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Review

Viral contamination of monoclonal antibody preparations: Potential problems and possible solutions

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Introduction

In recent years the transmission of the human immunodeficiency virus by blood transfusion and blood component therapy has focussed attention on the risks of viral contamination inherent in deriving therapeutic biologicals from biological sources. For those involved in the manufacture of viral vaccines, however, the biological substrates used to generate viruses have always been critically considered a safety risk. As Griffiths (1988) has recently described, the history of vaccine manufacture has seen an evolution correlated to the use of safer substrates – from animals to eggs, to primary cells and then to the use of human diploid cells in the 1970s. Today, as we approach the 1990s, continuous or immortal cell lines are being introduced in vaccine manufacture, e.g. the production of polio vaccine from VERO cells. In fact, the 1980s has seen a tremendous interest in the use of continuous cell lines for the production of not only vaccines but also therapeutic biologicals since bacteria were found to be unsuitable expression and production vehicles for many recombinant biologicals. Due in part to the large investment in research and development in mammalian cell technology during the 1980s a number of therapeutic biologicals, e.g. monoclonal antibodies and tissue plasminogen activator tPA, have now entered the market place.

The use of continuous cell lines has aroused controversy, with some scientists regarding it as a retrograde step in terms of safety due to the inherent oncogenic potential of these cells, whether from viruses, oncogenes or both. Others (Moyer and Moyer, 1987; Petricciani *et al.*, 1981, 1987) have argued that, provided adequate quality control assurance programs are designed and followed, the risks are negligible. This whole area is clearly extremely important from a medical health point of view and extensive debate is required to resolve the problems inherent in the use of continuous cell lines. The issues involved however are both complex and wide-ranging and it is therefore of paramount importance that discussions leading to the development of guidelines and quality assurance procedures are based on accurate scientific data and not on existing dogma.

This review focusses on the risks of viral contamination and hence transmission of viruses to patients and production personnel associated with the use and large-scale production of monoclonal antibodies (mabs). However, by implication it includes all biological materials used to produce therapeutic biologicals, particularly continuous cell lines.

Viral contamination: The scope of the problem

Murine hybridomas

The potential hazards of murine viral contamination of mab preparations intended for therapeutic

use were well reviewed recently by Carthew (1986) and are clearly significant. This problem is particularly acute when large-scale production includes the use of ascites preparations induced in either mice or rats. These problems have been recognised by the various national and international health authorities who have issued fairly comprehensive guidelines, e.g. Points to consider in the manufacture and testing of monoclonal products for human use, issued by the Office of Biologics, FDA, June 1987; Guidelines on the production and quality control of monoclonal antibodies of murine origin intended for use in man, Commission of the European Communities Committee for Proprietary Medicinal Products (1988); Guidelines for the production of mabs intended for therapeutic use, issued by The Australian National Biologicals Standards Laboratory, Department of Community Services and Health (1988). These guidelines have attempted to tackle the complex and wide-ranging problems created by the development of new biologicals using cell culture technology. These include not only the endogenous and adventitious viruses which might contaminate continuous cell lines and which are reviewed here, but also residual cellular DNA and other factors such as transforming proteins.

The scope of the viral problem is enormous with up to twenty-two viruses listed in the European guidelines as potential contaminants in the manufacture of murine mabs alone and twenty-four in the Australian guidelines. Some of these, such as the retroviruses, are known to be oncogenic while at least for five others, including the Hantaan virus and lymphocytic choriomeningitis virus, evidence exists for a capacity to infect man or primates. In addition there are other murine viruses which are capable of replicating in human cells *in vitro*. Many mouse hybridomas examined to date have contained viral particles, some of which expressed reverse transcriptase (RT) and were infectious (Bartal and Hirshaut, 1987; Levy, 1983; Weiss, 1982a). Weiss (1982b) found the NS-1 cell line was full of A-type particles but released no detectable C-type particles, whereas 12 of 17 hybridomas released large quantities of C-type particles as determined by EM or RT assay. In addition eight of the twelve C-type positive cell lines generated viruses infective for human cells although there is no evidence to date to indicate that these retroviruses infect humans. It has been reported that DNA sequences resembling retroviral genomes have been found in all mammalian and avian species that have been investigated (Bartal and Hirshaut, 1987). In a recent study of CHO cells, a popular expression vehicle for proteins of pharmaceutical interest, it was shown that all cell lines examined, both parental and recombinant, contain and express at least one family of retroviral sequences (Wurm *et al.*, 1989). Another more recent report concerning murine hybridomas estimated that at least twenty percent or more of the cell lines express one or more families of retroviruses (Lubiniecki, 1989).

