AUJESZKY'S DISEASE (INFECTION WITH AUJESZKY'S DISEASE VIRUS)

SUMMARY

Description and importance of the disease: *Aujeszky's disease, also known as pseudorabies, is caused by an alphaherpesvirus that infects the central nervous system and other organs, such as the respiratory tract, in a variety of mammals except humans and the tailless apes. It is associated primarily with suidae (pigs or wild boars), the natural host, which remain latently infected following clinical recovery (except piglets under 2 weeks of age, which die from encephalitis). The disease is controlled by containment of infected herds and by the use of vaccines and/or removal of latently infected animals.*

A diagnosis of Aujeszky's disease is established by detecting the agent (by virus isolation or polymerase chain reaction [PCR]), as well as by detecting a serological response in the live animal.

Identification of the agent: *Isolation of Aujeszky's disease virus can be made by inoculating a tissue homogenate, for example of brain and tonsil or material collected from the nose/throat, into a susceptible cell line such as porcine kidney (PK-15 or SK6), or primary or secondary kidney cells. The specificity of the cytopathic effect is verified by immunofluorescence, immunoperoxidase or neutralisation with specific antiserum. The viral DNA can also be identified using PCR; this can be accomplished using real-time PCR techniques.*

Serological tests: *Aujeszky's disease antibodies are demonstrated by virus neutralisation, latex agglutination or enzyme-linked immunosorbent assay (ELISA). A number of ELISA kits are commercially available world-wide. A WOAH International Standard Reference Serum defines the lower limit of sensitivity for routine testing by laboratories that undertake the serological diagnosis of Aujeszky's disease.*

It is possible to distinguish between antibodies resulting from natural infection and those from vaccination with gene-deleted vaccines.

Requirements for vaccines: *Vaccines should prevent or at least limit the excretion of virus from the infected pigs. Recombinant DNA-derived gene-deleted or naturally deleted live Aujeszky's disease virus vaccines, lack a specific glycoprotein (gG, gE, or gC), which enables the use of companion diagnostic tests to differentiate vaccinal antibodies from those resulting from natural infection.*

A. INTRODUCTION

Aujeszky's disease, also known as pseudorabies, is caused by *Suid herpesvirus 1* (SHV-1)*,* a member of the subfamily *Alphaherpesvirinae* and the family *Herpesviridae.* The virus should be handled with appropriate biosafety and containment procedures as determined by biorisk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*). The virus infects the central nervous system and other organs, such as the respiratory tract, of a variety of mammals (such as dogs, cats, cattle, sheep, rabbits, foxes, minks, etc.) except humans and the tailless apes. It is associated primarily with pigs, the natural host, which remain latently infected following clinical recovery (except piglets under 2 weeks of age, which die from encephalitis). In consequence, the pig is the only species able to survive a productive infection and therefore, serves as the reservoir host. In pigs, the severity of clinical signs depends on the age of the pig, the route of infection, the virulence of the infecting strain and the immunological status of the animal. Young piglets are highly susceptible with mortality rates reaching 100% during the first 2 weeks of life.

These animals show signs of hyperthermia and severe neurological disorders: trembling, incoordination, ataxia, nystagmus to opisthotonos and severe epileptiform-like seizures. When pigs are older than 2 months (grower-finisher pigs), the respiratory forms become predominant with hyperthermia, anorexia, and mild to severe respiratory signs: rhinitis with sneezing and nasal discharge that may progress to pneumonia. The frequency of secondary bacterial infections is high, depending on the health status of the infected herd. In this group of pigs, the morbidity can reach 100%, but in cases of the absence of complicated secondary infections, mortality ranges from 1% to 2% (Pejsak & Truszczynski, 2006). Sows and boars primarily develop respiratory signs, but in pregnant sows, the virus can cross the placenta, infect and kill the fetuses, inducing abortion, return to oestrus, or stillborn fetuses. Virus may be found in the semen of infected boars (van Rijn *et al.,* 2004). In the other susceptible species, the disease is fatal, the predominant sign being intense pruritus causing the animal to gnaw or scratch part of the body, usually head or hind quarters, until great tissue destruction is caused. For that reason, the disease was named "mad-itch" in the past.

Focal necrotic and encephalomyelitis lesions occur in the cerebrum, cerebellum, adrenals and other viscera such as lungs, liver or spleen. In fetuses or very young piglets, white spots on liver are highly suggestive of their infection by the virus. Intranuclear lesions are frequently found in several tissues.

Aujeszky's disease is endemic in many parts of the world, but several countries have successfully completed eradication programmes, e.g. the United States of America, Canada, New Zealand and many Member States of the European Union.

The disease is controlled by containment of infected herds and by the use of vaccines or removal of latently infected animals (Pejsak & Truszczynski, 2006). Stamping out has been or is used in several countries usually when the infected farms are small or when the threat to neighbouring farms is very high in free countries.

Whereas isolation of the Aujeszky's disease virus or detection of the viral genome by the polymerase chain reaction (PCR) are used for diagnosis in the case of lethal forms of Aujeszky's disease or clinical disease in pigs, serological tests are required for diagnosis of latent infections and after the disappearance of the clinical signs. Affected animals except suids, do not live long enough to produce any marked serological response. Serological tests are the tests to be used to detect subclinically or latently infected pigs, especially in the case of qualification of the health status of the animals for international trade or other purposes.

B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for the diagnosis of Aujeszky's disease and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Identification of the agent[a]						
Virus isolation	–	–	–	+++	–	–
Real-time PCR	–	+	+	+++	+	–
Detection of immune response						
Latex agglutination	+++	+++	+++	+	+++	+++
ELISA	+++	+++	+++	+	+++	+++
VN	+	+	+	+	+	+++

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
PCR = polymerase chain reaction; ELISA = enzyme-linked immunosorbent assay; VN = virus neutralisation.
[a]A combination of agent identification methods applied on the same clinical sample is recommended.

1. Identification of the agent

1.1. Virus isolation

The diagnosis of Aujeszky's disease can be confirmed by isolating the virus from the oro-pharyngeal fluid, nasal fluid (swabs) or tonsil swabs from living pigs, or from samples from dead pigs or following the presentation of clinical signs such as encephalitis in herbivores or carnivores. For post-mortem isolation of SHV-1, samples of brain, tonsil, and lung are the preferred specimens. In cattle, infection is usually characterised by a pruritus, in which case a sample of the corresponding section of the spinal cord may be required in order to isolate the virus. In latently infected pigs, the trigeminal ganglia is the most consistent site for virus isolation, although latent virus is usually non-infective unless reactivated, making it difficult to recover in culture.

