VIRUS VALIDATION STUDIES: THE DESIGN, CONTRIBUTION AND INTERPRETATION OF STUDIES VALIDATING THE INACTIVATION AND REMOVAL OF VIRUSES

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Additional Notes	**This guideline discusses the need for, and the contribution of, viral validation studies towards the viral safety of biological products, providing guidance on the design of a validation study including the choice of viruses to be used and on the interpretation of the data.** **This guideline was originally adopted in February 1991 under the title *Validation of Virus removal and Inactivation Procedures*. It was revised to take into consideration the ICH guideline Q5A *Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnological Products derived from Cell Lines of Human or Animal Origin* (CPMP/ICH/295/95)**

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1. INTRODUCTION

1.1 This guideline discusses the need for and the contribution of viral validation studies towards the viral safety of biological products. The principal aims of the guideline are to provide guidance on the design of a validation study including the choice of viruses to be used and on the interpretation of the ensuing data especially with respect to defining a process step which can be considered to be effective in the inactivation and/or removal of viruses.

1.2 The guideline concerns the validation of virus inactivation and/or removal procedures for all categories of medicinal biological products for human use with the exception of live viral vaccines including genetically engineered live vectors. The type of products covered include:

- products derived from in vitro culture of cell lines of human or animal origin,
- products derived from in vivo culture of cell lines, or from organs or tissues of human or animal origin,
- products derived from blood or urine or other biological fluids of human or animal origin.

1.3 The risk of viral contamination is a feature common to all biologicals whose production involves the use of material of animal or human origin. Viral contamination of a biological may arise from the source material, e.g. cell banks of animal origin, human blood, human or animal tissues, or as adventitious agents introduced by the production process, e.g. the use of animal sera in cell culture.

1.4 In the past, a number of biologicals administered to humans have been contaminated with viruses. In several instances, the virus was only identified many years after the product had been introduced into the market since contamination occurred prior to adequate knowledge concerning the presence of the infectious agents. The primary cause of these viral transmissions has been contamination of the starting or source materials. Examples include Yellow Fever vaccine which was contaminated by avian leukosis virus by virtue of its production in naturally infected hens eggs, whilst SV40 was a contaminant of poliovirus and adenovirus vaccines prepared in the 1950's on primary cultures of kidney cells obtained from Rhesus monkeys naturally harbouring a clinically inapparent infection with SV40. In addition, viruses present in human plasma, e.g. HIV and HCV, have contaminated blood products whilst human growth hormone extracted from the pituitaries of cadavers has been implicated in the transmission of the aetiological agent responsible for Creutzfeldt-Jakob disease. Contamination of a biological can also arise from the use of infected material during production or as an excipient. Perhaps the most notable was Yellow Fever vaccine contaminated with HBV present in human serum used as a stabiliser in the 1940's.

1.5 Three principal complementary approaches can be adopted to control potential viral contamination of biologicals:

(i) selecting and testing source material for the absence of detectable viruses,

(ii) testing the capacity of the production processes to remove or inactivate viruses,

(iii) testing the product at appropriate stages of production for freedom from detectable viruses.

No approach provides a sufficient level of assurance alone and this will only be achieved using a combination of the above.

1.6 Testing of starting materials is essential to minimise viral contamination. While tests may be able to detect one or more virus species, no single test will be able to demonstrate the presence of all known viruses. Moreover all test systems require a minimum level of viral contamination to record a positive and tests are also limited by statistical considerations in sampling. Some tests, e.g. the test for antibody to HCV in human plasma, may measure markers of infection which only become positive sometime after infection. Similar considerations apply to testing of the final product.

1.7 Therefore establishing the freedom of a biological from infectious virus will in many instances not derive solely from direct testing for their presence, but also from a demonstration that the manufacturing process is capable of removing or inactivating them. Validation of the process for viral inactivation/removal can play an essential and important role in establishing the safety of biological products especially when there is a high potential for the source material to be contaminated with a virus known to be pathogenic for man, e.g. plasma derived products. Also, since many instances of contamination in the past have occurred with agents whose presence was not known or even suspected at the time of manufacture, an evaluation of the process can provide a measure of confidence that a wide range of viruses including unknown, harmful viruses, may be eliminated.

