Avian influenza diagnostic manual

approval of the Diagnostic Guide to Avian Influenza, as stipulated in the Council Directive 2005/94/EC (notified under document number C (2006) 3477) (Text with relevance of the EEA)COMMISSION EUROPEANS, 2005/94/EC Directive of 20 December 2005, Community Action against Avian Influenza and the Repeal of Directive 92/40/EEC (1), and, in particular, Article 50 (1) of them,while:HAS ADOPTED THIS DECISION:Diagnostic guidance, as stipulated in the 2005/94/EC Directive and is uttered in the annex to this decision, is approved. Member States have been applying diagnostic guidance since the date of their postponement of the 2005/94/EC Directive or from 1 July 2007, depending on which date is an earlier date. This decision is addressed to Member States. DIAGNOSTIC DIAGNOSTIC FOR AVIAN INFLUENZA Chapter I Introduction, Purpose and Definition 1. In order to ensure unified procedures for diagnosing avian influenza (AI) in the Community, this diagnostic guide sets out: (a) guidelines and minimum requirements for diagnostic procedures, sampling methods and criteria for evaluating laboratory test results for correct AI diagnosis; (b) Laboratory tests to be used to diagnose AI and laboratory methods to be used for genetic input of AI virus isolates; (c) Minimum biosecurity requirements and quality standards to be met by diagnostic laboratories and for the transport of samples. 2. This diagnostic guide is addressed to the bodies responsible for controlling AI. Therefore, this is mainly the case with the principles and application of laboratory tests and the evaluation of their results, as well as laboratory methods. 3. For the purposes of this diagnostic manual, in addition to the definitions in article 2 of the 2005/94/EC Directive, the following definition is applied: a diagnostic sample means any material of animal origin, including a whole carcass transported for diagnostic or investigative purposes, but excluding live infected animals. 4. Confirmation of AI in poultry and other birds, dead birds, should be in accordance with procedures, sampling methods and criteria for evaluating laboratory test results established in this diagnostic manual, and based on one or more criteria in paragraphs (a), (b) and (c): (c) the detection of an infectious virus, antigen or specific genetic material in samples of poultry tissue or other birds, organs or other birds; (b) Detection of clinical signs and post-mortem lesions in these birds; (c) Demonstration of a specific antibody reaction in the blood samples of these birds. 5. Confirmation of mammals infection with avian influenza A virus, which is either highly pathogenic or if the low pathogenic subtype H5 or H7 should be based on one or criteria in the (a) or (b): (a) detection of an AI virus, antigen or specific genetic material in samples of tissue, organs, blood or mammalian excrement; (b) Demonstration of the specific response of antibodies to AI in mammalian blood samples. Procedures, sampling methods and criteria for evaluating laboratory test results should be: (a) procedures established in this diagnostic manual; or (b) Authorized by the competent authority, provided that: (i) the sensitivity and specificity of authorized laboratory tests have been demonstrated as effective after a comparative test organized by the Community Avian Influenza Reference Laboratory (community reference laboratory); or (ii) Where the Community Reference Laboratory has not organized such an assessment for a particular type of laboratory test, the sensitivity and specificity of the authorized laboratory has been confirmed by the national reference laboratory to ensure that the laboratory test meets the purpose for which it is used; the results of such a review should be submitted to the Community Reference Laboratory for consideration. Chapter II Description of AI with a focus on differential diagnosis 1. Etiology and VIRULENCE AI is a highly contagious viral infection caused by the orthomyxoviridae family viruses, a genus influenzavirus A. Influenza A viruses are the only orthomicsoviruses known for infecting birds. Many bird species have been shown to be infected with influenza A viruses; aquatic birds form the main reservoir of such viruses, but the vast majority of isolates have low pathogenicity in chickens and turkeys, the main birds of economic importance that will be affected by the disease. Influenza A viruses have antigenically associated nucleoproteins and antigenicly associated matrix proteins, but are classified into subtypes based on the antigenic kinship of surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). Currently, 16 HA subtypes (H1-H16) and 9 NA subtypes (N1-N9) have been recognized. Each Influenza A virus has one HA and one NA antigen, apparently in any combination. Influenza A viruses are divided into two groups based on their ability to cause disease in susceptible poultry: (a) highly pathogenic avian influenza viruses (HPAI), which cause a very serious disease, characterized by a generalized infection of infected poultry, where they can cause very high herd mortality (up to 100%); and (b) low pathogenic avian influenza (LPAI) viruses that cause mild herd mortality (up to 100%); primarily respiratory diseases in poultry unless there is an aggravation of other co-infections or factors. Wild birds, especially migratory waterfowl, play a very important role as a reservoir of influenza A virus, as evidenced by the isolation of almost all possible HA and NA subtypes from wild birds. Birds. except when hpAI has been overflowing from infected poultry, only LPAI viruses are detected in wild birds. The initial introduction of AI viruses at poultry farms is likely to come from direct or indirect contact with wild birds. Poultry is likely to have such LPAI viruses introduced from a wild reservoir may circulate undetected, as clinical signs are often mild or non-existent. After the introduction of H5 and H7 subtypes into the bird, the LPAI subtypes can mutate into HPAI strains. To date, it has been shown that only H5 and H7 subtype viruses are caused by HPAI. Although it seems that several mechanisms may be responsible for the mutation from LPAI to the HPAI virus, the factors that cause this mutation are not known. In some cases, mutations appear to have occurred rapidly in the primary area after introduction from wild birds, in other cases the LPAI virus circulates in poultry for months before mutating. Therefore, it is impossible to predict whether such a mutation will occur and when. However, it can reasonably be assumed that the more widespread the circulation of LPAI in poultry, the higher the probability of HPAI mutation. The incubation period is difficult to assess and probably varies depending on the strain of the virus and the host is usually five to six days cited, but the range for individual birds is probably from a few hours to seven days. 2. Clinical signs in birds infected with HPAI virus Clinical signs are very variable and are influenced by factors such as virulence of infectious virus, affected species, age, gender, comorbidities and environment. Early signs may include unappetizing, reduced water intake and relatively low mortality. However, as an alternative the disease may suddenly appear in the flock, and many birds may die either without premonitory signs or with minimal signs of depression, appcesitis, ruffled feathers and fever. As a rule, the longer the birds survive, the more visible are the clinical signs. The timing of the traits depends on the virus, the host and the initial dose of infection along with the husband's system. The virus spreads more slowly in the cells of layers or outdoor birds compared to in broiler homes. Chickens infected with the HPAI virus may first lay eggs with soft shells, but soon stop putting them away. Sick birds often sit or stand in a semi-comatosis state, with their heads touching the ground. Combs and wattles are cyanotic and edible, and may have petehial or echimotic bleeding at their tips. Profane water diarrhoea is often present and birds are over-craved. Breathing can be toiled and excessive lacriation can be seen. Hemorrhage can be seen on unfeasible areas of the skin. Mortality rates range from 50 to 100%. In broilers, the signs of HPAI are often less obvious than in other poultry and tend to severe depression, appetite, and a very noticeable increase in mortality may be the first anomaly observed. Facial and neck swelling and neurological signs such as toricolis and ataxia can also be seen. HPAI in turkeys is similar to that seen in poultry, but some HPAI viruses seem more virulent in turkeys, while others seem less virulent. In geese infected with the HPAI virus, the signs of depression, appetite and diarrhea are similar to those found in layers, although often with swollen sinuses. Young birds may exhibit neurological signs. Ducks may show no clinical signs when infected with HPAI viruses, but some strains are reported to cause signs similar to those in geese with some mortality. Clinical signs may be absent in HPAI and LPAI infections of ostriches. In HPAI outbreaks, such as in Italy in 1999 and 2000, seabirds and Japanese quail are reported to be prone to infections with signs and mortality resembling the disease in chickens or turkeys. However, in some experimental studies, quail is reported to be resistant to certain strains of HPAI. For all birds, the presence of antibodies to the same H subtype, whether vaccinated or a natural infection, may mean that infection with the HPAI virus does not lead to obvious clinical signs. 