Tissue samples are homogenised in normal saline or cell culture medium with antibiotics. The method used should be suitable for the subsequent diagnostic test. The amount of tissue homogenised should take into account a possible non-homogeneous distribution of the virus. A tissue homogenate of approximately 10% is recommended. The resulting suspension is clarified by low speed centrifugation, e.g. at 900 g for 10 minutes. The supernatant fluid is used to inoculate any sensitive cell culture system. Numerous types of cell line or primary cell cultures are sensitive to SHV-1, but a porcine kidney cell line (PK-15 or SK6) is generally employed. The overlay medium for the cultures should contain antibiotics (such as: 200 IU/ml penicillin; 100 µg/ml streptomycin; 100 µg/ml polymyxin; and 3 µg/ml fungizone).

SHV-1 induces a cytopathic effect (CPE) that usually appears within 24–72 hours, but cell cultures may be incubated for 5–7 days. The monolayer develops accumulations of birefringent cells, followed by complete detachment of the cell sheet. Syncytia also develop, the appearance and size of which are variable. In the absence of any obvious CPE, it is advisable to make one blind passage into further cultures. Additional evidence may be obtained by staining infected cover-slip cultures with haematoxylin and eosin to demonstrate the characteristic herpesviral acidophilic intranuclear inclusions with margination of the chromatin. Virus identity should be confirmed by immunofluorescence, immunoperoxidase, neutralisation using specific antiserum following the method described in Section B.2.1. or by PCR.

The isolation of SHV-1 makes it possible to confirm Aujeszky's disease, but failure to isolate does not guarantee freedom from infection.

1.2. Identification of virus by the polymerase chain reaction

The PCR can be used to identify SHV-1 genomes in secretions or organ samples. Many individual laboratories have established effective protocols, but there is as yet no internationally agreed standardised approach.

The PCR is based on the selective amplification of a specific part of the genome using two primers located at each end of the selected sequence. In a first step, the complete DNA may be isolated using standard procedures (e.g. proteinase K digestion and phenol–chloroform extraction) or commercially available DNA extraction kits. Using cycles of DNA denaturation to give single-stranded DNA templates, hybridisation of the primers, and synthesis of complementary sequences using a thermostable DNA polymerase, the target sequence can be amplified up to 10^6-fold. The primers must be designed to amplify a sequence conserved among SHV-1 strains, for example parts of the gB or gD genes that code for essential glycoproteins have been used (Mengeling *et al*., 1992; Van Rijn *et al*., 2004; Yoon *et al*., 2006). Real-time PCRs have been developed that can differentiate gE-deleted vaccine viruses from wild-type virus based on the specific detection of gB and gE genes (Ma *et al*., 2008; Wernike *et al*., 2014).

The amplified product may be identified from its molecular weight as determined by migration in agarose gel, with further confirmation where possible by sequencing the amplified product. More recent techniques include the use of fluorescent probes linked to an exonuclease action and real-time monitoring of the evolution of product, enabling simultaneous amplification and confirmation of the template DNA thus increasing the rapidity and specificity of the PCR assays.

In all cases, the main advantage of PCR, when compared with conventional virus isolation techniques, is its rapidity; with the most modern equipment, the entire process of identification and confirmation can be completed within one day. However, because of the nature of the test, many precautions need to be taken to avoid contamination of samples with extraneous DNA from previous tests or from general environmental contamination in the laboratory (see Chapter 1.1.9 *Tests for sterility and freedom from contamination of biological materials intended for veterinary use*). This may limit the value of the test for many laboratories unless care is taken to avoid DNA carry-over contamination. The use of an internal control is recommended so as to avoid false-negative results by ensuring adequate efficiency of DNA extraction and confirming the absence of PCR inhibitors in each sample. In practice, different systems can be used for detection of endogenous or exogenous genes (Hoffman *et al.,* 2009). Kits for the test are commercially available (Pol *et al.,* 2013).

2.   Serological tests

Virus neutralisation (VN) has been recognised as the reference method for serology (Moennig *et al*., 1982), but for general diagnostic purposes it has been widely replaced by the enzyme-linked immunosorbent assay (ELISA) because of its suitability for large-scale testing (Moennig *et al*., 1982). The tests can be performed on a variety of matrices (e.g. serum, whole blood, milk, muscular exudates, and filter paper), but the preferred matrix is serum.

A latex agglutination test has also been developed, and can be used for screening for antibodies. Kits for the test are commercially available (Schoenbaum *et al*., 1990).

Serological tests are carried out only for suids, as other animals (herbivores and carnivores) die too quickly to produce antibodies. In free areas where pigs are not vaccinated, an active epidemiological survey can be carried out using ELISA gB or gE or latex agglutination kits. As antibodies can be detected between 7 and 10 days post-infection, these serological tools can also be used to confirm infection in pigs in the case of a suspected outbreak. In area where pigs are vaccinated with gE deleted vaccines, the ELISA gE kits permit the differentiation between infected and vaccinated pigs (DIVA), but to assess the level of immunity induced by vaccination, gB ELISA, latex agglutination kits or viral neutralisation should be used.

Any serological technique used should be sufficiently sensitive to give a positive result with the WOAH International Standard Reference Serum or a calibrated secondary serum. Reference serum can be obtained from the WOAH Reference Laboratory for Aujeszky's Disease in France (see Table given in Part 4 of this *Terrestrial Manual*). For international trade purposes, the test should be sensitive enough to detect the standard serum diluted 1/2. To authorise pig movement from an area where deleted gE vaccines are used to a free area, serological assays should be able to detect at least the dilution of 1/8 for ELISA gE of the WOAH International Standard Reference Serum as prescribed by the European Commission (2008).

2.1.   Virus neutralisation

VN in cell culture can be performed in several ways, which vary according to the length of incubation of the virus/serum mixtures (e.g. 1 hour at 37°C or 24 hours at 4°C) and the presence or absence of complement. Most laboratories use a reaction period of 1 hour at 37°C in the absence of complement, because this is easy and rapid. However, the sensitivity can be improved by increasing the incubation period to 24 hours at 4°C, which facilitates the detection of antibody levels 10–15 times lower than in the 1-hour method. For international trade purposes, the test method should be validated as being sensitive enough to detect the WOAH Standard Reference Serum diluted 1/2.

