flowchart established:
 - 1.1. Negative result: send FAX or use other means for the immediate communication to the requesting official veterinarian, and to the aviary premises.

- 1.2. Positive result: send FAX or other type of immediate documentation to the DDA and to the SSA/DFA, where the establishment is located, who will do the same.

CHAPTER X CERTIFICATION OF PREMISES

1. When the result from the laboratory tests referred in Chapter V of these rules test negative, the official service may proceed with the certification of the aviary center and/or premises, as established in Chapter IV.
2. The collections for the monitoring and certification will be accepted when performed by the responsible technician before the MA and by the official service, being that the collections are exclusively performed by the federal inspector, or by the official veterinarian, or inspected and supervised by one of them.
3. Even after having complied with all previous requirements, in cases of death during the first days of the batch, the aviary establishment must forward material from thirty rejected or agonizing birds to a official or certified laboratory for isolation of mycoplasmas or PCR. If the diagnosis is confirmed, the poultry from the center will be sacrificed, when dealing with pure breeds, great granparent and grandparent hatchers, followed by an epidemiological investigation by the official service.
4. For parent hatchers, the treatment will be accepted and retested when positive for *Mycoplasma synoviae*.
5. The aviary establishment being certified as free will only be authorized to commercialize poultry or hatching eggs from this center. The aviary establishment that receives the infection free or controlled certificate, will be able to proceed with the commercialization of poultry and/or hatching eggs from all the centers.
6. The aviary establishment that is under surveillance and supervision for *Mycoplasma synoviae* cannot commercialize its products internationally (hatching eggs and chicks from the referred center).
7. A MA standard Health Certificate will be issued by the DFA of the State of the aviary establishment after performing the minimum of three tests, to the to the establishment or centers free or under surveillance and supervision for the agents treated in this rule.
8. This certificate will have its validity subject to performing the maintenance regarding the health issue of the aviary center or premises.
9. If the flock's health situation is altered, the certificate will be revoked, with the option of reinstating the certificate after the evaluation of the SSA/DFA, and/or of the State Secretariat of Agriculture of the state in which the aviary establishment is located.

CHAPTER XI GENERAL TERMS

1. The serological laboratory exams are always selected, with the possibility of occurring nonspecific crossed reactions. Therefore, only mycoplasmologic diagnostic is considered conclusive for the detection of the mycoplasmas referred in these rules.

2. If the centers that tested positive for the agents of these rules are to be slaughtered, it should be performed at slaughterhouses with the SIF, according to the rules of the DIPOA.
3. The monitoring of ratites will be performed according to the specific legislation of the MA for the hygiene-health registry and control for this species.
4. Biosafety measures for incubation:
 - 4.1. It is prohibited to incubate hatching eggs from pure breeds, great grandparent, grandparent, and parent hatchers that are under surveillance and official supervision in the same machine and in the same period, following the criterions of the superior provenance.
 - 4.2. It is prohibited to incubate eggs in the centers that are under surveillance and monitored for *Mycoplasma synoviae* in the same machine, and during the same period in which centers that are free of this agent are incubating.
5. The SSA/DFA at which the aviary establishment is located and the proper State Secretariat of Agriculture are responsible, for this field and jurisdiction, for the definition of appropriate measures for solving health issues, as established in the Regulation of Animal Health Protection and in the PNSA/SDA.
3. The relevant questions as to the application of this normative will explained by the Director of the Department of Animal Protection of the Secretariat of Animal and Plant Health and Inspection, of the Ministry of Livestock and Food Supply.

RECTIFICATION

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MAY 6th, 2002, SECTION 1 PAGE 85 (OF.EL No.No.55/2002)

In the Attachment of Normative Instruction No.44, of August 23rd, 2001, published in the Official Gazette of the Federal Executive No.163, Section 1, page 68, of August 24th, 2001, where it reads: TECHNICAL RULES FOR THE CONTROL AND CERTIFICATION OF AVIARY CENTERS AND ESTABLISHMENT FOR AVIARY MYCOPLASMOSIS (*Mycoplasma gallisepticum*, *synoviae* and *melleagridis*) please read: *Mycoplasma gallisepticum*, *M. synoviae* and *M. mellagridis*; in Chapter VII, item 1.2.4.1., where it reads: ... *Mycoplasma galisepticum*, *Mycoplasma mellagridis* and *Mycoplasma iowa*, please read: ... *Mycoplasma gallisepticum* and *Mycoplasma mellagridis*; and in Chapter VIII, where it reads: INTERPRETATION OF THE RESULTS, please read: INTERPRETATION OF THE RESULTS AND INTEGRATION OF MEASURES OF BIOSAFETY AND HEALTH CONTROL.

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