# 5.2. GENERAL TEXTS ON BIOLOGICAL PRODUCTS

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## 5.2.1. TERMINOLOGY USED IN MONOGRAPHS ON BIOLOGICAL PRODUCTS

For some items, alternative terms commonly used in connection with veterinary vaccines are shown in parenthesis.

**Seed-lot system**. A seed-lot system is a system according to which successive batches of a product are derived from the same master seed lot. For routine production, a working seed lot may be prepared from the master seed lot. The origin and the passage history of the master seed lot and the working seed lot are recorded.

**Master seed lot**. A culture of a micro-organism distributed from a single bulk into containers and processed together in a single operation in such a manner as to ensure uniformity and stability and to prevent contamination. A master seed lot in liquid form is usually stored at or below – 70 °C. A freeze-dried master seed lot is stored at a temperature known to ensure stability.

**Working seed lot**. A culture of a micro-organism derived from the master seed lot and intended for use in production. Working seed lots are distributed into containers and stored as described above for master seed lots.

**Cell-bank system (Cell-seed system)**. A system whereby successive final lots (batches) of a product are manufactured by culture in cells derived from the same master cell bank (master cell seed). A number of containers from the master cell bank (master cell seed) are used to prepare a working cell bank (working cell seed). The cell-bank system (cell-seed system) is validated for the highest passage level achieved during routine production.

**Master cell bank (Master cell seed)**. A culture of cells distributed into containers in a single operation, processed together and stored in such a manner as to ensure uniformity and stability and to prevent contamination. A master cell bank (master cell seed) is usually stored at – 70 °C or lower.

**Working cell bank (Working cell seed)**. A culture of cells derived from the master cell bank (master cell seed) and intended for use in the preparation of production cell cultures. The working cell bank (working cell seed) is distributed into containers, processed and stored as described for the master cell bank (master cell seed).

**Primary cell cultures**. Cultures of cells obtained by trypsination of a suitable tissue or organ. The cells are essentially identical to those of the tissue of origin and are no more than 5 *in vitro* passages from the initial preparation from the animal tissue.

**Cell lines**. Cultures of cells that have a high capacity for multiplication *in vitro*. In diploid cell lines, the cells have essentially the same characteristics as those of the tissue of origin. In continuous cell lines, the cells are able to multiply indefinitely in culture and may be obtained from healthy or tumoral tissue. Some continuous cell lines have oncogenic potential under certain conditions.

**Production cell culture**. A culture of cells intended for use in production; it may be derived from one or more containers of the working cell bank (working cell seed) or it may be a primary cell culture.

**Control cells**. A quantity of cells set aside, at the time of virus inoculation, as uninfected cell cultures. The uninfected cells are incubated under similar conditions to those used for the production cell cultures.

**Single harvest**. Material derived on one or more occasions from a single production cell culture inoculated with the same working seed lot or a suspension derived from the working seed lot, incubated, and harvested in a single production run.

**Monovalent pooled harvest**. Pooled material containing a single strain or type of micro-organism or antigen and derived from a number of eggs, cell culture containers etc. that are processed at the same time.

**Final bulk vaccine**. Material that has undergone all the steps of production except for the final filling. It consists of one or more monovalent pooled harvests, from cultures of one or more species or types of micro-organism, after clarification, dilution or addition of any adjuvant or other auxiliary substance. It is treated to ensure its homogeneity and is used for filling the containers of one or more final lots (batches).

**Final lot (Batch)**. A collection of closed, final containers or other final dosage units that are expected to be homogeneous and equivalent with respect to risk of contamination during filling or preparation of the final product. The dosage units are filled, or otherwise prepared, from the same final bulk vaccine, freeze-dried together (if applicable) and closed in one continuous working session. They bear a distinctive number or code identifying the final lot (batch). Where a final bulk vaccine is filled and/or freeze-dried on several separate sessions, there results a related set of final lots (batches) that are usually identified by the use of a common part in the distinctive number or code; these related final lots (batches) are sometimes referred to as sub-batches, sub-lots or filling lots.

**Combined vaccine**. A multicomponent preparation formulated so that different antigens are administered simultaneously. The different antigenic components are intended to protect against different strains or types of the same organism and/or different organisms. A combined vaccine may be supplied by the manufacturer either as a single liquid or freeze-dried preparation or as several constituents with directions for admixture before use.

