

2002/106/EC: Commission Decision of 1 February 2002 approving a Diagnostic Manual establishing diagnostic procedures, sampling methods and criteria for evaluation of the laboratory tests for the confirmation of classical swine fever (Text with EEA relevance) (notified under document number C(2002) 381)

Commission Decision

of 1 February 2002

approving a Diagnostic Manual establishing diagnostic procedures, sampling methods and criteria for evaluation of the laboratory tests for the confirmation of classical swine fever

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(Text with EEA relevance)

(2002/106/EC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 2001/89/EC of 23 October 2001 on Community measures for the control of classical swine fever⁽¹⁾, and in particular Article 17(3) and Article 29(1) thereof,

Whereas:

- (1) It is necessary to lay down at Community level diagnostic procedures, sampling methods and criteria for the evaluation of the results of laboratory tests for the confirmation of classical swine fever.
- (2) Annex IV to Directive 2001/89/EC lays down the functions and duties of the Community Reference Laboratory for classical swine fever in order to coordinate, in consultation with the Commission, the methods employed in the Member States for diagnosing the disease; these functions and duties include the organisation of periodic comparative tests and the supplying of standard reagents at Community level.
- (3) Classical swine fever virus is not considered to be a hazard for human health.
- (4) Laboratory tests have been recently developed to ensure a quick diagnosis of classical swine fever.
- (5) The experience gained in the control of classical swine fever in recent years has resulted in the identification of the most suitable sampling procedures and criteria for evaluation of the results of the laboratory tests for a proper diagnosis of this disease in different situations.
- (6) The measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION:

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Article 1

- 1 Member States shall ensure that the confirmation of classical swine fever is based on:
- a the detection of clinical signs and post-mortem lesions of disease;
 - b the detection of virus, antigen or genome in samples of pig tissues, organs, blood or excreta;
 - c the demonstration of a specific antibody response in blood samples,

in accordance with the procedures, sampling methods and criteria for evaluation of the results of laboratory tests laid down in the Manual annexed to this Decision.

2 However, the national diagnostic laboratories referred to in Annex III(1) to Directive 2001/89/EC may apply modifications to the laboratory tests referred to in the Manual annexed to this Decision, or use different tests, provided that an equal sensitivity and specificity can be demonstrated.

The sensitivity and specificity of these modified or different tests must be evaluated in the framework of the periodic comparative tests organised by the Community Reference Laboratory for classical swine fever.

Article 2

Annexes I and IV to Council Directive 80/217/EEC of 22 January 1980 introducing Community measures for the control of classical swine fever⁽²⁾, as last amended by the Act of Accession of Austria, Finland and Sweden, are hereby repealed.

Article 3

This Decision shall apply from 1 November 2002.

Article 4

This Decision is addressed to the Member States.

Done at Brussels, 1 February 2002.

For the Commission David Byrne Member of the Commission

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ANNEX

CLASSICAL SWINE FEVER DIAGNOSTIC MANUAL

CHAPTER I

Introduction, objectives and definitions

1. In order to ensure uniform procedures to diagnose classical swine fever, this Manual:
 - (a) provides guidelines and minimum requirements on diagnostic procedures, sampling methods and criteria for the evaluation of the results of clinical and post-mortem examinations and laboratory tests for a proper diagnosis of classical swine fever;
 - (b) establishes minimum bio-safety requirements and quality standards to be observed by the classical swine fever diagnostic laboratories and for transport of samples;
 - (c) establishes the laboratory tests to be used for the diagnosis of classical swine fever and the laboratory techniques to be used for the genetic typing of classical swine fever virus isolates.
2. This Manual is principally directed towards the authorities responsible for the control of classical swine fever. Therefore, emphasis is on the principles and applications of laboratory tests and evaluation of their results and not on detailed laboratory techniques.
3. For the purpose of this Manual, in addition to the definitions referred to in Article 2 of Directive 2001/89/EC, the following definitions shall apply:
 - (a) "suspected holding" means any pig holding which contains one or more pigs suspected of being infected with classical swine fever virus or a contact holding as defined in Article 2(v) of Directive 2001/89/EC;
 - (b) "singleton reactors" means any pig which yields a positive result in serological tests for classical swine fever but which has no history of contact with classical swine fever virus and from which there is no evidence of spread of infection to in-contact pigs ;
 - (c) "epidemiological sub-unit" or "sub-unit" means the building, place or land nearby in which groups of pigs within a holding are kept in such a way that they have frequent direct or indirect contact one to the other but, in the meantime, they are kept separated from other pigs kept in the same holding;
 - (d) "in-contact pigs" means the pigs which lived in a holding in direct contact with one or more pigs suspected to be infected with classical swine fever virus within the last 21 days.

CHAPTER II

Description of classical swine fever with emphasis on differential diagnosis

- A. Introduction
 1. Classical swine fever is caused by an enveloped RNA virus which belongs to the genus pestivirus of the flaviviridae family. This virus is related to the ruminant pestiviruses causing bovine viral diarrhoea (BVDV) and border disease (BDV). This relationship

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has serious diagnostic consequences as cross reactions occur and may lead to false positive results of the laboratory tests.

2. Classical swine fever virus is relatively stable in moist excretions of infected pigs, pig carcasses and fresh pig meat and some pig meat products. It is readily inactivated by detergents, lipid solvents, proteases and common disinfectants.
3. The main natural route of infection is oro-nasal by direct or indirect contact with infected pigs or by feeding of virus contaminated feed. In areas with a high density of pigs, spread of virus easily occurs between neighbouring pig holdings. Disease transmission via semen of infected boars may also occur.
4. The incubation period in individual animals is about one week to ten days, but under field conditions clinical symptoms may only become evident in a holding two to four weeks after virus introduction or even more if only adult breeding pigs or mild strains of virus are concerned.
5. The clinical signs of classical swine fever are extremely variable and it may be confused with many other diseases. Severity of symptoms depends mainly on the age of the animal and virus virulence. Usually young animals are affected more severely than older animals. In older breeding pigs the course of the infection is often mild or even sub-clinical.
6. Acute, chronic and prenatal forms of classical swine fever can be distinguished.

B. Acute form

1. Weaners and fattening pigs most often display the acute form of classical swine fever. The initial signs are anorexia, lethargy, fever, conjunctivitis, swollen lymph nodes, respiratory signs and constipation followed by diarrhoea.

The typical haemorrhages of the skin, are usually observed on the ear, tail, abdomen and the inner side of the limbs during the second and third week after infection until death. Neurological signs are frequently seen, such as a staggering hind limb gait, in coordination of movement, and convulsions.

A constant finding is fever. This is usually higher than 40 °C, but in adult pigs fever may not exceed 39,5 °C.

2. Classical swine fever virus causes severe leukopenia and immunosuppression, which often leads to enteric or respiratory secondary infections. The signs of these secondary infections can mask or overlap the most typical signs of classical swine fever and may mislead the farmer or the veterinarian.

Death occurs usually within one month. Recovery with production of antibodies does occur, most often in adult breeding animals which do not display severe clinical signs. Antibodies against classical swine fever virus are detectable from 2 to 3 weeks post infection onwards.

