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Medical devices utilizing animal tissues and their derivatives —

Part 3: Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents

Dispositifs médicaux utilisant des tissus animaux et leurs dérivés —

Partie 3: Validation de l'élimination et/ou de l'inactivation des virus et autres agents responsables d'encéphalopathie spongiforme transmissible (EST)

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 22442-3 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*, Subcommittee SC 1, *Tissue product safety*.

ISO 22442 consists of the following parts, under the general title *Medical devices utilizing animal tissues and their derivatives*:

— *Part 1: Application of risk management*

— *Part 2: Controls on sourcing, collection and handling*

— *Part 3: Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents*

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Introduction

Certain medical devices utilize materials of animal origin.

Animal tissues and their derivatives are used in the design and manufacture of medical devices to provide performance characteristics that were chosen for advantages over non-animal based materials. The range and quantities of materials of animal origin in medical devices vary. These materials can comprise a major part of the device (e.g. bovine/porcine heart valves, bone substitutes for use in dental or orthopaedic applications, haemostatic devices), can be a product coating or impregnation (e.g. collagen, gelatine, heparin), or can be used in the device manufacturing process (e.g. tallow derivatives such as oleates and stearates, foetal calf serum, enzymes, culture media).

It is important to be aware that the exposure to a properly validated and accurately controlled method of viral and TSE inactivation/elimination is not the only factor associated with demonstrating product safety. Attention has also to be given to a number of factors including sourcing, collecting, handling, storage, processing, testing of tissues and/or cells of animal origin, and to the control of the environment in which the product is manufactured, assembled and packaged. The manufacturer should consider the fact that each manufacturing phase can contribute to contamination as well as elimination and/or inactivation of viruses and TSE agents.

For the safety of medical devices there are two complementary approaches (see ISO 22442-1) that can be adopted to control the potential contamination of tissues. These typically are:

a) selecting source material for minimal contamination with viruses and/or TSE agents (see ISO 22442-1 and ISO 22442-2);

b) providing valid scientific evidence to demonstrate the ability of the production processes to eliminate or inactivate viruses and/or TSE agents (this part of ISO 22442).

Requirements for a quality system for medical devices for regulatory use are specified in ISO 13485. The standards for quality management systems recognize that, for certain processes used in manufacturing, the effectiveness of that process cannot be fully verified by subsequent inspection and testing of the product. The elimination and/or inactivation of viruses and TSE agents is an example of a special process because process efficacy cannot be verified by inspection and testing of the product. For this reason, the following need to be considered in particular:

— definition of the process(es) and materials to be used;

— adequate inactivation validation before routine use;

— performance monitoring of the process during manufacture;

— appropriate equipment maintenance;

— staff training, etc.

Historically there have been many instances of unknown or unsuspected viral contamination during manufacture. For this reason, evaluation of the manufacturing process can provide a measure of confidence that a wide number of viruses, including unknown pathogenic viruses are eliminated. Similar principles may apply to TSE agents.

NOTE To show compliance with this part of ISO 22442, its specified requirements should be fulfilled. The guidance given in the Notes and informative annexes is not normative and is not provided as a checklist for auditors.

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Medical devices utilizing animal tissues and their derivatives —

Part 3:
Validation of the elimination and/or inactivation of viruses and transmissible spongiform encephalopathy (TSE) agents

1 Scope

This part of ISO 22442 specifies requirements for the validation of the elimination and/or inactivation of viruses and TSE agents during the manufacture of medical devices (excluding *in vitro* diagnostic medical devices) utilizing animal tissue or products derived from animal tissue, which are non-viable or have been rendered non-viable. It applies where required by the risk management process as described in ISO 22442-1. It does not cover other transmissible and non-transmissible agents.

NOTE 1 Analysis and management of risk is described in ISO 22442-1. Conventional processes used for sterilization, when used for the treatment of animal tissues for medical devices, have not been shown to be completely effective in inactivating the causative agents of transmissible spongiform encephalopathy. Selective sourcing is extremely important (see ISO 22442-1 and ISO 22442-2).

NOTE 2 ISO 11135, ISO 11137, ISO 11737-1, ISO 13408, ISO 14160, ISO 14937 and ISO 17665 may be relevant for bacteria, moulds and yeast (see Bibliography).

This part of ISO 22442 does not cover the utilization of human tissues in medical devices.

This part of ISO 22442 does not specify a quality management system for the control of all stages of production of medical devices.

NOTE 3 It is not a requirement of this part of ISO 22442 to have a full quality management system during manufacture, but it does specify requirements for some of the elements of a quality management system. Attention is drawn to the standards for quality management systems (see ISO 13485) that control all stages of production or reprocessing of medical devices. The quality management system elements that are required by this part of ISO 22442 can form part of a quality management system conforming to ISO 13485.

This part of ISO 22442 does not consider the effect of any method of elimination and/or inactivation on the suitability of the medical device for its intended use.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 22442-1:2007, *Medical devices utilizing animal tissues and their derivatives — Part 1: Application of risk management*

ISO 22442-2, *Medical devices utilizing animal tissues and their derivatives — Part 2: Controls on sourcing, collection and handling*

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3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 22442-1 and the following apply.

3.1
model TSE agent
TSE agent that displays a known resistance to physical and/or chemical processing used as reference by analogy for the inactivation of relevant TSE agents, and thereby demonstrating the effectiveness of the process used for inactivation

3.2
model virus
virus that displays a known resistance to physical and/or chemical processing used as reference by analogy for the inactivation of relevant viruses, and thereby demonstrating the effectiveness of the process used for inactivation

NOTE This includes viral models (RNA, DNA, enveloped, non-enveloped) and bacteriophage models.