VN cannot be used to differentiate antibodies of vaccinal origin from those caused by natural infection. It is one of the two tests available that complies with the requirement in the WOAH *Terrestrial Animal Health Code* chapter when it refers to "a diagnostic test to the whole virus".

i)   Cells

Cells susceptible to infection with SHV-1 are used; they may be cell lines (e.g. PK-15, SK6, MDBK), or primary or secondary cell cultures (e.g. porcine kidney).

ii)   Cell culture medium

The medium depends on the type of cells. For example, the medium for PK-15 cells is Eagle's minimal essential medium (MEM) + 10% fetal bovine serum and antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin or, alternatively, 50 µg/ml gentamycin).

iii) Maintenance of the cells

The cells are cultured in cell culture vessels of, for example, 75 cm^2. They are trypsinised once or twice per week. For weekly trypsinisation, the cells are usually cultured in 50 ml of medium, with a multiplication rate of 5. For two trypsinisations a week, the cells are cultured in 30 ml of medium, with a multiplication rate of 3.

For trypsinisation, the growth medium is removed once the cell sheet is complete. The cell sheet is washed with about 5 ml of recently thawed trypsin/ethylene diamine tetra-acetic acid (EDTA) (0.25%) in an isotonic buffer. The washing fluid is discarded and the preparation is washed again, retaining only a few drops of trypsin. The container is placed in an incubator at 37°C for 5–10 minutes until the cells have become detached. Once the sheet is detached and the cells are well separated, for twice weekly passage they are suspended in 90 ml of growth medium, and this suspension is distributed into three 75 cm^2 cell culture vessels. For weekly trypsinisation the cells are suspended in 150 ml of growth medium and the suspension distributed into five 75 cm^2 cell culture bottles.

iv)   Virus

A suitable strain of SHV-1, such as the Kojnok strain or the NIA-3 strain, is stored at a temperature of –65°C or below, or in freeze-dried form at 4°C.

v)   Preparation of stock virus suspension

The culture fluid is removed from a cell culture vessel containing a complete cell sheet. About 1 ml of stock virus suspension of known titre (about 10^7 TCID$_{50}$/ml [50% tissue culture infective dose]) is added, and the vessel is incubated at 37°C±2°C for 1 hour. 30 ml of culture medium is added and the vessel is again incubated at 37°C±2°C. The vessel is examined frequently until there is about 75% cell destruction (after about 36–48 hours). It is then frozen at a temperature of –65°C or lower to disrupt the cells.

The vessel is then thawed and shaken vigorously. Medium is collected and centrifuged at 1500 *g* for 15 minutes. The supernatant fluid is divided into portions (of about 0.5 ml) in small tubes that are labelled (date and virus reference) before being stored at a temperature of –65°C or lower until required.

vi)   Titration of the stock virus suspension

Titration of the stock suspension is performed by the method of Reed & Muench or that of Kärber, and the titre is expressed per 50 µl and per ml.

The VN test requires an internal quality control serum with a known titre of neutralising antibody to SHV-1 (it can be calibrated against an international standard serum or a secondary standard prepared from that serum), and a negative control serum (from a specific antibody free pig, e.g. from an official Aujeszky's disease free herd). The test sera themselves should be of good quality, clearly labelled, of known provenance with clinical history, stored in refrigeration at all times, free from fungal or bacterial contamination, non-haemolysed and of sufficient quantity. Serum should be separated from the coagulum without delay, thereby preventing toxicity.

There are qualitative and quantitative procedures for VN, both of which are described below.

2.1.1. Qualitative virus neutralisation technique

i)   Complement in the serum samples is destroyed by heating in a water bath at 56–59°C for 30 minutes.

ii)   Each undiluted serum sample is placed in two to three wells, at 50 µl per well, of a 96-well cell-culture grade microtitre plate. Each serum can also be diluted 1/2 in the MEM, before being placed in two other wells.

iii)   50 µl of virus suspension containing 100 $TCID_{50}$ (or 2×10^3 $TCID_{50}$/ml), obtained by diluting stock virus suspension of known titre with MEM, is added to each well.

iv)   The plate is gently shaken and placed in an incubator for 1 hour at 37°C (±2°C) (5% CO_2 optional).

v)   150 µl of cell suspension containing about 150,000 cells/ml is added to each well.

vi)   The plate is covered (for incubation in CO_2), or a plastic sheet is sealed carefully around the edges of the plate (for incubation in air). The plate is shaken lightly to obtain an even distribution of cells at the bottom of the wells, and placed in the incubator at 37°C (±2°C) (CO_2 optional) for 3–5 days.

vii)   *Controls:* Each set of plates must include the following controls:

   a)   Virus control

   This is to verify the amount of virus actually used for the test. The virus dose used for VN (target titre 100 $TCID_{50}$/50 µl) is diluted with MEM at 1/10, 1/100 and 1/1000. Of each dilution, 50 µl is placed in at least four wells, to which 50 µl of medium is added before the wells are incubated for 1 hour at 37°C (±2°C). The cell suspension is added in the same way as for the sera under test.

   b)   Cell control

   150 µl cell suspension and 100 µl MEM are placed in each of at least four wells.

   c)   Positive serum control

   A serum of known SHV-1 neutralising antibody titre is used. Five dilutions are prepared in the same way as for the sera under test: a dilution corresponding to the serum titre, two-fold and four-fold dilutions, and 1/2 and 1/4 dilutions (equivalent to T, T/2, T/4, 2T and 4T, where T is the serum titre, i.e. undiluted serum for the qualitative test). Add 50 µl of virus suspension containing 100 $TCID_{50}$/50 µl to 50 µl of positive control sample dilutions. The cells are incubated and the cell suspension is added in the same way as for the sera under test.

   d)   Serum control

   This is to verify the absence of a toxic effect of the sera on the cells. Wells containing 50 µl of each serum are incubated for 1 hour at 37°C in the presence of 50 µl of medium. Then, 150 µl of cell suspension is added in the same way as for the sera under test.

   e)   Negative serum control

   This is done in the same way as for sera under test.

viii)   *Reading the results:* An inverted-image microscope (×100) is used to examine the wells for toxic effects and CPEs after 3 to 5 days. The controls must give the following results if the tests are to be considered valid:

   a)   Virus control

   The titre of the viral suspension should be between 30 and 300 $TCID_{50}$/50 µl.

   b)   Cell control

   The cell sheet must be intact.

   c)   Positive serum control

   The titre obtained must be equal to the predicted titre, within one dilution.

   d)   Serum control

   Examination for a CPE should take into account a possible toxic effect on cells.

e)   Negative serum control

A CPE should be present.