3. Pathological changes visible on post-mortem examination are most frequently observed in lymph nodes and kidneys. The lymph nodes become swollen, oedematous and haemorrhagic. Haemorrhages of the kidney may vary in size from hardly visible petechiae to ecchymotic haemorrhages. Similar haemorrhages can also be observed in the urinary bladder, larynx, epiglottis and heart and sometimes widespread over the serosa of the abdomen and chest. A non-purulent encephalitis is often present. Lesions due to secondary infections may also be seen which may mislead the veterinarian. Infarcts in the spleen are considered pathognomonic but are infrequently seen.

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4. In general the acute form of African swine fever leads to a clinical and pathological picture very similar to that of classical swine fever. When present, haemorrhages on the skin and ears are quite easy to detect and lead to suspicion of acute African or classical swine fever. Few other diseases cause similar lesions.

Acute classical swine fever must also be considered in case of suspected erysipelas, porcine reproductive and respiratory syndrome, coumarin poisoning, purpura haemorrhagica, post-weaning multisystemic wasting syndrome, porcine dermatitis and nephropathy syndrome, salmonella or Pasteurella infections or any enteric or respiratory syndromes with fever which do not respond to antibiotic treatment.

5. Classical swine fever virus is shed in saliva, urine and faeces from the onset of clinical signs until death. Classical swine fever virus can also be shed via semen.

C. Chronic form

1. The chronic course of infection occurs when pigs are not able to develop an effective immune response against classical swine fever virus. Initial signs of a chronic infection are similar to the acute infection. Later, predominately non-specific signs are present, i.e. intermittent fever, chronic enteritis and wasting. The typical haemorrhages of the skin are missing.

These pigs may show clinical signs of disease for 2 to 3 months before death. Classical swine fever virus is constantly shed from the onset of clinical signs until death. Antibodies may be temporarily detected in serum samples.

2. Pathological changes are less typical, especially haemorrhages in organs and serosa may not be observed. In animals showing chronic diarrhoea, necrotic lesions are common on the ileum, the ileocaecal valve and the rectum.
3. As clinical signs of chronic classical swine fever are rather non-specific, many other diseases must be considered for differential diagnosis. The increased body temperature is not necessarily present in every animal, but in an infected holding fever can be detected at least in some pigs.

D. Prenatal form and late onset of disease

1. Classical swine fever virus is able to pass across the placenta of pregnant animals, and infect foetuses, but in the sows the disease is often sub-clinical.

The outcome of trans-placental infection of foetuses depends largely on the stage of gestation and viral virulence. Infection during early pregnancy may result in abortions and stillbirths, mummification and malformations. All this leads to a reduction of the fertility index in the holding.

Infection of sows at up to 90 days of pregnancy can lead to the birth of persistently viraemic piglets, which may be clinically normal at birth and survive for several months. After birth, they may show poor growth, wasting or occasionally congenital tremor. This course of infection is referred to as "late onset classical swine fever". These piglets may play a crucial role in spreading the disease and in the maintenance of virus persistence within a population, as they constantly shed virus until death.

2. Detection of classical swine fever may be particularly difficult in breeding pig holdings, as the course of the infection may be very mild and may be confused with many other pathological conditions. Reduced fertility and abortions can be caused by classical swine fever virus as well as parvovirus infection, PRRS, leptospirosis, and

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Aujeszky's disease. Material aborted due to classical swine fever infection cannot be distinguished pathologically from abortions due to other disease agents.

In case of suspicion of an infectious disease of the reproductive tract, investigation for classical swine fever must be immediately carried out whenever the holding in question can be considered at risk (e.g. due to location of the holding in an area where classical swine fever occurs in feral pigs), and in any case as soon as more common infectious diseases of the reproductive tract have been excluded.

CHAPTER III

Guidelines on main criteria to be considered for the recognition of a holding as a classical swine fever suspected holding

The decision to recognise a holding as a suspected holding will be taken on the basis of the following findings, criteria and grounds:

- (a) clinical and pathological findings in pigs. The main clinical and pathological findings to be considered are:
 - fever with increased morbidity and mortality;
 - fever with haemorrhagic syndrome;
 - fever with neurological symptoms;
 - fever of unknown origin where treatment with antibiotics failed to improve the health state;
 - abortions and increased fertility problems during the last three months;
 - congenital tremor of piglets;
 - chronically diseased animals;
 - growth retarded (runted) young animals;
 - petechial and ecchymotic haemorrhages, especially in lymph nodes, kidneys, spleen, bladder and larynx;
 - infarction or haematomas, notably in the spleen;
 - button ulcers in the large intestine of chronic cases, particularly near the ileo-caecal junction.
- (b) epidemiological findings. The main epidemiological findings to be considered are:
 - where pigs had direct or indirect contact to a pig holding proven to have been infected with classical swine fever;
 - where a holding has supplied pigs that were subsequently shown to be infected with classical swine fever;
 - where sows have been artificially inseminated with semen originating from a suspect source;
 - where there has been indirect or direct contact with feral pigs of a population where classical swine fever occurs;
 - where pigs are kept outdoors in a region where feral pigs are infected with classical swine fever;
 - where pigs have been fed with swill and there is the suspicion that this swill has not been treated in such a way as to inactivate classical swine fever virus;
 - where possible exposure might have occurred, e.g. due to persons entering the holding, transports, etc.
- (c) findings related to results of serological tests. The main laboratory findings to be considered are:

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serological reaction caused by an unnoticed classical swine fever virus infection or by vaccination⁽⁵⁾;
cross-reaction between antibodies to classical swine fever and to other pestiviruses⁽⁶⁾;
detection of singleton reactors⁽⁷⁾.

CHAPTER IV

Checking and sampling procedures

- A. Guidelines and procedures for clinical examination and sampling on pigs in suspected holdings
1. Member States shall ensure that appropriate clinical examinations, sampling and laboratory investigations are carried out in suspected holdings to confirm or exclude classical swine fever, in accordance with the guidelines and procedures laid down in subparagraphs 2 to 7.

Irrespective of the adoption of the measures referred to in Article 4(2) of Directive 2001/89/EC in the holding in question, those guidelines and procedures shall also apply in cases of disease whenever classical swine fever is considered in the differential diagnosis. This will include occasions when the clinical signs and epidemiological pattern of disease that are observed in pigs suggest a very low probability of occurrence of classical swine fever.

In all other cases where one or more pigs are suspected of being infected with classical swine fever virus, the measures referred to in Article 4(2) of Directive 2001/89/EC shall be adopted in the suspected holding in question.

In case of suspicion of classical swine fever in pigs in a slaughterhouse or means of transport, the guidelines and procedures laid down in subparagraphs 2 to 7 shall also apply mutatis mutandis.

2. When an official veterinarian visits a suspected holding to confirm or rule out classical swine fever: a check of the production and health records of the holding must be carried out, if these records are available; an inspection in each sub-unit of the holding must be carried out to select the pigs to be clinically examined.

The clinical examination must include the taking of body temperature and must primarily concern the following pigs or group of pigs:

sick or anorexic pigs;
pigs recently recovered from disease;
pigs recently introduced from confirmed outbreaks or from other suspected sources;
pigs kept in sub-units recently visited by external visitors which had a recent close contact with classical swine fever suspected or infected pigs or for which other particularly risky contacts with a potential source of classical swine fever virus have been identified;
pigs already sampled and serologically tested for classical swine fever, in case the results of these tests do not allow to rule out classical swine fever, and in-contact pigs.