3.3
overall reduction factor
sum of the reduction factors of the individual process steps

3.4
permissive cell
cell that can become infected with the virus under study and in which that virus replicates

3.5
reduction factor
ratio of the virus or TSE agent load in the relevant material used or the device prior to the inactivation or elimination step and the virus or TSE agent load after the inactivation or elimination step when it is ready for the next step in the manufacturing process, expressed as the number of ten fold reduction ($\log_{10}$)

3.6
relevant TSE agent
TSE agent known to, or likely to, contaminate the source material or other materials used in the manufacturing process

3.7
relevant virus
virus known to, or likely to, contaminate the source material or other materials used in the manufacturing process

3.8
revalidation
set of documented procedures to confirm an established validation

3.9
scaled down process
scaling down
process at a specified reduced scale which simulates the performance parameters as used in the full scale production process

3.10
sterilization
validated process used to render a product free of all forms of viable microorganisms

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3.11
validation
documented procedure for obtaining, recording and interpreting the results required to establish that a process will consistently yield product complying with predetermined specifications

[ISO/TS 11139:2006, definition 2.55]

4 General requirements

4.1 Risk management

Analysis and management of risk shall be carried out in accordance with ISO 22442-1.

Due account shall be taken of manufacturing processes that are considered to be effective for certain animal materials as discussed in Annex C of ISO 22442-1:2007.

4.2 Sourcing and manufacturing process

A documented system shall be established and maintained to control the source of raw materials of animal origin. ISO 22442-2 shall be used to meet this requirement as far as applicable.

The manufacturing process shall be established to minimize the load of viruses and TSE agents in starting materials, intermediate products and finished products.

Appropriate documented protocols and procedures shall be established to ensure that the validated processing parameters will be applied during the routine manufacturing processes.

NOTE Employing a quality management system complying with ISO 13485 could be used to meet the requirements of this subclause.

4.3 General requirements related to validation

4.3.1 Documented procedures

The documented procedures and requirements of this part of ISO 22442 shall be implemented. Documentation and records shall be reviewed and approved by designated personnel (see 4.3.2).

Procedures for any literature review and/or any inactivation study shall be documented and records shall be retained for a period defined by the manufacturer.

4.3.2 Personnel

Responsibility for the implementation of this part of ISO 22442 shall be assigned to qualified personnel.

The requirements for the qualification, training or experience of personnel shall be documented and appropriate to the individual's work, responsibility and authority.

NOTE The level of qualification, training and experience required by personnel at various levels depends upon the activities being performed.

4.3.3 Calibration

An effective system shall be established, documented and maintained for the calibration of all controlling, indicating and recording instruments used for validation.

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4.3.4 Equipment

Appropriate equipment as specified in a protocol shall be used. All equipment requiring planned maintenance shall be maintained in accordance with documented procedures. Records of maintenance shall be retained.

In particular, any equipment shall be capable of delivering its intended process within defined limits. In addition, if the equipment used during validation is not identical to that used in normal production cycles, adequate documentation shall be available to demonstrate that the performance parameters are equivalent to those used in the production cycle.

4.3.5 Experimental systems

Additional parts of the experimental systems used for validation studies such as chemicals, cell systems and laboratory animals shall be adequately identified, justified, controlled and documented.

5 Literature review

5.1 Conduct of the literature review

A literature review shall be performed as specified in Annex A, in order to identify and analyse data on the elimination and/or inactivation of viruses and TSE agents.

5.2 Application of literature review output

Technical information from the literature review shall be used in optimizing the design of an inactivation and/or elimination study.

Any extrapolation based on the inactivation of viruses and TSE agents shall be justified and documented.

Intrinsic variability of materials of animal origin utilized in medical devices and of manufacturing processes can lead to misinterpretation of the validity of published data and shall be taken into account.

5.3 Viruses

The manufacturer shall demonstrate whether the literature review provides an indication of which inactivation and/or elimination steps are likely to be effective. A literature review is a prerequisite to performing a viral inactivation study. In exceptional cases, if a manufacturer chooses not to perform a study, this shall be justified and documented.

If the available information does not support the elimination and/or inactivation of viruses, then an alternative risk management strategy shall be implemented (see ISO 22442-1).

5.4 TSE agents

The literature review shall consider which of the published methods for elimination and/or inactivation of TSE agents are likely to be suitable for the medical device under consideration. In particular, the materials of animal origin and manufacturing processes referred to in the literature shall be comparable to those used for the medical device under consideration (see Annex A). A validated inactivation study shall be performed when the comparability of materials and processes cannot be demonstrated or specific claims are made for inactivation of TSE agents by the manufacturer (see Clause 6).

If the available information does not support the elimination and/or inactivation of TSE agents, then an alternative risk management strategy shall be implemented (see ISO 22442-1).

Special considerations for the manufacture of some animal materials are provided in ISO 22442-1:2007, Annex C.

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6 Elimination and/or inactivation study of viruses and TSE agents

6.1 General

If the need for an elimination and/or inactivation study is identified (see 5.3 and 5.4) this shall be performed so that it substantiates the effectiveness for the selected steps of manufacture against selected agents (see Annexes B and C).

If the manufacturer uses sterilization processes that have been validated for bacteria, moulds and yeast, these processes shall also be supported by relevant validation data for the elimination and/or inactivation of viruses and TSE agents.

