Media formulation options and manufacturing process controls to safeguard against introduction of animal origin contaminants in animal cell culture

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Abstract

Technical limitations and evolution of therapeutic applications for cell culture-derived products have accelerated elimination of animal-derived constituents to minimize inadvertent introduction of adventitious viral or prion agents. Practical considerations demand adequate emphasis both on design of the serum-free/protein-free culture environment and on nutrient media manufacturing process controls. Protein components may be acceptable, given adequate attention to synthetic process, sourcing (e.g., geographic location and endemicity, species and tissue/organ) and validated treatment method. Various options exist for re-engineering of traditional serum-free formulations (containing insulin, transferrin and other protein factors) with non-protein substitutes. Caution must also be exercised with sourcing of non-protein additives, particularly amino acids and lipids, to avoid introducing adventitious contaminants. Simple guidelines facilitate adaptation, cryopreservation and recovery of many cell types within a protein-free culture environment. Scrupulous maintenance of facility and equipment and monitoring of process water, air handling systems and technical personnel are required to ensure that approved raw materials are correctly formulated and dispensed. Validated sanitization processes provide additional assurance against cross-contamination from previous batches in a multi-use facility.

Abbreviations: CDM, chemically-defined medium; CHO, Chinese hamster ovary; CIP, clean-in-place; DHFR, dihydrofolate reductase; HEPA, high efficiency particulate air; HSA, human serum albumin; LVLM, large volume liquid manufacturing; PFM, protein-free medium; SFM, serum-free medium; SIP, sterilize-in-place; Tfn, transferrin; TOC, total organic carbon.

Introduction

Historically, efforts to reduce or eliminate serum supplementation from culture media have been motivated by technical or economic concerns. Classical 'serum-free' media were derived by enriching nutrient-fortified basal formulations with insulin, transferrin, albumin and other protein materials which were generally obtained from animal sources.

More recently, global awareness and concern regarding potential adventitious contaminants (e.g., mycoplasma, virus, prion) (Bader et al., 1998a; Ziomek,

1996) associated with media constituents derived from animal tissues and fluids has increased. The escalating frequency of clinical trials for biologicals intended for human therapy and *in vivo* diagnosis, coupled with the emergence of cell and gene therapy and tissue engineering applications, has accelerated efforts to eliminate all constituents of animal origin from these cell culture systems. Significant attention has also focused upon process controls to ensure against inadvertent contamination of nutrient media.

This article examines two facets of this effort: (1) Design and re-engineering of nutrient formula-

tions to identify and replace all medium constituents derived from animal sources (Fike et al., 1991; Gorfien et al., 1998; Price and Evege, 1997); and (2) Design, implementation and validation of media manufacturing process controls to eradicate potential contaminants of animal origin and to ensure against cross-contamination (Jayme et al., 1996; Darling and Spaltro, 1996).

Transition to animal origin-free cell culture environment

Table 1 provides a qualitative summary of cell culture trends observed with increasing degree of biochemical definition of medium constituents. Reduced serum media are generally fortified with insulin, transferrin and nutrient additives to permit significant reduction (but not total elimination) of supplemental serum. Serum-free media (SFM), as denoted by the name, do not require supplemental serum for specified applications; however, they may contain a broad range of additives, including protein factors which may be derived from animal fluids or tissues. Protein-free media (PFM) do not contain supplemental polypeptide factors which would test positive by conventional protein assays; however, they may contain various peptide fragments derived from enzymatic or acid hydrolysis of proteins obtained from animal or plant sources. Chemically-defined media (CDM) are usually also protein-free and are comprised exclusively of well-characterized, low molecular weight constituents.

As described in a subsequent section, non-protein constituents may also originate from animal sources, so elimination of animal-derived protein elements does not necessarily guarantee that all components of the medium formulation are animal origin free. The data summarized in Table 1 for PFM and CDM assume that non-protein constituents were obtained from qualified, non-animal sources.

Table 1 notes that serum reduction or elimination decrease the probability of introducing raw material-associated virus or prion contaminants to the culture environment. The potential for adventitious contamination decreases still further with progression to a protein-free formulation and becomes negligible with a chemically-defined formulation containing no constituents of animal origin.