If the inspection in the suspected holding has not indicated the presence of the pigs or group of pigs referred to in the above subparagraph, the competent authority, without prejudice to other measures that may be applied in the holding in question in accordance with Directive 2001/89/EC and taking into account the epidemiological situation, shall:

carry out further examinations in the holding in question in accordance with subparagraph 3 below; or

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ensure that blood samples for laboratory tests are taken from the pigs in the holding in question. In this case the sampling procedures laid down in subparagraph 5 and in F.2, shall be used for guidance purposes; or
adopt or maintain the measures laid down in Article 4(2) of Directive 2001/89/EC, pending further investigations in the holding in question; or
rule out the suspicion of classical swine fever.

3. When reference is made to this paragraph, the clinical examination in the holding in question must be carried out on pigs selected at random in the sub-units for which a risk of introduction of classical swine fever virus has been identified or is suspected.

The minimum number of pigs to be examined must allow for the detection of fever if it occurs at a prevalence of 10 % with 95 % confidence in these sub-units.

However, in case of:

breeding sows, the minimum number of sows to be examined must allow for the detection of fever if it occurs at a prevalence of 5 % with 95 % confidence;
at semen collection centres, all boars must be examined.

4. If dead or moribund pigs are detected in a suspected holding, post-mortem examinations must be carried out, preferably on at least five of these pigs and in particular on pigs that before death have shown or are showing very evident signs of disease; with high fever; recently dead.

If these examinations have not shown lesions suggesting classical swine fever but, due to the epidemiological situation, further investigations are deemed necessary:

a clinical examination, as laid down in subparagraph 3, and blood sampling as laid down in subparagraph 5 must be carried out in the sub-unit where the dead or moribund pigs were kept;
and

post-mortem examinations may be carried on 3 to 4 in-contact pigs.

Irrespective of the presence or absence of lesions suggesting classical swine fever, samples of the organs or tissues from pigs that have been subjected to post-mortem examination must be collected for virological tests in accordance with Chapter V B. 1 These samples must be preferably collected from recently dead pigs.

When post-mortem examinations are carried out the competent authority must ensure that: the necessary precautions and hygienic measures are taken to prevent any disease spread; and, in case of moribund pigs, they are killed in a humane way in accordance with Council Directive 93/119/EEC.

5. If further clinical signs or lesions that may suggest classical swine fever are detected in a suspected holding, but the competent authority deems that these findings are not sufficient to confirm an outbreak of classical swine fever and that laboratory tests are therefore necessary, blood samples for laboratory tests must be taken from the suspected pigs and from other pigs in each sub-unit in which the suspected pigs are kept, in accordance with the procedures laid down below.

The minimum number of samples to be taken for serological tests must allow for the detection of 10 % seroprevalence with 95 % confidence in the sub-unit in question.

However, in the case of:

breeding sows, the minimum number of sows to be sampled must allow for the detection of 5 % seroprevalence with 95 % confidence⁽⁸⁾;
a semen collection centre, blood samples must be taken from all boars.

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The number of samples to be taken for virological tests will be in accordance with the instructions of the competent authority, which will take into account the range of tests that can be performed, the sensitivity of the laboratory tests that will be used and the epidemiological situation.

6. If the suspicion of classical swine fever in the holding in question is related to the results of previous serological tests, in addition to the blood samples to be taken from the pigs referred to in 2, second subparagraph, fifth indent, the following procedures shall be applied: if the seropositive pigs are pregnant sows, some of them, preferably not less than three, shall be euthanased and subjected to a post-mortem examination. Prior to killing a blood sample must be taken for further serological tests. The foetuses shall be subjected to examination for classical swine fever virus, virus antigen or virus genome in accordance with Chapter VI to detect intrauterine infection; if the seropositive pigs are sows with suckling piglets, blood samples must be taken from all piglets and shall be subjected to examination for classical swine fever virus, virus antigen or virus genome as referred to in Chapter VI. Blood samples must also be taken from the sows for further serological tests.
 7. If, after the examination carried out in a suspected holding, clinical signs or lesions suggestive of classical swine fever are not detected, but further laboratory tests are deemed necessary by the competent authority to rule out classical swine fever, the sampling procedures laid down in subparagraph 5 shall be used for guidance purposes.
- B. Sampling procedures in a holding when pigs are killed following confirmation of disease
1. In order that the manner of introduction of classical swine fever virus into an infected holding and the length of time elapsed since its introduction may be established, when pigs are killed on a holding following confirmation of an outbreak in accordance with Article 5(1)(a) of Directive 2001/89/EC, blood samples for serological tests must be taken at random from the pigs when they are killed.
 2. The minimum number of pigs to be sampled must allow for the detection of 10 % seroprevalence with 95 % confidence in pigs in each sub-unit of the holding.

Samples for virological tests may also be taken in accordance with the instructions of the competent authority, which will take into account the range of tests that can be performed, the sensitivity of the laboratory tests that will be used and the epidemiological situation.

3. However, in case of secondary outbreaks, the competent authority may decide to derogate from subparagraphs 1 and 2 and establish ad hoc sampling procedures, taking into account the epidemiological information already available on the source and means of virus introduction into the holding and the potential spread of disease from the holding.
- C. Sampling procedures when pigs are killed as a preventive measure on a suspected holding
1. In order that classical swine fever may be confirmed or ruled out and additional epidemiological information is gained, when pigs are killed as a preventive measure on a suspected holding in accordance with the provisions of Article 4(3)(a) or Article 7(2) of Directive 2001/89/EC, blood samples for serological tests as well as blood or tonsils samples for virological tests must be taken in accordance with the procedure laid down in subparagraph 2.
 2. Sampling must primarily concern:

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pigs showing signs or post-mortem lesions suggesting classical swine fever and their in-contact pigs;

other pigs which might have had risky contacts with infected or suspected pigs or which are suspected to have been contaminated with classical swine fever virus.

These pigs must be sampled in accordance with the instructions of the competent authority, which will take into account the epidemiological situation. In this case, the sampling procedures laid down in the second, third and fourth subparagraphs below shall be used for guidance purposes.

Furthermore, pigs proceeding from each of the sub-units of the holding must be sampled at random⁽¹⁰⁾. In this case, the minimum number of samples to be taken for serological tests must allow for the detection of 10 % seroprevalence with 95 % confidence in the sub-unit in question.

However, in the case of:

breeding sows, the minimum number of sows to be sampled must allow for the detection of 5 % seroprevalence with 95 % confidence⁽¹¹⁾;

a semen collection centre, blood samples must be taken from all boars.

The type of samples to be taken for virological tests and the test to be used will be in accordance with the instructions of the competent authority, which will take into account the range of tests that can be performed, the sensitivity of these tests and the epidemiological situation.

D. Checking and sampling procedures before authorisation is given to move pigs from holdings located in protection or surveillance zones and in case these pigs are slaughtered or killed

1. Without prejudice to the provisions of Article 11(1)(f), second subparagraph of Directive 2001/89/EC, in order that authorisation may be given to move pigs from holdings located in protection or surveillance zones in accordance with Article 10(3) of the said Directive, the clinical examination to be carried out by an official veterinarian must: be carried out within the 24-hour period before moving the pigs; be in accordance with the provisions laid down in A. 2.
2. In the case of pigs to be moved to another holding, in addition to the investigations to be carried out in accordance with subparagraph 1, a clinical examination of pigs must be carried out in each sub-unit of the holding in which the pigs to be moved are kept. In case of pigs older than three to four months, this examination must include the taking of temperature of a proportion of pigs.