3. Postmortem lesions in birds infected with the HPAI Birds virus that die perakutico can show minimal rough lesions consisting of dehydration and congestion of the innards and muscles. In birds that die after a long clinical course, petechyal and ecchymic bleeding occur throughout the body, especially in the larynx, trachea, clear and epicardial fat, as well as on the serosal surfaces adjacent to the sternum. There is extensive subcutaneous swelling, especially around the head and hocks. Carcasses can be dehydrated. Yellow or gray necrotic pockets may be present in the spleen, liver, kidneys and lungs. An air bag may contain exudate. The spleen can be enlarged and hemorrhagic. AI is characterized by histological vascular disorders leading to swelling, bleeding and perivascular cuffs, especially in myocardial, spleen, lungs, brain, pancreas and wattles. Necrotic pockets are present in the lungs, liver and kidneys. Glyosis, vascular proliferation and neuron degeneration may be present in the brain. 4. Differential diagnosis of differential diagnosis of HPAI, the following diseases, in particular, should be considered: (a) other diseases causing sudden high mortality, such as: (ii) infectious laryngotrachitis; (b) Other diseases that cause swelling of ridges and wattles, such as: (i) acute bird cholera and other septic diseases; (ii) Bacterial cellulite ridge and wattles. 5. Clinical signs in LPAI virus-infected, the severity of the disease caused by LPAI viruses depends to a large extent on: (a) strain of the strain (b) The owner's appearance and age; (c) Host's immune status from the virus and, in particular, the presence of other infectious agents, such as: (ii) Newcastle disease viruses (including vaccine strains); (iii) Avian pneumovirus, infectious bronchitis virus; Immunodeficiency conditions; Environmental factors (such as excess ammonia, dust, hot or cold temperatures). On the one hand, the clinical signs of the disease seen may be mild or insignificant, producing only mild respiratory signs or egg production problems in bird laying. On the other hand, infections with LPAI viruses may be associated with severe clinical signs of the disease, especially in turkeys, usually with rales, cough, swelling of the infraorbital sinuses and feverish conditions associated with loss of appetite and high mortality. LPAI can be confused with, or complicated, many of the diseases with respiratory or enteric signs. AI should be suspected of any outbreak of the disease in poultry, which persists despite the use of preventive and therapeutic measures in other diseases. 6. Clinical signs in birds in captivity Spectrum clinical signs can be very broad and, like in poultry, can range from ignorant to severe signs of the disease leading to high mortality. Typically, the infection spreads more slowly in the collection of birds in captivity due to the variety of different species kept, with different susceptibility, incompatible levels of virus shedding and often relatively slow transmission due to low contact rate and relatively low stocking density. Chapter III guidelines, which should be considered in the event of suspicions of AI regarding the variability of clinical traits for both HPAI and LPAI, mean that a clear guide to the suspected outbreak is not possible. Sudden high mortality in poultry with any concomitant clinical traits described in Chapter II should be investigated by submitting samples for laboratory research, but in the absence of high mortality it is more difficult to suspect or exclude the presence of AI. Since the rapid diagnosis of HPAI or LPAI, caused by subtypes H5 and H7, is of paramount importance for their early control and eradication, AI should always be taken into account in the differential diagnosis of respiratory diseases, egg production problems and increased mortality in poultry and related samples presented for laboratory research. Figure Schematic review of diagnostic steps to confirm AI Chapter IV General Procedures for Collecting and Transporting Samples 1. The 2005/94/EC Directive and the Wheres Diagnostic Manual are made references in the 2005/94/EC Directive to diagnostic guidance, research, sampling and surveillance procedures, set in the current chapter of the Diagnostic Manual be fulfilled. Procedures to be followed where AI outbreaks Where an official veterinarian has a clinical suspicion of an AI outbreak or where the results of any laboratory test for the disease are not negative, the competent authority must ensure that the investigation, as outlined in this chapter of the diagnostic manual, is conducted in accordance with article 7 of the 2005/94/EC Directive and is satisfactorily completed before the presence of the disease is ruled out. 3. Interpretation of virological testing The competent authority may consider that the presence of the AI virus may be ruled out when an appropriate number of sick or dead birds and trachea/orie-vecheal or cloaca tampons were presented, in accordance with this chapter, to detect the virus or its genome and have produced negative results in testing using one of the indicated methods of detecting viruses referred to in Chapter V or VI or authorized by the competent authority in accordance with paragraph 6 (b) of Chapter I. A standard set of samples for virological or serological laboratory testing For the study of a holding suspected of being infected with the AI virus, it is necessary to take a standard set of samples for virological or serological testing, as stated in paragraphs (a) and (b) (standard samples) that must be taken and submitted directly for virological and serological laboratory tests. (a) A standard set of samples for virological testing: (i) at least five sick/dead birds, if any; and/or (ii) at least 20 trachea/orofaringia and 20 cloaca tampons. Carcasses must be taken by birds that have died recently or who are seriously ill or dying and have been killed humanely. The swabs must be taken from the number of birds mentioned at point (a) or from all birds on the proposed holding, where there are fewer birds. Birds with signs of clinical diseases should be directed to sampling. Cloak tampons should be covered with faeces (optimal 1 g). If for any reason it is impossible to take cloaca tampons from live birds, carefully collected fresh samples of faeces can serve as an alternative. Often, it is most practical to collect trachea/oropharyngeal tampons from the bushicle cavity. Once the characteristics of the virus growth are known, the competent authority may decide to choose either the trachea/oropharyngeal or cloacaly of the tampons rather than collect both depending on whether the virus replicates better in the respiratory or gastrointestinal tract, and given the species concerned. (b) The standard set of samples for serological testing is at least 20 blood samples. Samples must be taken from the number of birds mentioned at point (b) or from all birds on the holding, where there are fewer birds. Birds that appeared sick or, which, apparently, should be directed to sampling. The competent authority may decide that the entire range of standard sample samples not accepted, but instead can be taken a subset of standard samples. Special care is needed to store and transport samples to the laboratory for testing. The tampons should be immediately cooled on the ice or with frozen packets of gel and submitted to the laboratory as quickly as possible. Samples should not be frozen if absolutely necessary. If fast transportation to the laboratory for 24 hours is not guaranteed, samples should be immediately frozen, stored, and then transported on dry ice. Also, rather than as an alternative to cooling, tampons should be placed in an antibiotic or specific transport environment viruses at 4 oC, so that they are fully submerged. In the absence of such an environment, the tampons must be returned to their enclosure and transferred to the laboratory for testing. A number of factors can affect the storage and transport of samples, so the method chosen for transportation should be suitable for this purpose. 6. The medium medium antibiotic antibiotic mentioned at point 5 should be based on phosphate-buffer saline solution at 7.0 to 7.4 (tested after antibiotics are added). Protein media such as brain-heart-infusion or tris-buffer broth tryptoses can give additional stability to the virus, especially during transportation. Antibiotics and their concentrations can be varied according to local conditions and availability. Very high levels of antibiotics may be needed for fecal samples and corresponding levels: 10,000 IU/ml penicillin, 10 mg/ml streptomycin, 0.25 mg/ml of gentamicin and 5,000 IU/ml of nistatin. These levels can be reduced to five times for tissues and trachea tampons. If control of chlamydophiles is desirable, 0.05 to 0.1 mg/ml of oxytetracycline should be included. 7. Brain-heart-infusion medium solution should be prepared in water and contain 15% w/brain-heart-infusion broth powder, before sterilization (by autoclave at 121 oC/15 minutes). After sterilization, antibiotics should be added as follows: 10,000 IU/ml penicillin G, 20 micrograms of amphotericin B and 1,000 micrograms/ml of gentamicin. The media can be stored at 4 oC for no more than two months. Procedures to be followed in relation to the relevant provisions of the 2005/94/EC 8.A. Alleged outbreaks 8.1. Article 7 (1) - Measures to be applied to holdings where outbreaks are suspected, When an official veterinarian inspects the holding where the outbreak is suspected, the following measures should be taken: (a) checking the production and medical records of the holding, if such records exist. Daily mortality data and daily data on egg production, as well as water intake for the period beginning a week before the start of AI clinical signs, up to the date holding an official veterinarian should be official veterinarian. (b) Clinical inspection in each production unit, including an assessment of its clinical history and clinical studies of poultry or other birds, particularly sick birds. (c) If the competent authority is not satisfied that the alleged outbreak may be excluded through a clinical examination under paragraphs (a) and (b), standard samples should be taken from each production unit. (d) Regardless of the negative test results of standard samples and with local factors taken into account, a clinical inspection of poultry in each production unit is required before official surveillance can be abolished. 