ix)   For the sera under test if distributed in three wells, the following results may be seen:

a)   presence of a CPE in three wells = negative result;

b)   absence of a CPE in three wells on day 3 = positive result;

c)   presence of a CPE in one well but not in the others = inconclusive result, test must be repeated;

d)   small plaques indicating a CPE on day 3 = inconclusive result, test must be repeated;

e)   toxicity in serum control and test wells = unreadable result, test must be repeated. (NB replacement of medium with fresh medium after 16 hours' incubation will reduce the toxicity without affecting the titre of specific antibody.) Plates can be read until day 5 of incubation.

f)   If the serum was initially diluted 1/2 and distributed in two wells, it is considered positive if CPE is absent in one of the two wells, and it is highly recommended to retest using the quantitative technique. Diluting the serum to 1/2 can prevent the toxicity effect of the tested sera.

x)   *Interpretation of the results:* This test is capable of detecting the presence or absence of neutralising antibody to SHV-1. It is incapable of distinguishing vaccinated animals from infected animals.

The technique described (VN for 1 hour at 37°C) can give false-negative and false-positive results. The sensitivity can be increased (leading to fewer false negatives) by adopting a method based on neutralisation involving 24 hours of contact between virus and serum at 4°C, before the addition of cells.

A qualitative technique such as this one, which employs undiluted serum samples (1/2 final dilution), can give a false-positive result in certain cases due to nonspecific neutralisation of the virus. This problem can be addressed by carrying out a confirmatory test using the quantitative technique (see Section B.2.1.2 below).

Samples giving inconclusive results may be tested by an alternative technique with better sensitivity such as an ELISA or the animal should be re-bled to confirm status.

2.1.2. Quantitative virus neutralisation technique

The quantitative VN technique is similar to the qualitative procedure, but each serum is used both undiluted and in a series of dilutions. Depending on the desired precision, the purpose of testing and the expected titre, two wells are used for each dilution of serum, and a range of dilutions appropriate for the purpose. The procedure below describes the test for an initial maximum dilution of 1/16. It is possible to reach higher titres using more wells (e.g. A1 to A12 for 1/256 dilution).

i)   Complement in the serum samples is destroyed by heating in a water bath at 56–59°C for 30 minutes.

ii)   75 µl of MEM is added to well A2 and 50 µl of MEM is added to wells A1, and A3 to A6 of a 96-well cell-culture grade microtitre plate and continued for comparable wells in rows B, C, etc., for additional serum samples.

iii) 75 µl of undiluted serum sample is added to well A2, and continued for wells in rows B, C, etc., with other serum samples.

iv)   Using a multichannel pipette, the contents of wells in column 2 are mixed, then 50 µl is transferred to column 1 and 3, and so on to column 6 or further to a predetermined row, using the same nozzles. The 50 µl portions remaining after the last row are discarded.

v) 50 µl of virus suspension containing 100 $TCID_{50}$ (or 2×10^3 $TCID_{50}$/ml), obtained by diluting stock virus suspension of known titre with MEM, is added to each well in columns 2 to 6. No virus is added to wells in column 1, this is a control column of serum samples.

vi) The plate is shaken and placed in an incubator for 1 hour at 37°C (±2°C) (5% CO_2 optional).

vii) 150 µl of cell suspension containing about 150,000 cells/ml is added to each well.

viii) The plate is covered (for incubation in CO_2), or a plastic sheet is sealed carefully around the edges of the plate (for incubation in air). The plate is shaken lightly to obtain an even distribution of cells at the bottom of the wells, and placed in the incubator at 37°C (±2°C) (CO_2 optional) for 3–5 days.

ix) Controls are set up as described for the qualitative technique.

x) *Reading the results:* The neutralising titre of a serum is expressed by the denominator of the highest initial dilution that brings about complete neutralisation of the CPE of the virus in 50% of the wells. Neutralisation at any dilution (even undiluted, equivalent to a final dilution of 1/2) is considered to be positive. If the serum shows neutralisation only when undiluted (with growth of virus and CPE at the 1/2 and subsequent dilutions), it would be advisable to apply alternative tests (ELISA or latex agglutination) to provide confirmation of the result, or to request another sampling of the animal, at least 8 days after the first.

2.2. Enzyme-linked immunosorbent assay

The sensitivity of the ELISA is generally superior to that of the VN test using 1-hour neutralisation without complement. Some weak positive sera are more readily detected by VN tests using 24-hour neutralisation, while others are more readily detectable by ELISA.

ELISA kits, which are available commercially, use indirect or competitive techniques for detecting antibodies. They differ in their mode of preparation of antigen, conjugate, or substrate, in the period of incubation and in the interpretation of the results. Their general advantage is that they enable the rapid processing of large numbers of samples. This can also be automated and the results analysed by computer. Some of these kits make it possible to differentiate between vaccinated and naturally infected animals when used with a 'matching' vaccine (Eloit et al., 1989; Van Oirschot et al., 1986). Alternatively, non-commercial ELISA protocols may be adopted (Toma & Eloit, 1986) provided they are shown to detect the WOAH International Standard Reference Serum as positive at a dilution of 1/2 (the minimum sensitivity for international trade purposes). It is recommended to use a kit or in-house assay that has been validated to this standard or a secondary standard prepared against the International Reference Standard by external quality control tests by an independent laboratory. A suitable test protocol for whole virus antibodies is presented below (Toma & Eloit, 1986).

2.2.1. Preparation of antigen

i) A cell line sensitive to SHV-1, such as PK-15 or fetal pig testis, is used. It must be free from extraneous viruses, such as bovine viral diarrhoea virus. The cells should be split and seeded into fresh 75 cm^2 flasks the day before inoculation. A suitable medium such as MEM, without serum, is used to overlay the cultures.

ii) Virus inoculated, and control uninoculated flasks are processed in parallel throughout. A suitable well characterised strain of SHV-1 is used, e.g. Kojnock strain. When a confluent cell monolayer has developed (approximately 24 hours after seeding), it is inoculated with 10^8 $TCID_{50}$ SHV-1 in 5 ml medium; and 5 ml medium (without virus) is placed in control flasks. The cultures are left for adsorption for 30 minutes at 37°C, and then overlaid with 20 ml medium.

iii) When CPE is just beginning, the supernatant medium is discarded and 4 ml KCl (4 mM solution) and glass beads are added. The flasks are shaken gently to detach cells.

iv) Cells are washed by centrifuging three times at 770 *g* in 4 mM KCl. The pellet is resuspended in 4 mM KCl with 0.2% Triton X-100 (1 ml per flask) by applying 60 strokes with a glass homogeniser

v) The cell homogenate is layered on to 0.25 mM sucrose in 4 mM KCl and centrifuged for 10 minutes at 770 *g*.

vi)   The pellet is resuspended in antigen-diluting buffer, pH 9.6 (0.1 M Tris, 2 mM EDTA, 0.15 mM NaCl) at 1/50 the volume of the original culture medium. It may then be stored in small aliquots at –70°C. Antigen is stable in this form for 2 years.