The minimum number of pigs to be checked must allow for the detection of fever if it occurs at a prevalence of 10 % with 95 % confidence in these sub-units.

However, in the case of:

breeding sows, the minimum number of sows to be examined must allow for the detection of fever if it occurs at a prevalence of 5 % with 95 % confidence in the sub-unit where the sows to be moved are kept;

boars, all boars to be moved must be examined.

3. In case of pigs to be moved to a slaughterhouse, to a processing plant or to other places to be then killed or slaughtered, in addition to the investigations to be carried out in accordance with subparagraph 1, a clinical examination of pigs must be carried out in each sub-unit in which the pigs to be moved are kept. In case of pigs older than three to four months, this examination must include the taking of temperature of a proportion of pigs.

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The minimum number of the pigs to be checked must allow for the detection of fever if it occurs at a prevalence of 20 % with 95 % confidence in the sub-units in question.

However, in the case of breeding sows or boars, the minimum number of pigs to be examined must allow for the detection of fever if it occurs at a prevalence of 5 % with 95 % confidence in the subunit where the pigs to be moved are kept.

4. When the pigs referred to in subparagraph 3 are slaughtered or killed, blood samples for serological tests or blood or tonsils samples for virological tests must be taken from pigs proceeding from each of the sub-units from which pigs have been moved.

The minimum number of samples to be taken must allow for the detection of 10 % seroprevalence or virus prevalence with 95 % confidence in each sub-unit.

However, in the case of breeding sows or boars the minimum number of pigs to be sampled must allow for the detection of 5 % of seroprevalence or virus prevalence with 95 % confidence in the subunit where these pigs were kept.

The type of samples to be taken and the test to be used will be in accordance with the instructions of the competent authority, which will take into account the range of tests that can be performed, the sensitivity of these tests and the epidemiological situation.

5. However, if clinical signs or post-mortem lesions suggesting classical swine fever are detected when the pigs are slaughtered or killed, by way of derogation from subparagraph 4, the provisions on sampling laid down in C shall apply.

E. Checking and sampling procedures in a holding in relation to re-population

1. When pigs are re-introduced into a holding in accordance with Article 13(2)(a) or (2) (b) or Article 19(8), second subparagraph (b) of Directive 2001/89/EC, the following sampling procedures must be applied:

in case sentinel pigs are reintroduced, blood samples for serological tests must be taken at random from a number of pigs that allow for the detection of 10 % seroprevalence with 95 % confidence in each sub-unit of the holding;

in case of total re-population, blood samples for serological tests must be taken at random from a number of pigs that allow for the detection of 20 % seroprevalence with 95 % confidence in each sub-unit of the holding.

However, in the case of breeding sows or boars the number of samples to be taken must be such as to detect 10 % seroprevalence with 95 % confidence.

2. After re-introduction of pigs, the competent authority shall ensure that in case of any disease or death of the pigs in the holding due to unknown reasons, the pigs in question are immediately tested for classical swine fever. These provisions shall apply until the restrictions referred to in Article 13(2)(a), second subparagraph and Article 19(8), second subparagraph (b), second sentence of Directive 2001/89/EC are lifted in the holding in question.

F. Sampling procedures in holdings in the protection zone before lifting restrictions

1. In order that the measures referred to in Article 10 of Directive 2001/89/EC may be lifted in a protection zone, in all holdings in the zone:

a clinical examination must be carried out in accordance with the procedures laid down in A.2 and 3;

blood samples for serological tests must be taken as laid down in subparagraph 2.

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2. The minimum number of blood samples to be taken must allow for the detection of 10 % seroprevalence with 95 % confidence in pigs in each sub-unit in the holding.

However, in the case of:

breeding sows, the minimum number of samples to be taken must allow for the detection of 5 % seroprevalence with 95 % confidence;

a semen collection centre, blood samples must be taken from all boars.

- G. Sampling procedures in holdings in the surveillance zone before lifting restrictions

1. In order that the restrictions referred to in Article 11 of Directive 2001/89/EC may be lifted in a surveillance zone, a clinical examination must be carried out in all holdings in the zone in accordance with the procedures laid down in A.2.

In addition, blood samples for serological tests must be taken from pigs:

in all the holdings where no pigs of between two and eight months of age are kept;

whenever the competent authority deems that classical swine fever might have spread unnoticed amongst breeding sows;

in any other holding where sampling is deemed necessary by the competent authority;

in all semen collection centres.

2. Whenever blood sampling for serological tests is carried out in holdings located in the surveillance zone, the number of blood samples to be taken in these holdings must be in accordance with F.2. However, if the competent authority deems that classical swine fever might have spread unnoticed amongst breeding sows, sampling may only be carried out in the sub-units where these animals are kept.

- H. Serological monitoring and sampling procedures in areas where classical swine fever is suspected to occur or has been confirmed in feral pigs

1. In the case of serological monitoring in feral pigs in areas where classical swine fever has been confirmed or is suspected to occur, the size and the geographical area of the target population to be sampled should be previously defined in order to establish the number of samples to be taken. Sample size must be established as a function of the estimated number of living animals and not as a function of shot animals.

2. If data on population density and size are not available, the geographical area within which to sample must be identified taking into account the continuous presence of feral pigs and the presence of natural or artificial barriers efficient to prevent large and continuous movement of the animals. When such circumstances do not occur, or in case of large areas, it is recommended to identify sampling areas of not more than 200 km², where population of about 400 to 1000 feral pigs may usually live.

3. Without prejudice to the provisions of Article 15(2)(c) of Directive 2001/89/EC, the minimum number of pigs to be sampled within the defined sampling area must allow to detect 5 % seroprevalence with 95 % confidence. For this purpose at least 59 animals must be sampled in each area which has been identified.

It is also recommended that:

in areas where hunting pressure is higher and regularly performed, or selective hunting is carried out as a disease control measure, approximately 50 % of the sampled animals belong to the three months to one year age class, 35 % to one to two years age class and 15 % to more than 2 years age class;

in areas where hunting pressure is very low or absent, at least 32 animals are sampled for each one of the three age classes;

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sampling is performed in a short time, preferably not more than one month; the age of sampled animals is identified according to the teeth eruption.

4. Collection of samples for virological tests from feral pigs shot or found dead must be carried out as laid down in Chapter V B.1.

When virological monitoring on shot feral pigs is deemed necessary, it must be primarily carried out on animals three months to one year old.

5. All samples to be sent to the laboratory must be accompanied by the questionnaire referred to in Article 16(3)(1) of Directive 2001/89/EC.

CHAPTER V

General procedures and criteria for collection and transport of samples

A. General procedures and criteria

1. Before sampling is carried out in a suspected holding, a map of the holding must be prepared and the epidemiological sub-units of the holding must be identified.
2. Each time that it is deemed that re-sampling of pigs might be necessary, all pigs which are sampled must be uniquely marked in such a way that they can be easily re-sampled.
3. Without prejudice to Chapter IV A. 5.b, samples for serological testing must not be taken from piglets less than eight weeks old.
4. All samples must be sent to the laboratory accompanied by appropriate forms, in accordance with the requirements established by the competent authority. These forms will include details of the history of the pigs sampled and the clinical signs or post-mortem lesions observed.

In the case of pigs kept in holdings, clear information on age, category and holding of origin of the pigs sampled must be provided. It is recommended that the location of each pig sampled in the holding be recorded together with its unique identification mark.