8.2. Article 10 (3) - Additional measures based on the epidemiological request of Standard Samples must be taken from birds or other birds that die in each production unit. 8.B. Highly pathogenic avian influenza (HPAI) 8.3. Article 11 (4) - Measures to be applied in cases where a bird has hatched from eggs collected from stocks where outbreaks have been confirmed, When an official veterinarian inspects a holding where a bird is present that has already hatched from eggs collected during the incubation period at the holding, where HPAI has been confirmed, the following measures must be carried out: (a) a check of the production and medical records of the holding. Daily mortality data and daily data on feed and/or water consumption, if any, for the period beginning a week before the start of HPAI clinical signs before the farm inspection date, the official veterinarian must be documented in the farm inspection report by an official veterinarian. Clinical inspection in each production unit and clinical examination of poultry, in particular those that appear sick or who do not grow as expected. (c) Standard samples must be taken from poultry between the ages of two and three weeks. (d) Official supervision of the holding may be withdrawn after a clinical examination of poultry over 21 days and negative test results for standard samples. 8.4. Article 13 (2) (b) - Retreats in respect of certain possessions When an official veterinarian inspects a holding that has received a derogation from the first subparagraph of Article 11 (2) of the 2005/94/EC Directive, the following measures must be taken: (a) to check the holding's production and medical records, if such records exist. (b) Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of domestic or other birds in captivity, particularly patients. (c) Instead of standard samples, the following samples must be taken for laboratory testing, 21 days after the date of the last HPAI positive finding from each production unit and within 21 days of the day (i) Samples of any dead poultry or other birds present in captivity during sampling; (ii) Where practical, tracheal/orofaring and cloaccal tampons are found in at least 60 birds or other birds in captivity or from all such birds or other birds in captivity, where there are fewer than 60 in the holding; or if the birds are small, exotic and not accustomed to processing or processing them it would be dangerous for humans, samples of fresh faeces should be collected. However, the competent authority may provide deviations from the sample size referred to in (i) and ii, based on the results of the risk assessment. (d) The sample referred to in paragraph (c) and laboratory testing of such samples should continue until two consecutive negative laboratory results are achieved, which should be at least 21 days apart. Article 15(1) and (3) - Measures to be applied in contact holdings When checking the contact holding by an official veterinarian, it is necessary to carry out the following measures: (a) checking the production and medical records of the holding, if such records are available. Daily mortality data and daily data on feed and/or water consumption, if any, for the period beginning a week before the date of contact with the pack suspected of AI infection, prior to the date of the examination of the holding by the official veterinarian should be documented in the report on the inspection of the farm by the official veterinarian. (b) Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of domestic or other birds in captivity, particularly patients. (c) If there are clinical signs present in poultry or other birds in captivity, or signs of an increase in daily mortality (3 times normal herd mortality) or depression in daily egg production (5%) or a reduction in daily feed and/or water consumption (5%), standard samples must be taken immediately from each production unit. d) if there are no signs referred to in paragraphs (b) and c, standard samples must be taken 21 days after the date of the last alleged contact with an infected holding company or when a bird or other birds in captivity die. 8.6. Article 18, paragraphs (b) and (c) - Census and inspection of the official veterinarian and supervision at the security sites In the security area When the official veterinarian inspects the commercial holding, the following activities are required: (a) inspection of the production and medical records of the holding. If there are signs of an increase in daily mortality (3 times the normal mortality of the herd) or depression in the daily production of eggs (5%) or a reduction in daily feed and/or water consumption (5%), standard samples must be taken from each production unit. (b) clinical in each production unit, including an assessment of its clinical history and examinations of poultry and other birds in captivity, particularly the sick. (c) Where poultry or other birds in captivity should not clearly express clinical signs of the disease or, in the case of vaccinated birds, the competent authority may decide on the basis of the results of the risk assessment that standard samples should be taken from each production unit. (d) Based on the results of the risk assessment, the competent authority must decide on additional formal surveillance through clinical inspections and sampling for laboratory tests in targeted holdings, compartments or product types. 8.7. Article 19, paragraph (f) - Measures to be applied to holdings in the security areas When the official veterinarian of the holding, where increased morbidity, mortality or changes in production data have been registered, must be taken as follows: (a) verification of the production and medical records of the holding. If there are signs of an increase in daily mortality (3 times the normal mortality of the herd) or depression in the daily production of eggs (5%) or a reduction in daily feed and/or water consumption (5%), standard samples must be taken from each production unit. (b) clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of domestic or other birds in captivity, particularly patients. 8.8. Article 23, paragraph (b) - Retreats from direct transport of poultry for immediate slaughter When verified by the official veterinarian of the holding, which was exempted from article 22 of the 2005/94/EC Directive, should be taken as follows: (a) inspection of the holding's production and medical records. (b) Clinical examination in each production unit, including an assessment of its clinical history and clinical trials of any poultry, particularly those who became ill less than 24 hours before the poultry were released. (c) Based on the results of the risk assessment by the competent authority and instead of standard samples, at least 60 trachea/oropharyngeal and/oropharynitary and/or 60 cloak tampons must be taken from the poultry from each production unit to be sent for slaughter less than 48 hours before the bird leaves. 8.9. Article 25, paragraph (b) - Retreats from the direct transport of ready-to-carry poultry When examined by the official veterinarian of the holding, which has been deviated from article 22, to the direct transport of the ready-to-carry bird, must be carried out the following measures: (a) inspection of the production and sanitary documentation of the holding. (b) Clinical examination in each production unit, including an assessment of its clinical history and clinical studies of poultry, particularly those that appear sick less than 24 hours before the time of departure of the bird. (c) Based on the results of the risk assessment by the competent authority and instead of standard samples, at least 60 trachea/orofaringia and/or cloaca smears must be taken from poultry from each production unit for transport less than 48 hours before poultry is shipment. 8.10. Article 26 (1) - Retreat from the direct transport of incubation and table eggs When the parent of the herd, who had the right to deviate from article 22, to the direct transport of the incubation egg, must carry out the following measures: (a) the inspection of the production and medical documentation of the holding. Clinical inspection in each production unit every 15 days. (c) Standard samples must be taken from each production unit. Article 29(1) - The duration of measures in the security zone under Article 3 of Chapter IV of the 2005/94/EC Directive, can be cancelled no earlier than 21 days after the pre-cleaning and disinfection date of the infected holdings, provided that: (a) All commercial holdings located in the security zone have been checked by the official veterinarian and all checks and clinical checks, laboratory tests and laboratory tests as specified in paragraph 8.6 (a), b) and (c) and paragraph 8.7 have given negative results. (b) All identified non-profit holdings in the security zone were checked by an official veterinarian, and neither the clinical examination nor the results of any laboratory tests led to suspicion of AI infection. (c) Any additional official monitoring, as cleared in paragraph 8.6 d, has yielded negative results. 