As the cells used in the production of biologicals may contain activated oncogenes, assays of cell transformation with DNA derived from a continuous cell line at the limit for *in vitro* culture age for production should be considered in order to determine whether or not activated oncogenes can be detected. The 3T3 assay system has been found useful for *ras* assays. Additional tests may also be considered as new techniques are developed for the detection of a broader range of oncogenes.

B.2.3.8 *Tests on cells carrying a recombinant-DNA expression system*

Data shall be obtained demonstrating that a continuous cell line can be used for its intended purpose. If a continuous cell line contains an expression construct to produce a recombinant DNA-derived protein, data shall be obtained to demonstrate the consistent quality and quantity of the protein it produces throughout the proposed *in vitro* culture age range for production (*14*, *15*). Studies shall be performed to determine whether manipulation of the cell line in order to produce a product by transfection changes its biological characteristics significantly, for instance conversion to the tumorigenic phenotype. Any such change must be taken into account in product development and in assessing approaches taken to assure an acceptable product.

The International Conference on Harmonisation has issued additional useful information (*43*).

### B.2.4 *Production cell cultures*

Characterization of the product and routine monitoring for adventitious agents during the production process are part of the quality control of biological products.

The choice of method for quality control of the production cell substrate depends on the nature of the propagation system used. Cell substrates are propagated as monolayer cultures, in suspension cultures or in bioreactors, and can be maintained on a short-term, a long-term or even on a potentially indefinite basis. The product is obtained either from a single harvest of cell culture fluid or from multiple harvests. In some cases, quality control testing may need to be performed on each harvest before pooling into a bulk lot. The management of cell substrates for the purposes of quality control testing should be designed to optimize sensitivity of the testing.

B.2.4.1 *Serum used in cell-culture media*

Serum used in cell-culture media shall be tested as specified in section A.3.2.

B.2.4.2 *Trypsin used for preparing cell cultures*

Trypsin used for preparing cell cultures shall be tested as specified in section A.3.3.

B.2.4.3 *Identity test*

For viral vaccines, an identity test shall be performed on the control cell culture as described in section B.2.3.1. For recombinant DNA proteins and monoclonal antibodies, the presence of the protein at consistent levels in the harvest is an adequate confirmation of identity and purity.

B.2.4.4 *Tests for bacteria, fungi and mycoplasmas at the end of production*

Tests for bacteria, fungi and mycoplasmas shall be conducted on the production culture supernatant or lysate as specified in section A.3.4.

B.2.4.5 *Tests for adventitious viruses at the end of production*

Tests for adventitious viruses shall be conducted on the production culture supernatant or lysate as specified in section A.3.5.

# Part C. Requirements for diploid cell substrates

## C.1 General considerations

Two human diploid cell lines, WI-38 and MRC-5, derived from embryo lung tissue, have been in widespread use for many years for the production of live virus vaccines, including oral poliomyelitis, measles, mumps, rubella and varicella vaccines, and inactivated vaccines, for example, rabies and hepatitis A vaccines. In addition, a rhesus diploid cell line, FRhL-2, has been in limited use for rabies vaccine production. These substrates have been found to be safe and to produce vaccines that stimulate effective immunity without untoward reactions attributable to the cell substrate.

The following requirements concern the characterization and testing of diploid cell lines used for the production of biologicals. They should be read in conjunction with the general manufacturing requirements applicable to all cell cultures contained in Part A of these Requirements.

## C.2 Manufacturing requirements

### C.2.1 *Certification of diploid cell lines for use in the production of biologicals*

A diploid cell line used for biologicals production shall be approved by the national control authority and shall be identified by historical

records that include information on the origin of the cell line, its method of development and the range of passage levels at which it can be used in biologicals production.

A new diploid cell line (e.g. other than WI-38, MRC-5 and FRhL-2) used for biologicals production shall be characterized with respect to genealogy, genetic markers (e.g. HLA, DNA fingerprinting), or other markers of identity acceptable to the national control authority, as well as for viability during storage. In addition, data must be obtained to establish the cell line's diploid character and growth characteristics at *in vitro* culture ages equivalent to, or beyond, those of the master and working cell banks, and of the cell cultures used for production.

