5.2. GENERAL TEXTS ON VACCINES

5.2.1. TERMINOLOGY USED IN MONOGRAPHS ON VACCINES

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 trypsinization 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.

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

INTRODUCTION

Where specified in a monograph, 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.

GENERAL PRINCIPLES AND PROCEDURES

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.
For SPF flocks established on a rolling basis, all replacements are hatched and reared in the controlled environment house. Subject to the agreement of the competent authorities, SPF embryos derived from a tested SPF flock from another house on the same site may be introduced. From 8 weeks of age, these replacement birds are regarded as a flock and monitored monthly in accordance with the Subsequent Testing requirements. At point of lay, all these replacement birds are tested in accordance with the Initial Testing requirements.

The flock is housed so as to minimise the chance of contamination. It is not sited near to non-SPF flocks of birds and is housed in an isolator or on wire in a building with filtered air under positive pressure. Appropriate measures are taken to prevent access of rodents, wild birds, insects and unauthorised people.

Personnel authorised to enter must have no contact with other birds or with agents likely to infect the flock. It is advisable for personnel to shower and change clothing or to wear protective clothing before entering the chicken house.

Items taken into the flock are sterilised. The feed is suitably treated to avoid the introduction of undesirable micro-organisms and water is obtained from a chlorinated supply. No medication is given that could interfere with detection of disease in the flock.

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. Dirty eggs are discarded; clean eggs may be surface-disinfected whilst warm.

The flock originates from chickens shown to be free from vertically-transmitted agents. In particular, each chicken from which the flock is derived is tested repeatedly to ensure freedom from leucosis viruses and their antibodies. In order to establish the SPF status of a flock, it is kept under SPF conditions for a test period of not less than 4 months. Each bird in the entire flock is shown to be free from evidence of infection with the agents listed below under Initial Testing after 6 weeks and at the end of the test period.

For each new generation in an established flock, all of the birds in the flock are tested at not later than 20 weeks of age, using the tests prescribed below under Initial Testing. After the initial test, monthly tests are carried out on a representative 5 per cent sample (but not less than ten and not more than two hundred birds), using the tests prescribed below under Subsequent Testing, with a final test at 4 weeks after the last collection of eggs.

For all tests, blood samples are collected from an appropriate number of birds at the specified time. The resultant serum samples are examined for antibodies against the relevant agents. Serum-neutralisation tests are done on pools of not more than five sera. All other tests are done on each individual serum. Positive and negative controls are used in all tests. The reagents used in the tests are standardised against international or European standard reagents where these are available. For avian leucosis virus, in addition to tests for antibodies carried out on serum samples, appropriate samples are taken for testing for the virus.

In addition to serological tests, clinical examination is carried out at least once per week to verify that the birds are free from fowl-pox and signs of other infections. Necropsy and, where necessary to confirm diagnosis, histopathological examination are carried out on any bird that dies to verify that there is no sign of infection. The absence of *Salmonella* spp. is determined by cultural examination of faecal samples at least once every 4 weeks; a pool of up to ten samples may be used for the tests.

If a positive result is obtained in any test carried out to establish the SPF status of a flock, the flock may not be designated as an SPF Flock. If a positive result is obtained in any test carried out on an established flock, the flock loses its SPF status. Special provisions apply to chick anaemia agent (CAA) as described below. Any chickens, embryos or cell cultures collected since the previous negative test are not suitable for use: any product made from them must be discarded and any quality control tests done with them are invalid and must be repeated.

In order to regain SPF status, the flock is maintained under SPF conditions and routine 5 per cent monthly testing shall continue except that every bird in the entire flock is tested every month for infection with the particular agent that gave the positive result. Infected birds and their progeny are removed from the flock. SPF status is regained after two such consecutive tests have yielded completely negative results.

A positive result for CAA does not necessarily exclude use of material derived from the flock, but live vaccines for use in birds less than 7 days old must be produced using material from CAA-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 CAA, provided it has been demonstrated that the inactivation process inactivates CAA.

Permanenent records of mortality and of results of flock testing are kept for a minimum of five years. Details of any deterioration in egg production or hatchability, except for accidental cases identified as being of non-infectious origin, and of any test results indicating infection with a specified agent, are immediately submitted to the user of the eggs.

**INITIAL TESTING**

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>
<thead>
<tr>
<th>Micro-organism</th>
<th>Type of test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avian adeno-viruses</td>
<td>Enzyme-linked immuno-sorbent assay</td>
</tr>
<tr>
<td>Avian encephalomyelitis virus</td>
<td>Enzyme-linked immuno-sorbent assay</td>
</tr>
<tr>
<td>Avian infectious bronchitis virus</td>
<td>Enzyme-linked immuno-sorbent assay</td>
</tr>
<tr>
<td>Avian infectious laryngotracheitis virus</td>
<td>Serum neutralisation</td>
</tr>
<tr>
<td>Avian leucosis viruses</td>
<td>Enzyme-linked immuno-sorbent assay for virus and serum neutralisation for antibody</td>
</tr>
<tr>
<td>Avian nephritis virus</td>
<td>Enzyme-linked immuno-sorbent assay</td>
</tr>
<tr>
<td>Avian reoviruses</td>
<td>Enzyme-linked immuno-sorbent assay</td>
</tr>
<tr>
<td>Avian reticuloendotheliosis virus</td>
<td>Fluorescent antibody</td>
</tr>
<tr>
<td>Chick anaemia agent</td>
<td>Fluorescent antibody</td>
</tr>
<tr>
<td>Haemagglutinating avian adeno-virus (Egg drop syndrome 76 EDS 76 virus)</td>
<td>Haemagglutination inhibition</td>
</tr>
<tr>
<td>Infectious bursal disease virus</td>
<td>Serum neutralisation against each serotype present in the country of origin</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>Enzyme-linked immuno-sorbent assay</td>
</tr>
<tr>
<td>Marek’s disease virus</td>
<td>Enzyme-linked immuno-sorbent assay</td>
</tr>
</tbody>
</table>

See the information section on general monographs (cover pages)
5.2.3. Cell substrates for production of vaccines for human use

This general chapter deals with diploid cell lines and continuous cell lines used for the production of vaccines for human use; specific issues relating to vaccines prepared by recombinant DNA technology are covered by the monograph on *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.

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 and 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 animal and human origin.** The composition of media used for isolation and all subsequent culture is recorded in detail and if substances of animal origin are used they must be free from extraneous agents.

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

Bovine serum used for the preparation and maintenance of cell cultures is tested and shown to be sterile and free from bovine viruses, notably bovine diarrhoea virus and mycoplasmas.

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 and parvoviruses.

**Cell seed.** The data used to assess the suitability of the cell seed comprise 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 and 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);