8.12. Article 30, paragraph ((g) - Measures to be taken in the observation areas When the official veterinarian of the holding, which has recorded an increased incidence, mortality or change in production data, must be carried out as follows: (a) the inspection of the production and medical records of the holding. (b) Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of domestic or other birds in captivity, particularly patients. (c) Standard samples must be taken from each production unit. Article 35- Investigation of THE alleged presence of HPAI in abattoirs and vehicles When an official veterinarian checks the origin of birds in abattoirs or vehicles, the following activities must be carried out: (a) check of the holding's production and medical records, if such records are available. Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of poultry or other birds, consultation with an official veterinarian at the abattoir, who must provide details of any previous inspection data and the results of ant- and post-mortem examinations. (c) If the competent authority is not satisfied that the alleged presence of CPAI may be excluded on the basis of a veterinary investigation under paragraphs (a) and (b), standard samples must be taken from each production unit. (d) In addition to standard laboratory samples, samples of at least five sick, dead or slaughtered birds in a slaughterhouse with pathological finds should be presented. 8.14. Article 36 (1) - Measures to be applied at abattoirs after the completion of the studies mentioned in paragraph 8.13 and provided that the results of laboratory trials are negative and that there is no clinical suspicion that HPAI is present in the presence of origin and at the slaughterhouse, official supervision may be revoked. 8.15. Articles 37 (1) and (2) - Measures to be applied at border posts or vehicles 8.15.1. When an official veterinarian inspects birds or other birds that are in isolation, which have been moved from a border inspection post or vehicle, in connection with the suspicion or confirmation of HPAI, the following measures must be taken: (a) check of relevant documents and records if such documents or records exist. (b) Clinical examination of such birds or other captive birds that are isolated and clinical examination of any other birds or other birds in captivity, particularly the sick. (c) Standard samples must be taken from poultry or other birds selected from different transport boxes or cages. 8.15.2. When an official veterinarian checks the identified origin in the event of slaughter of a bird or other birds in captivity, the following activities must be carried out: (a) check of the production and medical records of the holding, if such records are available. (b) Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of poultry or other birds in captivity, based on consultation with an official veterinarian at the abattoir, who must provide details of any previous inspection data and the results of antenatal and post-mortem examinations. (c) If the competent authority is not satisfied that the alleged presence of CPAI may be excluded on the basis of a veterinary investigation under paragraphs (a) and (b), standard samples must be taken from each production unit. (d) In addition to the standard samples mentioned in paragraph C, samples of at least five sick, dead or slaughtered birds at a slaughterhouse with pathological finds should be submitted for laboratory testing. (e) Provided that the results of laboratory The samples mentioned at points (c) and d) are negative and that there is no clinical suspicion of HPAI on retention of origin and at the slaughterhouse, official supervision can be abolished. 8.C. Low pathogenic avian influenza (LPAI) 8.16. Article 39 (6) (b) and (h) - Measures to be applied to holdings where LPAI outbreaks are confirmed When conducting an official veterinary inspection of the holding before transporting the bird to the slaughterhouse, or in a holding where the bird has already hatched from eggs collected during the incubation period, it is necessary to carry out the following measures: (a) inspection of the production and medical documentation of the holding. Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of poultry or other birds in captivity. (c) Standard samples must be taken from birds from each production unit to be sent to slaughter less than 48 hours before the bird leaves. (d) Standard samples must be taken from each poultry production unit already hatched from eggs harvested during the incubation period. 8.17. Article 40 (2) (b) - The departure of some holdings from measures to be taken when outbreaks are confirmed, when an official veterinarian inspects a holding that has been granted a derogation from Article 39 (2) and paragraph (b) of article 39 (5) of the 2005/94/EC Directive, should be followed by: (a) production checks and records of a medical holding, if such records are available. (b) Clinical inspection in each production unit at regular intervals, including an assessment of its clinical history and clinical trials of poultry or other birds in captivity, particularly patients. (c) Instead of standard samples for laboratory testing, the following samples should be taken within 21 days of the date of the latest positive results of the LPAI from each production unit and at intervals of 21 days: (i) samples of any dead birds or other birds present in captivity during sampling; (ii) Tracheal/orofaring and cloacal tampons from 60 birds and other birds in captivity or from all poultry and other birds in captivity, where there are fewer than 60 birds in the holding; Or if the bird or other birds in captivity are small, exotic and not accustomed to processing or processing them it would be dangerous for humans, samples of fresh faeces should be collected. However, the competent authority may provide deviations from the sample size referred to in (i) and (ii) based on the results of the risk assessment. (d) The sample referred to in paragraph (c) and laboratory testing of such samples should continue until two consecutive negative laboratory results are achieved, which should be at least 21 days apart. Articles 42 (1) and (3) - Measures to be applied in checks the contact holding, the following measures should be carried out: (a) check of the production and medical records of the contact holding, if such records exist. (b) Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of domestic or other birds in captivity, particularly patients. (c) Standard samples must be taken from each production unit or when birds or other birds in captivity are killed. 8.19. Article 44 (1) (b) - Measures to be applied in restricted areas when an official veterinarian

inspects a commercial holding in the exclusion zone, the following measures must be carried out: (a) verification of the production and medical records of the holding. (b) Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of domestic or other birds in captivity, particularly patients. (c) Standard samples must be taken from each production unit. (d) Based on the results of the risk assessment, the competent authority must decide on additional formal surveillance through clinical inspections and sampling for laboratory tests in targeted holdings, compartments or product types. Article 45, paragraphs (a) and (b) - The duration of measures in the exclusion zone, in accordance with Article 3 of Chapter 3 of the 2005/94/EC Directive, can be cancelled no earlier than 21 days after the date of pre-cleaning and disinfection of infected holdings after the depopulation of the holding or no earlier than 42 days after the date of confirmation of the LDAI, provided that: (a) all commercial holdings in the exclusion zone have been checked by the official veterinarian and all laboratory tests of samples mentioned in paragraphs (c) and d) paragraph 8.13 have been conducted and are available; (b) There are results of any additional clinical inspections and laboratory tests, which may include non-commercial holdings to determine the risk of spread of LDAI; (c) The competent authority is satisfied with the results of the risk assessment, taking into account the epidemiological situation and the results of the laboratory tests mentioned in paragraphs (a) and (b), that the risk of spreading LPAI is low; such an assessment may conclude that, in the case of positive serological findings and negative virological findings, restrictions may be lifted. 8.D. Measures to prevent the spread of avian influenza viruses to other types of 8.21. Article 47 (1) and (6) - Laboratory tests and other measures relating to pigs and other species When an official veterinarian inspects the holding where the pig is kept, after the AI is confirmed, the following measures should be carried out: (a) Inspection of production and medical records if such records exist. Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of pigs, particularly patients. (c) Nose/oropharyngeal smears of at least 60 pigs from each production unit or all pigs with fewer than 60 pigs in the production unit must be taken before or on the day of the cull of an infected bird or other birds in captivity. At least 60 blood samples must be collected from pigs, two to four weeks from the cull date. Samples should be collected in such a way that at least one sample is obtained from groups of pigs that are in direct contact with each other. (d) Moving pigs to other holdings may be permitted if at least 60 nasal/oropharyngeal smears and 60 pig blood samples from each production unit within 14 days of the date of the positive conclusion of the presence of AI have yielded negative results. The movement of pigs to the abattoir may be allowed if at least 60 nasal/rotary tampons, from each production unit, 14 days after the date of positive findings about the presence of AI have yielded negative results. In the case of inconclusive or positive laboratory results, any further research is needed to eliminate infection or AI transmission among pigs. (e) Where the official veterinarian suspects that other domestic mammals on the holdings, in particular those who have been diagnosed with Susceptibility to H5 and H7 AI viruses, may have contact with an infected bird or other birds in captivity, it is necessary to take samples for laboratory tests. 