6.2 Protocol

The protocol for a study to demonstrate the elimination and/or inactivation of viruses and TSE agents during manufacture shall detail the following including, if applicable, values and acceptability criteria:

a) the identified risks associated with the tissue concerned (see ISO 22442-1);

b) identification of the relevant agent(s);

c) the rationale for the choice of the particular combinations of model agents: the models for an elimination and/or inactivation study shall be chosen by the manufacturer; the justification for the choice of model(s) shall be documented;

NOTE 1 Such models include viral models (RNA, DNA, enveloped, non-enveloped, see also Table B.1), and TSE agent models.

NOTE 2 As part of the studies, it is possible to use bioassay of TSE agents (mouse or hamster models) for the validation of the inactivation of agents by the manufacturing process(es) of the medical device or components. Such studies have been considered to be predictive of inactivation efficacy for TSE agents which may cause disease, e.g. bovine spongiform encephalopathy, scrapie and Creutzfeldt-Jacob disease.

d) identification and definition of the manufacturing stage(s) chosen to eliminate and/or inactivate the relevant viruses and TSE agents;

e) documentation of any scaling down, including demonstration of the validity of the scaled down version of the manufacturing process;

NOTE 3 Guidance on scaling down is given in Annex D.

NOTE 4 Consideration should be given to the potential for one processing stage to have an adverse effect on the inactivation/elimination efficacy of subsequent processing stages. Reliance on literature information on individual processing stage efficacy might be inappropriate if the available information does not relate to the same sequence of processes intended for use by the manufacturer.

NOTE 5 The overall reduction factor is unlikely to equal the sum of the reduction factors from individual processing stages which use similar physical, chemical, enzymatic or thermal mechanisms or reagents to reduce the viral or TSE agent load. There may be a loss of efficacy in subsequent application of the same processing stage.

f) the methods of calculation for the reduction factors;

g) the method of the estimation of reduction kinetics, if applicable (see Annexes E and F).

NOTE 6 Careful consideration should be given to the statistical and physical limitations in sampling, and limits of sensitivity of detection methods (see also B.3.5 and Annexes C, E and F).

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6.3 Conduct of the study

The study shall be conducted in accordance with the protocol.

6.4 Interpretation of data

The reduction factor shall be determined (see B.3.5 and Annexes C, E and F). The efficacy of the identified manufacturing steps for the elimination and/or inactivation of viruses and TSE agents shall be reviewed. Scaling down and other variables which may influence the results shall be addressed.

NOTE Reduction factors are typically calculated for each step within a controlled study.

7 Final report

A final report shall be compiled containing:

— the literature review (see Clause 5 and Annex A);

— and/or a critical evaluation of the data obtained during any elimination;

— and/or inactivation study undertaken (see Clause 6);

— an overall conclusion;

— reference to this part of ISO 22442.

The report shall identify manufacturing parameters that are critical to the effectiveness of the inactivation or elimination process. Acceptable limits shall be determined and specified for such parameters.

An overview shall be given showing all relevant processing steps with a statement of agent reduction factors (see B.3.5 and Annex C).

NOTE This can take the form of a flow chart.

The report shall be signed by persons designated as responsible for its preparation, review and approval. The report shall be retained and included in the risk management file [for revalidation(s), see Clause 8].

8 Review of final report

Procedures for a review of the final report by persons designated as responsible shall be documented.

A review of the final report shall be conducted when significant changes in the manufacturing process(es) occur and/or when relevant information not previously considered in the final report, becomes available, e.g. valid scientific evidence, scientific literature and authoritative publications. If necessary, corrective actions and/or additional studies shall be undertaken and reported to revalidate the manufacturing process.

Records of any review of the final report shall be retained.

9 Routine monitoring and control of critical process parameters

The manufacturer shall assure that all critical parameters identified in the final report are monitored and controlled during manufacture.

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Annex A
(normative)

Requirements related to literature review

A.1 General

NOTE ISO 14155 contains relevant information on literature review. Developments in the revision of that document will be kept under review.

The literature review shall identify investigations into the elimination and/or inactivation capacity of the manufacturing process for viruses and TSE agents. The literature review shall be carried out by person(s) suitably qualified in the relevant field, knowledgeable in the “state of the art” and able to demonstrate objectivity.

A.1.1 Methodology

A.1.1.1 General

A precise definition of the questions to be answered shall be established. This shall include at least:

— identification of the relevant viruses and TSE agents that will be the basis for the search (see Annexes B and C);

— identification of the manufacturing steps which may inactivate and/or eliminate the relevant viruses and TSE agents;

— identification of effects of preceding manufacturing steps on process efficacy;

— identification of the relevant parameters of the manufacturing steps;

— review of the potential efficacy of the process for the elimination and/or inactivation of viruses and TSE agents;

— evaluation of the effect of the animal tissue or derivative on the efficacy of the process.

A protocol for the identification, selection and review of relevant reports shall be written and preferably be based on recognised practice for systematic review for literature.

A.1.1.2 Objective

The objective of the literature review shall be clearly defined. The types of report which are relevant to the objective of the literature review shall be specified.

A.1.1.3 Identification of data

Data shall be taken from scientific publications, e.g. valid scientific evidence, scientific literature and authoritative publications. A systematic search of the literature shall be conducted in order to reduce the risk of introducing bias. Unpublished data shall also be taken into account.

The literature review shall identify:

— the sources of data and the extent of the searches of databases or other sources of information;

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- the rationale for the selection/relevance of the published literature;
- the rationale for concluding that all relevant references, both favourable and unfavourable, have been identified;
- the criteria for exclusion of particular references together with a justification for this exclusion.