1.8 The intention of this note for guidance is to provide a general framework for validation studies and the virological approach which should be used in the design of virus validation studies. Manufacturers should apply the recommendations presented here to their specific product taking into consideration the nature of the source material, the procedures used for production and purification and any other factors which can have consequences on this safety issue. The approach used by manufacturers in studies for evaluating virus elimination should be explained and justified.

2. SOURCES OF VIRAL CONTAMINATION

Viral contamination of biologicals can arise in the following ways:

2.1 Source material may be contaminated with a virus indigenous to the species of origin. Blood can harbour many viruses and the use of products derived from human plasma has caused infections by HBV, HCV, HIV, parvovirus B19 and occasionally HAV. Murine viruses, some of which are pathogenic for man, may contaminate murine hybridomas. Cell lines which are intended to be used for genetic manipulation may be contaminated by viruses and, therefore, they should be chosen carefully and tested for freedom from detectable adventitious agents even before genetic manipulation, in order to start with a well characterised cell line.

2.2 Cells may have a latent or persistent infection, for example, a herpesvirus or a retrovirus, which may be transmitted vertically from one cell generation to the next as a viral genome and which may be expressed intermittently as infectious virus.

2.3 The process of construction of a production cell line may introduce a contaminant virus indigenous to another species, e.g. an EBV transformed human lymphoblastoid cell line secreting a monoclonal antibody can be infected with a murine retrovirus after fusion with a murine myeloma.

2.4 Adventitious viruses may be introduced by the use of contaminated animal products in the production process e.g. cell cultures may be contaminated with bovine viruses through the use of bovine sera or a murine monoclonal antibody used in affinity chromatography may contaminate a product with a murine virus.

2.5 Other sources of contamination, e.g. operating personnel or raw materials of non-biological origin, are possible.

3. THE VALIDATION PROCESS

3.1 The aim of viral validation studies is:

(i) to provide evidence that the production process will effectively inactivate/remove viruses which are either known to contaminate the starting materials, or which could conceivably do so, and

(ii) to provide indirect evidence that the production process might inactivate/remove novel or unpredictable virus contamination.

This is achieved by deliberately adding ('spiking') a virus to material at various production steps and measuring its removal or inactivation during the subsequent individual step or steps. This will identify production steps which are effective in reducing the level of infectious virus and provide an estimate of the overall ability of the process to eliminate contaminating viral infectivity.

3.2 Virus validation studies, as with direct testing of materials at appropriate steps, contribute to confidence in the virological safety of the product. However, all virus validation studies must be regarded as an approximation to the true capacity of the process since it may be difficult or impossible to conduct a perfect validation study of a process because of the large numbers of complex variables involved. Results have shown that even small modifications in procedure or the particular laboratory strain of virus used can have a large effect on virus removal or inactivation.

3.3 Where the starting or source material is less well characterised, such as blood, tissues and organs of human or animal origin, or when cells have been cultured by in vivo techniques, there is a higher possibility of viral contamination and the manufacturing process will normally incorporate one or more effective virus inactivation/removal steps. Products derived from human plasma raise particular viral safety concerns and specific guidance is given in the guideline on *Medicinal Products Derived From Human Plasma*.

3.4 In the past, where the starting material posed a lower virological risk, such as a fully characterised cell bank, the purification process often did not contain a specific virus inactivation/removal step and a validated purification process was considered to give sufficient levels of viral inactivation/removal. Clinical experience has not revealed any problems with this approach. However, some manufacturers of monoclonal antibodies

(mAbs) are introducing specific viral inactivation/removal steps into their production process since mAb producing cell lines of murine origin inevitably secrete variable quantities of retroviruses which may be infectious.

3.5 It should be borne in mind that cell culture systems inherently support virus replication. Therefore, a distinct low level of risk of viral contamination of the culture persists despite a high level of cell bank characterisation and occasional cases of adventitious virus contamination have been reported.

3.6 The justification for, and the extent of, the required validation studies will vary depending on the manufacturing process and type of product (e.g. species of origin of starting material, whether source material is variable or defined, stability of the active material, etc.). The appropriateness of the studies will be reviewed on a case by case basis.

4. THE CHOICE OF VIRUSES FOR VALIDATION

4.1 Viruses for validation should be chosen firstly to resemble viruses which may contaminate the product as closely as possible and secondly to represent as wide a range of physico-chemical properties as possible in order to test the ability of the system to eliminate viruses in general.