8.E. Re-population 8.22. Article 49 (3) (b) and c) - Re-filling of holdings When checking the official veterinarian of the commercial holding, which was overcrowded, it is necessary to carry out the following measures: (a) check of the production and medical records of the holding. Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of poultry or other birds in captivity, particularly patients. (c) Instead of standard samples, the following samples should be taken from each production unit: (i) at least 20 blood samples immediately after the bird has been placed in the holding, with the exception of day chicks; If necessary, such a sample can be carried out on the basis of the bird's origin before moving to a holding for the re-population; (ii) Samples of dead birds or tampons taken from their carcasses from no more than 10 dead birds per week within 21 days of the re-population date. (d) Where the holding has previously been infected with HPAI 20 trachea/oropharyngeal and 20 cloaca tampons must also be taken from waterfowl (ducks/geese) from each production unit if appropriately, during the last week of the week 21-day period from the date of the re-population. (e) In cases where the holding company has previously been infected with LDAI, 20 tracheia/orofarings and 20 cloacas and 20 blood samples from each production unit must be taken. 8.F. Vaccination 8.23. Article 56 (2) (i) - Preventive vaccination of birds or other birds in captivity Laboratory tests, as stipulated in Chapter IX Directive 2005/94/EC, must be conducted on vaccinated birds or other birds in captivity using approved diva tests where the field virus is known. When using watchdog birds, they must be present in each vaccinated herd, clinically examined and tested with a hemagglutination inhibition test (HI). To do this, you must take at least every 60 days 20 blood samples from unvaccinated marines in each vaccinated holding. 8.24 Annex IX - Requirements for the movement of birds or other birds in captivity and poultry products applicable in connection with emergency vaccination should apply strict controls on the movement of live poultry and other birds in captivity and their eggs in order to minimize the risk of further spread of AI infection. To this end, at the beginning of the emergency vaccination campaign, the same monitoring measures should be taken to monitor the movement of live poultry and other birds in captivity and their eggs in order to minimize the risk of further spread of AI infection within and beyond. (a) Before the first movement within and out of the vaccination zone of incubation eggs and table eggs, and then at least every 30 days, the official veterinarian must carry out the following measures: (i) clinical examination of unvaccinated parent or layered poultry in each production unit, including an assessment of its clinical history and clinical studies of poultry, in particular those that appear to be sick; Standard samples must be taken from the bird from each production unit; or (ii) Clinical examination of vaccinated parent or layered poultry in each production unit, including an assessment of its clinical history and clinical trials of sentinel birds present in these flocks; standard samples must be taken from these guard birds. (b) In order to move live vaccinated poultry or other birds to other locations or to move live vaccinated birds within and outside its territory, the official veterinarian must conduct the following activities: (i) a review of the production and medical records of the holding. Clinical inspection in each production unit, including an assessment of its clinical history and clinical trials of poultry or other birds in captivity within 72 hours of departure with special attention to Sentinel birds; (iii) Where the results of inspections and clinical inspections and examinations in (i) (ii) are not satisfactory, standard samples must be taken from sentry; however, where these results are satisfactory, The following samples must be taken: - vaccinated bird or other birds in captivity: at least 20 trachea/oropharyngeal and 20 cloaca tampons and 20 blood samples for use of appropriate DIVA analysis for 72 hours prior to departure time, and - watchdog birds: 20 trachea/rotary swallows and 20 cloaca tampons and 20 samples for blood serology using HI test. Chapter V Diagnostic Virological Tests and Score Results 1. Prior to the introduction and development of molecular tests, viral isolation by egg-inoculation of embryonic birds was considered the most sensitive diagnostic test for AI to date and necessary for the subsequent identification and identification of the infectious virus. The main steps set in this chapter. 2. Example of Swabs treatment if presented dry should be placed in sufficient antibiotic environment to ensure full immersion. Samples can be combined in batches of five, provided they come from the same species, time and epidemiological unit. Carcasses submitted to the laboratory should be subjected to a post-mortem examination, as well as samples of the following organs: feces or contents of the intestines, brain tissue, trachea, lungs, liver, spleen and other clearly affected organs. These organs and tissues can be combined, but separate treatment of fecal material is important. Samples and organs of faeces should be homogeneous (in a closed blender or using pestle and solution and sterile sand) in the environment of antibiotics and made up to 10 to 20% w / in suspension in the environment. Submerged tampons and suspensions should be left for about two hours at ambient temperature (or longer periods at 4 oC) and then refined by centrifuge (e.g. 800 to 1,000 x g for 10 minutes). 3. Viral insulation in the embryonic eggs of birds Refined supernathic fluid should be inoculated in quantities of 0.1 to 0.2 ml in the allantoy cavity of each of at least four embryonic eggs of birds that are incubated within nine to 11 days. Ideally, these eggs should be derived from a specific pathogen free (SPF) herd, but when it is unfeasible eggs derived from the herd it is shown to be free of antibodies to AI (antibody negative serum - SAN) can be used. Vaccinated eggs should be carried out at 37 oC and candles daily. Eggs with dead or dying embryos as they arise, and all remaining eggs six days after vaccination must be cooled to 4 oC and allanto-amniotic fluids tested for hemagglutination activity. If hemagglutination is not detected, this should be repeated using undiluted allanto/amniotic fluid as an inoculum. If hemagglutination is detected, the presence of bacteria should be ruled out by culture. If bacteria bacteria Fluids can pass through the membrane filter 450 nm, additional antibiotics are added and grafted into embryonic eggs, as above. To speed up diagnosis, some labs used two 3-day passes or 2-day and four-day passes and reported comparable results with two 6-day passes, but this has not yet been fully evaluated. Positive fluids should be tested for freedom from bacteria. If the bacteria present the liquid can be transferred through a 450-nm membrane filter or centrifuged to remove bacteria and re-pass in the eggs after adding more antibiotics. 4. Differential Diagnosis (a) Preliminary differentiation Since it is important that control measures aimed at limiting the spread of the AI virus are implemented as soon as possible, each national reference laboratory that has isolated the hemagglutinous virus should be able to identify it if it is influenza A virus subtype H5 or H7 or Newcastle disease virus. Hemagglutinating fluids should be used in hemagglutination inhibition tests, as described in Chapter IX. Positive braking, such as titre within 2-3 logs2 of positive control, with polyclonal antisers specific to H5 or H7 influenza subtypes, can serve as a pre-identification, allowing the introduction of interim control measures. (b) Confirming identification Since each of them has 16 subtypes of hemaglutinin and 9 subtypes of influenza viruses and variations can be neither practical nor cost-effective for each national antiserata reference laboratory, which can allow the full sub-type identification of influenza isolates. However, each national reference laboratory must at least: (i) confirm that isolate is influenza A virus using an immunodo-bbleditfusia test to detect group antigens; To determine whether isolate is a subtype of H5 or H7, positive identification requires control measures for subtypes of LPAI H5 and H7; (iii) Immediately submit all HPAI and all H5 and H7 isolates to the Community Reference Laboratory for confirmation and full specification, unless the retreat is provided in accordance with paragraph (d). It is also desirable in laboratories with appropriate means: (iv) to conduct an intravenous pathogenicity index test in six-week-old chickens, as learned in Chapter VII. Intravenous pathogenicity indicators, more than 1.2, indicate the presence of a virus requiring full implementation of control measures for HPAI. National reference laboratories should also consider putting in place experiments and equipment that allow nucleotide sequencing of the hemaggluin gene to determine whether there are several essential amino acids at the site of hemagglutin protein cleavage for LPAI H5 or H7 virus. While the Community Reference Laboratory will conduct defining as a priority in the responsibilities mentioned in Annex VII, paragraph 2 (b) of the 2005/94/EC Directive, this characterization of the virus at the national level would significantly reduce the time for diagnosis and, in the case of positive, to fully implement the CPAI control