NOTE Possible data sources for a systematic literature review are, for example:

- medical and paramedical databases;
- technical papers from relevant Standards Committees;
- foreign language literature;
- “grey literature” (theses, internal reports, non-peer-reviewed journals, the internet, industry files);
- references listed in primary sources;
- other unpublished sources known to experts in the field (obtained by personal communication);
- raw data from published trials (obtained from personal communication).

A.1.1.4 Relevance of data

A literature review shall clearly establish the extent to which the literature relates to the specific characteristics and features relating to the manufacturing process for the device under consideration.

If included published reports do not directly refer to the manufacturing process for the device under consideration, the following shall apply.

The manufacturer shall demonstrate equivalence of the manufacturing process which is the subject of the published reports in relation to that applied to the device under consideration. This includes demonstration of:

- same tissue;
- same geometry;
- same quality of material;
- same process conditions (e.g. time, temperature, concentration, pH, pressure, scale, solvents),

unless differences can be justified.

A.1.1.5 Assessment

The literature review shall make clear the significance that is attached to particular references based on a number of factors. These include:

- the relevance of the author’s background and expertise in relation to the particular device and/or manufacturing process involved;
- whether the author’s conclusions are substantiated by the available data;
- whether the literature reflects the current practice and the generally acknowledged “state of the art” technologies;

NOTE Historical studies that no longer reflect the state of the art sometimes contain relevant information.

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- whether references are taken from recognized scientific publications;
- whether or not they have been reported in peer-reviewed journals;
- the extent to which the published literature is the outcome of a study/studies that have followed scientific principles in relation to design, for example, in having demonstrable and appropriate endpoints, and identifying an appropriate statistical plan of analysis.

If “grey literature” or unpublished data are being included in the assessment, the literature review needs to weigh the significance that is attached to each report. When such information is used, sufficient scientific information (which may be a summary) shall be included from controlled studies that allow for a scientific review.

The evidence shall not consist of:

a) reports lacking sufficient detail to permit scientific evaluation (including lack of accepted and validated statistical design if this is relevant to the design of the intended study);

b) unsubstantiated opinions.

A.1.2 Critical evaluation

The literature review shall contain a critical evaluation of the literature. This critical evaluation shall:

- be written by a person suitably qualified in the relevant field, knowledgeable in the “state of the art” and able to demonstrate objectivity;
- contain a short description of the medical device;
- contain an analysis of all the available data considered, both favourable and unfavourable;
- establish the extent to which the literature relates to the specific characteristics and features of the tissue being assessed, taking due account of the extent of similarity between the tissue(s) covered by the literature and the device under assessment;
- analyse the identified hazards, the associated risks and the appropriate safety;
- contain a description of the methods of weighting of different papers; in order to avoid overweighting, particular attention should be given in circumstances where there are repeated publications by the same authors;
- contain a list of publications appropriately cross-referenced in the evaluation;
- if the data relate to an equivalent device, contain a statement that equivalence with all the relevant characteristics has been demonstrated.

The critical evaluation shall be signed and dated by the author.

A.2 Conclusions

As a result of a literature review, the manufacturer needs to be able to answer the following questions.

- Are the stated conclusions valid?
- Are the data sufficient to demonstrate compliance with the relevant essential requirements?

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A.3 Report

The results of the literature review shall be compiled in a report. This shall include a critical evaluation of the literature, conclusions and all relevant information as required in this annex (see Clause 7).

NOTE Insufficient content or quality in any of the above steps or the availability of relevant new information logically results in re-examination of the information and, if necessary, a return to an appropriate earlier stage, making the necessary adjustments and repeating the process.

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Annex B
(informative)

Guidance on the elimination and/or inactivation study for viruses

B.1 General

Elimination and/or inactivation of viruses is assumed to follow probabilistic concepts and it is therefore not possible to guarantee absolute freedom of contamination in products.

All testing suffers from the inherent limitation of quantitative virus assays, when the ability to detect low virus concentrations depends, for statistical reasons, on the size of the sample (see Annex G).

Establishing the freedom from relevant viruses in or on tissue will not derive solely from direct testing for their presence but also from a demonstration that the manufacturing steps are capable of inactivating or removing them.

B.2 Selection of viruses

B.2.1 The selection of viruses to be used when performing the elimination and/or inactivation study is critical. Whenever possible the relevant virus should be included. If the use of relevant viruses does not demonstrate a wide range of properties where required, then validation should be performed with model viruses.

B.2.2 Model viruses for elimination and/or inactivation studies should be chosen to represent as closely as possible the relevant viruses which may contaminate the product and to represent as wide a range of physico-chemical properties as possible in order to test the ability of the process to inactivate viruses. The choice of the model viruses should also be influenced by the quality and characteristics of the source material and the manufacturing process.

B.2.3 Unless otherwise justified, where two possible viruses could be used for an elimination and/or inactivation study of a particular step either because of their equal resemblance to possible contaminants or similarities in their physico-chemical properties, the more resistant should be chosen.

NOTE Although the source material may not necessarily be a host for specific model viruses, reduction values obtained for such viruses provide useful information on the ability of the manufacturing process to eliminate and/or inactivate viruses in general.

B.2.4 Consideration should be given to the continued recoverability of the model virus during the inactivation study.

B.2.5 Where possible, the choice of the viruses should be such that the viruses can be grown to high titre.

B.2.6 There should be an efficient, sensitive and reliable assay for the detection of the chosen viruses before and after processing through a manufacturing step.

B.2.7 Consideration should be given to health hazards, which certain viruses may pose to the personnel performing the validation studies, and to the use of suitable protective measures.

B.2.8 Examples of model viruses, which have been used or have been recommended for use in inactivation validation studies, are given in Table B.1.

