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5.2.2. CHICKEN FLOCKS FREE FROM SPECIFIED PATHOGENS FOR THE PRODUCTION AND QUALITY CONTROL OF VACCINES

Where specified, chickens, embryos or cell cultures used for the production or quality control of vaccines are derived from eggs produced by chicken flocks free from specified pathogens (SPF). The SPF status of a flock is ensured by means of the system described below. The list of micro-organisms given is based on current knowledge and will be updated as necessary.

A flock is defined as a group of birds sharing a common environment and having their own caretakers who have no contact with non-SPF flocks. Once a flock is defined, no non-SPF birds are added to it.

Each flock is housed so as to minimise the risk of contamination. The facility in which the flock is housed must not be sited near to any non-SPF flocks of birds with the exception of flocks that are in the process of being established as SPF flocks and that are housed in facilities and conditions appropriate to SPF flocks. The SPF flock is housed within an isolator or in a building with filtered air under positive pressure. Appropriate measures are taken to prevent entry of rodents, wild birds, insects and unauthorised personnel.

Personnel authorised to enter the facility must have no contact with other birds or with agents potentially capable of infecting the flock. It is advisable for personnel to shower and change clothing or to wear protective clothing before entering the controlled facility.

Wherever possible, items taken into the facility are sterilised. In particular it is recommended that the feed is suitably treated to avoid introduction of undesirable micro-organisms and that water is at least of potable quality, for example from a chlorinated supply. No medication is administered to birds within the flock that might interfere with detection of any disease.

A permanent record is kept of the general health of the flock and any abnormality is investigated. Factors to be monitored include morbidity, mortality, general physical condition, feed consumption, daily egg production and egg quality, fertility and hatchability. Records are maintained for a period of at least 5 years. Details of any deviation from normal in these performance parameters or detection of any infection are notified to the users of the eggs as soon as practicable.

The tests or combination of tests described below must have suitable specificity and sensitivity with respect to relevant serotypes of the viruses. Samples for testing are taken at random.

A positive result for chicken anaemia virus (CAV) does not necessarily exclude use of material derived from the flock, but live vaccines for use in birds less than 7 days old shall be produced using material from CAV-negative flocks. Inactivated vaccines for use in birds less than 7 days old may be produced using material from flocks that have not been shown to be free from CAV, provided it has been demonstrated that the inactivation process inactivates CAV.

ESTABLISHMENT OF AN SPF FLOCK

A designated SPF flock is derived from chickens shown to be free from vertically-transmissible agents listed in Table 5.2.2-1. This is achieved by testing of 2 generations prior to the designated SPF flock. A general scheme for the procedure to be followed in establishing and maintaining an SPF flock is shown diagrammatically in Table 5.2.2.-2. In order to establish a new SPF flock, a series of tests must be conducted on 3 generations of birds. All birds in the first generation must be tested at least once before the age of 20 weeks for freedom from avian leucosis group-antigen and tested by an enzyme immunoassay (EIA) or by virus neutralisation (VN) for freedom of antibodies to avian leucosis virus subtypes A, B and J. All birds must also be tested for freedom from antibodies to the vertically-transmissible agents listed in Table 5.2.2-1. From the age of 8 weeks the flock is tested for freedom from *Salmonella*. Clinical examination is carried out on the flock from 8 weeks of age and the birds must not exhibit any signs of infectious disease. The test methods to be used for these tests are given in the table and further guidance is also given in the section below on routine testing of designated SPF flocks. From 20 weeks of age, the flock is tested as described under Routine testing of designated SPF flocks. All stages of this testing regime are also applied to the subsequent 2 generations, except the testing of every bird before lay for vertically-transmissible agents. All test results must indicate freedom from pathogens in all 3 generations for the flock consisting of the third generation to be designated as SPF.

SPF embryos derived from another designated SPF flock contained within a separate facility on the same site may be introduced. From 8 weeks of age, these replacement birds are regarded as a flock and are tested in accordance with test procedures described above.