measures. (c) Further input and characterization of isolates, the Community Reference Laboratory should receive all hemagglutinating viruses from the National Reference Laboratories for further antigenic and genetic research, so that the epizootics of the disease (s) within the Community Framework can be better understood in accordance with the functions and responsibilities of the Community Reference Laboratory, as established in Annex VII of The 2005/94/EC Directive. In addition to these functions and responsibilities, the Community Reference Laboratory should carry out a complete antigenic type for all influenza viruses received. For H5 and H7 viruses that do not have intravenous pathogenicity indicators greater than 1.2, nucleotide sequencing of the hemagglutinin gene to determine whether there are several essential amino acids at the site of the breakdown of the hemagglutin precursor protein, should also be conducted immediately, and the national reference laboratory and the competent authority in the country of origin should be informed as soon as the results are available to (d) in view of the changing epidemiological situation in relation to HPAI/LPAI, it may be possible, in consultation with both the Commission and the Community, to assess the retreats to laboratories that have every capacity to characterize the virus quickly, to provide a subset of these viruses after the verification of the data. Such a retreat should only be permitted if the data can be quickly obtained by the national reference laboratory and transferred to the Community Reference Laboratory. CHAPTER VI Molecular tests and evaluation of the results of the current definition of HPAI, allows molecular identification of virulence factors and confirms the use of molecular methods in the diagnosis of AI. Recently, there have been developments in their application to detect and characterize the AI virus directly from clinical samples of infected birds. Conventional RT-PCR methods on clinical samples can, with properly defined primer, lead to rapid detection and subtype (at least H5 and H7) identification, as well as a PCR amplinone product that can be used for nucleotide sequencing and have been demonstrated to have an important use by quickly detecting subsequent outbreaks after the primary infected premises have been detected and the virus characterized. A single-step RT-PCR in real time using primer/probe systems (rRT-PCR) allows for even faster and faster detecting AI viruses and identifying the H5 or H7 subtype in clinical samples. An important problem with RT-PCR and RRT-PCR systems is that to date various laboratories have developed different systems that, although perfectly legal, have not been tested or tested with a large number of samples in different laboratories. The Community Reference Laboratory and these national reference laboratories are addressing this problem as part of a Community-funded project (EU AVIFLU) to draw up ratified protocols for conventional RT-PCR and RRT-PCR that can be adopted by other national reference laboratories. If the testing parameters, such as cycling and ramp time, differ from those recommended in these protocols, they should be demonstrated as suitable for purposes before use under paragraph 6 of Chapter I of this diagnostic guide. Standard protocols for these molecular tests and their assessments, used by the Community Reference Laboratory, can be found on the following website: Chapter VII In vivo pathogenicity test and evaluation of virulence results for chickens of influenza A viruses isolated from birds, should be evaluated using an intravenous pathogenicity index (IVPI) test, which should be conducted as follows (a) Fresh infectious allanto fluid with a titrem ha qgt;1/16 (qgt;24 or qgt;log2 4, expressed as reciprocal) from the lowest level of passage, preferably from the initial insulation without any selection diluted 1/10 in sterile salt. (b) 0.1 ml of diluted virus is administered intravenously in each of the ten six-week-old SPF or SAN chickens. (c) Birds are screened at 24-hour intervals for 10 days. With each observation, each bird scored 0 if normal, 1 if sick, 2, if severely ill, 3 if dead. The decision of sick and seriously ill birds is a subjective clinical assessment. Typically, sick birds show one of the following signs and are seriously ill over one of the following signs: respiratory involvement, depression, diarrhea, open skin cyanosis or wattles, swelling of the face and/or head, nerve signs. Dead birds should be slaughtered as 3 on each of the remaining daily observations after death. For welfare reasons, when birds are too sick to eat or drink, they should be killed humanely and slaughtered like dead on the next observation as they die within 24 hours without intervention. This approach is acceptable to accreditation bodies. (d) IVPI is an average score per bird per observation over a 10-day period. The 3.00 index means that all birds died within 24 hours, and the 0.00 index means that no bird has shown any clinical during a 10-day observation period. A simple method of recording results and calculating indices is shown in the following Clinical Signs Day After Vaccination General Score 1 2 3 4 5 6 7 8 9 10 Normal 10 2 0 0 0 0 0 0 0 0 0 12 x 0 0 Sick 0 4 2 0 0 0 0 0 0 6 x 1 6 Seriously Ill 0 2 2 2 0 0 0 0 0 0 0 6 x 2 x 12 Dead 0 2 2 6 8 10 10 10 10 10 76 x 3 : 3 10 birds observed over 10 days and 100 observations Index - average score per bird per bird per observation - 246/100 - 2.46 Any influenza A virus, regardless of subtype, more than 1.2 in the IVPI test is considered to be the HPAI virus. Chapter VIII Serological Tests and Evaluation results Preferred method used to show the presence of influenza A virus to demonstrate the presence of nucleoprotein or matrix antigens that share all influenza A viruses. The preferred methods used for serological tests for antibodies to the AI virus are hemagglutination (HA) and inhibition of hemagglutination (HI). Chapter 2.7.12 of the World Organization for Animal Health (IWE) Guide to Diagnostic Trials and Vaccines for Terrestrial Animals (IWE) provides detailed information on laboratory methods and evaluation of results. Standard serological test protocols and evaluation of their results by the Community Reference Laboratory can be found on the following website: Chapter IX Monitoring Systems related to vaccination 1. The 2005/94/EC Directive and the Diagnostic Sections of The Manual 2 and 3 of Chapter IX of the 2005/94/EC Directive allow the use of emergency and preventive vaccination under certain conditions. One such condition is that the DIVA (differentiation of those infected from vaccinated animals) strategy is used. Vaccination should be aimed at preventing infection and the subsequent spread of the virus between the packs. There is overwhelming evidence that vaccination increases the amount of virus needed to infect birds and reduces the amount of the virus. However, although vaccinated birds no longer develop clinical signs, they can still spread the virus when challenged. Thus, HPAI subtypes H5 and H7 viruses may circulate undetected for some time in a pack with suboptimal immunity levels just as LPAI viruses could do in an unvaccinated flock. It is therefore necessary to be able to identify positively viral vaccinated herds infected with the field virus so that other control measures, such as eradication of the virus, can be implemented. 2. Using sentry to monitor infection At herd level, a simple method is to regularly monitor guard birds left unvaccinated in each vaccinated flock, but this approach has management problems, especially in identifying especially in large flocks. Contact must be made between sentinels and vaccinated birds. 3. A laboratory test of DIVA to monitor infection As an alternative or, in addition, field exposure testing can be conducted on vaccinated birds themselves through DIVA laboratory tests. In recent years, several testing systems have been developed to identify problems at the field of vaccinated birds. One method that has proven useful is the use of a vaccine containing a virus of the same subtype of hemaglutinin (H) but another neuraminidase (N) from the prevailing field virus. Antibodies to N field virus act as natural markers of infection. This system was used in Italy after the re-emergence of the LPAI H7N1 virus in 2000. In addition to direct control measures, DIVA's strategy of using the H7N3 vaccine to fight field infection H7N1 has been implemented. Vaccinated and field-open birds were differentiated by a serological test to detect specific antibodies against N1. The same strategy was used to combat LPAI caused by H7N3 in Italy in 2002-2003, in this case with the H7N1 vaccine and the serological antibody detection test against N3 in particular. In both cases, vaccination with the elimination using this DIVA strategy has led to the eradication of the field virus. Problems with this system arise if there is a field virus that has the same antigen N as the existing field virus, but has a different subtype H than H5 or H7, or if subtypes with the same N antigens are already circulating in the field. Especially ducks, as it is known, carriers more than one subtype. It is also necessary to develop a suitable test that would allow the regular control of herds for antibodies to anti-nayrominidase. In Italy, a special serological test based on indirect analysis of fluorescent antibodies was developed and used, using proteins expressed by baculovirus recombinants as an antigen N. This may have a wider and simpler application in the development of the ELISA test. The use of HA-only vaccines, such as recombinant vector vaccines, allows the use of classic AGID tests or ELISA tests based on nucleoprotein, non-structural protein or matrix proteins to detect infection