Accumulated experience suggests that WI-38 and MRC-5 can be used for production until 10 generations before senescence.

### C.2.2 ***Cell banks***

#### C.2.2.1 *Master cell bank and working cell bank*

Tests shall be performed on the master and working cell banks as described in section C.2.3, where appropriate and approved by the national control authority. In addition, for a new diploid cell line (e.g. other than WI-38, MRC-5, and FRhL-2) the cells of the working cell bank shall be shown to be diploid and stable with respect to karyology by the tests outlined in section C.2.3.5.

### C.2.3 ***Identification and characteristics of diploid cell lines***

The characterization of a diploid cell intended for use in the manufacture of biologicals shall include information on: the history and general characteristics of the cell line; the cell bank system; and quality control testing. These data shall be made available to the national control authority.

#### C.2.3.1 *Identity tests*

An identity test shall be performed on the master cell bank by a method approved by the national control authority.

Methods for identity testing include, but are not limited to, biochemical tests (e.g. isoenzyme analyses), immunological tests (e.g. HLA assays), cytogenetic tests (e.g. for chromosomal markers), and tests for genetic markers (DNA fingerprinting).

Tests to ensure that the master cell bank is not contaminated with a continuous cell line shall be performed.

Tests of identity such as DNA fingerprinting of appropriate sensitivity, karyology at different levels of passage or studies of lifespan in culture may be used for this purpose if approved by the national control authority.

C.2.3.2 *Sterility tests*

Tests for bacteria, fungi and mycoplasmas shall be conducted in cell cultures as specified in section B.2.3.2.

C.2.3.3 *Tests for viral agents using cell cultures*

Tests for viral agents shall be conducted in cell cultures as specified in section B.2.3.3.

C.2.3.4 *Tests for viral agents using animals and eggs*

Tests for viral agents shall be conducted in animals and eggs as specified in section B.2.3.4.

C.2.3.5 *Chromosomal characterization of a diploid cell line*

The usefulness of chromosomal characterization depends on the nature of the product and the manufacturing process. In general, products that might contain live cells or which have little "downstream" purification will require chromosomal characterization of the cell line. Such products manufactured in cells identified to be WI-38, MRC-5 or FRhL-2 cells do not require recharacterization of the cell substrate by karyology, unless the cells have been genetically modified.

The utility of chromosomal monitoring of the cell substrate for unpurified products manufactured in other cell lines shall be evaluated on a case-by-case basis. However, products that contain no cells and are highly purified will not require this test.

For the determination of the general character of a new diploid cell line (i.e. other than WI-38, MRC-5 and FRhL-2), samples from the master cell bank shall be examined at approximately four equally spaced intervals over the life span of the cell line during serial cultivation through to senescence. Each sample shall consist of a minimum of 200 cells in metaphase and shall be examined for exact counts of chromosomes and for frequency of hyperdiploidy, hypoploidy, polyploidy, breaks and structural abnormalities. The acceptablity of any new diploid cell line shall be determined by the national control authority.

It is recommended that photographic reconstruction should be employed to prepare chromosome-banded karyotypes of an additional ten metaphase cells.

Stained slide preparations of the chromosomal characterization of the diploid cell line, or photographs of these, shall be maintained permanently as part of the cell line record.

C.2.3.7 *Tests for tumorigenicity*

The tumorigenic potential of a new diploid cell line (i.e. other than WI-38, MRC-5 and FRhL-2) shall be tested as specified in section B.2.3.7 as part of the characterization of the cell line, but is not required on a routine basis.

If satisfactory data from at least two independent laboratories are available, further tumorigenicity testing may not be required. The adequacy of tumorigenicity testing of a new diploid cell line should be discussed with the national control authority. Positive results should be discussed with the authority, taking into consideration the purity of the product, including residual cellular DNA.