4.2 Most validation studies employ laboratory strains of virus which can be produced and assayed conveniently. However, experience has shown, and manufacturers should be aware, that different laboratory strains of virus may have different properties from each other and from naturally occurring viruses. Consequently, any virus used in a validation study is actually a model virus. The manufacturer should justify the choice of viruses in accordance with the aims of the validation study and the principles laid down in this guideline. Unless otherwise justified, where two similar viruses could be used for validation studies either because of their equal resemblance to possible contaminants or similarities in their properties, the virus considered to be the more resistant should be used.

4.3 Examples of the choice of viruses are:

(i) Human plasma-derived clotting factor concentrates have been contaminated by HIV. Thus the production process for such materials must be evaluated for its ability to inactivate/remove infectious HIV.

(ii) Cell lines derived from rodents usually contain endogenous retroviral particles which may be infectious (C-type particles) or non-infectious (A-type particles). Where the source material is obtained from rodent cell lines, the production process should be evaluated for its ability to inactivate/remove one of the closely related laboratory murine retroviruses.

(iii) Examples of viruses representing a range of physico-chemical properties which have been used to evaluate the general ability of a process to remove virus infectivity include:

a) SV40, poliovirus or an animal parvovirus as small non-enveloped viruses,

b) a parainfluenza or a murine retrovirus as large enveloped RNA viruses,

c) a herpesvirus as a large DNA virus.

Examples of viruses which have been used in the past in validation studies are given in Table 1. However, since these and the viruses mentioned above are merely examples, the use

of any of them is not mandatory and manufacturers are invited to consider other viruses especially those which may be more appropriate for their individual processes. Further guidance on the choice of viruses for the validation of manufacturing processes of plasma derivatives is provided in the guideline *Medicinal Products Derived From Human Plasma.*

4.4 There should be an efficient, sensitive and reliable infectivity assay for the viruses used. Viruses which can be grown to high titre will be desirable, although this may not always be possible.

4.5 Products derived from ovine, caprine or bovine tissues raise the problem of contamination by agents of transmissible spongiform encephalopathy, such as scrapie, which accumulate in the central nervous system and lymphoid tissue. These agents are the subject of a separate note for guidance on *Minimising the Risk of Transmitting Agents causing Spongiform Encephalopathy via Medicinal Products.*

5. DESIGN OF VALIDATION STUDIES

5.1 Validation studies involve the deliberate addition of a virus at various production steps and measuring the extent of its removal/inactivation during the subsequent individual step or steps. It is not necessary to validate every individual step of a manufacturing process. Only those steps which are likely to contribute to inactivation/removal of a virus need to be subject to a validation study.

5.2 GMP restraints prevent the deliberate introduction of any virus into the production facilities. Therefore, the validation should be conducted in a separate laboratory equipped for virological work on a scaled-down version of the production process and performed by staff with virological expertise in conjunction with the production bioengineers. Studies should be carried out in accordance with the principles of GLP.

5.3 The comparability of the model and full scale procedures is the premise on which the results obtained with the scaled-down system can be accepted in evaluating the virus safety of the product. Therefore, the validity of the scaling down should be demonstrated, by comparison of process parameters such as pH, temperature, concentration of protein and other components, reaction time, column bed height, linear flow rate, flow rate to bed height ratio, elution profile and step efficiency (e.g. yield, balance, specific activity, composition). Deviations which cannot be avoided should be discussed with regard to any potential influence on the results.

5.4 Whenever possible, it should be shown whether the reduction in virus infectivity is accomplished by inactivation of virus or by removal of virus particles. This may be achieved by establishing the kinetics of loss and/or a balance of infectivity, as appropriate. Processes which reduce virus infectivity by inactivation are potentially more easily modelled than those which physically remove particles. For a viral inactivation step, the kinetics of inactivation should be studied and included in both tabular and graphical form in reports. Where the inactivation is too rapid to plot the kinetics using process conditions, further studies should be performed in order to prove that infectivity is indeed lost by inactivation. Thus appropriate controls should be introduced to detect possible interference with the assay from the sample or the matrix into which it is introduced and the limits of detection should be established.

5.5 Production parameters which influence the effectiveness of a process step to inactivate/remove viruses should be explored and the results used in setting appropriate in-process limits. Critical parameters include:

- mechanical parameters such as flow rates, mixing rates, column dimensions, column reuse, etc.
- physico-chemical parameters such as protein content, pH, temperature, moisture content, etc.