Virus-like particles resembling type A and type C retroviruses have been observed in CHO-K1 cells (Heine *et al.*, 1979 and Petricciani, 1981). The type A-like particles are thought to be associated with the ventricle in rDNA modified cells (Heine *et al.*, 1979). Budding type C-like particles are reported to occur infrequently in a small percentage of sections from CHO-K1 cells and cell bank materials (Manley *et al.*, 1978). In their detailed characterization of recombinant CHO cells Lubiniecki and May (1985) detected virus-like particles resembling type A and C retroviruses by electron microscopy but the particles had no biological properties, i.e. they were found to be defective. Thus the presence of retroviral genomic sequences and the detection of virus-like particles in the cytoplasm of cells does not necessarily pose an infection risk although clearly this does not negate the need for well designed strategies for eliminating these potential viral contaminants.

Human monoclonals

The generation of human mabs has not solved the viral problem but rather changed the range of viruses that have to be considered in quality

assurance programs. Human mabs have been generated by a number of different techniques including human-human fusions, human-murine fusions, human-human-murine fusions and finally direct immortalization of human lymphocytes by the Epstein-Barr virus (EBV). There are a number of viruses which are known to infect human lymphocytes, including HTLV, HIV, Herpes 6, CMV, Hepatitis B, Hepatitis non-A non-B and, of course, EBV, which all have to be considered as potential contaminants of human mab preparations. Thus the source of human lymphocytes would need to be extensively screened for these different viruses. The generation of human mabs often involves the use of rosetting techniques using sheep red blood cells, which could be contaminated with viruses such as the Lentivirus and, although much more unlikely, prions such as Scrapie. Also many procedures for producing human mabs employ mouse feeder layers in generating EBV-transformed cells which could theoretically generate recombinant human-mouse retroviruses. This is also a problem with human-murine heteromyelomas although the significance of these, at present purely theoretical, problems has yet to be established. The problems inherent in using EBV-transformed cell lines were first discussed in association with interferon production from Namalwa cells. The safety concerns relevant to interferon have been discussed in detail elsewhere (Finter and Fantes, 1980) and were clearly resolved to the satisfaction of the licencing authorities since interferon from these cells is now available for therapeutic use.

Other potential sources of viral contamination

There are other potential viral risks which are not directly related to the cells themselves. For example, the use of bovine serum in cell culture carries with it the risk of bovine viral diarrhoea virus and infectious bovine rhinotracheitis virus. The recent discovery of bovine spongiform encephalopathy in some cattle in the U.K. increases the pressure to develop serum-free media. However, even serum-free media usually incorporate products of bovine origin, such as insulin, transferrin and bovine albumin, and thus cannot be considered free of associated viral risks. In a recent review on pestiviruses, Collett *et al.*, (1989) have discussed the difficult task of trying to distinguish unequivocally viral components from host cell contaminants. They state that this problem is a direct consequence of the low level of pestivirus growth in cultured cells and the problem is compounded by the fact that pestiviruses often contaminate cell cultures undetectably and are present in tissue culture supplements (especially bovine sera). MacLeod (1988) has suggested the use of human plasma supplements to replace material of bovine origin. Although there are clear advantages in that the sensitization of patients to foreign proteins is avoided, the viral risk remains as a major problem.

Other problems involve the potential contamination of cell lines by adventitious viruses during the production process. As yet detailed information concerning murine hybridoma cell lines has not appeared in the literature, although in a recent comprehensive study Wiebe *et al.* (1989) found that 7 out of 44 viruses infected CHO-K1 cells; these included mumps and parainfluenza types 1, 2 and 3. As with the endogenous viruses, these potential contaminants must be considered in the design of the overall production process with particular emphasis on appropriate purification schemes. Some of these measures are discussed in the following section.

Viral contamination: solutions to the problem?

Preventive measures

These measures are designed so that, on the one hand, all input materials to the process are tested to ensure minimal viral risk and, on the other, all output or end product from the process does not contain any evidence of contaminating viruses. The guidelines issued by the various health authorities with regard to the generation of mabs for therapeutic use have addressed these prob-

lems in some detail and have concentrated on the following major areas:

(a) Screening of all materials used in the process including the cell substrate (e.g. testing for Hantaan LCM and retroviruses, etc.) and the media components, particularly serum supplements. With regard to the screening of the cell substrate it is important to establish a primary cell seed, a master cell bank and then the manufacturer's working cell bank (WCB). This approach allows for the WCB to be characterized for identity, stability and microbial contaminants, including mycoplasmas, in addition to the endogenous and adventitious viruses previously mentioned. From the data presented earlier concerning the contamination of cell lines with human viruses, Wiebe *et al.*, (1989) it is clear that processes should be designed to avoid direct contact between personnel and cell substrates and products. In addition, it is equally as important to protect operators from potential viral contamination risks and this is an area which has received little attention despite the fact that process workers could now be involved in handling large quantities of antibodies during their working lives.