Elimination of serum-associated bulk protein or other protein additives which may co-purify with target product facilitates downstream purification with

PFM and CDM, possibly reducing the number or complexity of purification steps or resulting in improved product recovery. Pilot cultures should be evaluated to ensure that protein-free environments do not have an adverse impact on yield due to non-specific adsorption, sensitivity to biophysical stresses, or proteolytic degradation. Note: iron chelates frequently used as transferrin substitutes may interfere with ion exchange resins or produce artifactual results with tracking devices.

Elimination of serum negatively impacts the universality of the culture environment. The investigator should anticipate that a given SFM, PFM or CDM will be ideally-suited for a narrower range of cell types and culture applications and may require nutritional adjustment for optimal performance. In some instances, it may be more challenging to adapt parental strains to PFM or CDM (e.g., DHFR⁻ CHO strains) than derived transfectants.

Assuming that the culture has been thoroughly adapted to an optimized nutrient environment, improved biological performance may be observed with conversion to novel, serum-free and protein-free culture conditions. Elimination of serum-associated inhibitors, neutralizing antibodies, and proteolytic enzymes are commonly implicated in this enhancement of cell growth and biological yield. Care must be exercised to minimize biophysical forces and adsorption of product to culture vessel surfaces in the absence of serum, albumin and other bulk protein.

Critical considerations to eliminate risk of animal origin constituents

Protein constituents

Depending upon the application, native protein components may still be acceptable medium constituents. Conventional laboratory research and many *in vitro* diagnostic applications may consider using purified protein components for the foreseeable future.

Regulatory risk, interference with downstream product purification, and the high cost associated with protein-supplemented media have influenced many biopharmaceutical manufacturers to re-engineer culture media and production cell types to eliminate a requirement for protein constituents.

Key factors to evaluate regarding use of protein constituents include the origin of the animal source material (including thorough documentation of chain

Table 1. Cell culture points to consider with component substitution

Cell culture parameter	Reduced serum medium ^a	Serum-free medium ^b	Protein-free medium ^c	Chemically-defined medium ^d
Potential for introducing adventitious contaminants	+++	++	+	--
Complexity of downstream product purification	+++	++	+	+
Improved bioperformance (e.g., growth, product yield)	+/-	++	++	++
Ease of cell culture adaptation to novel formula	+++	+	+/-	+/-

^a *Reduced serum medium*: Basal formulation enriched with nutrients and animal-derived factors to reduce supplemental serum requirement.

^b *Serum-free medium (SFM)*: Basal formulation containing protein factors (which may be animal-derived) which does not require serum to support cellular function.

^c *Protein-free medium (PFM)*: Basal formulation which does not contain any polypeptide materials (may contain animal protein hydrolysate).

^d *Chemically-defined medium (CDM)*: Basal formulation which may also be protein-free and is comprised solely of biochemically-defined low molecular weight constituents.

((--)= negligible; (+/-)= slight; (+)= significant; (++)= much greater; (+++)= very much greater).

of custody), integrity of the collection and purification processes, and validated treatment methods to remove or inactivate potential contaminants.

Source material

The geographic location and pathogen endemicity clearly impacts the security of animal-derived products. Certain nations or regions, by virtue of their geographic isolation and/or absence or low incidence of specified pathogens may be preferred sourcing locations. In addition, certain animal species may be regarded as less susceptible to specified contaminants, or less probable to generate materials of human host pathogenicity. Homologous proteins may also be derived from non-mammalian and non-avian species. Even in an infected animal, certain pathogens predominate in specified organs, tissues or fluids, while other source materials may be pathogen-free. Isolation of a low-risk starting material reduces the probability of infection of derived products (Bader et al., 1998b).