2.2.2. Coating microtitre plates

i)   Virus antigen and control (no virus) antigen are diluted in diluting buffer, pH 9.6 (see above) to a dilution predetermined in chequerboard titrations.

ii)   200 µl of antigen is dispensed into each well of 96-well ELISA-grade plates, coating alternate rows with SHV-1 positive and control antigen. Incubation is for 18 hours at 4°C.

iii)   The plates are washed three times with washing solution (Tween 20, 0.5 ml/litre).

iv)   Coated plates are stored at –20°C or –70°C. They are stable for several months.

2.2.3. Test procedure

i)   Test serum samples are diluted 1/30 in PBS/Tween buffer, pH 7.2 (137 mM NaCl, 9.5 mM phosphate buffer, 0.5 ml/litre Tween 20).

ii)   Diluted samples are added to virus and control antigen coated wells, and incubated at 37°C for 30 minutes.

iii)   The plates are washed three times with washing solution (0.5 ml/litre Tween 20).

iv)   Protein A/peroxidase conjugate is added to all wells at a predetermined dilution in PBS/Tween buffer, pH 7.2 (see above), with added bovine serum albumen fraction V (10 g/litre), and the plates are incubated at 37°C for 30 minutes.

v)   The plates are washed three times with washing solution (0.5 ml/litre Tween 20).

vi)   A suitable chromogen/substrate mixture, such as tetra methyl benzidine (TMB)/hydrogen peroxide, is added to each plate.

vii)   The reaction is stopped with 2 M sulphuric acid. The absorbance is read at 492 nm.

The test must be fully validated using known positive and negative sera, and calibrated against the WOAH International Standard Reference Serum. It is highly recommended to carry out a batch control for each batch of the test, to determine sensitivity and specificity in relation to the original validation criteria (criteria to accept or refuse the batch have to be set). For routine analysis, all tests must include positive and negative internal controls, including at least one weak positive sample that, when diluted at the appropriate dilution for the test, has equivalent activity to a 1/2 dilution of the WOAH International Standard Reference Serum. Internal controls are also used to monitor the sensitivity, specificity and reproducibility of the test over time. For further details see Toma & Eloit, 1986 and Chapter 1.1.6 *Validation of diagnostic assays for infectious diseases of terrestrial animals*. Commercial ELISA kits also have to be validated in the setting in which they are going to be used.

As well as testing sera, the ELISA can be adapted to test pools of sera, filter paper disks that have been moistened with a small quantity of blood obtained by puncturing a superficial vein (Banks, 1985; Toma *et al.,* 1986), or muscle exudates (Le Potier *et al.,* 1998). These techniques make it convenient to collect blood samples from large numbers of pigs (Vannier *et al.,* 2007). The disks are air-dried before shipment to the laboratory. The (analytical) sensitivity may be lower than for a standard ELISA due to the type of sample or unavoidable dilution of the sample. Use of an adapted ELISA is therefore more appropriate for testing at the population level rather than for individual testing (e.g. prior to animal movement), unless a validation study has shown a comparable (analytical) sensitivity to the standard ELISA.

Requirements for the detection of gE antibodies by ELISA in pigs destined for slaughter that are to be introduced into zones free from Aujeszky's disease have been defined by several control authorities. For example, in the European Union, ELISA gE kits must be able to detect activity at least equivalent to a 1/8 dilution of the WOAH International Standard Reference Serum (European Commission, 2008). The WOAH *Terrestrial Animal Health Code* specifies circumstances in which gE-specific tests may be used. The gE ELISAs can also be adapted to test blood on filter paper disks depending on its sensitivity.

C. REQUIREMENTS FOR VACCINES

1. Background

1.1. Rationale and intended use of the product

Aujeszky's disease may be controlled by the use of vaccines containing either modified live or inactivated virus antigens. In addition, these conventional vaccines have been supplemented by recombinant DNA-derived gene-deleted or naturally deleted live SHV-1 vaccines. These vaccines, referred to as marker or DIVA-vaccines, are made with a virus that lacks a specific glycoprotein (most commonly gE-, although gG- or gC-deleted vaccines have also been described, as have vaccines with multiple deletions[1]). These gene-deleted DIVA-vaccines have the advantage over conventional whole virus vaccines that it is possible to distinguish infected animals from non-infected vaccinated animals. This is done by testing for the antibodies directed against the protein coded for by the deleted gene, which will be absent in non-infected DIVA-vaccinated pigs but present in field-infected pigs. Therefore, in countries with infected pigs, where the eradication of Aujeszky's disease is planned, these DIVA-vaccines are the vaccines of choice (Pensaert *et al.*, 2004). Standards applicable to the manufacture of live and inactivated virus vaccines are described. For DIVA-vaccines, the tests should include demonstrable absence of a serological response in vaccinated pigs to the protein coded for by the deleted gene, and in addition a demonstrable response to the same protein in vaccinated pigs that become infected by field virus.

Other vaccines are inactivated and constituted of adjuvanted, viral subunit of purified and concentrated immunogenic glycoproteins (except the gE) allowing differentiation of vaccinated from infected pigs.

Guidelines for the production of veterinary vaccines are given in Chapter 1.1.8 *Principles of veterinary vaccine production*. The guidelines given here and in chapter 1.1.8 are intended to be general in nature and may be supplemented by national and regional requirements.

2. Outline of production and minimum requirements for conventional vaccines

2.1. Characteristics of the seed

2.1.1. Biological characteristics

Vaccines are made using a seed-lot system in which a master seed virus (MSV) is prepared from a suitable strain of Aujeszky's disease virus. A number of strains are used for vaccine manufacture. The antigen in an inactivated vaccine can be one of a number of wild-type strains, or the naturally deleted Bucharest virus. Modified live conventional vaccines use numerous strains, such as Bartha or are derived from Aujeszky's original isolate or from other field isolates, such as the NIA-3 strain (Marchioli *et al.*, 1987; McFerran & Dow, 1975; Van Oirschot *et al.*, 1990; Visser & Lutticken, 1988).