(b) Screening of all product batch lots and final products to ensure that there is no evidence of viral contamination. The Commission of the European Communities' working party for murine mabs (1988) recommends testing for twenty-two viruses while the Australian guidelines suggest twenty-four viruses should be assayed. In the "Points to consider in the manufacture and testing of mabs for human use" (issued by the USA Dept. Health and Human Services, 1987) a mouse antibody production test (map) is recommended for the detection of the following viruses: retrovirus type 3, Hantaan virus, polyoma, pneumonia virus of mice, mouse adenovirus, minute virus of mice, mouse hepatitis, ectromelia, Sendai and mouse encephalomyelitis virus (GD VII viruses). In a recent report it was stated that the map test is suitable for detecting 13 viruses (Shek, 1987). Other assay procedures are suggested for lymphocytic choriomeningitis virus (LCMV), mouse salivary gland virus (murine CMV), mouse rotavirus (EDIM), thymic virus and LDH viruses. LCMV, for example, does not necessarily induce the formation of antibodies. In addition, tests should also be included for murine leukaemia viruses, such as the XC test (Klement *et al.*, 1969) for ecotropic viruses, the S+L-test (Peebles, 1975) and the MCF assay (Cloyd et al., 1981) for xenotropic viruses. It is also suggested that reverse transcriptase assays for retroviruses should be performed utilizing both Mg^{++} (Kacian and Speigelman, 1974) and Mn^{++} (Liu *et al.*, 1977). More recently (Onions *et al.*, 1989) the adaption of the polymerase chain reaction to the assay of retroviruses has increased the sensitivity of detection of these viruses.

The map test procedure as reported by Collins and Parker (1972) involves the inoculation of mice 4 weeks of age or older with the specimen plus appropriate controls. Virus-specific antibodies produced as a response to any viruses present in the specimen are then detected by serological tests, usually ELISAs, performed approximately 4-weeks post-inoculation. It is important that the animals used for these tests are either viral antibody-free or gnotobiotic. In addition to the detection of murine viruses, cell cultures capable of detecting a wide range of human and bovine viruses should also be inoculated. Finally, the European guidelines recommend that there should be tests in animals for adventitious agents involving the inoculation of different groups, e.g. suckling mice, adult mice and guinea pigs, with test material and/or disrupted cells from the seed lot propagated beyond the maximum level. Thus very comprehensive and expensive screening and testing procedures are recommended to ensure the viral safety of mabs and recombinant biologicals. It is axiomatic, however, that these tests, as comprehensive and as well

designed as they are, cannot, with absolute certainty, detect as yet unknown viruses. That such viruses exist is surely the major lesson to be learnt from the AIDS epidemic! Thus process control strategies designed to remove viruses are equally as important as those designed to prevent viral contamination.

Removal of viruses

Downstream processing technologies were developed to purify mabs to a level largely determined by the ultimate use of the mab, clearly less demanding for diagnostic use than for therapeutic use. More recently the various licencing authorities have required that purification techniques should have the capacity to remove not only unwanted immunoglobulins, e.g. bovine from serum and murine from ascites, and DNA but also viruses. Data from Levy *et al.* (1984) showed that purification of mabs, produced in ascites using protein A, completely eliminated all detectable murine leukaemia viruses and nucleic acids, even when these agents were added in quantities far in excess of those normally expected. It is now apparent that the murine retroviruses are particularly sensitive to low pH and a reduction in pH to 3.5 can cause a 3 to 4 log kill (Martin, 1985). It is therefore quite likely that the low pH buffer systems required for elution of mabs from protein A leads to significant viral kill and thus the reduction in viral titre associated with the use of protein A is due to an indirect inactivation mechanism rather than virus removal.

It is also important that the purification be validated to demonstrate that the procedures employed should be both effective and reproducible in removing viruses. The validation process usually involves the 'spiking' of a crude preparation in pilot-scale studies using a representative selection of appropriate viruses, some of which may be radiolabelled. Similar studies are performed to validate DNA removal. The purification scheme should result in an acceptable reduction in viral load. A major problem, however, inherent to this approach is the choice of viruses for use in the validation process since viruses vary so greatly in their structure, biophysical and biochemical properties. Clearly it would not be possible to test all potential contaminants and hence there is a need for some standardization of this type of work which could be accepted internationally. The WHO would be an appropriate international body to consider this comply issue since it was the WHO who took the initiative in facilitating the acceptance of continuous cell lines as substrates for biologicals production in the early '80s, i.e. approval of a polio vaccine derived from a continuous cell lines (WHO Tech. Report, 1987).