5.6 Antibodies present in the starting material may affect the behaviour of a virus in partition or inactivation steps. Validation studies should take this into account.

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

5.8 Published work concerning the ability of related or generic processes to inactivate/remove viruses may provide an indication of which steps are likely to be effective. However, the variability intrinsic to validation studies arising from the need to model the process, choose viruses to be used and explore full scale production parameters on a laboratory scale, means that validation data must be based on experimental studies provided by the

5.9 The amount of virus added to the starting material for the production step which is to be studied should be as high as possible in order to determine the capacity of the production step to inactivate/remove viruses adequately. However, the virus spike should be added such that the composition of the production material is not significantly altered (typically the volume of the virus spike will be less than 10%). Whenever possible, calculated reduction factors should be based on the virus which can be detected in the spiked starting material and not on the amount of virus added.

5.10 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 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. Effects of the sample on the detection system, including toxic effects, should be recorded as they influence the limits of detection.

5.11 Quantitative infectivity assays should be performed according to the principles of GLP and may involve plaque formation, detection of other cytopathic effects such as syncytia or foci formation, end point titrations (e.g. $TCID_{50}$ assays), detection of virus antigen synthesis or other methods. The method should have adequate sensitivity and reproducibility and should be performed with sufficient replicates and controls to ensure adequate statistical accuracy of the result (see Appendix I).

5.12 Nucleic acid amplification methods, e.g. PCR, are a promising approach capable of great sensitivity in detecting viral genomes and also can detect viruses such as hepatitis B and C for which culture systems are not available. However, an important limitation of the technology is that inactivated virus may still score positive in a genome amplification assay and thus may underestimate the degree of virus inactivation obtained by a potentially effective step. On the other hand, PCR may be of value in studies of processes which depend on virus removal. The use of this technology poses major problems in terms of quantification, standardisation, quality control and interpretation of results. Validation and

standardisation of these assays must be unambiguously demonstrated before they are acceptable and extreme caution used in interpretation of both positive and negative results.

5.13 Assurance should be provided that any virus potentially retained by the production system will be adequately destroyed prior to reuse of the system, e.g. by sanitisation of columns, etc.

6. INTERPRETATION OF DATA

6.1 A combination of factors must be considered when judging the effectiveness of a virus inactivation/removal step. Assessment of a step based solely on the quantity of virus inactivated/removed can lead to the conclusion that a process meeting specified levels of virus reduction will produce a safe product. This is not necessarily the case. The following factors all contribute in defining the effectiveness of a step and the data must be carefully evaluated in each case:

(i) The appropriateness of the test viruses used (see Section 4).

(ii) The design of the validation studies (see Section 5).

(iii) The $\log_{10}$ reduction achieved. Log reductions of the order of 4 logs or more are indicative of a clear effect with the particular test virus under investigation. However, it is emphasised that log number reduction cannot be used as the single, absolute measure of the effectiveness of a step.

(iv) The kinetics of inactivation. This will indicate whether or not the measured log reduction is a conservative estimate. Virus inactivation is usually not a simple first order reaction and often has a fast initial phase followed by a slower phase. However, a dramatic reduction in the rate of inactivation with time may suggest a loss of effectiveness of the inactivating agent or that a residual virus fraction is resistant to the inactivating agent and implies that the step is neither highly effective nor robust.

(v) The nature of inactivation/removal and whether it is selective for only certain classes of virus. A process step may be highly effective for some viruses but ineffective against others, e.g. S/D treatment is effective against lipid-containing but not lipid-free viruses.

(vi) The susceptibility of virus inactivation/removal to small variations in-process parameters will affect the confidence placed in a step.

(vii) The limits of assay sensitivities.

It is the combined evaluation of the above factors that will lead to a decision on whether a process step can be regarded as effective, moderately effective or ineffective in the inactivation/removal of viruses.

6.2 The following examples are intended to illustrate some of these principles and are neither definitive nor all encompassing:

(i) Where a process step is challenged with 6 logs of virus and 4 logs are recovered, the step cannot be claimed to be effective, although it may contribute to overall removal.

(ii) Where a process step is challenged with 6 logs of virus, but because of the cytotoxicity of the product the limit of assay sensitivity in the product is 4 logs, only 2 logs of removal have been demonstrated, and the step cannot be claimed to be effective. The process step

may in fact be able to remove far greater quantities of virus, which might be demonstrated by a different experimental design.