in vaccinated birds. A test has been described for inactivated vaccines that detect antibodies to non-structural viral proteins that are produced only during a natural infection. Such a system has yet to be tested in the field, but has a limitation that a natural pack infection with any influenza virus, regardless of subtype leads to the production of antibodies against non-structural protein. Develop fast and sensitive methods for detecting viruses, especially those that may be such as RT-PCR in real time, means they can be used for simple extensive and regular testing of vaccinated vaccinated the presence of a field virus. The detection of the agent, however, will be limited to a short window into the acute phase of infection and cannot be used to conclude that the herd has not been exposed to the virus in the past. This approach is most suitable for testing vaccinated birds prior to movement to demonstrate freedom from active infection. The number of samples to be tested by the selection systems should eliminate the prevalence of AI virus infection in the pack of more than 15% with a confidence level of 95%. Chapter X's AI diagnostic strategy, as stated in Annex IV to The 2005/94/EC Directive, decisions about the application of measures in specific areas or contact rooms, and the severity of these measures may vary greatly depending on the level of risk. Similarly, the necessary diagnostic evidence of the disease is likely to be balanced with the current situation, the scale of the risk and the degree of risk. Veterinary authorities must make decisions on diagnostic evidence that balances rapid control and eradication of diseases with the potential consequences of misdiagnosis. Such judgements should be made against the background of many factors of the time, but certain situations can be predicted. Disease Situation Potential Problem Diagnostic Criteria Non-Specific Signs, No Official Suspicion Isolated Conduct to Conduct Rapid Detection Based on M Gene RT-PCR. Differential diagnosis as needed Primary suspected outbreak is isolated conducting full diagnostic testing, viral isolation and characteristics Primary suspected outbreak Holding in the densely populated poultry zone Conduct full diagnostic testing, isolation and characterization of the virus, but focus on rapid detection and characteristics, especially those based on RT-PCR and sequencing (1). The second and subsequent suspected outbreaks of isolated holdings, epidemiologically related to the primary suspected outbreak, focus on rapid detection and performance techniques, especially on the basis of RT-PCR and sequencing (1). Secondly, subsequent suspected outbreaks in a densely populated poultry area or with many epidemiological links rely on rapid detection techniques that provide the earliest data on the presence of any AI virus (1). Numerous suspected outbreaks of disease or disease that are spreading rapidly, including surveillance, will become uncontrollable without rapid intervention, relying on rapid detection techniques that provide the earliest evidence of any AI virus or rely on clinical traits (1). Chapter XI Diagnosis of AI virus infection in pigs and other mammals 1. AI in pigs AI viruses are easy to infect pigs and although replication in most relatively limited, there is the potential that infected pigs can transmit disease to poultry and other susceptible animals. To date, there is no evidence that pigs transmit AI viruses of H5 and H7 subtypes. Experience during the 2003 outbreak in the Netherlands showed that infected H7N7 pigs show no clinical signs that may be associated with H7N7 infection. In addition, no sick pigs appear to have been reported in Asia and other countries during the H5N1 outbreak to date. Thus, clinical signs cannot be used to indicate whether pigs are infected, although a clinical representation due to the infection of pigs with other avian influenza viruses may occur after the virus has become adapted to the host. Diagnosis of viral infections of AI pigs is essentially similar to the diagnosis for avian species, relying on virus insulation, molecular methods and detection of specific antibodies through hemagglutination inhibition tests. There are, however, certain differences, and none of the tests are fully tested for use in pigs to confirm infection with AI viruses. 2. Samples of viral INFECTIONS of the AI virus in pigs are usually limited to respiratory tracts and samples must be airway tissues and, if necessary, oropharyngeals or nasal tampons, preferably taken from pigs with signs of the disease. These samples and tampons can be processed to isolate the virus or molecularly detect the virus using the same techniques described above for bird samples. However, the use of PCR methods should be properly monitored to ensure that the amplification does not suppress substances in pig samples. 3. Egg vaccination and incubation To isolate mammalian influenza viruses in eggs of 9-11-day embryonic birds is commonly practiced to vaccinate each egg through the allantoi cavity and in the amniotic cavity. However, when testing pigs in contact with AI viruses when the virus had little room to adapt, allanto cavity vaccination is probably enough. Similarly, 35 oC is generally recommended for incubation temperatures to isolate mammalian influenza viruses, but again for viruses ill-adapted to pigs, 37 OC does not harm the isolation of the virus. 4. Testing for specific antibodies in HI tests for virus insulation or molecular detection is likely to be the most sensitive to detect viral AI infections of pigs. However, serological reactions in pigs were detected in the absence of isolation or detection of the virus. Hi-tests using pork serum require some changes in the test used for avian serum referred to in Chapter VIII. Pig sulfur is renowned for its non-specific inhibition properties in HI tests and therefore each serum sample must be treated with a destructive enzyme receptor (RDE) to prevent this. The following method should be used: (a) Before mll pork antisczema add 400 ml RDE (predetermined working dilution) and mix thoroughly. (b) Incubate at 37 oC for one hour. c) Then then 30 minutes at 56 oC. (d) Cool samples at 4 oC for at least 15 minutes. (e) Add 10 ml of 30% (v/v packed cells) of chicken red blood cells and stir vigorously. (f) Incubate at 4 oC overnight. Also, if it is important to use samples on the same day, incubate at 37 oC for one hour and centrifuges at 300 x g for five minutes. Processed serum is then used in HI tests, as described for avian serum in paragraph .. initial dilution of 1:10. A set of swine sulfur with known gray-negative AI status should be used to assess the specifics of the HI test for the use of the virus strain (see use of the virus strain for serology derived from the outbreak; Chapter VIII). During an outbreak in the Netherlands in 2003, a swine serum HI test found up to 2.6 per cent of non-specific reactors collected independently of outbreak 5. A selection of pigs In particular, on farms where pigs and birds are kept mixed or in individual homes, pigs are at risk of contracting AI directly or indirectly when exposed to poultry or poultry products. To prevent such infection, bare or nasal tampons and blood samples must be collected in accordance with the procedures described in paragraph 8.21 of Chapter IV. Samples must be obtained from pigs that show clinical signs of the disease. However, when they do not show any clinical signs, samples can be collected at random across all sections of the house. If there are smears in the laboratory, they must be tested during rapid molecular tests and/or virus isolation. RT-PCR should be properly tested and have sensitivity at least equivalent to the isolation of the virus in eggs for influenza A viruses. Two to four weeks after culling infected AI poultry, at least 60 blood samples must be collected from pigs in such a way that at least some samples are obtained from groups of pigs that are in direct contact with each other. Samples should be tested in the HI test using the virus from the outbreak in poultry. Samples of both acute and convalescent phases should be tested in the same test. Positive samples can be confirmed by virus analysis and/or western blot. If tested positive, any of these samples should be epidemiologically investigated at all pig farms within the protection zone, whether they are of mixed type or not. AI viruses in other mammals other than pigs are studies in other mammals other than pigs that are susceptible to AI, including cats should be undertaken. As for the specific reference to HPAI H5N1, to test cats you need to sell the following: rough lesions associated with viral replication are concentrated on the lungs and liver, so samples for virological research are desirable to take from these organs of dead animals. In living animals, preferably trachea / orofaringia orofaringia must be taken to detect the virus. In addition, fecal tampons can be taken separately. Blood samples to be examined in HI tests require heat treatment for 56 oC for 30 minutes and RDE treatment can be omitted. CHAPTER XII Minimum safety requirements for the transport of samples 1. The transport of samples that are known to be present or suspected of being present is subject to strict national and international rules that must be observed at any time. Viral isolates are not classified as diagnostic samples, but must be packaged in accordance with international standards. The instructions set in this chapter are for air transport, but similar packaging should be used for land or sea transport samples. 2. The packaging of diagnostic samples for the transport of diagnostic samples carried under IATA Rules is assigned to UN identification numbers 2814, 2900 or 3373. The shipper, not the shipping company, is responsible for shipping until the package reaches the shipper. 