Recently Builder *et al.* (1989) described process development and regulatory approval of tissue-type plasminogen activator and discussed the removal of virus-like particles during the purification process. They implied that multistep procedures were required for virus removal and that the total viral clearance was the product of the individual step clearances. Thus, in the example they quoted, if each step in the process removed brought about a four-log removal of virus and the process contained three steps, there would be a twelve-log clearance of virus. The process details for tPA were not described but the design of a three-step process for mab purification could involve ion-exchange chromatography, followed by affinity separation using protein A and size exclusion chromatography. Each step could reduce viral loads significantly as does the use of protein A purification (Levy *et al.*, 1984). However at this stage there is no firm data to our knowledge describing the mechanism(s) by which viruses are 'removed'. In order to assume that the total viral clearance was a product of the removal at each step the mechanisms of removal would need to be different for each step. It is possible, as we stated earlier, that the large reduction in viral load observed with protein A by Levy *et al.* (1984) was due to an indirect effect of the chemistry of the process and, since mabs differ significantly in the chemical conditions required for their purification, it could be assumed the viral removal would also vary according to the

conditions used. Crawford *et al.*, (1983, 1987) have described a purification process for a human monoclonal antibody (UCH D4 produced by a cloned, EB virus immortalized B cell line which is specific for the RhD antigen on human red blood cells) which removes 'spiked' EB virus and DNA to the limits of detection. The purification scheme involved filtration via 0.22 and 0.1 μm filters, treatment with DNAase and affinity chromatography using anti-human IgG. Affinity purification procedures using monoclonal antibodies have also been developed for the large-scale production of Factor VIII preparations (Lusher *et al.*, 1988). The manufacturers have claimed that this approach significantly reduces viral load (Mannucci *et al.*, 1988) thus confirming the earlier work by Levy *et al.* (1984) with mabs. Thus well designed removal strategies can play an important role in reducing the risks of viral transmission from biologicals.

Filtration has been used widely and successfully to remove bacteria from biological preparations but, as yet, has proved impractical for virus removal due to the slow flow rates in the presence of high protein concentrations. A recent report, however, has shown that with a novel porous regenerated cellulose hollow fiber, filtration can remove HIV infectivity completely from a virus-rich culture supernatant while maintaining a high filtration rate and a high permeability for proteins (Hamamoto *et al.*, 1989). Thus it may be feasible to incorporate filtration steps for viral removal in the overall purification process using specifically designed filters.

Inactivation strategies

From the authors' point of view the large array of complex testing and quality assurance procedures described in this review does not provide an absolute guarantee of safety with regard to viral contamination. While it is recognised that absolute safety can never be guaranteed, we believe the use of inactivation procedures in addition to removal strategies would significantly increase the safety margin. The inclusion of inactivation procedures would take account of our previously stated major concern – the presence of as yet unknown and hence undetectable viruses. In addition, if the inactivation process could be designed to operate at the terminal stage of the production process, i.e. when the product has been added to the final container, then it would avoid further contamination from adventitious viruses. The European guidelines state that consideration should be given to incorporating procedures which inactivate potential viral contaminants and the Australian guidelines (1988) actually recommend and describe a chemical inactivation method for possible use with mabs. Although we recognise that this is another complex area in that it is difficult to inactivate viruses without compromising the biological activity of the product, we would argue most strongly that inactivation procedures should be included as routine procedures in the overall production process.

If the inactivation approach is to be adopted then it is clearly important to establish appropriate strategies whereby potential viral contaminants are inactivated without affecting the biological activity and, or, structure of the product. Inactivation strategies can be divided into two very broad categories:

a. Physical methods
b. Chemical methods (including radiation).

Although there is no data in the literature directly relevant to the inactivation of viruses in mab preparations, there exists a considerable body of knowledge relevant to viral inactivation from vaccine production. Viral vaccine manufacturers have over the years developed a number of techniques for inactivating viruses, including chemicals such as formaldehyde, the aziridines and beta-propiolactone (BPL).

Recently it was reported (Jiang *et al.*, 1986) that recovery of poliovirus D-antigen after virus inactivation using BPL was significantly higher than when formalin was used. A previous study had also shown that use of BPL when compared to formalin for viral inactivation required less time to complete and antigen loss was less likely (Lo Grippo, 1960). BPL has been shown effectively to inactivate a wide range of viruses in-

cluding encephalomyocarditis virus, rabies virus, poliovirus, ECHO viruses, Herpes viruses, measles, mumps, influenza (A and B) and hepatitis. Lo Grippo and Hartman (1955) reported that BPL inactivation of the murine encephalomyelitis virus resulted in relatively small loss of antigenicity compared to live virus. BPL has also been used in the production of an inactivated rabies vaccine for human use. Therefore this body of data should prove useful in the design and testing of inactivation procedures for mabs although there are likely to be significant differences between the effects of chemicals on viruses compared to their effects on mabs. There are other sources of information in this area however which could prove as, if not more, relevant to the study of mabs, that is the data derived from the inactivation of viruses in proteins derived from human blood.