B. Collection of samples for virological tests

1. For detection of classical swine fever virus, antigen or genome from dead or euthanised pigs, tonsils, spleen and kidney tissues are the most suitable samples. In addition, it is recommended to collect two samples of other lymphatic tissues, such as the retro-pharyngeal, parotid, mandibular or mesenteric lymph nodes and a sample of ileum. In case of autolysed carcasses, an entire long bone or the sternum is the specimen of choice.
2. Anticoagulated blood or clotted blood samples must be collected from pigs showing signs of fever or other signs of disease, in accordance with the instructions of the competent authority.
3. Virological tests are recommended in the case of sick animals. They are usually of limited value when used for monitoring purposes on animals which do not show clinical signs. However, if the objective of a large-scale sampling is to detect classical swine fever virus when the pigs are in their incubation period, the tonsils are the most appropriate samples.

C. Transport of samples

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1. It is recommended that all samples are transported and stored in leak-proof containers; are not frozen but kept cool at refrigerator temperature; are delivered to the laboratory as quickly as possible; are kept in a package where ice packs rather than wet ice is used inside to keep them cool; if tissue or organs are placed in a separate sealed plastic bag and properly labelled. They must be then placed in larger strong outer containers and packed with sufficient absorbent material to protect from damage and absorb leakage; whenever possible, are directly transported to the laboratory by competent personnel in order that a rapid and reliable transport is ensured.
2. The outside of the package must be labelled with the address of the recipient laboratory and the following message should be prominently displayed: Animal pathological material; Perishable; Fragile; Do not open outside a classical swine fever laboratory.
3. The laboratory receiving the samples must be informed in advance of the time and mode of the arrival of the samples.
4. For air transport of samples to the Community Reference Laboratory for classical swine fever from Member States other than Germany or from third countries the package has to be labelled according to IATA regulations.

CHAPTER VI

Principles and use of virological tests and evaluation of their results

A. Detection of virus antigen

1. Fluorescent antibody test (FAT)

The principle of the test is the detection of viral antigen on thin cryosections of organ material from pigs suspected of being infected with classical swine fever virus. The intracellular antigen is detected by using a FITC conjugated antibody. A positive result should be confirmed by repeating the staining with a specific monoclonal antibody.

Suitable organs are tonsils, kidney, spleen, different lymph nodes and ileum. A smear of bone marrow cells might also be used in case of feral pigs, if these organs are not available or are autolysed.

The test can be performed within one day. As organ samples can only be obtained from dead animals its use for screening purposes is limited. Confidence in the test result may be limited by doubtful staining, particularly where considerable experience in performing the test has not been acquired or if the organs tested are autolysed.

2. ELISA for antigen detection

Viral antigen is detected by using various ELISA techniques. The sensitivity of the antigen ELISA should be high enough to score a positive result from animals showing clinical signs of classical swine fever.

The use of ELISA for antigen detection is recommended on samples from animals with clinical signs or pathological lesions of disease. It is not suitable for the investigation of individual animals. Suitable samples are leukocytes, serum, non-coagulated blood as well as suspensions of the organs referred to in subparagraph 1 taken from pigs suspected of being infected with classical swine fever virus⁽¹³⁾.

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The ELISA can be carried out within one day and can be performed by automatic equipment. The most important advantage is that large numbers of samples can be processed in a short period of time. It is recommended that ELISA antigen which give satisfactory results on reference material are used. However, at present all commercial ELISA are less sensitive than the virus isolation on cell culture and their sensitivity is significantly better on blood samples from piglets than from adult pigs.

B. Virus isolation

1. Virus isolation is based on the incubation of sample material on susceptible cell cultures of porcine origin. If classical swine fever virus occurs in the sample, it will replicate in the cells to an amount that can be detected, by immunostaining of the infected cells with conjugated antibodies. Classical swine fever specific antibodies are required for differential diagnosis with respect to other pestiviruses.
2. The preferred samples for isolation of classical swine fever virus are leukocytes, plasma or whole blood obtained from non-coagulated blood samples or the organs referred to in A.1.
3. Virus isolation is best suited for the investigation of samples from small numbers of animals rather than mass surveillance. The virus isolation procedure is labour intensive and requires at least three days before results are available. Two further cell culture passages may be necessary in order that a small amount of virus in the sample is detected. This may lead to an investigation time of up to 10 days before a final result is obtained. Autolysed samples can be cytotoxic to the cell culture and consequently limit its use.
4. It is recommended to perform virus isolation also in case of previous confirmation of classical swine fever by other methods. It must be used as reference test for the confirmation of positive results of prior antigen ELISA, PCR or FAT, indirect peroxidase-staining methods respectively.

Classical swine fever virus isolates obtained in this way are useful for virus characterisation including genetic typing and molecular epidemiology.

5. All classical swine fever virus isolates from all primary outbreaks, primary cases in feral pigs or cases in slaughterhouse or means of transport must be genetic typed by a national reference laboratory in the Member States, or by any other laboratory authorised by the Member State in question or by the Community Reference Laboratory, in accordance with E.

In any case, these virus isolates must be sent to the Community Reference Laboratory for virus collection without delay.

C. Detection of virus genome

1. The polymerase chain reaction (PCR) is applied to detect virus genome in blood, tissues or organ samples. Small fragments of viral RNA are transcribed into DNA fragments which are amplified by PCR to detectable quantities. Since this test detects only a genome sequence of the virus, the PCR may be positive, even when there is no infectious virus present (e.g. in autolysed tissues or samples from convalescent pigs).
2. PCR can be used on small numbers of samples which have been carefully selected from suspect animals or on material from aborted fetuses. In carcasses from wild boar it might be the method of choice, if the material is autolysed and virus isolation is not possible any more due to cytotoxicity.

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3. Suitable sample material for diagnostic PCR are the organs described for virus isolation or unclotted blood.
4. PCR can be performed within 48 hours. It requires appropriate laboratory equipment, separated facilities and skilled staff. An advantage is that infectious virus particles need not be replicated in the laboratory. The method is highly sensitive, but contamination may easily occur, which leads to false positive results. Therefore stringent quality control procedures are essential. Some methods are pestivirus rather than classical swine fever specific, requiring further confirmatory tests, such as sequencing of the PCR product.

D. Evaluation of the results of virological tests

1. Virological tests are essential for the confirmation of classical swine fever.

Virus isolation must be considered as the reference virological test and must be used as confirmatory test when necessary. Its use is particularly recommended in case positive FAT, ELISA or PCR results are not associated with the detection of clinical signs or lesions of disease and in any other doubtful case.

However, a primary outbreak of classical swine fever can be confirmed if clinical signs or lesions of disease have been detected in the pigs in question and at least two antigen or genome detection tests have given a positive result.

A secondary outbreak of classical swine fever can be confirmed if, in addition to the epidemiological link to a confirmed outbreak or case, clinical signs or lesions of disease have been detected in the pigs in question and an antigen or genome detection tests has given a positive result.

A primary case of classical swine fever in feral pigs can be confirmed after virus isolation or if at least two antigen or genome detection tests have given a positive result. Further cases of classical swine fever in feral pigs for which an epidemiological link with previously confirmed cases have been found can be confirmed if an antigen or genome detection test has given a positive result.

2. A positive result for classical swine fever to a genome or antigen detection test requires that the test in question has been performed using classical swine fever virus-specific antibodies or primers. If the test used was not specific for classical swine fever virus but only pestivirus specific, it must be repeated using classical swine fever specific reagents.