Collection and purification processes

Three primary categories for qualification of raw material suppliers do not eradicate prospective contaminants, but serve to contain their impact and minimize cross-contamination. Products should be maintained within a closed, integral system which precludes ex-

posure to environmental contaminants throughout processing, storage, delivery and formulation. Collection and processing vessels should be sanitized by validated procedures to ensure against residual carryover and subsequent introduction of contaminants. Separation media and other downstream processing components should be treated by validated sanitization methods to ensure against introduction of contaminants while preserving purification matrix functionality.

Treatment method

Various approaches have been reported and are under current investigation to eliminate adventitious contaminants while preserving biological function. Of these, gamma irradiation has been most widely-accepted. Regardless of the treatment employed, two interdependent elements of the validation design remain critical: (1) The proposed treatment must be validated against a broad spectrum challenge panel of representative virus, prion or mycoplasma contaminants in a technically-extrapolatable, scaled-down model system to document eradication of adventitious organisms while preserving biological activity. (2) There must be adequate trend analysis of the incoming bioburden to define, both qualitatively and quantitatively, the expected contaminants and to establish safety margin and confidence levels for proposed treatment methods.

Table 2. Common media constituents and cell culture reagents derived from animal sources

Constituent	Animal source	Non-animal substitute
Insulin	Bovine/porcine pancreas	Bovine or human recombinant
Transferrin	Bovine, porcine or human plasma fraction	Inorganic iron carriers/chelates
Serum protein fractions (e.g., albumin, fetuin, lipoproteins)	Bovine or other animal serum	Lipid delivery alternatives Plant-derived hydrolysates
Protein hydrolysates	Lactalbumin, peptones, casein	Plant-derived hydrolysates
Lipids/sterols	Ovine/human cholesterol Piscine lipids, Porcine liver	Plant-derived sterols; Synthetic and plant-derived fatty acids
Growth and attachment factors	Murine/bovine organ digests	Recombinant factors Collagen precursors
Amino acids (e.g., tyrosine, cyst(e)ine, hydroxyproline)	Human hair; Avian feathers; Bovine collagen; Bovine/porcine bone gelatin	Recombinant, synthetic or plant-derived amino acids
Surfactants (e.g., Tween 80)	Bovine tallow	Plant-derived polysorbate
Dissociating enzymes (e.g., trypsin)	Porcine pancreas	Plant-derived enzymes Microbial enzymes

Non-animal origin/non-protein substitutes

To re-engineer a classical serum-free formulation to ensure against any constituents of animal origin, there are two categories of concern: (1) replacement of any proteinaceous elements of the serum-free formulation with non-protein substitutes of non-animal origin and equivalent bioactivity; and (2) identification of intermediary metabolite constituents of animal origin and qualification of reliable sources of non-animal alternatives. Table 2 illustrates common medium constituents which are traditionally obtained from mammalian sources and lists non-animal origin substitutes which we have qualified for various cell culture applications.

Protein substitution by non-protein components

Transferrin: Various alternative methods have been examined for efficient, non-toxic delivery of iron to cells. Figure 1 illustrates results obtained in serum-free culture of 293 cells. The positive reference (squares) was supplemented with human transferrin, while cells were unable to proliferate in transferrin-free deletion medium (circles). Supplementation of the negative reference medium with various inorganic iron carriers (two are illustrated in this figure; triangles) restored normal proliferation. Note that, for this assay system, 50% higher levels of iron carrier A were required than for iron carrier B to provide equivalent growth. These data also suggest that iron carriers should be titrated to determine the optimal concentration for each system.

Albumin: Serum albumins perform a variety of cell culture functions and represent the predominant protein constituent of many mammalian sera. Some functions may be replaced by alternative lipid binding and cellular delivery options and through medium supplementation by other intermediary metabolites, shear protectants and antioxidants. However, these replacements may not be sufficient for some culture systems. Plant-derived hydrolysates may be utilized to generate a nominally protein-free formulation, while still retaining a formulation devoid of animal origin constituents. Figure 2 shows the preliminary results of such an experiment, using a Vero cell bioassay. Deficient medium lacking albumin was prepared and performance was contrasted in a formulation supplemented with human serum albumin. Four plant-derived hydrolysates were added to the deficient medium and screened at comparable concentrations against the HSA control. Hydrolysate D was subsequently titrated to yield a formulation with growth performance equivalent to HSA-supplemented controls (data not shown).

