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Appendix XV J (Vet) 1. Cell Cultures for the Production of Vaccines for Veterinary Use

(Ph. Eur. general texts 5.2.4)

Cell cultures for the production of vaccines for veterinary use comply with the requirements of this general chapter.

For most mammalian viruses, propagation in cell lines is possible and the use of primary cells is then not acceptable.

Permanently infected cells used for production of vaccines for veterinary use comply with the appropriate requirements described below. The cells shall be shown to be infected only with the agent stated.

CELL LINES

Cell lines are normally handled according to a cell-seed system. Each master cell seed is assigned a specific code for identification purposes. The master cell seed is stored in aliquots at -70 °C or lower. Production of vaccine is not normally undertaken on cells more than 20 passages from the master cell seed. Where suspension cultures are used, an increase in cell numbers equivalent to approximately 3 population doublings is considered equivalent to 1 passage. If cells beyond 20 passage levels are to be used for production, it shall be demonstrated, by validation or further testing, that the production cell cultures are essentially similar to the master cell seed with regard to their biological characteristics and purity, and that the use of such cells has no deleterious effect on vaccine production.

The history of the cell line shall be known and recorded in detail (e.g. origin, number of passages and media used for multiplication, storage conditions).

The method of storing and using the cells, including details of how it is ensured that the maximum number of passages permitted is not exceeded during product manufacture, are recorded. A sufficient quantity of the master cell seed and each working cell seed are kept for analytical purposes.

The main characteristics of the cells are assessed (as prescribed in Table 5.2.4.-1) using a culture of the master cell seed and the working cell seed or using cell cultures from the working cell seed at the highest passage level used for production and derived from a homogeneous sample demonstrated to be representative.

Table 5.2.4.-1. – *Cell culture stage at which characteristics are assessed*

	Master cell seed	Working cell seed	Cell from working cell seed at highest passage level
General microscopy	+	+	+
Karyotype	+	-	+
Identification of species	+	-	+
Bacteria and fungi	+	+	-
Mycoplasmas	+	+	-
Extraneous viruses (for those tested)	+	+	-
Endogenous retroviruses (mammals)	+	-	+
Tumorigenicity	+	-	-

General microscopy

The appearance of cell monolayers, before and after histological staining, is described. Information, if possible in the form of numerical data, is provided especially on the speed and rate of growth. Similarly, the presence or absence of contact inhibition, polynucleated cells and any other cellular abnormalities are specified.

Karyotype

A chromosomal examination is carried out on not fewer than 50 cells undergoing mitosis in the master cell seed and at a passage level at least as high as that to be used in production. Any chromosomal marker present in the master cell seed must also be found in the high passage cells and the modal number of chromosomes in these cells must not be more than 15 per cent higher than of cells of the master cell seed. The karyotypes must be identical. If the modal number exceeds the level stated, if the chromosomal markers are not found in the working cell seed at the highest level used for production, or if the karyotype differs, the cell line shall not be used for manufacture.

Identification of species

It shall be shown, by a validated method, that the master cell seed and the cells from the working cell seed at the highest passage level used for production come from the species of origin specified. When a fluorescence test is carried out and the serum corresponding to the species of origin of the cells is used and shows that all tested cells are fluorescent, it is not necessary to carry out other tests with reagents able to detect contamination by cells of other species.

Bacteria and fungi

The cells comply with the test for sterility (*2.6.1*). The sample of cells to be examined consists of not less than the number of cells in a monolayer with an area of 70 cm^2 or, for cells grown in suspension, an approximately equivalent number of cells. The cells are maintained in culture for at least 15 days without antibiotics before carrying out the test.

Mycoplasmas (*2.6.7*)

The cells comply with the test for mycoplasmas. The cells are maintained in culture for at least 15 days without antibiotics before carrying out the test.

Extraneous viruses

The cells must not be contaminated by viruses. General requirements for managing the presence of extraneous viruses in cells are given in general chapter *5.2.5*. *Management of extraneous agents in immunological veterinary medicinal products*. In light of the results of the risk assessment, testing may be reduced or omitted.

Retroviruses

A validated *in vitro* test is carried out to detect the presence of retroviruses in mammalian cell lines. If the presence of a retrovirus is known or established by testing, such as an end-point product-enhanced reverse transcriptase (PERT) assay (*2.6.21*), then infectivity assays should be carried out. A PERT assay may be suitable to detect infective retroviruses after passage on permissive cells.