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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 1st 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 3rd 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.

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.

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 |



## 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 0.2 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. 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 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.



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## 5.2.3. CELL SUBSTRATES FOR THE PRODUCTION OF VACCINES FOR HUMAN USE

This general chapter deals with diploid cell lines and continuous cell lines used as cell substrates for the production of vaccines for human use; specific issues relating to vaccines prepared by recombinant DNA technology are covered by the monograph *Products of recombinant DNA technology (0784)*. Testing to be carried out at various stages (cell seed, master cell bank, working cell bank, cells at or beyond the maximum population doubling level used for production) is indicated in Table 5.2.3.-1. General provisions for the use of cell lines and test methods are given below. Where primary cells or cells that have undergone a few passages without constitution of a cell bank are used for vaccine production, requirements are given in the individual monograph for the vaccine concerned.

**Diploid cell lines**. A diploid cell line has a high but finite capacity for multiplication *in vitro*.

**Continuous cell lines**. A continuous cell line has the capacity to multiply indefinitely *in vitro*; the cells often have differences in karyotype compared to the original cells; they may be obtained from healthy or tumoral tissue either from mammals or from insects.

For injectable vaccines produced in continuous cell lines, the purification process is validated to demonstrate removal of substrate-cell DNA to a level equivalent to not more than 10 ng per single human dose, unless otherwise prescribed.

**Cell-bank system**. Production of vaccines in diploid or continuous cell lines is based on a cell-bank system. The *in vitro* age of the cells is counted from the master cell bank. Each working cell bank is prepared from one or more containers of the master cell bank. The use, identity and inventory control of the containers is carefully documented.

**Media and substances of human or animal origin**. The composition of media used for isolation and all subsequent culture is recorded in detail, and if substances of human or animal origin are used they must be free from extraneous agents (*2.6.16*) and must comply with the general chapter on *5.1.7. Viral safety*.

If human albumin is used, it complies with the monograph *Human albumin solution (0255)*.

If bovine serum is used, it complies with the monograph *Bovine serum (2262)*.

Trypsin used for the preparation of cell cultures is examined by suitable methods and shown to be sterile and free from mycoplasmas and viruses, notably pestiviruses, circoviruses and parvoviruses.

**Cell seed**. The data used to assess the suitability of the cell seed comprises information, where available, on source, history and characterisation.

*Source of the cell seed*. For human cell lines, the following information concerning the donor is recorded: ethnic and geographical origin, age, sex, general physiological condition, tissue or organ used, results of any tests for pathogens.

For animal cell lines, the following information is recorded concerning the source of the cells: species, strain, breeding conditions, geographical origin, age, sex, general physiological condition, tissue or organ used, results of any tests for pathogens.

Cells of neural origin, such as neuroblastoma and P12 cell lines, may contain substances that concentrate agents of spongiform encephalopathies and such cells are not used for vaccine production.

*History of the cell seed*. The following information is recorded: the method used to isolate the cell seed, culture methods, any other procedures used to establish the master cell bank, notably any that might expose the cells to extraneous agents.

Full information may not be available on the ingredients of media used in the past for cultivation of cells, for example on the source of substances of animal origin; where justified and authorised, cell banks already established using such media may be used for vaccine production.

*Characterisation of the cell seed*. The following properties are investigated:

(1) the identity of the cells (for example, isoenzymes, serology, nucleic acid fingerprinting);

(2) the growth characteristics of the cells and their morphological properties (optical and electron microscopes);

(3) for diploid cell lines, karyotype;

(4) for diploid cell lines, the *in vitro* life span in terms of population doubling level.

**Cell substrate stability**. Suitable viability of the cell line in the intended storage conditions must be demonstrated. For a given product to be prepared in the cell line, it is necessary to demonstrate that consistent production can be obtained with cells at passage levels at the beginning and end of the intended span of use.

**Infectious extraneous agents**. Cell lines for vaccine production shall be free from infectious extraneous agents. Tests for extraneous agents are carried out as shown in Table 5.2.3.-1 using the methods described below.

For cell lines of insect origin, tests for specific viruses relevant to the species of origin of the insect cells and for arboviruses (arthropod - borne viruses) are applied. The panel of viruses tested is chosen according to the current state of scientific knowledge.

Cell lines that show the presence of retroviruses capable of replication are not acceptable for production of vaccines.