E. Genetic typing of classical swine fever virus isolates

1. Genetic typing of classical swine fever virus isolates is achieved by determining the nucleotide sequence of portions of the virus genome, namely specific parts of the 5'noncoding region and/or of the E2 glycoprotein gene. The similarity of these sequences with those already obtained from previous virus isolates can indicate whether or not outbreaks of disease are caused by new or already recognised strains. This can support or refute hypotheses on transmission routes that have been provided by epidemiological tracing.

Genetic typing of classical swine fever virus isolates is of major importance to determine the source of disease. However, a close relationship between viruses obtained from different outbreaks is not an absolute proof for a direct epidemiological link.

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2. If virus typing cannot be performed in a national laboratory or in any other laboratory authorised to diagnose classical swine fever within a short delay, the original sample or the virus isolate must be sent to the Community Reference Laboratory for typing as soon as possible.

The data on typing and sequencing of classical swine fever virus isolates available to the laboratories authorised to diagnose classical swine fever must be forwarded to the Community Reference Laboratory in order that this information is entered into the database kept by this laboratory.

The information included in this database must be available to all national reference laboratories in the Member States. However, for the purpose of publication on scientific journals, if requested by the laboratory in question, the Community Reference Laboratory shall guarantee confidentiality of these data until they are published.

CHAPTER VII

Principles and use of serological tests and evaluation of their results

- A. Basic principles and diagnostic value
 1. In classical swine fever virus infected pigs, antibodies are usually detectable in serum samples from two to three weeks after infection. In pigs that have recovered from the disease, protective neutralising antibodies can be detected for several years or even for their lifetime. Antibodies are also sporadically detectable in the terminal stage of lethally diseased animals. In some pigs with chronic form of classical swine fever, antibodies might be detectable for a few days at the end of the first month post-infection.

Pigs infected in utero may be immunotolerant against the homologue classical swine fever virus and produce no specific antibodies. However, antibodies of maternal origin can be detected during the first days of life. The half-life of maternal antibodies in non-viraemic healthy piglets is about two weeks. If found in piglets older than three months, classical swine fever antibodies are very unlikely to be of maternal origin.

2. The detection of antibodies against classical swine fever virus in serum or plasma samples is carried out to assist the diagnosis of classical swine fever in suspected holdings, for establishing the age of infection in case of a confirmed outbreak and for monitoring and surveillance purposes. However, serological tests are of limited value for the detection of classical swine fever in the case of a recent infection in a holding.

A few seropositive pigs with a low neutralisation titre can be indicative of a recent infection (two to four weeks). Many pigs with high neutralisation titre could indicate that virus entered the holding more than one month before. The location of seropositive pigs in the holding can provide valuable information on how classical swine fever virus entered the holding.

However, an accurate evaluation of the results of the serological tests must be carried out taking into account the whole clinical, virological and epidemiological findings, in the framework of the enquiry to be carried out in case of suspicion or confirmation of classical swine fever, in accordance with Article 8 of Directive 2001/89/EC.

- B. Recommended serological tests

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1. The virus neutralisation test (VNT) and the ELISA are the tests of choice for the serological diagnosis of classical swine fever.

The quality and efficiency of the serological diagnosis performed by the national laboratories must be regularly checked in the framework of the inter-laboratory comparison test periodically organised by the Community Reference Laboratory.

2. The VNT is based on the determination of the virus neutralising activity of the antibodies of the serum sample, expressed as neutralising 50 % end point.

A constant amount of classical swine fever virus is incubated at 37 °C with diluted serum. For screening purposes, the sera are initially diluted 1/10. When a full titration is necessary two-fold dilutions of serum starting at 1/2 or 1/5 can be prepared. Each dilution is mixed with an equal volume of a virus suspension containing 100 infectious doses (TCID 50).

After incubation the mixture is inoculated onto cell cultures which are incubated for three to five days. After this incubation period the cultures are fixed and any viral replication in the infected cells is detected by an immune labelling system. Either the neutralisation peroxidase-linked antibody (NPLA) or the neutralisation-immunofluorescence (NIF) assays may be used.

The results of the VNT are expressed as the reciprocal of the initial serum dilution at which half the inoculated cell cultures (50 % end point) fail to show viral replication (no specific labelling). A point between two dilution levels is estimated. The final dilution system is based on the actual dilution of serum during the neutralisation reaction, i.e. after addition of virus, but before adding the cell suspension.

3. The VNT is the most sensitive and reliable test to detect antibodies against classical swine fever virus. Therefore, it is recommended for the serological examination of a single animal as well as on a herd basis. However, cross-neutralising antibodies specific for ruminant pestiviruses infections of pigs may be detected by this test.

The VNT for the detection of antibodies against BVD virus and BD virus follow the same principals mentioned above and are conducted for the differential diagnosis of classical swine fever.

4. The pestiviruses strains to be used in the neutralisation tests shall be in accordance with the recommendation of the Community Reference Laboratory.
5. Several ELISA techniques using specific monoclonal antibodies have been developed, which are based on two formats: competitive or blocking ELISA and non-competitive ELISA.

The competitive or blocking ELISA is usually based on monoclonal antibodies. If the serum sample contains antibodies to classical virus, the binding of a selected peroxidase-conjugated monoclonal antibody to virus antigen will be inhibited resulting in a reduced signal.

In non-competitive ELISA the binding of serum antibodies to antigen is measured directly using peroxidase-conjugated anti-pig antibodies.

6. Quality control on sensitivity and specificity of each batch of an ELISA must be regularly performed by the national laboratories, making use of the panel of reference sera provided by the Community Reference Laboratory. This panel shall include: sera from pigs in the early phase of classical swine fever virus infection (before 21 days post infection); sera from convalescent pigs (after 21 days post infection); sera from pigs infected with ruminant pestiviruses.

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The ELISA to be used for the serological diagnosis of classical swine fever must recognise all reference sera from the convalescent pigs. All results obtained with the reference sera must be repeatable. It is further recommended that they detect all positive sera from the early phase and to show a minimum of cross-reactions with the sera from pigs infected with ruminant pestiviruses.

The results obtained with the reference sera from pigs in the early phase of infection give an indication of the sensitivity of the ELISA.

7. The sensitivity of the ELISA is regarded as lower than that of the VNT, and it is recommended to use it as a screening test on a herd basis. However, the ELISA require less-specialised facilities and can be performed much more rapidly, thanks to automated systems, than the VNT.

The ELISA must ensure identification of all classical swine fever infections at the convalescence stage and need to be as free as possible from interference by cross-reacting antibodies to ruminant pestiviruses.

- C. Interpretation of serological results and differential diagnosis with infections due to ruminant pestiviruses (BVDV and BDV)

1. Without prejudice to the provisions of Article 4(3)(a) or Article 7(2) of Directive 2001/89/EC, in case of detection of a classical swine fever virus neutralisation titre equal to or higher than 10 ND50 in serum samples collected from one or more pigs or a positive ELISA result in serum samples from a group of pigs, the measures referred to in Article 4(2) of Directive 2001/89/EC shall immediately apply or shall continue to apply in the holding in question.

The samples already collected from this holding must be re-tested by VNT by comparative end point titration of the neutralising antibodies against classical swine fever virus and ruminant pestiviruses.