Other protein additives: Many protein additives may be replaced by recombinant factors or hydrolysates or by adjustment of the low molecular weight composition with appropriate trace elements and precursors. Two materials warrant particular mention.

Insulin is among the most ubiquitous protein additives for serum-free formulations. The most convenient

Comparison of Iron Carriers A, B and Human Transferrin

Figure 1. Transferrin replacement by inorganic iron carriers. Two inorganic iron carriers were evaluated in a mammalian cell bioassay using human embryonic kidney (HEK 293) cells. A complete, serum-free nutrient formulation lacking transferrin was supplemented with either Inorganic Iron Carrier A (ferrous sulfate EDTA; 60 μ M), Inorganic Iron Carrier B (ferrous citrate complex; 40 μ M) or Human Transferrin (5 mg l⁻¹). A fourth culture without any iron carrier or transferrin served as a negative growth control. Cell count was determined electronically with viability assessment by trypan blue dye exclusion.

substitute for bovine pancreatic insulin is recombinant human or bovine insulin. Most bioassay systems find the native or truncated forms to be indistinguishable. Protein-free media have successfully replaced the insulin requirement by appropriate augmentation of the basal formulation by trace metals (principally, zinc) and other constituents.

Fetuin is an alpha globulin fraction of fetal bovine serum which may be obtained by various purification techniques and which has been utilized for serum-free culture of various mammalian cell types. The fetuin requirement of CHO cells may also be replaced in cell culture by protein-free and chemically-defined alternatives without loss of biological performance and with substantial cost benefit.

Substitutes for animal-derived low molecular weight nutrients

Amino acids: There exists only a relatively small number of manufacturers of amino acids of sufficient

purity for cell culture applications. One primary manufacturer clearly states that their starting materials are of animal origin, but utilizes validated treatment methods to inactivate adventitious contaminants. The other primary manufacturers implied synthetic origin for all amino acid products, but further investigation revealed that several amino acids (e.g., tyrosine, cyst(e)ine and hydroxyproline) are derived from various animal source materials, including human hair, avian feathers, bovine collagen, and bovine or porcine gelatin. Alternate suppliers have been qualified who manufacture these amino acids by fermentation techniques or from plant materials, rather than by purification from animal origin starting materials.

Lipids: Animal-sourced lipids have been problematic to replace for some culture systems without some loss of bioactivity. Typically, lipids used for cell culture are derived from ovine 'wool grease' (lanolin), marine oils, and porcine liver. Progress is be-

Comparison of Different Plant Hydrolysates on Growth of Vero Cells

Figure 2. Albumin replacement by plant hydrolysates. Four hydrolysates derived from different plant species were evaluated in a mammalian cell bioassay using Vero cells (Price and Evege, 1997). Hydrolysates were purchased from Quest International: A = wheat #1; B = wheat #2; C = soybean; D = rice. Each hydrolysate was titrated and the data reflect the optimal concentration for each substituted hydrolysate. A complete nutrient formulation lacking albumin was supplemented either with a hydrolysate sample or with human serum albumin (500 mg l^{-1} ; positive control). Qualitative prioritization of candidate hydrolysates preceded a second optimization step to obtain performance equivalent to the HSA control materials.

ing made to qualify plant-derived sterols to replace animal-derived cholesterol and other lipids, but some formulations still require piscine oil lipids.

Facility and process controls

While surveillance of incoming raw materials is critical to ensuring product integrity, deficiencies in facility or process design and personnel function may also inadvertently introduce contaminants.

Facility design

Work area configuration

One decision driving the work area configuration is whether it will be dedicated to a single task or qualified for multiple applications. Multi-use configurations require point-of-use contamination controls due to air

flow dynamics and higher perceived risk of material cross-over. Single-use areas limit operational flexibility, resulting from segregated/dedicated rooms and equipment.