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B.3 Design and implications of elimination and/or inactivation studies

B.3.1 General

Elimination and/or inactivation studies involve the deliberate addition (spiking) of a known titre of virus at various production manufacturing stages and measuring the extent of the inactivation during the subsequent individual manufacturing stage or manufacturing stages. It is not necessary to validate each individual manufacturing stage of a manufacturing process. Only those manufacturing stages that are likely to or could conceivably contribute to elimination and/or inactivation of viruses need to be the subject of an elimination and/or inactivation study. In the case where a process has a number of manufacturing stages each with a small reduction factor, the validation of the overall process may be necessary (see B.3.5).

Careful consideration should be given to the exact definition of an individual manufacturing stage.

The deliberate introduction of any virus into the production facility should be avoided. The validation should be conducted in a separate laboratory equipped for this work, usually on a scaled down version of the production process and performed by suitably qualified and experienced staff. For guidance on scaling down, see Annex D.

B.3.2 Design of the study

B.3.2.1 The study should be designed such that the quantity of virus available at the end of the study is detectable given the limits of sensitivity of the assay (see Annex G).

B.3.2.2 The effectiveness of inactivation/elimination of viruses will depend on the structure, size and shape of the material and on their distribution in it. The study should be designed to take this into account.

B.3.2.3 The amount of virus added to the starting material for the production step that is to be studied should be as high as possible in order to adequately determine the capacity of the production step. However, the volume of the added suspension containing the virus should not exceed 10 % of the total product to be spiked so that the test sample remains similar in composition to the production material. Calculated reduction factors should be based on the virus that can be detected in the spiked starting material and not on the amount of virus added.

B.3.2.4 If possible, virus in samples from model experiments should be titrated without further manipulations such as ultra-centrifugation, dialysis or storage. Where further treatments are unavoidable, e.g. to remove or neutralize inhibitors or toxic substances, or storage for a period to ensure that all samples are titrated together, appropriate controls should be included to determine what effect the procedures have on the result of the study, e.g. dilution effects. Effects of the sample on the detection system, including toxic effects, should be recorded, as they influence the limits of detection. Storage of viruses and viral-spiked samples should be under stated conditions.

B.3.2.5 Whenever possible, kinetics of inactivation of viruses should be obtained in order to measure the slope of the curve and to determine the theoretical time necessary to inactivate the total virus population. Inactivation studies should be planned in such a way that samples are taken at different times allowing an inactivation curve to be constructed. Often virus inactivation has a fast initial phase followed by a slower phase. For the characterization of the inactivation process, the study of the second phase is particularly important [see B.4.1 d)].

B.3.2.6 In case of elimination, e.g. reduction of virus infectivity by partition into precipitates or removal of certain fractionates, the sample that is removed should always be studied as well. A balance of the distribution of virus in the different fractions should be drawn up wherever possible.

B.3.2.7 The method of quantitative infectivity assay of viruses should have adequate sensitivity and reproducibility and should be performed with sufficient replicates and controls to ensure adequate statistical accuracy of the result (see Annex E).

B.3.2.8 The validity of the log reduction achieved should be established from investigation of the effects of variation in critical process parameters used to set in-process limits.

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B.3.3 Culturing model viruses

Model viruses used to validate the inactivation process are preferably produced in cell cultures where these are available. The cell culture system chosen should not alter the properties of the model virus.

B.3.4 Conduct of cell culture tests

B.3.4.1 Model viruses used to validate the inactivation processes are preferably tested in cell cultures. A permissive cell model for testing the inactivation potential of the different manufacturing step(s) should be used.

Prior to assessing viral titres after inactivation, one needs to first assess if there is any toxicity of the inactivating agents or process samples on the cell culture system used to grow the virus. Typically the samples must be neutralized or diluted to a non-toxic dose in order to be assayed in cell culture systems.

B.3.4.2 Intra-cellular viruses are usually more difficult to inactivate than extra-cellular viruses. The permissive cell approach allows testing the effectiveness of the processing parameters in inactivating both intra-cellular and extra-cellular viruses.

B.3.4.3 The testing should be extended to the point where no infective viruses can be recovered from the cell cultures infected with the selected model viruses.

If possible, some measure of the antibody or other factor of interference on the native agent assay should be performed on the samples that are expected to have all agents removed or inactivated. In some instances it is not possible to separate the inactivation effects from the interference effect.

B.3.5 Reduction factors

B.3.5.1 The objective of the elimination and/or inactivation study is to identify steps effective in the inactivation and/or elimination of viruses and to obtain a measure of the overall capacity of the manufacturing process to inactivate/remove them. An overall reduction factor is generally expressed as the sum of the individual factors. However, a simple summing of the individual reduction factors may not be justifiable, especially in the case of viruses for which the resistance characteristics are not fully known. Reductions in virus titre during a process stage of the order of less than 1 log are considered to be negligible and should be ignored. Manufacturers should differentiate effective stages from process stages which may contribute to removal but upon which less reliance can be placed. Consideration should also be given to whether viruses surviving one stage would be resistant to a subsequent stage or alternatively have increased susceptibility. In general, a single stage having a large effect gives a better risk reduction assurance than several stages having the same overall effect.

B.3.5.2 For all viruses, manufacturers should justify the acceptability of the reduction factors obtained. An overall testing of the manufacturing process should be taken into consideration, where appropriate, to substantiate the overall capacity of the process as regards inactivation and/or elimination of viruses (test of the product after the process run following addition to the starting material of a spike with maximum contamination).