C.2.3.8 *Tests on cells carrying a recombinant-DNA expression construct*

Data shall be obtained demonstrating that a diploid cell line can be used for its intended purpose. If a cell line contains an expression construct to produce a recombinant-DNA-derived protein, data shall be obtained to demonstrate the consistent quality and quantity of the protein produced throughout the proposed *in vitro* culture age range for production (*33*, *34*).

The International Conference on Harmonisation has issued additional useful information (*43*).

### C.2.4 *Production cell cultures*

C.2.4.1 *Serum used in cell-culture media*

Serum used in cell-culture media shall be tested as specified in section A.3.2.

C.2.4.2 *Trypsin used for preparing cell cultures*

Trypsin used for preparing cell cultures shall be tested as specified in section A.3.3.

C.2.4.3 *Identity test*

An identity test shall be performed on the control cell culture as specified in section B.2.3.1.

C.2.4.4 *Tests for bacteria, fungi and mycoplasmas at the end of production*

Tests for bacteria, fungi and mycoplasmas shall be conducted on the production culture supernatant or lysate as specified in section A.3.4.

C.2.4.5 *Tests for adventitious viruses at the end of production*

Tests shall be conducted on the product at the end of production but before further processing as specified in section A.3.5. If the presence of the product interferes, tests shall be performed on the control cell culture as specified in section A.3.5.

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Appendix

# Validation of viral elimination from monoclonal antibodies and biologicals prepared using recombinant DNA technology (except viral vaccines)

Traditionally, cell lines used as cell substrates for the production of biologicals have been tested to ensure the absence of contamination with adventitious viruses. As continuous cell lines have been introduced, it has become necessary to approve for production cell lines that produce virus-like particles and even infectious viruses. These efforts have resulted in an enhanced understanding of the significance of virus-like particles in cell lines and have demonstrated that certain findings, such as the presence of intracisternal A particles, are only of remote theoretical concern. As experience has been gained with monoclonal antibodies produced in cell lines that produce murine retroviruses, evidence has accumulated that such products can be safe, and methods have been developed to minimize both the potential for contamination of the products with retroviruses and the theoretical risk associated with such contamination. In particular, manufacturers have used manufacturing procedures that include steps that inactivate and/or remove viruses from the product, and have performed studies to validate the effectiveness of these procedures. When the manufacturing process is known to eliminate significantly more virus than is present in the unprocessed bulk, and when the purified product is tested for the presence of virus, there is reasonable assurance of freedom from contamination.

Validation studies assist in the quantification of risk, but do not of themselves prove absence of risk. They are relevant for the evaluation of production using cell lines potentially carrying any type of virus (e.g. Epstein-Barr virus, papillomavirus), but risk assessment also includes consideration of the type of virus and the potential use of the product. Validation studies are not a means of demonstrating that introduction of an adventitious virus during manufacture is acceptable. Validation is accomplished by evaluating the ability of downstream processing steps to remove and/or inactivate virus from the bulk harvest: virus is added to test the efficacy of selected steps in a scaled-down model of the manufacturing process.

## Design

The design of procedures to validate the elimination of virus during processing should take into account the following variables.

***Selection of appropriate virus or viruses***

The virus or viruses to be used may be the virus which is known or suspected to contaminate the cell line, or it may be a model virus (or viruses) selected because of its similarity to the virus of concern and because of practical considerations, such as availability of material of high titre and the ease of assay. The viral contaminant may be added in a labelled (i.e. radioactive) or non-labelled form. It may be necessary to use more than one virus when, for example, the use of a single virus does not provide an adequate basis for the evaluation of the purification process.

***Scaled-down manufacturing system***

If a scaled-down model of the purification process is used for validation, it should accurately reflect the actual manufacturing process. Bed height, flow rate, flow-rate to bed-height ratio, types of buffer, pH, and the concentration of protein, buffer and product should all be evaluated, and equivalence to the full-scale manufacturing system demonstrated.