The decision regarding process compartmentalization vs. open area processing will determine spatial arrangement of physical barriers and point-of-use contamination controls. Over-compartmentalization ('maze effect') may impede efficiency and create potential safety hazards. Open area processing requires strict discipline of personnel and equipment movement to avoid violating work flow paths.

Air-handling systems

Environmental controls to minimize cross-contamination may be categorized into general and micro-environment considerations. General decisions regarding single-pass vs. multi-pass HEPA and the rate of air ex-

changes per unit volume will have profound cost implications, requiring cost-to-value analysis of real vs. perceived benefits. Considerations impacting the localized micro-environment include determination of centralized vs. point-of-use dust collection and requirements for systems validation and assurance monitoring.

Process water systems

Process water may be a frequent source of contamination, either by microorganisms or trace contaminants from the municipal system. Piping configurations generally specify a continuous, recirculating loop, but may prefer dead-ends in 'sensitive' processing areas. Such specialized delivery systems may be justifiable, but increase the cost and complexity of routine water sampling. Usage drop points also impact quality and economy, since a poorly selected site could increase the potential for introduction of systemic contamination. Sampling ports must be situated at locations which are both effective and representative.

Process design

Material flow

Key issues for effective materials flow are safeguards to eliminate the potential for component mix-up or gross cross-contamination and to segregate 'sensitive' materials. Usage reconciliation is indicated for all controlled, costly or otherwise 'sensitive' process components or materials. As before, analysis of real vs. perceived benefits may facilitate rationalization of cost and flexibility vs. process control considerations.

Equipment: Process design will be impacted by the decision to use dedicated vs. general use equipment. Cleaning validation and control mechanisms will vary with the option to re-use or discard previously utilized equipment (e.g., disposable tank liners or encapsulated filters).

Sanitization of equipment and process work areas: Consideration of cost vs. benefit must be pro-actively considered during protocol and validation design phases. Rinsate confirmation is minimally required. Validation should focus on specific process claims (e.g., protein removal, virus inactivation). Process design must select appropriate sanitizing agents and identify the challenge material, probable contaminants and representative contact surfaces. The validation study design must translate to an effective and

economical operational activity (i.e., an 'overkill' approach can accelerate initial validation, but may demand higher-than-necessary routine cleaning costs).

Monitoring of equipment and work areas: Equipment must undergo batch-dependent confirmatory testing of the sanitization process via rinsate analysis (e.g., conductivity, TOC). Archivable print-outs of sanitizing and sterilizing equipment can document equipment cleaning activity. Work area monitoring may require continuous data collection or periodic confirmation of procedural effectiveness depending upon application, with supplemental confirmation for processing areas associated with 'sensitive' materials.

Personnel

Given adequate facility and process design and effective systems controls, training, outfitting, deployment and monitoring of technical personnel remain a critical focus to ensure against cross-contamination.

Training: Employees new to the area should be provided with a general awareness orientation, which is reinforced periodically through in-service training. Proficiency assessment should be routinely documented.

Outfitting: To ensure against transfer of contaminating materials, various levels of captive uniforms should be considered. General plant uniforms would be required at the lowest level of control, with captive gowning and storage locations for work area or process-specific functions representing more stringent controls. The decision to use disposable vs. recycled uniforms becomes a cost vs. benefit issue.

Deployment: Conflicting priorities may dictate preferences between two beneficial deployment strategies. Dedication of personnel to a specific work area or process may offer control benefits, while cross-functional capability provides additional flexibility and quality of life benefits.

Monitoring: Monitoring activities should be scheduled to capture realistic 'worst case' scenarios, such as the beginning and ending of a shift, defined break-points during 'time' or 'activities' within the shift, or via continuous monitoring. Parameters should be established for acceptability, alert, and action.

Case study

The Large Volume Liquid Manufacturing (LVLM) System was developed by Life Technologies, Inc., in partnership with bioKinetics, for high precision preparation of large batches (5000–50 000 l) of liquid media and other fluids from concentrated intermediates. The system integrates programmed dilution of multiple concentrated (30–100×) components using a mixing skid, sterile filtration, and automated dispensing into bulk containers.