(iii) Where a process step is challenged with 6 logs of virus and 2 logs are recovered, substantial amounts of virus have been removed. The product is not virologically sterile. However, if this reduction is reproducible and not influenced by process variables, the step is of some efficacy. It contributes to overall reduction of virus load and may be counted as such.

(iv) Where a process step is challenged with 6 logs of virus and no virus is detected in the product with a limit of sensitivity of the order of 2 logs, approximately 4 logs of removal have been demonstrated. This is substantial and the step may in fact remove far greater quantities than can be quantified or claimed.

(v) Where virus is inactivated, the kinetics of loss of infectivity are important. If a process step involves prolonged incubation, e.g. heating for ten hours, and infectivity reaches the limits of detection rapidly, the process is likely to have a greater virucidal effect than can often be demonstrated. On the other hand, if infectivity is lost slowly and the limits of detection are reached towards the end of the treatment period, the step provides less assurance of viral safety.

6.3 In general, partition processes are not considered to be effective viral removal steps although it is recognised that they can contribute to virus removal. Partition processes usually have a number of variables that are difficult to control and are often difficult to scale down for validation purposes. Furthermore, partitioning is dependent on the extremely specific physico-chemical properties of a virus which influence its interaction with gel matrices and precipitation properties. Thus a model virus may be partitioned in a completely different manner to a target virus because of relatively minor differences in surface properties such as glycosylation. Even a relevant virus propagated in the laboratory may act differently from the wild-type virus in this respect. However, if a partition process gives reproducible reduction of virus load and if manufacturing parameters influencing the partition can be properly defined and controlled and if the desired fraction can be reliably separated from the putative virus-containing fraction, then it could fit the criteria of an effective step.

6.4 The objective of the validation is to identify steps effective in the inactivation/removal of viruses and to obtain an estimate of the overall capacity of the manufacturing process to inactivate/remove them. An overall reduction factor is generally expressed as the sum of individual factors (see Appendix II). However, a simple summing of low individual reduction factors may be misleading. Reductions in virus titre of the order of 1 log or less are considered to be unreliable because of the limitations of virus validation studies and should be ignored. Manufacturers should differentiate effective steps from process steps which may contribute to removal but upon which less reliance can be placed. Consideration should also be given to whether virus surviving one step would be resistant to a subsequent step or alternatively have increased susceptibility. In general, a single step having a large effect gives more assurance of viral safety than several steps having the same overall effect.

6.5 If little reduction of infectivity is achieved by the production process, and the removal of virus is considered to be a major factor in the safety of the product, a specific, additional inactivation/removal step or steps should be introduced.

6.6 For all viruses, manufacturers will be expected to justify the acceptability of the reduction factors obtained. Results will be considered on a case by case basis.

6.7 The GMP principle that material subjected to an effective virus inactivation/removal step should be segregated from untreated material should be rigorously applied.

7. LIMITATIONS OF VALIDATION STUDIES

Validation studies are useful in contributing to the assurance that an acceptable level of safety in the final product is established and do not by themselves establish safety. A number of factors in the design and execution of virus validation experiments may lead to an incorrect estimate of the ability of the process to remove naturally occurring virus infectivity. These factors include the following points.

7.1 Experience has shown that different laboratory strains of virus may differ in their sensitivity to the same treatment. The particular virus chosen may therefore not resemble the virus for which it has been chosen as a model. Native viruses may have unpredicted properties, for example association with lipid, which may affect their properties. Virus preparations used to validate a production process are likely to be produced in tissue culture. The behaviour of tissue culture virus in a production step may be different from that of the native virus for example if native and cultured viruses differ in purity or degree of aggregation. The strains of virus, their cultivation and assay, and details of sampling and storage should all be documented.

7.2 There are some situations in which it may not be valid to add logarithmic reductions. For example, if a matrix is able to adsorb 10^4 infectious units of a virus and then cannot adsorb further material with comparable affinity then it will remove all virus when challenged with 10^4 infectious units, but only 1% when challenged with 10^6. The clearance measured may therefore differ with the challenge titre.

7.3 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 virus escaping a first inactivation step may be more resistant to subsequent steps. As a consequence, the overall reduction factor is not necessarily the sum of reduction factors calculated from each individual step in which a fresh virus spike suspension is used. For example if the resistant fraction takes the form of virus aggregates, infectivity may be resistant to a range of different chemical treatments and to heating.