2. If the comparative tests show antibodies to ruminant pestiviruses and no or evidently lower (less than three-fold) antibody titres to classical swine fever virus, the suspicion for classical swine fever shall be ruled out, unless other reasons exist which warrant the continued application of the measures referred to in Article 4(2) of Directive 2001/89/EC in the holding in question.
3. If the comparative tests show a virus neutralisation titre in more than one pig equal to or higher than 10 ND50 and this titre is equal or higher than the titres to other pestiviruses, the competent authority shall ensure that classical swine fever shall be confirmed, provided that epidemiological evidence of disease has been found in the holding in question.
4. Without prejudice to the provisions of Article 4(3) of Directive 2001/89/EC, if epidemiological evidence of disease has not been found or if the results of the previous tests are inconclusive, the competent authority shall ensure that in the holding in question:

the measures referred to in Article 4(2) of Directive 2001/89/EC shall continue to apply; further investigations are carried out as quickly as possible to confirm or rule out classical swine fever, in accordance with Chapter IV.

5. However, if the further checks and tests referred to in subparagraph 4 do not allow classical swine fever to be ruled out, further blood sampling for serological testing shall be carried out in the holding after at least two weeks have elapsed from the previous checks.

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In the framework of this further sampling, the pigs already sampled and tested shall be re-sampled for a comparative serological testing with the previously collected samples to detect sero-conversion for classical swine fever virus or for ruminant pestiviruses, if any.

If these further checks and tests do not allow classical swine fever to be confirmed, the measures referred to in Article 4 of Directive 2001/89/EC may be lifted.

CHAPTER VIII

Discriminatory tests in case of emergency vaccination

[A. Basic principles

1. A discriminatory serological ELISA test (discriminatory test) is available to successfully distinguish pigs which have been vaccinated with marker vaccines, that induce antibodies only against the E2 glycoprotein of classical swine fever virus, from pigs which have been infected with the wild type of classical swine fever virus. This test is designed to detect antibodies against the glycoprotein Erns of classical swine fever virus. It is based on the principle that non-infected animals vaccinated with marker vaccines only produce antibodies against the glycoprotein E2 of classical swine fever virus, whilst animals infected with field virus react and produce antibodies against other virus antigens, too.

This discriminatory test is sensitive and specific ⁽¹⁴⁾. However, also pigs which have become infected with Pestiviruses other than classical swine fever virus, such as BVD virus and BD virus, will also react Erns-positive. Furthermore, the sensitivity of the test is not ideal, as some marker-vaccinated and then infected animals may not react Erns-positive.

The data currently available suggest that the discriminatory test cannot be reliably used to test serum samples from feral pigs.

2. The discriminatory test is a liquid phase blocking enzyme-linked immunoassay. The samples to be tested are incubated onto microtitre plates precoated with monoclonal anti-Erns antibodies together with a defined amount of Erns antigen. Any antibody specific for Erns binds to the defined amount of Erns antigen in the solution and an antigen/antibody complex is formed, which does not react with the anti-Erns antibodies on the microtitre plate. After washing of the plates to remove unbound material a peroxidase labelled anti-Erns conjugate is added which binds to the Erns antigen complexed with the antibody coated on the surface of the microtitre plate. Unbound conjugate is removed by washing and chromogen-containing substrate is added. The degree of colour, which develops, is reversely proportional to the amount of antibody specific for Erns present in the sample. If the sample does not contain antibodies (negative sample) much of the defined amount of Erns antigen that was added can bind to the anti-Erns antibodies on the plate surface and a strong colour reaction is observed.

A result is obtained by comparing the optical density (OD) in wells containing test samples with those of wells containing the negative and positive controls.

- #### B. Guidelines for the use of the discriminatory test in the context of an emergency vaccination with a marker vaccine in pig holdings in the framework of Article 19 of Directive 2001/89/EC

The discriminatory test is designed to verify the presence or absence of classical swine fever virus circulation on a pig population vaccinated with a marker vaccine. The available data

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suggest that it can be successfully used for that purpose on herd bases, but it cannot reliably exclude that individual pigs are infected with classical swine fever virus. In particular, the specificity of the discriminatory test might not be sufficient to reliably discriminate marker vaccinated pigs from infected pigs in case of vaccination of adult pigs. In case of doubtful results, however, the pigs in question must be slaughtered or killed in a humane way in accordance with Directive 93/119/EC and their organs tested for classical swine fever virus. Virus isolation and the PCR are the most suitable tests for that purpose.

These aspects have to be taken into full account when designing an emergency marker-vaccination strategy and then interpreting the results of a classical swine fever virus survey onto the marker-vaccinated population.

The procedure for sampling and testing the vaccinated pig population before lifting the restrictions to be applied in the vaccinated area in accordance with Article 19 of Directive 2001/89/EC, should depend on the age of the vaccinated pigs, the category of pigs (fattening/slaughter, breeding) and the desired level of safety as regards the absence of virus circulation in the population.

Details on the procedure for sampling and testing shall therefore be laid down in the emergency vaccination plan to be submitted to the Commission pursuant to Article 19(3) of Directive 2001/89/EC."]

CHAPTER IX

Minimum safety requirements for classical swine fever laboratories

1. The minimum requirements laid down in Table 1 must be fulfilled in any laboratory where classical swine fever virus is to be manipulated, even if only in a small amount, as required by the virus isolation and neutralisation tests. However, post-mortem examinations, processing of tissues for FAT and serology using inactivated antigen, may be carried out at a lower containment level provided that basic hygiene and post-operational disinfection with safe disposal of tissues and sera apply.
2. The additional requirements laid down in Table 1 must be fulfilled by any laboratory where procedures involving extensive multiplication of virus are carried out.
3. The requirements laid down in Table 2 must be fulfilled by any laboratory where animal experiments with classical swine fever virus are carried out.
4. In any case, all stocks of classical swine fever virus must be kept in secure storage, whether deep-frozen or freeze-dried. It is recommended that freezers and refrigerators are not used for viruses other than classical swine fever, or for other materials unrelated to classical swine fever diagnosis. All individual ampoules must be clearly labelled, and comprehensive records maintained of virus stocks together with dates and results of quality-control checks. Records must also be kept of viruses added to stock, with details of the source, and of viruses issued to other laboratories.
5. It is recommended that the bio-safe unit for classical swine fever virus work should be supported by areas where classical swine fever virus is not manipulated. These other areas should be available for the preparation of glassware and media, the maintenance and preparation of non-infected cell cultures, the processing of sera and serological testing (other than methods using live classical swine fever virus), and the provision of administrative and clerical support.

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Table 1

PRINCIPLES OF BIOLOGICAL CONTAINMENT
APPROPRIATE FOR DIAGNOSTIC LABORATORIES

	<i>Additional requirements</i>	<i>Minimal requirements</i>
General environment	Normal atmospheric pressure Double HEPA filtration of exhaust air. Dedicated rooms, used exclusively for classical swine fever diagnostic procedures.	Normal atmospheric pressure. Dedicated rooms limited to defined procedures.
Laboratory clothing	Complete change of clothes on entry. Laboratory clothing used only in the classical swine fever virus unit. Disposable gloves for all manipulations of infected material. Clothing sterilised before removal from unit, or washed within unit.	Dedicated outer clothing used only in the classical swine fever virus unit. Disposable gloves for all manipulations of infected material. Outer clothing sterilised before removal from unit, or washed within unit.
Control of personnel	Entry to unit limited to named, trained personnel. Wash and disinfect hands on leaving unit. Personnel not permitted near pigs for 48 hours after leaving unit.	Entry to unit limited to named, trained personnel. Wash and disinfect hands on leaving unit. Personnel not permitted near pigs for 48 hours after leaving unit.
Equipment	Biological safety cabinet (Class I or II) used for all manipulations of live virus. Cabinet should have double HEPA filtration of exhaust air. All equipment needed for laboratory procedures to be available within the dedicated laboratory suite.	

