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Validation and Quality Assurance: Risk and Potential of Process Changes

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Abstract. The development of biotechnical processes is a very complex operation. It involves the creation and verification of a strong expression system, selection and characterization of an appropriate host cell, optimization of a fermentation media, design of a fermentation process yielding high product titers, development of a tailor-made recovery process resulting in high yields and product quality, scale up of the biotechnical process to the commercial scale, formulation of the final product, validation of the entire process, analytical product characterization, and a comprehensive stability programme of the cell line and the final product.

In many cases the time frame for such a development programme is determined by the competitive situation, therapeutic needs, patent life-span, and the return on investment. In addition, host vector systems, manufacturing technologies, and protein analytical methods improve rapidly and as a result regulatory standards become tighter.

As a consequence of the rapid evolution in this field, a process can lose its economic value within five years after launch of the product and product quality might no longer correspond to the actual state of the art. Both facts can be the driving force for process changes in the vector system, the host cell, the cell culture media, fermentation technology, scale, purification process and the manufacturing site.

In order to comply with the clinical results of the Process License Application (PLA) for the product, extensive development-chemistry is required to demonstrate product identity and efficacy after process changes.

In cases where product quality is influenced by the manufacturing process, which is often the case for the production of complex glycoproteins, the question of the pharmacological significance of different glycoforms is raised. Process changes with this impact certainly require bioequivalence studies with the product derived from the modified process in comparison to the product derived from the previous process. If the results of such studies would show a significant difference between the products of both processes, phase III studies with an appropriate number of patients would have to be carried out.

1. Process Changes on the Level of the Genetic Construct

Changes in the genetic construct are directed towards enhanced product expression levels. Changes of the genetic construct can increase the productivity at least 2–10 fold. According to regulatory guidelines all such changes on the nucleotide level have to be carefully verified to ensure that the primary amino-acid sequence of the product is not altered, because changes in the amino-acid sequence of the active protein may significantly affect biological activity and may contribute to an antigenic effect.

As a consequence, the FDA recommends the verification of the genetic construct at various stages of the host cell (*Table*).

How important a verification of the genetic construct for ensuring the fidelity of the amino-acid sequence can be, and what impact amino-acid sequence changes in the biological activity can have can be seen by comparing the two tissue plasminogen activator molecules Alteplase and Duteplase.

The fibrin specificity of tissue plasminogen activator is facilitated by the kringle structures and enhanced by the finger region of the molecule.

Alteplase, the *native* tissue plasminogen activator molecule, has valin in position 245 in the fibrinspecific kringle 1 (*Fig. 1*). In Duteplase, the valin in this position is replaced by methionine. Although this change can be detected at the level of the nucleotide sequence, protein analytical chemistry by tryptic peptide mapping in combination with amino acid sequencing is a qualified tool to detect this change too.

If the relative specific activities of Alteplase and Duteplase are compared in a clot-lysis assay, a direct chromogenic assay or an indirect chromogenic assay, Duteplase demonstrates only 50–60% of the activity of Alteplase (*Fig. 2*). This demonstrates that minor changes in the amino-acid sequence can have tremendous effects on the potency of the protein molecule.

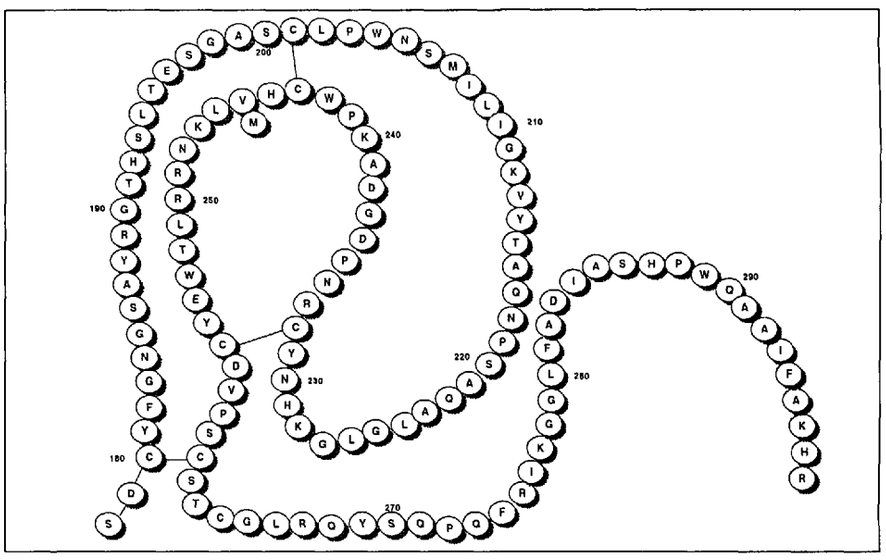

Fig. 1. *Kringle 1 of tissue plasminogen activator*

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Table. *Verification of Genetic Construct at Various Stages of the Host Cell*[a])