In recent years medical scientists engaged in blood transfusion and plasma fractionation have had to investigate viral inactivation procedures for biologicals with respect to several major problems in the viral field, i.e. HBV, NANB and HIV-1 and -2. There is now a considerable body of data referring to the methods that have been developed for viral inactivation in blood products. Pasteurization procedures were adopted to inactivate HBV in albumin preparations (60°C for 10 hours) and products treated in this way have had an excellent safety record. Unfortunately this process was not as successful with the more heat resistant NANB virus and also proved more difficult to use with the more heat labile blood products such as immunoglobulins and coagulation factors, i.e. Factor VIII and Factor IX. The AIDS problem refocussed researchers' attention on the problems associated with these products and, while sensitive screening tests and donor selection strategies significantly reduced the viral transmission risks associated with coagulation therapy, they did not eliminate them completely. As a result a number of inactivation strategies have been developed and are now used by different producers in an attempt to prevent viral transmission via blood product therapy. These have included:

(a) physical methods such as dry heating of freeze-dried products at temperatures ranging from 60° to 80°C for 30–96 hours; heating of freeze-dried material in a slurry containing N-heptane; steam heating of free-dried product; heating in solution in the presence of stabilizers such as different sugars and glycine, i.e. modified pasteurization (Helderbrandt *et al.*, 1985; Horowitz *et al.*, 1985; Levy *et al.*, 1985; Petriciani *et al.*, 1985; Quinan *et al.*, 1985; Smith *et al.*, 1985).
(b) Chemical treatments such as treatment in solution with solvent and detergent mixtures; treatment in solution with BPL with or without UV irradiation (Horowitz *et al.*, 1988; Lo Grippo *et al.*, 1964; Manucci and Colombo, 1988; Prince *et al.*, 1983; Prince *et al.*, 1986; Prince *et al.*, 1984; Stephan and Kotitschk, 1987).

The majority of these procedures have proven effective for inactivating HIV-1 but hepatitis non-A, non-B has proved more difficult to inactivate.

Despite this it is possible that some of these strategies could prove effective for use with mab preparations. There is, however, little background literature available specifically concerning viral inactivation with human immunoglobulins upon which to design a rational process for mabs. This is because the various methods described were not developed for human immunoglobulin due to their excellent safety record with regard to HIV infection when manufactured by the cold-ethanol procedure. Recently, however, this belief that Ig manufactured by the cold-ethanol procedure is inherently non-infective has required modification in the light of reports of NANB transmission by some intravenous (iv) immunoglobulin products (Lever *et al.*, 1984; Ochs *et al.*, 1985). The manufacture of Ig for iv use involves procedures designed to produce a product with low levels of aggregates, anti-complementary activity and contamination with enzymes of the contact activation system. This has been achieved by treating Cohn fraction 11 with traces of pepsin at pH4. It has been proposed

(Welch *et al.*, 1983) that pH4/pepsin treatment may provide a significant degree of viral inactivation and more recently Reid *et al.* (1988) showed that this treatment did indeed inactivate a range of lipid-enveloped viruses including HIV-1.

Some of the techniques that have been used with blood products were initially used for the preparation of killed viral vaccines, e.g. BPL with or without the UV irradiation (Lo Grippo, 1960; Prince *et al.*, 1983). As described previously, BPL has been widely and successfully used to inactivate viral vaccines although there have been reports of a tailing effect associated with its use (Doel, 1985). This agent has been employed to inactivate viruses in blood products without apparent problems (Heinrich *et al.*, 1987) although there is some evidence that BPL treatment significantly affects the reactivity of polyclonal IgG with Fc receptors (Jungi *et al.*, 1986). This is not perhaps surprising since BPL was not employed initially in order to inactivate viruses but to eliminate anti-complementary activity in immunoglobulin preparations intended for iv use (Nelson, 1988). Thus the use of BPL involves some kind of chemical modification of the immunoglobulin molecule, although to what degree is not really known. BPL readily interacts with proteins and thus the inactivation kinetics would be influenced by both the nature of the proteins in the preparation and the protein concentrations present. In view of the problems encountered in the past due to viral inactivation during vaccine manufacture it would be essential to develop accurate kinetics of viral inactivation if this, or any other chemical, were to be used for the treatment of mab preparations.