It is recommended that for differentiating between infected and vaccinated animals, deleted strains should be used.

A virus identity test (using either a fluorescent antibody test, neutralisation test, [constant serum/decreasing virus method], or any other suitable identity test) must be conducted on the MSV.

2.1.2. Quality criteria (sterility, purity, freedom from extraneous agents)

Most of the cell lines used to propagate SHV-1 are continuous lines, such as the PK-15 line. A master cell stock (MCS) is established at a specified passage level. The MCS and the highest passage level (MCS × n) intended for use in the preparation of a biological product is specified in

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[1]   The nomenclature for the genes changed several years ago, but the old designation is still in the literature. The old and the new nomenclature is: gII = gB; gIII = gC; gp50 = gD; gI = gE; gX = gG; gp63 = gI. Note that some commercial serological kits may still be named by the old nomenclature.

an Outline of Production. Both MCS and MCS × n are monitored by a variety of procedures to characterise the cell line and to ensure freedom from adventitious agents. The extraneous agents to be detected are generally defined in monographs or guidelines (e.g. European Pharmacopoeia, US Code of Federal Regulations, EU guidelines, etc.). In general, the type of agents to be looked for is founded on a risk analysis depending on the history of the viral strain and cells on which the vaccinal strain was isolated and on which it is cultivated. The MCS must be monitored for species of origin. A minimum of 50 mitotic cells should be examined at both the MCS and MCS × n passage levels. The modal number in the MCS × n must not exceed 15% of the modal number of the MCS. Any marker chromosomes in the MCS must also be present in the highest cell passage.

If there is evidence that the cell line may induce malignancies in the species for which the product is intended, the cell line is tested for tumorgenicity and oncogenicity.

Both the MSV and the MCS must be shown to be free from mycoplasma, bacteria, fungi, cytopathogenic or haemadsorbing viruses, porcine parvovirus, cytopathic and noncytopathic ovine and bovine pestiviruses and other extraneous agents, such as circovirus, as determined by culturing and by fluorescent antibody procedures or others, such as PCR.

2.2. Method of manufacture

2.2.1. Procedure

Only MSV that has been established as pure, safe and immunogenic may be used as seed for a vaccine product. Cells from the MCS are propagated in a variety of growth media. All batches of vaccine must be from the first to the twentieth passage of MCS.

2.2.2. In-process controls

It is necessary to carry out tests at each critical step of the manufacturing process. The control tests are also carried out on intermediate products with a view to verifying the consistency of the production process and the final product.

2.2.3. Final product batch tests

It is essential to differentiate the tests that are carried out on a routine basis to release batches of final product from those that are performed to define the biological properties of a vaccine. The trials carried out for batch release are not the same as the ones carried out once only to determine the safety and efficacy of a vaccine. The batch release controls are always short-term trials, as inexpensive as possible, and not always carried out in pigs. Their purpose is mainly to attest the reproducibility of the quality of the finished product, which has to be in compliance with the quality initially defined in the application for marketing authorisation.

i) Sterility and purity

Tests must be carried out for sterility and freedom from contamination (see chapter 1.1.9 and Section C.2.1.2 of this chapter).

Each batch of SHV-1 vaccines must be tested for freedom from extraneous viruses. Using a minimum amount of a monospecific antiserum, the live vaccinal strain is neutralised and inoculated into cell cultures known to be sensitive to viruses pathogenic for pigs. No CPE and no haemadsorbing agents should be detected. The vaccines have to be free from pestiviruses.

ii) Inactivation

For inactivated vaccines, inactivation must be checked using two passages in the same type of cell culture as used in the production of the vaccine. Tests can be carried out by vaccinating susceptible animals such as rabbits.

iii) Identity

Where necessary, a specific test for virus identification should be carried out.

iv)   Safety

Safety tests in target animals are not required by many regulatory authorities for the release of each batch. Where required, standard procedures should be statistically relevant and target the smallest number of animals required for the relevant regulatory approval.

v)   Batch potency

The potency of the vaccine must be demonstrated using a suitable method, the results of which have to be correlated with the efficacy tests described previously.

In this kind of test, the most difficult point is to determine an acceptability threshold for using or rejecting the batch according to the results that are obtained.

Virus content tests should be carried out using each of at least three containers. The virus titre of the vaccine must be determined and must normally not be higher than 1/10 of the dose at which the vaccine has been shown to be safe, and not lower than the minimum release titre.

vi)   Preservatives

If no preservative is included in the final product, the manufacturer must demonstrate that the product remains acceptable for its recommended period of use after opening the vial.

The efficacy of preservatives in multidose containers must be demonstrated. The concentration of the preservative in the final filled vaccine and its persistence throughout shelf life must be checked.

vii)   Precautions (hazards)

All information about possible adverse reactions induced by the vaccine must be indicated. Any putative risk for human health if the user is accidentally given a small quantity of the product has to be indicated. The manufacturer should indicate all the conditions of use of the vaccine: mixing, reconstitution, storage, asepsis, length of needle, route of administration and health status of the vaccinated animals.

2.2.4. Stability tests

Tests have to be carried out to verify the shelf life proposed by the manufacturer. These tests must always be real-time studies; they must be carried out on a sufficient number of batches (at least three) produced according to the described production process and on products stored in the final container, and normally include biological and physicochemical stability tests. The manufacturer has to provide the results of analyses that support the proposed shelf life under all proposed storage conditions. Usually, the proposed shelf life corresponds to the period for which the product is considered to be stable minus 3 months.

2.3. Requirements for authorisation

2.3.1. Safety requirements

Local and general reactions must be examined. When a live vaccine is used, it is necessary to differentiate the exact safety properties of the vaccinal strain from those of the finished product if this includes an adjuvant.

Objective and quantifiable criteria to detect and measure adverse reactions should be used; these would include temperature changes, weight gain, litter size, reproductive performance, etc., of vaccinated and control groups. The tests must be performed by administering the vaccine to the pigs in the recommended dose and by each recommended route of administration.

In general, safety is tested initially under experimental conditions, following the requirements of the WOAH *Terrestrial Animal Health Code,* Chapter 7.8 *Use of animals in research and education*.

When the results of these preliminary tests are known, it is necessary to increase the number of animals vaccinated in order to evaluate the safety of the vaccine under practical conditions.

i)   **Laboratory testing**

All tests must be carried out on pigs that do not have antibodies against Aujeszky's disease virus or against a subunit of the virus.

a)   **General effects**

1.   Live vaccines

Intranasal tests and vaccination of 3- to 5-day-old piglets are very useful for ascertaining the degree of safety of a strain. At least five piglets should be used.