7.4 Model scale processing is likely to differ from full scale processing despite care taken to design the scaled down process.

7.5 The presence of antibodies to a native virus may affect partition of the virus or its susceptibility to chemical inactivation; but it may also complicate the design of the study by neutralising infectivity. The appropriateness of the study design may be difficult to judge. The level of antibody present may be considered a significant process variable.

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

8. RE-EVALUATION STUDIES

8.1 Changes to the production process may necessitate a new validation study.

8.2 As scientific experience accumulates, processes will require re-examination to ensure that they remain of an acceptable standard.

APPENDIX 1

STATISTICAL EVALUATION OF VIRUS TITRES AND REDUCTION FACTORS AND ASSESSMENT OF THEIR VALIDITY

Virus titrations suffer the problems of variation in 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 are 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 ± 0.5 $\log_{10}$ or better. Between assay variation can be monitored by the inclusion of a 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 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 $\pm\sqrt{(s^2 + a^2)}$, where ± s is the 95% confidence limits for the viral assays of the starting material, and ± a for the viral assays 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 estimated minimum reduction factor of an effective inactivation process as large as possible, as much processed undiluted material as possible should be sampled.

APPENDIX II

CALCULATION OF REDUCTION FACTORS

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

$$R = \log \frac{V1 \ x \ T1}{V2 \ x \ T2}$$

where, *R* = the reduction factor,

V1 = volume of starting material,

T1 = concentration of virus in starting material,

V2 = volume of material after the step, and

T2 = concentration of virus 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 virus infectivity may be greatly reduced, it will never be reduced to zero. The European Pharmacopoeial convention* with respect to methods of sterilisation is that processes which deliver a sterility assurance level (SAL) of 10^{-6} or better for bacteria, moulds and yeasts are considered adequate. A SAL of 10^{-6} denotes a probability of not more than one viable micro-organism in 1 x 10^{-6} sterilised items of the final product.

* "Methods of Preparation of Sterile Products" monograph of the European Pharmacopoeia

TABLE 1
EXAMPLES OF VIRUSES WHICH HAVE BEEN USED IN VIRUS VALIDATION STUDIES

Virus	Family	Genus	Natural Host	Genome	Env	Size	Shape	Resistance to Physico-chemical Treatment*
Vesicular stomatitis virus	Rhabdo	Vesiculovirus	Equine Bovine	RNA	Yes	70x175 nm	Bullet shaped	Low
Parainfluenza virus	Paramy xo	Paramyxovirus	Various	RNA	Yes	100-200nm	Pleo/Spher	Low
Human immuno-deficiency virus	Retro	Lentivirus	Man	RNA	Yes	80-100nm	Spherical	Low
Murine leukaemia virus (MuLV)	Retro	Type C oncovirus	Mouse	RNA	Yes	80-110nm	Spherical	Low
Sindbis virus	Toga	Alphavirus	Man?	RNA	Yes	60-70nm	Spherical	Low
Bovine viral diarrhoeal virus (BVDV)	Toga	Pestivirus	Bovine	RNA	Yes	50-70nm	Pleo-Spher	Low
Pseudorabies virus	Herpes	Varicellovirin ae	Swine	DNA	Yes	120-200nm	Spherical	Med
Poliovirus, Sabin type 1	Picorna	Enterovirus	Man	RNA	No	25-30nm	Icosahedral	Med
Encephalomyocard itis virus (EMC)	Picorna	Cardiovirus	Mouse	RNA	No	25-30nm	Icosahedral	Med
Reovirus 3	Reo	Orthoreovirus	Various	RNA	No	60-80nm	Spherical	Med
Hepatitis A	Picorna	Hepatovirus	Man	RNA	No	25-30nm	Icosahedral	High
SV40	Papova	Polyomavirus	Monkey	DNA	No	40-50nm	Icosahedral	V.High
Parvoviruses (canine, porcine)	Parvo	Parvovirus	Canine Porcine	DNA	No	18-24nm	Icosahedral	V.High

This Table gives an incomplete list of viruses which have been used in validation studies. Consequently, the use of any of the viruses in the Table is not mandatory and manufacturers are invited to consider other viruses especially those which may be more appropriate for their individual production processes.

* This general classification is based on validation studies of production processes