There is as yet little data available concerning the application of viral inactivation strategies to mab preparations. In a recent report we have demonstrated (Harbour *et al.*, 1989) that it is possible to inactivate a particularly heat resistant virus (vaccinia) while retaining mab reactivity with its specific antigen. The procedure involved freeze-drying of the mab and subsequent heating at 80°C for 72 hours. The preliminary results from the application of this physical process were most encouraging but further detailed development work is required to assess the effect of the procedure on mab structure and function. The process that was developed has certain advantages over chemical methods, particularly the fact that it is an endstage process in that inactivation can be performed in the final product vial and thus further contamination by adventitious viruses cannot occur. However, this type of process, being dependent on sophisticated equipment, would be more expensive than a chemical approach. In addition the procedure could prove difficult to optimize if different mabs, as one would expect, have different freeze-drying characteristics and heat susceptibilities, although these problems should not prove insurmountable. Perhaps an ideal process for a large producer would involve the preliminary use of a viral-inactivating chemical prior to mab purification in order to protect downstream processing personnel, followed by a heat inactivation step designed to inactivate viruses as the final process step.

As stated previously, we believe viral inactivation procedures are essential components of the downstream processing of mabs. There is, however, an urgent need for standards or appropriate models that can be agreed upon with whatever process is employed whether physical or chemical. These include, on the one hand, viral models that can be used to assess the effectiveness of a particular inactivation program and, on the other hand, appropriate mab models with which to assess the effect of the process on the structure and functional integrity of the molecule.

References

1. Australian drug evaluation committee (1988) Guidelines for the production of monoclonals intended for therapeutic use, National Biological Standards Laboratory, Commonwealth Department of Health.
2. Bartal AH, Feit C, Erlandson R and Hirshaut Y (1982) The presence of virus particles in clones secreting monoclonal antibodies. New Med. 306(23): 1423.
3. Bartal AH and Hirshaut Y (1987) Retrovirus and hybridoma formation, bystanders or active participants? In: Bartal and Hirshaut (eds) Methods of Hybridoma formation (pp. 41–61) Humana Press, USA.