It is also essential to assess the properties of a vaccine, especially live ones, in the target animals under normal conditions of use and at the youngest age intended for vaccination, e.g. fattening pigs, which are generally vaccinated when they are between 9 and 12 weeks old, and pregnant sows when this use of the vaccine is claimed by the manufacturer and is authorised. No clinical signs, including significant thermal reactions (data have to be recorded before vaccination and on a schedule such as 6 hours, 24 hours and 48 hours later, then on a daily basis during the observation period), should be observed after vaccination. These assays have to be performed on at least ten vaccinated pigs, with five unvaccinated pigs as controls.

Reversion to virulence following serial passage must be examined. Primary vaccination is done by the intranasal route. Series of at least four passages in piglets are made. No fewer than two fully susceptible animals must be used for each passage.

The object of these assays is to test the genetic stability of live vaccine strains. The tests appear to be less necessary when a genetically modified live strain is concerned, especially if it is produced by gene deletion.

It is recommended to test for possible excretion of the vaccine strain. For this purpose, no fewer than 14 piglets, 3–4 weeks old each receive one dose of vaccine by the recommended route and at the recommended site (except for vaccines administered by the intranasal route). Four unvaccinated piglets are kept as controls. Suitably sensitive tests for the virus are carried out individually on the nasal and/or oral secretions of vaccinated and in-contact pigs as follows: nasal and oral swabs are collected daily from 1 day before vaccination to 10 days after vaccination. Vaccine strains that are isolated from the nasal/oral secretion collected from pigs in which the vaccine was administered by the parenteral route are not recommended for eradication purposes.

The ability of the Aujeszky's disease vaccine strain to spread from vaccinated pigs to unvaccinated ones (lateral spread) must be tested by using the recommended route of administration that presents the greatest risk of spread (except for vaccines administered by the intranasal route). A repetition of the assays (four times) is necessary as this phenomenon is difficult to detect. Four piglets should be used each time for vaccination and placed in contact, 1 day later, with two unvaccinated piglets. It may also be necessary to examine the spread of the strain to nontarget species that may be susceptible to the vaccine strain.

Live attenuated vaccine strains are tested with regard to their general effects by administering to 5- to 10-day-old piglets ten times the field dose. This administration of an overdose makes it possible to detect reactions not produced under normal conditions of use. Such reactions may be produced inadvertently when large numbers of animals are vaccinated. If vaccines are administered by the intranasal route, the manufacturer has to indicate clearly that the vaccine will spread from vaccinated pigs to unvaccinated ones.

2.   Inactivated vaccines

It is essential to test inactivated vaccines in the target animals under normal conditions of use for fattening pigs and for sows when this use is claimed by the manufacturer and authorised (European Pharmacopoeia, 2008; Vannier *et al.*, 2007). As described previously, it is fundamental to use objective and quantifiable criteria to detect and to measure adverse reactions, such as temperature changes, weight performance, litter size, reproductive performance, etc., on vaccinated and control groups. The tests must be performed by administering the vaccine in the recommended dose and by each recommended route of administration to the pigs for which it is intended.

Pigs or sows are usually observed until there is no further evidence of vaccine reaction. The period of observation must not be fewer than 14 days from the day of administration. This period has to be extended when, for example, the vaccine is used in pregnant sows and it is necessary to assess the possible effects of the vaccine on reproductive performance. In this case, the period of observation lasts the full duration of the pregnancy.

Control authorities generally request vaccination with a double dose so that adverse reactions, which may be at the limit of detection when a single dose is administered, are more likely to be detected.

b)   **Local reactions**

Local reactions are often associated with the use of inactivated vaccines, as these side-effects can be induced by adjuvants, particularly oil adjuvants. However, some Aujeszky's disease live vaccines are mixed with different adjuvants, which modify what has been observed in the past.

Local reactions are mainly inflammatory and can be more or less complicated (necrotic or suppurative), depending on the nature of the adjuvants used and the aseptic conditions of the vaccination. Oil adjuvants can induce a variety of effects including muscular degeneration, granuloma, fibrosis and abscessation. In addition to the nature of the oil used (the intensity of the reaction is reduced when metabolisable oils are used in the vaccine), the type of emulsion used (water/oil, oil/water, water/oil/water) induces these reactions to a greater or lesser extent. In consequence, it is necessary to observe the site of injection not only from the outside, but also by dissection after slaughter, especially for growing and finishing pigs.

ii)   **Field testing**

Field trials are necessary to assess the safety of an Aujeszky's disease vaccine in a large number of pigs or sows. In Europe (European Pharmacopoeia, 2008), tests must be carried out in each category of animals for which the vaccine is intended (sows, fattening pigs). At least three groups of no fewer than 20 animals each are used with corresponding groups of no fewer than 10 controls. The rectal temperature of each animal is measured at the time of vaccination, 6, 24 and 48 hours later. At slaughter, the injection site must be examined for local reactions. If the vaccine is intended to be used in sows, reproductive performances have to be recorded. Field trials are supplemented by laboratory studies of efficacy correlated to vaccine potency.

2.3.2. Efficacy requirements

i)   **Laboratory trials**

All tests must be carried out on pigs that do not have antibodies against Aujeszky's disease virus or against a subunit of the virus, except that some tests may be done using maternally immune animals.

a)   **Assessment of passive immunity**

To test the efficacy of vaccines, it is important to mimic the natural infection conditions (European Commission, 2008). SHV-1 infection gives rise to important losses of young piglets from nonimmune sows. Thus, when vaccinating sows, the main goal is to protect the young piglets through passive immunity conferred by the colostrum ingested immediately after birth, with the secondary objective of preventing abortion.

To measure this passive immunity and the protection induced by vaccinating the sows, experimental models have been established. The sows are vaccinated according to the vaccinal protocol during pregnancy. When the piglets are, for example, 6–10 days old they are given an intranasal challenge exposure with a virulent SHV-1 strain. It is preferable to use a strain titrated in median lethal doses (LD_{50}). Pigs should be inoculated by the nasal route, 10^2 LD_{50} per pig in 1 ml. The efficacy of the vaccine is assessed by comparing clinical signs, but also and more importantly, mortality, or humane euthanasia, in piglets from unvaccinated dams with that observed in piglets from vaccinated sows.