INITIAL TESTING REQUIREMENTS FOR SUBSEQUENT GENERATIONS DERIVED FROM A DESIGNATED SPF FLOCK

Where a replacement flock is derived exclusively from a fully established SPF flock the new generation is tested prior to being designated as SPF. In addition to the tests for *Salmonella* and monitoring of the general health and performance of the flock, further specific testing from the age of 8 weeks is required. Tests are performed on two 5 per cent samples of the flock (minimum 10, maximum 200 birds) taken with an interval of at least 4 weeks between the ages of 12-16 weeks and 16-20 weeks.

All samples are collected and tested individually. Blood samples for antibody tests and suitable samples for testing for leucosis antigen are collected. The test methods to be used are as described under Routine testing of designated SPF flocks. Only when all tests have confirmed the absence of infection may the new generation be designated as SPF.

ROUTINE TESTING OF DESIGNATED SPF FLOCKS

General examination and necropsy. Clinical examination is carried out at least once per week throughout the life of the flock in order to verify that the birds are free from fowl-pox virus and signs of any other infection. In the event of mortality exceeding 0.1 per cent per week, necropsy is performed on all available carcasses to verify that there is no sign of infection. Where appropriate, histopathological and/or microbiological/virological studies are performed to confirm diagnosis. Specific examination for tuberculosis lesions is carried out and histological samples from any suspected lesions are specifically stained to verify freedom from *Mycobacterium avium*. Caecal contents of all available carcasses are examined microbiologically for the presence of *Salmonella* spp. using the techniques described below. Where appropriate, caecal samples from up to 5 birds may be pooled.

Cultural testing for Salmonella spp. Cultural testing for *Salmonella* spp. is performed either by testing samples of droppings or cloacal swabs or by testing of drag swabs.

Table 5.2.2.-1

Agent	Test to be used**	Vertical transmission	Rapid/slow spread
Avian adenoviruses, group 1	AGP, EIA	yes	slow
Avian encephalomyelitis virus	AGP, EIA	yes	rapid
Avian infectious bronchitis virus	HI, EIA	no	rapid
Avian infectious laryngotracheitis virus	VN, EIA	no	slow
Avian leucosis viruses	EIA for virus, VN, EIA for antibody	yes	slow
Avian nephritis virus	IS	no	slow
Avian orthoreoviruses	IS, EIA	yes	slow
Avian reticuloendotheliosis virus	AGP, IS, EIA	yes	slow
Chicken anaemia virus	IS, EIA, VN	yes	slow
Egg drop syndrome virus	HI, EIA	yes	slow
Infectious bursal disease virus	Serotype 1: AGP, EIA, VN Serotype 2: VN	no	rapid
Influenza A virus	AGP, EIA, HI	no	rapid
Marek's disease virus	AGP	no	rapid
Newcastle disease virus	HI, EIA	no	rapid
Turkey rhinotracheitis virus	EIA	no	slow
Mycoplasma gallisepticum	Agg and HI to confirm a positive test, EIA, HI	yes	slow
Mycoplasma synoviae	Agg and HI to confirm a positive test, EIA, HI	yes	rapid
Salmonella pullorum	Agg	yes	slow

Agg: agglutination
AGP: agar gel precipitation; the technique is suitable where testing is carried out weekly
EIA: enzyme immunoassay
HI: haemagglutination inhibition
IS: immunostaining
VN: virus neutralisation

**Subject to agreement by the competent authority, other types of test may be used provided they are at least as sensitive as those indicated and of appropriate specificity.

Where droppings or cloacal swabs are tested, a total of 60 samples within each 4-week period is tested throughout the entire life of the flock. Tests may be performed on pools of up to 10 samples. Where drag swabs are tested, a minimum of 2 drag swabs are tested during each 4-week period throughout the entire life of the flock. Detection of *Salmonella* spp. in these samples is performed by pre-enrichment of the samples followed by culture using *Salmonella*-selective media.

Tests for avian leucosis antigen. Prior to the commencement of laying, cloacal swabs or blood samples (using buffy coat cultivation) are tested for the presence of group-specific leucosis antigen. A total of 5 per cent (minimum 10, maximum 200) of the flock is sampled during each 4-week period. During lay, albumen samples from 5 per cent (minimum 10, maximum 200) of the eggs are tested in each 4-week period. Tests are performed by EIA for group-specific antigen using methods that are capable of detecting antigen from subgroups A, B and J.