B.4 Elimination and/or inactivation study limitations

B.4.1 Elimination and/or inactivation studies are useful in contributing to the application of risk management to ensure that an acceptable level of safety is established for the medical device. However, a number of factors in the design and execution of elimination and/or inactivation studies may lead to an incorrect estimate of the ability of the process to eliminate and/or inactivate viruses. These factors include the following.

a) Different laboratory strains of virus may differ in their sensitivity to the same treatment.

b) When virus preparations used to validate an elimination and/or inactivation step are produced in tissue culture, the behaviour of tissue-culture-derived virus in the elimination and/or inactivation study may be

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different from that of the native virus (for example if native and culture viruses differ in purity or degree of aggregation).

c) The ability of the overall process to reduce infectivity is often expressed as the sum of the logarithm of the reductions of each stage. This is a useful way of calculating the overall reduction factor, though there are some situations in which it may not be valid to add logarithmic reductions, (e.g. where reduction depends on virus adsorption by a matrix).

d) Inactivation of virus infectivity frequently follows a biphasic curve in which a rapid initial phase is followed by a slower phase. It is possible that viruses surviving a particular inactivation stage may be more resistant to subsequent stages. As a consequence, the overall reduction factor is not necessarily the sum of reduction factors calculated from each individual stage, each time using a fresh virus inoculum.

e) For example, if the resistant fraction takes the form of virus aggregates, infectivity may be resistant to a range of different treatments like fixation and sterilization.

f) If the inactivation curve is atypical by comparison to historical data this should be given particular consideration and explained.

g) The expression of reduction factors as logarithmic reductions in titre implies that, while residual virus infectivity may be greatly reduced, it will never be reduced to zero.

B.4.2 Scaled down processing is likely to differ from full scale processing despite care taken to design the scaled down process (see Annex D).

B.4.3 In certain circumstances, antibodies or other molecules interfering/interacting with the model virus may be present in the material. This may affect partition of the model virus or its susceptibility to inactivation; but it may also complicate the design of the study by neutralizing the infectivity. The appropriateness of the study design may be difficult to judge. The level of antibody present may be considered as a significant process variable.

When relevant, some measure of the antibody or other molecular interference/interaction with the model virus assay should be performed on the samples that are expected to have all model virus removed or inactivated. In some instances it is not possible to separate the inactivation effects from the interference/interaction effect.

B.4.4 Small differences in production parameters such as protein content or temperature can produce large differences in the reduction of virus infectivity by whatever mechanism.

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Table B.1 — Examples of viruses which have been used in virus validation studies

Virus	Family	Natural host	Genus	Genome	Envel-oped	Size nm	Shape	Resistance to physico-chemical treatment
Poliovirus, Sabin type 1	Picorna	Man	Enterovirus	RNA	No	25 to 30	Icosahedral	Medium
Encephalomyocarditis virus (EMC)	Picorna	Mouse	Cardiovirus	RNA	No	25 to 30	Icosahedral	Medium
Reovirus 3	Reo	Various	Orthoreovirus	RNA	No	60 to 80	Spherical	Medium
SV 40	Papova	Monkey	Polyomavirus	DNA	No	40 to 50	Icosahedral	Very high
Parvoviruses (canine, porcine)	Parvo	Canine Porcine	Parvovirus	DNA	No	18 to 24	Icosahedral	Very high
Murine leukaemia virus (MulV)	Retro	Mouse	Type C Oncovirus	RNA	Yes	80 to 110	Spherical	Low
Bovine viral diarrhoeal virus (BVDV)	Toga	Bovine	Pestivirus	RNA	Yes	50 to 70	Pleo/ spherical	Low
Human immunodeficiency virus	Retro	Man	Lentivirus	RNA	Yes	80 to 100	Sperical	Low
Vesicular stomatitis virus	Rhabdo	Equine Bovine	Vesiculovirus	RNA	Yes	70 × 175	Bullet shaped	Low
Hepatitis A	Picorna	Man	Hepatovirus	RNA	No	25 to 30	Icosahedral	High
Parainfluenza virus	Paramyxo	Various	Paramyxovirus	RNA	Yes	100 to 200	Pleo/ spherical	Low
Sindbis virus	Toga	Man	Alphavirus	RNA	Yes	60 to 70	Spherical	Low
Pseudorabies virus	Herpes	Swine	Varicellovirinae	DNA	Yes	120 to 200	Spherical	Medium

This table gives an incomplete list of viruses that have been used in elimination and/or inactivation studies either as relevant agents, i.e. agents considered to be potential contaminants of the source material, or as model agents. Consequently, the use of any of the viruses in the table is not obligatory and manufacturers are invited to consider other viruses, especially those that may be more appropriate for their individual production processes.

Consideration should be given to health hazards, which certain viruses may pose to the personnel performing the validation studies, and to the use of suitable protective measures.

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Annex C
(informative)

Guidance on the elimination and/or inactivation study for TSE agents

C.1 General

Validation studies of removal/inactivation procedures for TSE agents are difficult to design and perform, and the results are difficult to interpret. It is necessary to take into consideration the nature of the spiked material and its relevance to the natural situation, the design of the study (including scaling-down of processes) and the method of detection of the agent (*in vitro* or *in vivo* assay). *In vitro* assays can be useful for spiking experiments to investigate manufacturing processes but it is important to correlate such results with those from infectivity assays, as has already been reported in publications in this area. Further research is needed to develop an understanding of the most appropriate “spike preparation” for validation studies. Therefore, validation studies are currently not generally required.

In addition to appropriate sourcing, manufacturers are encouraged to continue their investigations into removal and inactivation methods to identify steps/processes that would have benefit in assuring the removal or inactivation of TSE agents. In any event, a production process wherever possible should be designed taking account of available information on methods that are thought to inactivate or remove TSE agents.