Piglets from vaccinated sows can be found to have 80% protection against mortality compared with those from the control sows. In order for the results to be significant, it is recommended that eight vaccinated sows and four control sows be used (subject to satisfactory numbers of piglets from each sow).

b)   **Assessment of active immunity**

1.   Clinical protection

Several criteria can be considered when measuring active immunity induced by vaccinating pigs. Generally, pigs are vaccinated at the beginning of the growing period, i.e. when they are between 9 and 12 weeks old. Laboratory trials are performed by challenging pigs at the end of the finishing period, when they weigh between 80 and 90 kg.

In general, at least three criteria, such as rectal temperature, weight loss and clinical signs, along with mortality, are used to measure the clinical protection of pigs after vaccination and challenge (De Leeuw & Van Oirschot, 1985). The antibody titres have little predictive value for the efficacy of the vaccines. Weight loss compared between the vaccinated and control groups is the most reproducible and reliable parameter when the challenge conditions are well standardised. The measure of the difference in weight gain or loss between the two groups of pigs and, in the interval of time between challenge (day 0 and day 7), has a very good predictive value for the efficacy of the vaccines (Stellmann *et al.*, 1989). Significant results can be obtained when weight performances are compared between one group of at least eight vaccinated pigs and another group of eight unvaccinated control pigs

For challenge, it is usually preferable to use a high titre of a virulent strain, as this makes it possible to obtain a more marked difference between vaccinated and control pigs. On the basis of previous work, a challenge dose with at least 10^6 $TCID_{50}$/ml virulent strain having undergone not more than three passages on primary cells can be sufficient, but a higher titre ($10^{7.5}$ $TCID_{50}$/ml) is recommended. The oro-nasal route should be used to challenge the pigs by introducing the virulent strain in an appropriately high volume ($\geq$4 ml).

This method of evaluating the efficacy of SHV-1 vaccines is now well tested and has made it possible to establish an objective index for determining the efficacy of a vaccine. This index, which compares the relative weight losses between vaccinated and control pigs, can also be used for potency testing batches before release and for batch efficacy testing. However, the value of the cut-off index will be different as the conditions of the assay will not be identical. The influence of passively acquired, maternally derived antibodies on the efficacy of a vaccine must be evaluated adequately.

2. Virulent virus excretion

Additionally, it is desirable that vaccines should prevent or at least limit viral excretion from infected pigs (Vannier *et al.*, 1991). When a control programme against Aujeszky's disease is based on large-scale vaccination, it is essential to choose the vaccines or the vaccinal scheme that best limits the replication of virulent virus in infected pigs. Several assays have been performed to compare vaccines on that basis.

Generally, the pigs are vaccinated and challenged at different periods. It is better, but more time-consuming, to infect pigs at the end of the finishing period. To measure the virus excretion, nasal swabs (taken at 10 cm depth in the nostrils) are taken daily from each pig from the day before challenge to at least 12 days after challenge. The swabs can be weighed before the sampling and immediately after to calculate the exact weight of collected mucus. Medium is then added to each tube containing a swab. The virus is titrated from the frozen and thawed medium.

Different indexes can be used to express the quantity of virulent virus excreted by pigs, taking into consideration the duration and the level of viral excretion, and the number of pigs excreting virulent virus.

3. Duration of immunity

It is recommended that any claims regarding the onset and duration of immunity should be supported by data from trials. Assessment of duration of immunity can be based on challenge trials or, as far as it is possible, on immunological and serological tests.

ii) Field trials

In general terms, it is extremely difficult to assess vaccine efficacy in animal populations. In order to do this, it would be necessary to vaccinate the animals in the absence of the pathogen that the vaccine protects against, then to await the moment of infection and to compare the effects of infection in vaccinated animals (or the offspring of vaccinated dams) with the effects in the unvaccinated animals of the same age, in the same building and in the same batch as the vaccinated animals (or those protected passively). As all these conditions are difficult to achieve in the field, field trials are certainly more appropriate to safety testing than to efficacy testing, except for the development of DIVA-vaccines that offer the opportunity to evaluate the effectiveness of vaccines under field conditions (Bouma, 2005).

2.3.3. Stability

Tests have to be carried out to verify the shelf life proposed by the manufacturer. These tests must always be real-time studies; they must be carried out on a sufficient number of batches (at least three) produced according to the described production process and on products stored in the final container, and normally include biological and physicochemical stability tests. The manufacturer has to provide the results of analyses that support the proposed shelf life under all proposed storage conditions. Usually, the proposed shelf life corresponds to the period for which the product is considered to be stable minus 3 months.

3. Vaccines based on biotechnology

3.1. Vaccines available and their advantages

Biotechnology combined with a better knowledge of the functions and characteristics of the SHV-1 glycoproteins helped to develop new vaccines. For example, Quint *et al.* (1987) deleted glycoprotein E-coding sequence from the NIA3 strain. This resulted in an efficient DIVA-vaccine against Aujeszky's disease, allowing differentiation of vaccinated from infected animals (DIVA vaccines). Most of the vaccines used at the moment are obtained from recombinant DNA-derived gene-deleted virus. The deletion of the genes coding for the glycoprotein E is the most commonly used, allowing an attenuated live virus vaccine to be obtained but still protecting against the clinical signs and reducing significantly

the level of the viral excretion by the pigs vaccinated and infected. Because of the ability of some glycoproteins of SHV-1 to induce strong immune responses, efficiencies of DNA vaccines, consisting of plasmids encoding these glycoproteins, were tested. Indeed, DNA vaccination has a number of advantages: ease of construction and standardised production of plasmids, no handling of infectious particles, induction of humoral and cellular immune responses, bypass of the maternal derived immunity. The pioneering study on DNA vaccination against Aujeszky's disease infection was published in 1997 (Gerdts *et al.,* 1997). The use of a novel generation of plasmid amplifying the level of gene transcription of the proteins of interest (Dory *et al.,* 2005) have been shown to be efficient strategies. These vaccines are not yet commercialised.

3.2. Special requirements for biotechnological vaccines, if any

Criteria to assess quality, safety and efficacy of the vaccines derived from the biotechnology are the same as the ones defined for conventional vaccines (see section C.2). Nevertheless special attention has to be paid to the stability of the recombinant DNA construction.

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NB: There are WOAH Reference Laboratories for Aujeszky's disease (please consult the WOAH Web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the WOAH Reference Laboratories for any further information on
diagnostic tests, reagents and vaccines for Aujeszky's disease

NB: FIRST ADOPTED IN 1991. MOST RECENT UPDATES ADOPTED IN 2018.