Since the sensitivity of PERT assays is very high, interpretation of a positive signal may be equivocal.

Cell seeds that show the presence of infectious retroviruses are not acceptable for the production of vaccines. However, in exceptional cases, it may be justified and authorised to use cells for which a positive or equivocal result has been obtained in the infectivity assay. Justification for such use is based on a risk assessment, which includes all available data and any downstream processing steps up to the final product stage. The results of the risk assessment must demonstrate that the risk associated with the presence of infectious retroviruses is negligible in the final product.

Tumorigenicity

The risk of a cell line for the target species must be evaluated and, if necessary, tests are carried out.

PRIMARY CELLS

For most mammalian vaccines, the use of primary cells is not acceptable for the manufacture of vaccines because cell lines can be used. If there is no alternative to the use of primary cells, the cells are obtained from a herd or flock free from

specified pathogens, with complete protection from introduction of diseases (e.g. disease barriers, filters on air inlets, suitable quarantine before introduction of animals). Chicken flocks comply with the requirements prescribed in general chapter 5.2.2. *Chicken flocks free from specified pathogens for the production and quality control of vaccines*. For all other species, the herd or flock is shown to be free from relevant specified pathogens. All the breeding stock in the herd or flock intended to be used to produce primary cells for vaccine manufacture is subject to a suitable monitoring procedure including regular serological checks carried out at least twice a year and 2 supplementary serological examinations performed on 15 per cent of the breeding stock in the herd between the 2 checks mentioned above.

Wherever possible, particularly for mammalian cells, a seed-lot system is used with, for example, a master cell seed formed after less than 5 passages, the working cell seed being no more than 5 passages from the initial preparation of the cell suspension from the animal tissues.

Each master cell seed, working cell seed and cells of the highest passage of primary cells are checked in accordance with Table 5.2.4.-2 and the procedure described below. The sample tested shall cover all sources of the cells used for the manufacture of the batch. No batches of vaccine manufactured using the cells may be released if any one of the checks performed produces unsatisfactory results.

Table 5.2.4.-2. – *Cell culture stage at which characteristics are assessed*

	Master cell seed	Working cell seed	Highest passage level
General microscopy	+	+	+
Identification of species	+	-	-
Bacteria and fungi	+	+	-
Mycoplasmas	+	+	-
Extraneous viruses (for those tested)	+	+	-
Endogenous retroviruses (mammals)	+	+	-

General microscopy

The appearance of cell monolayers, before and after histological staining, is described. Information, if possible in the form of numerical data, is recorded, especially on the speed and rate of growth. Similarly, the presence or absence of contact inhibition, polynucleated cells and any other cellular abnormalities are specified.

Identification of species

It shall be demonstrated by a validated test that the master cell seed comes from the specified species of origin.

When a fluorescence test is carried out and the serum corresponding to the species of origin of the cells is used and shows that all tested cells are fluorescent, it is not necessary to carry out other tests with reagents able to detect contamination by cells of other species.

Bacteria and fungi

The cells comply with the test for sterility (2.6.1). The sample of cells to be examined consists of not less than the number of cells in a monolayer with an area of 70 cm^2 or, for cells grown in suspension, an approximately equivalent number of cells. The cells are maintained in culture for at least 15 days without antibiotics before carrying out the test.

Mycoplasmas (2.6.7)

The cells comply with the test for mycoplasmas. The cells are maintained in culture for at least 15 days without antibiotics before carrying out the test.

Extraneous viruses

The cells must not be contaminated by viruses. General requirements for managing the presence of extraneous viruses in cells are given in general chapter 5.2.5. In light of the results of the risk assessment, testing may be reduced or omitted.

Retroviruses

A validated *in vitro* test is carried out to detect the presence of retroviruses in mammalian primary cells. If the presence of a retrovirus is known or established by testing, such as an end-point product-enhanced reverse transcriptase (PERT) assay (*2.6.21*), then infectivity assays should be carried out. A PERT assay may be suitable to detect infective retroviruses after passage on permissive cells.

Since the sensitivity of PERT assays is very high, interpretation of a positive signal may be equivocal.

Primary cells that show the presence of infectious retroviruses are not acceptable for the production of vaccines. However, in exceptional cases, it may be justified and authorised to use cells for which a positive or equivocal result has been obtained in the infectivity assay. Justification for such use is based on a risk assessment which includes all available data and any downstream processing steps up to the final product stage. The results of the risk assessment must demonstrate that the risk associated with the presence of infectious retroviruses is negligible in the final product.