Table 2

BIO-SAFETY REQUIREMENTS FOR EXPERIMENTAL ANIMAL ROOMS

	<i>Requirements</i>
General environment	Negative pressure controlled ventilation. Double HEPA filtration of exhaust air. Facility for complete fumigation/disinfection at end of experiment. All effluents treated to inactivate classical swine fever virus (heat or chemical).
Laboratory clothing	Complete change of clothes on entry. Disposable gloves for all manipulations.

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	<i>Requirements</i>
	Clothing sterilised before removal from unit, or washed within unit.
Control of personnel	Entry to unit limited to named, trained personnel. Full shower on exit from unit. Personnel not permitted near pigs for 48 hours after leaving unit.
Equipment	All equipment required for animal procedures to be available within the unit. All materials to be sterilised on removal from unit or, in the case of animal samples, to be double wrapped in leakproof container which is surface disinfected for transport to the classical swine fever laboratory.
Animals	All animals to be slaughtered before leaving the unit, post mortem examinations to be completed within the bio-safe area, and carcasses incinerated on completion of examinations.

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- (1) OJ L 316, 1.12.2001, p. 5.
- (2) OJ L 47, 21.2.1980, p. 11.
- (3) When deciding the number of samples to be taken for laboratory testing, the sensitivity of the tests that will be used shall also be considered. The number of animals to be sampled shall be higher than the one indicated in this Manual, if the sensitivity of the test to be used is not very high.
- (4) Singleton reactors may have titres of virus neutralising antibodies ranging from borderline (which is more often the case) to strongly positive. On re-sampling, singleton reactors may show a decreasing or constant titre. In general only few pigs in a herd give rise to these false/positive reactions.
- (5) If pigs have been vaccinated against classical swine fever with a conventional vaccine they can be found seropositive due to the vaccination alone, or due to a silent infection in vaccinated animals.
- (6) Under certain circumstances up to 10 % of the pigs within a herd may have antibodies against ruminant pestiviruses causing bovine viral diarrhoea and border disease. For example, when pigs have direct contact with cattle or sheep infected with BVD virus or BD virus, or when pigs have contact with materials contaminated with ruminant pestiviruses.
- (7) In all of the current serological tests for classical swine fever a small proportion of sera give false/positive results either due to the lack of specificity of the test-system or due to sera from the singleton reactors.
- (8) In certain cases, e.g. when classical swine fever is suspected in a holding with a limited number of young pigs, the proportion of infected sows may be very small. In these cases a higher number of sows must be sampled.
- (9) However, if the derogation provided in Article 6(1) of Directive 2001/89/EC has been applied, sampling must concern the sub-units of the holding where pigs have been killed, without prejudice to the further examinations and sampling to be carried out on the remaining pigs in the holding, which shall be carried out in accordance with the instructions of the competent authority.
- (10) However, if the competent authority has limited the application of preventive killing only to the part of the holding where the pigs suspected of being infected or contaminated with classical swine fever virus were kept, in accordance with Article 4(3)(a) of Directive 2001/89/EC, sampling must concern the sub-units of the holding where this measure has been applied, without prejudice to the further examinations and sampling to be carried out on the remaining pigs in the holding, which will be carried out in accordance with the instructions of the competent authority.
- (11) In certain cases, e.g. when classical swine fever is suspected in a holding with a limited number of young pigs, the proportion of infected sows may be very small. In these cases a higher number of sows must be sampled.
- (12) The Community Reference Laboratory has an unlimited permit to receive diagnostic samples and classical swine fever virus isolates. Copy of the import permit may be requested from this laboratory before transport and attached in an envelope to the outside of the package.
- (13) Several Classical swine fever ELISA antigen are commercially available, which are validated with different types of samples.
- (14) [In accordance with the results of a study carried out by the Community reference laboratory for classical swine fever and the national classical swine fever laboratories, the sensitivity of the discriminatory test is about 94 % and the specificity is about 98 %.]

Changes to legislation:

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Changes and effects yet to be applied to :

- Art. 1(1) words substituted by [S.I. 2018/1410 reg. 7\(2\)\(a\)](#)
- Art. 1(2) substituted by [S.I. 2018/1410 reg. 7\(2\)\(b\)](#)
- Art. 4 omitted by [S.I. 2018/1410 reg. 7\(3\)](#)

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

- Annex Ch. 6 s. E omitted by [S.I. 2018/1410 reg. 7\(7\)\(b\)\(i\)](#)
- Annex Ch. 6 s. E word substituted by [S.I. 2018/1410 reg. 7\(7\)\(b\)\(ii\)\(bb\)](#)
- Annex Ch. 7 s. B word substituted by [S.I. 2018/1410 reg. 7\(8\)\(a\)\(i\)](#)
- Annex Ch. 7 s. B word substituted by [S.I. 2018/1410 reg. 7\(8\)\(b\)](#)
- Annex Ch. 7 s. B word substituted by [S.I. 2018/1410 reg. 7\(8\)\(c\)\(i\)](#)
- Annex Ch. 6 s. E words inserted by [S.I. 2018/1410 reg. 7\(7\)\(b\)\(ii\)\(aa\)](#)
- Annex Ch. 5 s. C words omitted by [S.I. 2018/1410 reg. 7\(6\)](#)
- Annex Ch. 6 s. E words omitted by [S.I. 2018/1410 reg. 7\(7\)\(b\)\(iii\)](#)
- Annex Ch. 7 s. B words omitted by [S.I. 2018/1410 reg. 7\(8\)\(a\)\(ii\)](#)
- Annex Ch. 7 s. B words omitted by [S.I. 2018/1410 reg. 7\(8\)\(c\)\(iii\)](#)
- Annex Ch. 4 s. A words substituted by [S.I. 2018/1410 reg. 7\(5\)](#)
- Annex Ch. 6 s. B words substituted by [S.I. 2018/1410 reg. 7\(7\)\(a\)](#)
- Annex Ch. 7 s. B words substituted by [S.I. 2018/1410 reg. 7\(8\)\(c\)\(ii\)](#)
- Art. 1(3) inserted by [S.I. 2018/1410 reg. 7\(2\)\(c\)](#)
- Art. 1(3)(a)(ii)(iii) Art. 1(3)(a)(iii)(iv) renumbered as Art. 1(3)(a)(ii)(iii) in earlier amending provision [S.I. 2018/1410, reg. 7\(2\)\(c\)](#) by [S.I. 2020/1388 reg. 25\(4\)\(a\)](#)
- Art. 1(3)(a)(ii) omitted in earlier amending provision [S.I. 2018/1410, reg. 7\(2\)\(c\)](#) by [S.I. 2020/1388 reg. 25\(4\)\(a\)](#)
- Art. 1(3)(b) words substituted in earlier amending provision [S.I. 2018/1410, reg. 7\(2\)\(c\)](#) by [S.I. 2020/1388 reg. 25\(4\)\(b\)](#)