4. Builder SE, Ogez JR, Van Reis R, Paoni NF, Arathoon R and Lubiniecki AS (1989) Process development and regulatory control of tissue type plasminogen activator. In: Spier RE, Griffiths JB, Crooy P and Stephenne J (eds) Advances in animal cell biology and technology (pp. 452–464) Butterworths, Guildford.
5. Carthew P (1986) Is rodent virus contamination of monoclonal antibody preparations for use in human therapy a hazard? J. Gen. Virol. 67: 963–974.
6. Cloyd MW, Hartley JW and Rowe WP (1981) Genetic study of lymphoma induction by AKR mink cell focus-inducing virus in AKRxNFS crosses. J. Exp. Med. 154: 450–459.
7. Collet MC, Moennig V and Hurtinek MC (1989) Recent advances in pestivirus research. J. Gen. Virol. 70: 253–266.
8. Collins MJ and Parker JC (1972) Murine viral contaminants of leukemia viruses and transplantable tumours. J. Nat. Cancer Inst. 49: 1139–1143.
9. Commission of the European Communities (1988) Guidelines on the production and quality control of monoclonal antibodies of murine origin intended for use in man. TIBTECH 6: G5–G8.
10. Crawford DH, Huehns ER and Epstein MA (1983) Therapeutic use of antibodies. Lancet i: 1040.
11. Crawford DH and Azim T (1987) The use of the Epstein-barr virus for the production of human monoclonal antibody secreting cell lines. In: Brown J (ed) Human monoclonal antibodies (pp. 1–6). IRL Press, Oxford, Washington DC.
12. Doel JR (1985) Inactivation of viruses produced in animal cell culture. In: Spier RE and Griffiths JB (eds) Animal cell biotechnology (pp. 129–149). Academic Press, London.
13. Finter MB and Fantes KH (1980) The purity and safety of interferons prepared for clinical use (: the case for lymphoblastoid interferons) In: Gresser (ed) Interferon 2 1980 (pp. 65–80). Academic Press, New York.
14. Griffiths JB (1988) Cell substrates and world health. Cytotechnology 1: 111–112.
15. Harnomoto Y, Kobayashe S, Yamaguchi K, Iikima H, Manabe S, Tsurumi T, Aizawa H and Yamamoto N (1989) A novel filtration method for removal of human immunodeficiency virus: filtration with porous polymeric membranes. Vox Sang. 56:230–236.
16. Harbour C, MacLeod AL, Reid KG and McIntosh RV (1989) Viral inactivation strategies for monoclonal antibodies: dry heating. Biotech. Techniques 3: 261–264.
17. Heine UI, Kramarsky B, Wendel E and Suskind RG (1979) Enhanced proliferation of endogenous virus in Chinese hamster cells associated with microtubules and the mitotic apparatus of the host cell. J. Gen. Virol. 44: 45–55.
18. Heinrich D, Sugg U, Brackman HH, Stephan W and Lissener R (1987) Virus safety of beta-propiolactone treated plasma preparations. Clinical experiences. Develop. Biol. Standard. 67: 311–317.
19. Heldebrant CM, Gomperts ED, Kasper CK, McDougal JS, Friedman AE, Hwang DS, Mushmore E, Jordan S, Miller R, Sergis-D E and Lam W (1985) Evaluation of two viral inactivation methods for the preparation of safer Factor VIII and Factor IX concentrates. Transfusion 25: 510–515.
20. Horowitz B, Piet MPJ, Prince AM, Edwards CA, Lippin A and Walakouits L (1988) Inactivation of lipid enveloped viruses in labile blood derivatives by unsaturated fatty acids. Vox Sang. 54: 14–20.
21. Horowitz B, Wiebe ME, Lippin A, Vandersa J and Stryker MH (1985) Inactivation of viruses in labile blood derivatives. Transfusion 25: 523–527.
22. Jiang Shu de, Bye D and Cox JC (1986) Inactivation of poliovirus with beta-propiolactone. J. Biol. Standard. 14: 103–109.
23. Jungi TW, Santer M, Lerch P and Barandun S (1986) Effect of various treatments of gamma-globulin (IgG) for achieving intravenous tolerance on the capacity to interact with human monocyte receptors. Vox Sang. 51: 18–26.
24. Kacian DL, and Spiegelman S (1974) Purification and detection of reverse transcriptase in viruses and cells. Meth. Enzymol. 29E: 150–173.
25. Klement W, Rowe WP, Hartley JW and Pugh WE (1969) Mixed culture cytopathogenicity: a new test for growth of murine leukemia viruses in tissue culture. Proc. Nat. Acad. Sci. 63: 753–758.
26. Kuwert W, Rowe WP, Hartley JW and Pugh WE (1977) Post-exposure use of human diploid cell culture rabies vaccine. Devel. Biol. Standard 37: 123–127.
27. Lever AML, Webster ADB, Brown D and Thomas HC (1984) Non-A, non-B hepatitis occurring in gammaglobulamic patients after intravenous immunoglobulin. Lancet II: 1062–1064.
28. Levy JA (1983) Mouse plasmacytoma cells produce infectious type C particles. Lancet II: 522.
29. Levy JA, Lee HM, Kawahata RT, and Spitler LE (1984) Purification of monoclonal antibodies from mouse ascites eliminates contaminating infectious mouse type C viruses and nucleic acids. Clin. Exp. Immunol. 56(1): 114–120.
30. Levy JA, Mitra GA, Wong MF and Mozen MM (1985) Inactivation by wet and dry heat of AIDS associated retroviruses during factor VIII purification from plasma. Lancet I: 1456–1457.
31. Liu WT, Natori T, Chang KS and Wu AM (1977) Reverse transcriptase of foaming virus. Purification of the enzymes and immunological identification. Arch. Virol. 55(3): 187–200.
32. Le Grippo GA (1960) Investigation of the use of beta-propiolactone in virus inactivation. Ann. N.Y. Acad. Sci. 83: 578–594.
33. Le Grippo GA and Hartman FW (1955) Antigenicity of beta-propiolactone inactivated by virus vacines. J. Immunol. 75: 123–127.
34. Lo Grippo GA, Wolfram BR and Rupe C (1964) Human plasma treated with ultraviolet and propiolactone, a six year clinical evaluation. J.A.M.A. 178: 722–726.
35. Lubiniecki AS (1989) Endogenous retrovirus of continuous cell substrates. In: Spier RE, Griffiths JB, Crooy P and