***Analysis of step-wise elimination of virus***

In many cases it is desirable to evaluate the individual contribution to virus elimination of different manufacturing steps. Sufficient virus should be present before each critical step so that an adequate evaluation of the effectiveness of each step is obtained. In some cases, the addition of high-titre virus to the unpurified bulk and the testing of its concentration between steps will be sufficient. In other cases, the addition of virus to material during the manufacturing process will also be necessary. The virus titre should be determined before and after each tested step.

***Determining physical removal or inactivation***

The type of contribution (removal or inactivation) of each step should be identified by determining, when feasible, what portion of the reduction in titre is due to virus inactivation and what portion is due to physical removal of the virus from the product.

***Kinetics of inactivation***

In some cases, the kinetics of virus inactivation at the critical inactivation step should be determined. This is particularly important where the virus is known to be a human pathogen and it is necessary to design a completely effective inactivation process.

***Estimation of combined effects***

The combined effect of each tested step on the reduction of virus titre should be calculated in order to establish the total virus inactivation/removal capacity of the purification procedure. Where a process involves several steps that achieve a reduction in titre by the same mechanism, unless otherwise justified the results of only one such step should be considered in calculating the overall titre reduction.

***Regeneration of columns***

When chromatographic procedures are used for virus elimination, it is critical that validation studies should employ columns that are representative of those actually used in manufacturing. Routine procedures for the regeneration of columns should be such that the design of the validation study is relevant to the manufacturing process.

***Specific precautions***

- Validation usually takes place outside the manufacturing facility in order to prevent possible viral contamination of the facility.
- Care should be taken in preparing virus preparations to avoid the aggregation of viral particles. This may facilitate physical removal and hinder inactivation, thereby reducing comparability with the actual manufacturing process.
- The virus preparation to be added to the product should constitute a small volume so as not to dilute or change the characteristics of the product.
- Care should be taken to avoid even small differences in, for example, buffers, media or reagents as these can substantially affect virus clearance and comparability with the manufacturing process.
- As virus removal/inactivation is time dependent, the amount of time the product remains in a buffer solution or on a chromatography column should reflect the conditions of the full-scale manufacturing process.
- Buffers and product should be evaluated independently for interference with the assays used to determine the virus titre, since these components may adversely affect the indicator cells. If the buffer solutions are toxic for the indicator cells, dilution, adjustment of pH or dialysis of the virus-containing buffer may be necessary. If the biological product itself has an antiviral activity, it may be possible to perform the validation study without the product, although omission of product or substitution of a similar protein without antiviral activity could affect behaviour of the virus in some manufacturing steps.
- Many purification schemes repeatedly use the same or similar buffers or chromatography columns. The effects of this approach

should be taken into account when analysing the data. The effectiveness of virus removal/inactivation by a particular process may vary according to the stage in manufacture at which it is used.

### Interpretation

The purpose of a validation study is to show that a process, when conducted according to standard operating procedures, will reliably produce a certain result. For viral contaminants, it is important to show that not only is the virus removed and/or inactivated, but also that there is excess capacity for this built into the purification process that will assure an appropriate level of safety for the final product. It is recommended that a purification process should include at least one viral inactivation step when infectious virus is known to be routinely present in the unpurified bulk product.

The following potential limitations of validation studies for virus removal or inactivation should be addressed when interpreting results.

- A model virus may not behave identically to relevant potential viral contaminants.
- The full-scale manufacturing process may differ from the scaled-down process used for validation purposes.
- Unrecognized differences in the materials or procedures used for validation as compared with those used for manufacturing may overestimate virus removal or inactivation.
- The effects of repeated steps, particularly of those with little individual effect, may not be additive, and summation of the effects of such steps may result in overestimation.
- The efficacy of chromatography columns and other devices used in the purification scheme may change on repeated use.

### Statistics

Validation studies should analyse data statistically. Validation studies should be duplicated, and the statistical variation within and between them evaluated.

The design of the validation study should be statistically valid, i.e. it should be capable of supporting the conclusions reached.