For appropriate validation studies, the manufacturer should set up a specific inactivation and/or elimination study on a scientific basis and the following need to be considered:

— the identified hazard associated with the tissue;

— identification of the relevant model agents;

— the rationale for the choice of the particular combination of model agents;

— identification of the stage chosen to eliminate and/or inactivate the transmissible agents;

— calculation of the reduction factors.

A final report should identify manufacturing parameters and limits that are critical to the effectiveness of the inactivation or elimination process.

Appropriate documented procedures should be applied to ensure that the validated processing parameters are applied during routine manufacture.

Examples of TSE agent models that have been used or have been recommended for use in inactivation validation studies are scrapie strains 263K, 139A, 22C, ME7, 87A; and 301V mouse-passaged BSE.

In the controlled study with model TSEs, the animal studies give some measure of quantification.

C.2 TSE agents surviving inactivation steps

Consideration should also be given to whether TSE agents surviving one stage would be resistant to a subsequent stage or alternatively have increased susceptibility. For example, it has been shown that formaldehyde treatment of TSE agents enhanced their resistance to heat. In general, a single stage having a large effect gives a better risk reduction assurance than several stages having the same overall effect.

Reductions in TSE titre during a process step of less than 1 log are considered to be negligible.

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Annex D
(informative)

Guidance on scaling down

As it is hazardous to introduce infectious agents into production areas, validation of the elimination and/or inactivation study should be conducted in a separate laboratory equipped for virological work and performed by staff with the appropriate expertise. It may be necessary for practical reasons to use a process that is scaled down. The following points are intended as guidance if scaled down processes are used:

a) the scaled down model should simulate the production process as far as practicable and should be designed in conjunction with the production personnel responsible for the full scale process;

b) the validity of each relevant step of the scaled down process should be documented by comparison of process parameters, such as pH, concentration, volume, temperature, equipment, reaction time, composition;

c) the scaled down process should be designed in such a way as to represent worst-case conditions regarding the ability of the manufacturing process to eliminate and/or inactivate viruses and TSE agents;

NOTE Particular attention should be given to the determination of worst-case conditions. Evaluation of the literature or experiments on the operational limits of the process can assist in doing this.

d) deviation(s) from the full manufacturing process which cannot be avoided should be justified with regard to their potential influence on the results;

e) the rationale, the study protocol and the acceptance criteria for the scaled down process should be documented.

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Annex E
(informative)

Statistical evaluation of virus titres and reduction factors and assessment of their validity [1)]

Virus titrations suffer the problems of variation common to all biological assay systems. Assessment of the accuracy of the virus titrations and reduction factors derived from them and the validity of the assays is therefore necessary to define the reliability of a study. The objective of statistical evaluation is to establish that the study has been carried out to an acceptable level of virological competence.

1) Assay methods may be either quantal or quantitative. Quantal methods include infectivity assays in animals or in tissue culture infectious dose (TCID) assays, in which the animal or cell culture is scored as either infected or not. Infectivity titres are then measured by the proportion of animals or cultures infected. In quantitative methods, the infectivity measured varies continuously with the virus input. Quantitative methods include plaque assays where each plaque counted corresponds to a single infectious unit. Both quantal and quantitative assays are amenable to statistical evaluation.

2) Variation can arise within an assay as a result of dilution errors, statistical effects and differences within the assay system which are either unknown or difficult to control. These effects are likely to be greater when different assay runs are compared (between-assay variation) than when results within a single assay run are compared (within-assay variation).

3) The 95 % confidence limits for within-assay variation and for between-assay variation normally should be of the order $\pm$ 0,5 $\log_{10}$ or better. Between-assay variation can be monitored by the inclusion of an in-house reference preparation, the estimate of whose potency should be within approximately 0,5 $\log_{10}$ of the mean estimate established in the laboratory for the assay to be acceptable. Within-assay variation can be assessed by standard textbook methods. In any particular experiment, if the precision of the titration is less than these target figures, the study may still be acceptable if justified.

4) The reduction in the virus load should be calculated from the experimentally determined virus titres. The 95 % confidence limits of the reduction factors should be obtained wherever possible. They can be approximated by $\pm (s^2 + a^2)$, where $\pm s$ is the 95 % confidence limit for the viral assays of the starting material, and $\pm a$ the 95 % confidence limit for the viral assay of the material after the step.

If after an inactivation/removal step no sample shows signs of infectivity, a reduction factor cannot be estimated by statistical means. To obtain an estimate of a minimum reduction factor, the titre should be expressed as less than or equal to one infectious unit in the volume of the highest concentration tested. Especially after potent inactivation processes, it can be expected that no sample shows signs of infectivity. To make the estimate minimum reduction factor of an effective inactivation process as large as possible, as much processed undiluted material as possible should be sampled.

Due account should be taken of these recommendations for TSE agents.

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1) This annex includes text which was originally applied to viruses (CPMP/BWP 268/95, 14 February 1996).

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Annex F
(informative)

Calculation of reduction factors

The reduction factor, R, for an individual inactivation or removal step is given by the expression:

$$R = \log_{10} \frac{V_1 \times T_1}{V_2 \times T_2} \quad \text{(F.1)}$$

where

V_1 is the volume of starting material;

T_1 is the concentration of model virus or model TSE agent in starting material;

V_2 is the volume of material after the step;

T_2 is the concentration of model virus or model TSE agent after the step.

This formula takes into account both the titre and the volume of the material before and after the step.