Test for antibodies to other agents. Tests for antibodies to all agents listed in Table 5.2.2.-1 are performed throughout the laying period of the flock. In each 4-week period, samples are taken from 5 per cent (minimum 10, maximum 200) of the flock. It is recommended that 1.25 per cent of the flock is sampled each week since some test methods for some agents must be conducted on a weekly basis. Table 5.2.2.-1 classifies the agents into those that spread rapidly through the flock and those that spread slowly or may not infect the entire flock. For those agents listed as slowly spreading, each sample is tested individually. For those agents listed as rapidly spreading, at least 20 per cent of the samples collected in each 4-week period are tested individually or, where serum neutralisation or ELISA tests are employed, all of the samples may be tested individually or by preparing pools of 5 samples, collected at the same time.

Suitable methods to be used for detection of the agents are shown in Table 5.2.2.-1. Subject to agreement by the competent authority, other test methods may be used provided they are shown to be at least as sensitive as those indicated and of appropriate specificity.

TESTS TO BE CONDUCTED AT THE END OF THE LAYING PERIOD

Following the last egg collection, final testing to confirm the absence of vertically-transmissible agents indicated in Table 5.2.2.-1 is performed. After the last egg collection, a minimum of 5 per cent of the flock (minimum 10, maximum 200) is retained for at least 4 weeks. Blood samples are collected from every bird in the group during the 4-week period with at least 1.25 per cent of the birds (25 per cent of the sample) being bled not earlier than 4 weeks after the final egg collection. Serum samples are tested for vertically-transmissible agents (as defined by Table 5.2.2.-1) using the methods indicated. Where sampling is performed on a weekly basis, at least 1.25 per cent of the birds (25 per cent of the sample) are tested each week during this period. Alternatively, within 4 weeks of the final egg collection blood and/or other suitable sample materials are collected from at

Table 5.2.2-2. – *Schematic description of the establishment and maintenance of SPF flocks*

NEW STOCK	Establish freedom from vertically-transmissible agents
	Test all birds for avian leucosis antigen and antibodies prior to 20 weeks of age
	Test for *Salmonella* spp. and perform general clinical observation from 8 weeks of age
	Carry out routine testing for specified agents from 20 weeks of age
2nd GENERATION	Test all birds for avian leucosis antigen and antibodies prior to 20 weeks of age
	Test for *Salmonella* spp. and perform general clinical observation from 8 weeks of age
	Carry out routine testing for specified agents from 20 weeks of age
3rd GENERATION	Test all birds for avian leucosis antigen and antibodies prior to 20 weeks of age
	Test for *Salmonella* spp. and perform general clinical observation from 8 weeks of age
DESIGNATE FLOCK AS SPF IF ALL TESTS ARE SATISFACTORY	
3rd GENERATION	Carry out routine testing for specified agents from 20 weeks of age
	Carry out post-lay testing for vertically-transmissible agents
SUBSEQUENT GENERATIONS	Test two 5 per cent samples for avian leucosis antigen and for antibodies against specified agents between 12 and 20 weeks of age
	Test for *Salmonella* spp. and perform general clinical observation from 8 weeks of age
	Carry out routine testing for specified agents from 20 weeks of age
	Carry out post-lay testing for vertically-transmissible agents

least 5 per cent of the flock and tested for the presence of vertically-transmissible agents using validated nucleic acid amplification techniques (*2.6.21*).

ACTION TO BE TAKEN IN THE EVENT OF DETECTION OF A SPECIFIED AGENT

If evidence is found of contamination of the flock by an agent listed as slowly spreading in Table 5.2.2.-1, all materials derived from the flock during the 4-week period immediately preceding the date on which the positive sample was collected are considered unsatisfactory. Similarly, if evidence is found of contamination of the flock by an agent listed as rapidly spreading in Table 5.2.2.-1, all materials derived from the flock during the 2-week period immediately preceding the date on which the positive sample was collected are considered unsatisfactory. Any product manufactured with such materials, and for which the use of SPF materials is required, is considered unsatisfactory and must be discarded; any quality control tests conducted using the materials are invalid.

Producers must notify users of all eggs of the evidence of contamination as soon as possible following the outbreak.

Any flock in which an outbreak of any specified agent is confirmed may not be redesignated as an SPF flock. Any progeny derived from that flock during or after the 4-week period prior to the last negative sample being collected may not be designated as SPF.