The features and benefits of this system for large-scale manufacture have been described previously (Jayme et al., 1996). In-process monitoring and end product evaluation previously demonstrated homogeneity of biochemical composition and biological activity throughout the LVLM manufacturing process.

We present this case study as an overview of required process validation which has been completed or initiated to commission the LVLM system for multiple uses and to manage the risk of cross contamination by residual medium constituents or adventitious agents (Table 3).

Discussion

Table 2 summarized the scope of cell culture medium constituents identified in our study as derived from animal origin. Naively, perhaps, we assumed that our effort to eliminate animal-derived constituents would be limited exclusively to proteinaceous additives. In reality, our investigation opened a Pandora's box of issues, the resolution of which can best be described as a 'work in progress'.

From a positive perspective, serum-free, protein-free and biochemically-defined culture formulations are commercially-available to support various relevant biotechnology applications. Serum-free formulations have achieved total protein concentrations substantially below 20 $\mu\text{g ml}^{-1}$. Protein-free and chemically-defined formulations have eliminated the presumed-axiomatic requirement for insulin and transferrin without loss of performance or stability.

Further, suppliers of common non-proteinaceous additives (e.g., amino acids, lipids, sterols) have been identified and qualified, so that many catalog and customized medium formulations may be commercially manufactured so that none of the constituents are animal-derived.

However, some 'synthetic' proteins, amino acids and other constituents may derive from microbial fer-

mentation broths and purification processes which contain crude animal-derived fractions (e.g., peptones, infusions, extracts, and homogenates). Suppliers of these raw materials are well aware of these concerns and many are actively working to eliminate animal components from their fermentation broths. Even so, risk assessment suggests that the probability of residual virus or prion contamination from these processes is minimal (Bader et al., 1998b). The observation that many such amino acid and lipid constituents have been components of hyperalimentation solutions administered intravenously without adverse findings to humans over decades adds some assurance to this approach.

While the ultimate objective may be a chemically-defined medium with no ill-defined constituents or components of animal origin, other interim approaches may be useful. Some cell types have proved difficult to adapt to CDM without significant decrease in proliferative rate, maximal cell density, or product expression. For such applications, a protein-free medium supplemented with hydrolysates of plant origin may be effective.

Lipids and sterols of non-animal origin have been problematic to source in sufficient quantity, purity and cost for industrial biotechnology applications. Plant-derived sterols and fatty acids of synthetic or plant origin may replace animal origin materials, including cholesterol. For cell types with a critical nutritional requirement for cholesterol, we suggest replacing sheep-derived cholesterol with concentrated additives of cholesterol and other lipids derived from fish. Although fish, obviously, are animals, the pragmatic assumption is that viral susceptibility of cells derived from mammals and other warm-blooded species is substantially reduced for residual viral contaminants of piscine origin.

A discussion of elements within the cell culture environment which are derived from animal materials and, thus, allow a finite risk of adventitious agent contamination would be incomplete without mention of several other factors. Trypsin remains the most frequently-used enzyme for tissue dissociation and removal of adherent cells from surfaces. Derived from porcine pancreas, trypsin is screened for parvovirus; however, treatment methods and non-mammalian alternatives should be considered to eliminate risk of parvovirus contamination. Collagen, typically of bovine origin, and other extracellular matrix elements from murine or bovine organ extracts may be used to coat surfaces (e.g., plasticware, microcarrier