3. The primary packaging (a) the primary vessel (s) should be waterproof, for example, screw caps must be sealed with steamfilm or duct tape or similar protective protection. (b) Several primary vessels must be wrapped individually to prevent breakdown. (c) Viral media should be taken into account when determining the volume of diagnostic samples shipped. (d) The primary vessel must not contain more than 500 ml or 500 g. All contents of the main vessel is a diagnostic sample. 4. Secondary packaging (a) Enough absorbent material in a secondary container to absorb all the contents of all primary vessels in the event of leakage or damage. (b) Secondary packaging must meet IATA's packaging requirements for diagnostic samples, including a 1.2 metre (3.9-foot) drop test. Because the packaging of infectious substances exceeds the requirements for diagnostic packaging of samples, it can be used in IATA's 602 packaging instructions. (c) The packaging of infectious substances should have the necessary specification markings on the packaging (UN will be in a circle), for example: UN 4G/CLASS 6.2/99/GB/2450 (d) Secondary packaging should be waterproof. The manufacturer of packaging or other instructions of the authorized party on the packaging included in the secondary packaging must be observed. Secondary packaging should be at least 100 mm (four inches) in the smallest overall external dimension. (f) The secondary packaging should be large enough to deliver documents such as air travel. 5. External packaging (a) external packaging should not contain more than 4 litres or 4 kg. b) if necessary dry ice or wet ice be placed outside the secondary packaging. When using dry ice, the packaging should allow carbon dioxide emissions and prevent Packaging. When wet ice is used, the packaging should be ineduly important. Each package and air pathbill should be marked with the following exact formulation: UN 3373 DIAGNOSTIC SPECIMEN PACKAGING IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650 (c) a detailed list of contents must be concluded between the secondary packaging and the external packaging. (d) External packaging should be placed in airtight plastic bags to protect against moisture. (e) A declaration of dangerous goods by the shipper is not required. Chapter XIII Sending Viruses and Samples to the Community 1 Reference Laboratory. Samples to be sent to the Community Reference Laboratory must comply with the recommendations for the transport of dangerous pathogens within the Community, as well as the rules and regulations in force in the United Kingdom. The instructions set in this chapter must be followed. 2. Sending viruses or other materials to the Community Reference Laboratory (a) All materials must be packaged in accordance with the instructions set out in this chapter. (b) External packaging should be marked AS FOLLOWS: ANIMAL PATHOGEN - PACKAGE ONLY TO BE OPENED AT THE AVIAN VIROLOGY SECTION, VLA, WEYBRIDGE. IMPORTS ALLOWED BY LICENSE NUMBER..... IT IS ISSUED IN ACCORDANCE WITH THE ORDER ON IMPORT OF PATHOGENS OF ANIMALS. (c) One of the following license numbers must be inserted: AH/2232/2002/5 (ii) for fabrics and other materials: AH/2074C/2004/3 Since these license numbers change from time to time, laboratories, submit samples, must ensure that they use current license numbers before sending parcels. (d) The package should be addressed to: Avian Virology VLA Weybridge, New Haw, Addlestone, Surrey KT15 3NB UK (e) The letter must accompany the parcel with as many stories as possible about isolates such as species and age, area/country isolation, any clinical history. Packages must be sent by air or air. If parcels are sent by air, the air bill number must be transferred to the Community Reference Laboratory by fax, telephone or email before the materials arrive. Packages shipped by air must be clearly labelled CARE OF TRANSGLOBAL to ensure quick processing at the airport. Community reference laboratory contacts Jan H. Brown, Director of the Direct Tel. Reference Laboratory (44-1932) 35 73 39; Direct Fax (44-1932) 35 72 39; Email: i.h.brown@vla.defra.gsi.gov.uk Ruth Manwell, Reference Laboratory Manager Direct Tel. (44-1932) 35 77 36 or (44-1932) 35 77 08 Direct Fax (44-1932) 35 78 56 E-mail: r.manvell@vla.defra.gsi.gov.uk CHAPTER XIV Minimum Safety Requirements for Diagnostic Laboratory I 11. Security requirements laboratories working with AI viruses should cover both the containment of viruses as a threat to animal health and the protection of those who work in the laboratory (and those outside) from any zoonous risk. In the Community, there are several Directives that set minimum laboratory safety requirements. In addition, operational aspects are described and described in the fundamental European norms. There are additional rules for diagnostic work, such as good laboratory practice. 2. Community Directives on the Laboratory Council Directive 89/391/EEC of 12 June 1989 to take measures to promote the safety and health of workers (OJ L 183, 29.6.1989, p. 1). Council Directive 90/679/EEC of 26 November 1990 to protect employees from the risks associated with exposure to biological agents at work (seventh Individual Directive for the meaning of Article 16 (1) Directive 89/391/EEC) (OJ L 374, 31.12.1990, p. 1). If there is a diagnosis made by polymerase chain reaction (PCR) and cloning PCR products into bacterial plasmid for distribution, for example, for the purposes of DNA sequencing, the following Directives and European Regulations (EN) are applied to these two Directives: The Council Directive 90/219/EEC of 23 April 1990 on how genetically modified microorganisms are contained (OJ L 117, 8.5.1990, p. 1). In addition to the Community Directives, European regulations should be recognized: EN 12128 Biotechnology. Laboratories for research, development and analysis. Levels of deterrence of microbiological laboratories, risk areas, settlements and physical safety requirements EN 12738 Biotechnology. Laboratories for research, development and analysis. A guide to containment of animals vaccinated with microorganisms in experiments EN 12740 Biotechnology. Laboratories for research, development and analysis. Guide to processing, inactivating and testing waste EN 12741 Biotechnology. Laboratories for research, development and analysis. The Guide to Biotechnology Laboratory Operations For the Operation/Management of the Laboratory applies the following conditions: 4. Laboratory requirements (deterrence levels 1 to 4) in accordance with the European Parliament and Council Directive 2000/EC of 18 September 2000 on the protection of workers from risks, Related to the impact of biological agents at work (seventh individual directive meaning Article 16 (1) Directive 89/391/EEC) (OJ L 262, 17.10.2000, p. 21), Directive 90/219/EEC and European Regulations: EN 12128; RU 12740; RU 12741. Containment measures Containment level 1 2 3 4 Laboratory suite: isolation no yes yes yes Laboratories separated by doors no yes yes yes An observation window or alternative must be present so that occupants can be seen optional optional optional yes Hand washing facilities must be provided for personnel yes yes yes yes Disinfecting facilities (hands) must be provided optional yes yes yes access no yes yes yes Specific measures to control aerosol no Yes minimise Yes prevent Yes prevent Biohazard sign on the door no yes yes yes Shower no no optional yes Eye irrigation yes yes yes yes Laboratory: sealable for fumigation no no yes yes Surfaces resistant to water, acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean Yes (bench) Yes (bench) Yes (bench, floor) Yes (bench, floor) Entry to lab via airlock no no optional yes Negative pressure relative to the pressure of the immediate environment no no optional yes Extract and input air from laboratory must be HEPA-filtered no no yes (extract air) yes Autoclave on site in the building en suite in lab, double ended Protective clothing Suitable protective clothing Suitable protective clothing Suitable protective clothing (optional footwear) Complete change of clothing Gloves no optional yes yes Efficient vector control (e.g. for rodents and insects) optional yes yes yes Safe Storage of a biological agent yes Yes yes yes Lab to maintain its own equipment is not recom-mended yes there are additional European regulations that relate to the management and organization of laboratories. There are other national and international norms and recommendations to be followed. WHO has published its Biosecurity Laboratory Guide on its website: 5. Veterinary authorities in Member States should take action on animal health regulations relating to the containment of AI viruses, especially HPAI, as well as all AI viruses in the H5 and H7 subtypes. Some recommendations are provided by the World Organization for Animal Health (IEG) in Chapter 1.4.5 of the 2005 Land Animal Health Code, and HPNAI is seen as a pathogen by the 4 MEI Deterrent Group. Although the rules governing the handling of AI viruses will be put in place by the veterinary authorities of the Member State. The minimum security requirements applied by the Community Reference Laboratory, which are national rules of the United Kingdom, can be found on the following website: 6. Human health laboratories working with AI viruses should always know that these are at least potential human pathogens, and conduct laboratory work to avoid infecting those working in the lab and anyone escaping the virus outside. Guidelines for the processing of samples suspected of containing the AI A virus can be found on the World Health Organization (WHO) website: (1) Samples should be conducted and stored for a later assessment for later assessment. Evaluation. oie diagnostic manual avian influenza