Reduction factors are normally expressed on a logarithmic scale which implies that, while residual model virus or model TSE agent infectivity may be greatly reduced, it will never be reduced to zero.

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Annex G
(informative)

Probability of detection of agents at low concentrations

G.1 At low agent concentrations (e.g. in the range of 10 infectious particles per litre to 1 000 infectious particles per litre) the probability, P, that a sample of a few millilitres does not contain infectious agents is:

$$P = \left\{ \frac{(V - v)}{V} \right\}^{n} \tag{G.1}$$

where

V is the overall volume of the material to be tested, expressed in litres;

v is the volume of the sample, expressed in litres;

n is the absolute number of infectious particles statistically distributed in the overall volume.

G.2 With $V >> v$ this equation approximates to the Poisson distribution:

$$P = \mathrm{e}^{-cv} \tag{G.2}$$

where

c is the concentration of infectious particles per litre;

v is the volume of the sample, expressed in litres.

G.3 If a sample volume of 1 ml is tested, the probability, P, at agent concentration, c, ranging from 10 infectious particles to 1 000 infectious particles is given in Table G.1

Table G.1 — Probability at agent concentration

P	c
0,99	10
0,90	100
0,37	1 000

NOTE This indicates the probability that for a concentration of 1 000 agents per litre, in 37 % of sampling, 1 ml will not contain an agent particle.

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Bibliography

[1] ISO 11135, *Medical devices — Validation and routine control of ethylene oxide sterilization*

[2] ISO 11137 (all parts), *Sterilization of health care products — Radiation*

[3] ISO/TS 11139:2006, *Sterilization of health care products — Vocabulary*

[4] ISO 11737-1, *Sterilization of medical devices — Microbiological methods — Part 1: Determination of a population of microorganisms on products*

[5] ISO 13408-1, *Aseptic processing of health care products — Part 1: General requirements*

[6] ISO 13408-2, *Aseptic processing of health care products — Part 2: Filtration*

[7] ISO 13408-3, *Aseptic processing of health care products — Part 3: Lyophilization*

[8] ISO 13408-4, *Aseptic processing of health care products — Part 4: Clean-in-place technologies*

[9] ISO 13408-5, *Aseptic processing of health care products — Part 5: Sterilization in place*

[10] ISO 13408-6, *Aseptic processing of health care products — Part 6: Isolator systems*

[11] ISO 13485, *Medical devices — Quality management systems — Requirements for regulatory purposes*

[12] ISO 14155-1, *Clinical investigation of medical devices for human subjects — Part 1: General requirements*

[13] ISO 14155-2, *Clinical investigation of medical devices for human subjects — Part 2: Clinical investigation plants*

[14] ISO 14160, *Sterilization of single-use medical devices incorporating materials of animal origin — Validation and routine control of sterilization by liquid chemical sterilants*

[15] ISO 14937, *Sterilization of health care products — General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices*

[16] ISO 17665-1, *Sterilization of health care products — Moist heat — Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices*

[17] CHALMERS, I. and ALTMAN, D.G. (eds), Systematic reviews, BMJ Publishing, London, 1995

[18] OXMAN, A.D., Checklists for review articles, BMJ Publishing, London, 309, pp 648-651, 1994

[19] RHEINBABEN, F.V., BANSEMIR, K.-P. and HEINZEL, M., *Virucidal effectiveness of some commercial disinfectants for chemothermal disinfection procedures tested against temperature resistant viruses and bacteriophages — Evaluation of a test model*, Zentrallblatt für Hygiene, **192**, pp 419-431, 1992

[20] WOOLWINE, J.D. and GERBERDING, J.L., *Effect of testing method on apparent activities of viral disinfectants and antiseptics*, Antimicrobial Agents and Chemotherapy, **39**, pp 921-923, 1995

[21] Council Directive 93/88/EEC of 12 October 1993 amending Directive 90/697/EEC on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16 (1) of Directive 89/391/EEC)

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[22] CPMP/BWP/268/95 – revised 14 February 1996, Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses (http://www.emea.eu.int/pdfs/human/bwp/026895en.pdf)

[23] EMEA/410/01 rev2 – October 2003, Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and vetinary medicinal products adopted by the Committee for Proprietary Medicinal Products (CPMP) and by the Committee for Veterinary Medicinal Products (CVMP) – Official Journal of the European Union, 28.1.2004 (http://www.emea.eu.int/pdfs/human/bwp/TSE%20NFG%20410-rev2.pdf)

[24] EMEA/BWP/5136/03 – October 2004, Guideline on the investigation of manufacturing processes for plasma-derived medicinal products with regard to VCJD Risk adopted by the Committee for Medicinal Products for Human Use (CHMP)

[25] EMEA/CPMP/BWP/2879/02/rev – CHMP position statement on Creutzfeldt Jacob disease and plasma derived and urine derived medicinal products adopted by the Committee for Medicinal Products for Human Use (CHMP) (http://www.emea.eu.int/pdfs/human/press/pos/287902en.pdf)

[26] European Pharmacopoeia Chapter 5.2.8 *Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and Veterinary Medicinal Products*

[27] Notification No. 177 of the Ministry of Health, Labour and Welfare on the standard for biological ingredients, 31 March 2005 on *Standards for Raw Materials Originating from Living Organisms* (http://www.nihs.go.jp/cgtp/cgtp/guidline/03052001.pdf) (in Japanese)

[28] Qualification of Animals as Origin of Animal-Derived Medicinal Products provided in the General Notices 39 of Japanese Pharmacopoeia and Other Standards

[29] ICH Q5AR1 Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin (http://www.ich.org/lob/media/media425.pdf)

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