Table 3. Large volume liquid manufacturing (LVLM) process validation outline

Validation category	Validation objective	Validation activities
General processing controls	Aseptic processing	Compendial guidelines for processing of aseptic materials (modified to fit this application), targeting maximum batch size, environmental classification and monitoring for all aseptic connections, media fill validation of nutrient broths under 'worst case' conditions
	Filter validation	Generation of a matrix of filter sizes and combinations to accommodate prospective protein binding and incoming bioburden at production scale; definition of acceptable forward flow diffusion parameters for post-SIP and post-production integrity confirmation
	Automated dispensing	Incorporated into aseptic processing validation, under operating parameters demonstrated to deliver acceptable fill tolerances
	Packaging integrity	Classical shipping and 'drop' tests to demonstrate durability of primary and secondary packaging assemblies and connections; microbial ingress testing of transfer sets
Sanitization and sterilization	CIP chemical residuals	Rinsate studies to demonstrate endpoint conductance and endotoxin and significant and acceptable reduction in TOC concentration; Adhesion and drying of proteinaceous 'soiling' solution, followed by proposed CIP cycle and swab studies to confirm absence of residuals
	Adventitious agent eradication	Downscale system challenge with model viruses and prion panel, followed by sanitizing cycle exposure to quantitate kinetics of agent eradication; Correlation with 'worst case' incoming bioburden
	SIP validation	Steam distribution and cold spot mapping with multiple thermocouples; Steam penetration studies with spore indicators

beads, artificial substrates) to promote cell attachment, spreading and three-dimensional structure. Recombinant attachment factors, synthetic peptides and collagen precursors may be utilized to minimize risk of adventitious agent contamination. Surfactants (e.g., Tween 80) may be derived from bovine tallow or other animal-derived lipid fractions. Consideration may be given to replacing these detergents with amphipathic molecules derived from plants.

For all of these potential contributors to contamination, a cautionary note may be advisable. While our original goal was to eliminate all raw materials of animal origin, we found it necessary to strike an interim balance between this ultimate objective and the pragmatic realities of product availability and the perceived acceptability of negligible contamination risks (Federal Bulletin, 1994; Bader et al., 1998b).

Conclusion

Regulatory concern has increased regarding potential contamination of biologicals by adventitious agents introduced via medium constituents or manufacturing process defects. Nutrient formulations used in

cell culture bioreactors may be engineered to eliminate constituents of animal origin without sacrificing biological performance. Process controls must also be implemented in nutrient media kitchens, purification suites and product formulation and storage areas to provide an integral manufacturing process and to minimize risk of cross contamination.

References

- Bader F, Davis G, Dinowitz M, Garfinkle B, Harvey J, Kozak R, Lubiniecki A, Rubino M, Schubert D, Wiebe M and Woollett G (1998a) Assessment of risk of bovine spongiform encephalopathy in pharmaceutical products, Part 1. *BioPharm* 11: 20–31, 56.
- Bader F, Davis G, Dinowitz M, Garfinkle B, Harvey J, Kozak R, Lubiniecki A, Rubino M, Schubert D, Wiebe M and Woollett G (1998b) Assessment of risk of bovine spongiform encephalopathy in pharmaceutical products, Part 2: Example risk assessment for a hypothetical product. *BioPharm* 11: 18–30.
- Darling AJ and Spaltro JJ (1996) Process validation for virus removal: Considerations for design of process studies and viral assays. *BioPharm* 9: 42–50.
- Federal Ministry of Health (Germany) (1994) Guidelines on safety measures in connection with medicinal products containing body materials obtained from cattle, sheep, or goats from minimizing

- the risk of transmission of BSE and scrapie. Federal Bulletin No. 40.
- Fike RM, Pfohl JL, Epstein DA, Jayme DW and Weiss SA (1991) Hybridoma growth and monoclonal antibody production in protein-free hybridoma medium. *BioPharmacology* 4: 26–29.
- Gorfien SF, Dzimian JL, Tilkins ML, Godwin GP and Fike RM (1998) Recombinant protein production by CHO cells cultured in a chemically defined medium. In: Nagai K and Wachi M (eds), *Animal Cell Technology: Basic and Applied Aspects*, Vol. 9, (pp. 247–252). Kluwer Academic Publishers, Dordrecht.
- Jayme DW, Kubiak JM, Battistoni TA and Cady DJ (1996) Continuous, high capacity reconstitution of nutrient media from concentrated intermediates. *Cytotechnology* 22: 255–261.
- Price PJ and Evege EK (1997) Serum-free medium without animal components for virus production. *Focus* 19: 67–69.
- Ziomek CA (1996) Minimization of viral contamination in human pharmaceuticals produced in the milk of transgenic goats. *Dev Biol Stand* 88: 265–268.