Stephenne J (eds) Advances in animal cell biology and technology for bioprocesses (pp. 85–92). Butterworths, Guildford.
36. Lubiniecki AS and May LH (1985) Cell bank characterization for recombinant DNA mammalian cell lines. Develop. Biol. Standard. 60: 141–146.
37. Lusher JM, Lamon KD and the monoclate study group (1988) A multicentre study to determine the hepatitis safety of monoclate, a new highly purified FVIII:C preparation. XVIII international congress of the world federation of haemophilia: Madrid, May 26–31, 1988: abstr p. 124.
38. MacLeod AJ (1988) The use of plasma protein fractions as medium supplements for animal cell culture. In: A Flechter (ed) Advances in biochemical engineering and biotechnology (37: 41–56) Springer Verlag.
39. Manley KF, Givens JF, Taber RL and Ziegel FF (1978) Characterization of virus like particles released from the hamster cell line CHO-K1 after treatment with 5-bromodeoxyuridine. J. Gen. Virol. 39(3): 505–517.
40. Mannucci PM, and Colombo M (1988) Virucidal treatment of clotting factor concentrates. Lancet II: 782–785.
41. Martin DW (1985) An overview of risk association with viruses endogenous to cell substrates. In: Hopps HE and Petricciani JC (eds) Abnormal cells, new products and risks (pp. 29–35). Tissue culture association, Gaithersburg.
42. Moyer MP and Moyer RC (1987) Potential risks of tumour viruses subgenomes in the production of biologicals. Develop. Biol. Standard. 68: 51–62.
43. Nelson JM (1988) Intravenous immunoglobulin: silver bullet of the 80s. Lab. Medicine 19: 799–805.
44. Ochs HD (1985) Non-A, non-B hepatitis and intravenous immunoglobulin. Lancet I: 404.
45. Onions DE, Lees GM and Sear CHJ (1989) Retroviruses: new problems and new solutions in the safety testing of biologicals for regulatory approval. In: Spier RE, Griffiths JB, Crooy P and Stephenne J (eds) Advances in animal cell biology and technology for bioprocesses (pp. 72–75). Butterworths, Guildford.
46. Peebles PT (1975) An in vitro induction assay for xenotropic murine leukaemia viruses, feline leukaemia virus C, and the feline primate viruses RD-114/CC/M-7. Virology 67: 288–296.
47. Petricciani JC, McDougal JS and Evatt BL (1985) Case for concluding that heat-treated licensed anti-haemophilic factor is free from HTLV-III. Lancet II: 890–891.
48. Petricciani JC and Regan PJ (1987) Risk of neoplastic transformation from cellular DNA: calculation using the oncogene model. Develop. Biol. Standard. 68: 43–49.
49. Petricciani JC, Salk PL, Salk J and Noguchi PD (1981) Theoretical considerations and practical concerns regarding the use of continuous cell lines in the production of biologics. Develop. Biol. Standard. 50: 15–25.
50. Prince AM, Stephan W and Brotman B (1983) Beta-propiolactone ultraviolet irradiation: a review of its effectiveness for inactivation of viruses in blood derivatives. Rev. Infect. Dis. 5: 92–107.
51. Prince AM, Horowitz B and Brotman B (1986) Sterilization of hepatitis and HTLV-III viruses by exposure to tri(-n-butyl)phosphate and sodium cholate. Lancet I: 706–710.
52. Prince AM, Horowitz B, Brotman B, Huima T, Richards L and Van der Ende M (1984) Inactivation of hepatitis B and Hutchinson strain non-A, non-B hepatitis viruses by exposure to tween 80 and ether. Vox Sang. 46: 36–43.
53. Quinnan GV, Wells MA, Wittek AE, Phelan MA, Mayner RE, Feinston S, Purcell RH and Epstein JS (1985) Inactivation of human T-cell lymphotropic virus, type III by heat, chemicals and irradiation. Transfusion 26: 485–483.
54. Reid KG, Cuthbertson B, Jones ADL and McIntosh RV (1988) The potential contribution of mild pepsin treatment at pH 4 to the viral safety of human immunoglobulin products. Vox Sang. 55: 75–80.
55. Shek WR (1987) Detection of murine viruses in biological materials by the mouse antibody production test. In: Seaver SS (ed) Commercial production of antibodies (pp. 3–16). Marcel Dekker, Inc. NY & Basel.
56. Smith JK, Winkleman L, Evans DR, Haddon ME and Sims G (1985) A pasteurized antithrombin III concentrate for clinical use. Vox Sang. 48: 325–332.
57. Stephan W and Kotitschk (1987) Safety of beta-propiolactone/UV treated PPSB (factor IX concentrate). Thromb. Res. Suppl. VII: 58.
58. Weiss RA (1982a) Retroviruses produced by hybridomas. New Eng. J. Med. 307: 1587.
59. Weiss RA (1982b) Hybridomas produce viruses as well as antibodies. Immunol. Today 3: 292–294.
60. Welch AG, McIntosh RV and Foster PR (1983) Non-A, non-B hepatitis from intravenous immunoglobulin. Lancet II: 1198–1199.
61. WHO Technical report series (1987) No. 747.
62. Wiebe ME, Beker F, Lazar R, May L, Casto B, Semense M, Fautz C, Garnick R, Miller C, Masover G, Bergmann D and Lubiniecki AS (1989) A multifaceted approach to assure that recombinant tPA is free of adventitious virus. In: Spier RE, Griffiths JB, Crooy P and Stephenne J (eds) Advances in animal cell biology and technology for bioprocesses (pp. 68–71). Butterworths, Guildford.
63. Winkleman L, Owen NE, Haddon ME and Smith JK (1985) Treatment of a new high specific activity factor VIII concentrate to inactivate viruses. Thromb. Haemostas. 54: 19.
64. Wurm FM, Williams SR, Anderson K, Dinowitz M, Obijeski J and Arathoon R (1989) Presence and transcription of endogenous retroviral sequences in chinese hamster ovary cells. In: Spier RE, Griffiths JB, Crooy P and Stephenne J (eds) Advances in animal cell biology and technology for bioprocesses (pp. 76–81). Butterworths, Guildford.

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