Initial MCB	MWCB	PPCB	New MCB
Origin and source of nucleic acid	Identity assessment by restriction endonuclease mapping	Integrity of the expression construct	Acceptance criteria for new clone and protein produced
Assembly of expression construct	Copy number	Consistency of yield from full scale	Determination of integrity of expression construct
Description of promotors, termination region, selection marker, enhancer, fusion protein	Insertions or deletions	Criteria for rejection of culture lots	Verification of nucleotide sequence encoding the protein – single genomic copy: sequencing of total genomic DNA – Multiple chromosomal insertions: sequencing of mRNA or cDNA – extrachromosomal: sequencing of the isolated expression construct
Restriction endonuclease digestion map			Integrated expression system – copy number – large insertions and deletions – number of integration sites
Nucleotide sequence analysis of coding region			Extrachromasomal expression system – Percentage of retained expression construct under selecting and non-selecting conditions
Alterations from native sequence and consequences for pharmacological features			
Characterization of additional sequences: introns, flanking region			
Transfer of expression construct			
Amplification of expression construct			
Copy number of expression construct			

[a]) MCB: master cell bank; MWCB: master working cell bank; PPCB: post production cell bank; New MCB: new master working cell bank.

2. Changes in Culture Media

In cell-culture fermentation a goal for process changes can be the avoidance or the reduction of foetal calf serum in order to minimize the protein load for the downstream process and to reduce the protease content during fermentation and downstream processing.

This has also been a goal for the process improvements in the manufacturing process for tissue plasminogen activator. Manufacturing of tissue plasminogen activator in serum containing media leads to a predominantly two chain-molecule by cleavage of tissue plasminogen activator between the amino acids 275 and 276. Following this initial cleavage, the new C-terminus is further proteolytically processed to some degree which leads to a heterogeneous population of molecules. Again this change in primary structure can be detected by tryptic peptide mapping of the two tissue plasminogen activator variants (*Fig. 3*). This underlines that peptide mapping is a powerful tool for analyzing the primary structure of proteins and glycoproteins. However, although there is a detectable biochemical change of the tissue plasminogen activator molecule the *in vitro* enzymatic activity of single- and two-chain tissue plasminogen activator is not different.

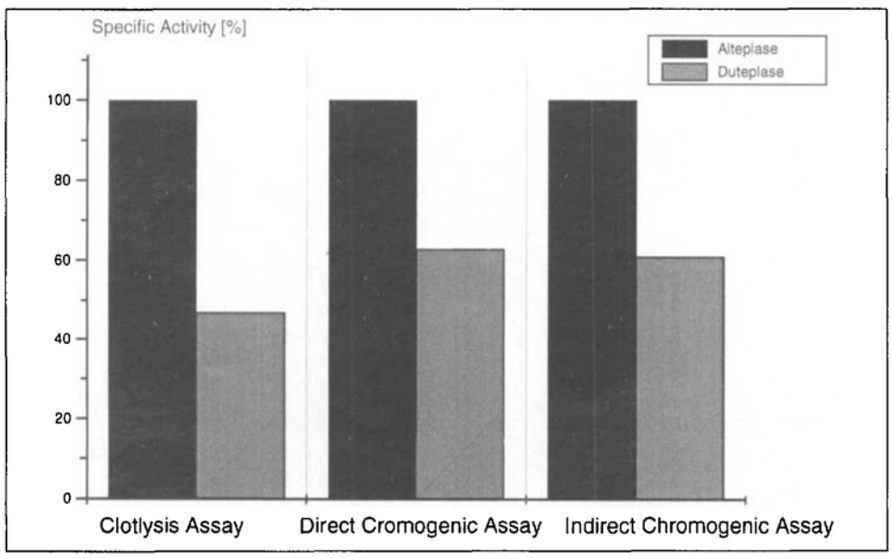

Fig. 2. *Fibrinolytic activity of Alteplase and Duteplase*

Obviously, also the physical state of cell cultures influences glycosylation, because cultivation of adherent CHO cells in the presence of serum *vs.* suspended CHO cells in the absence of serum causes a shift in the microheterogeneity of the glycosylation pattern. This change in glycosylation can be detected in the HPLC profiles of the corresponding glycopeptides (*Fig. 4*).

Because tissue plasminogen activator is eliminated in the liver tissue, plasminogen activator from adherent cells, comprising a higher sialylated oligosaccharides moiety has a longer half-life than tissue plasminogen activator from suspended cells.

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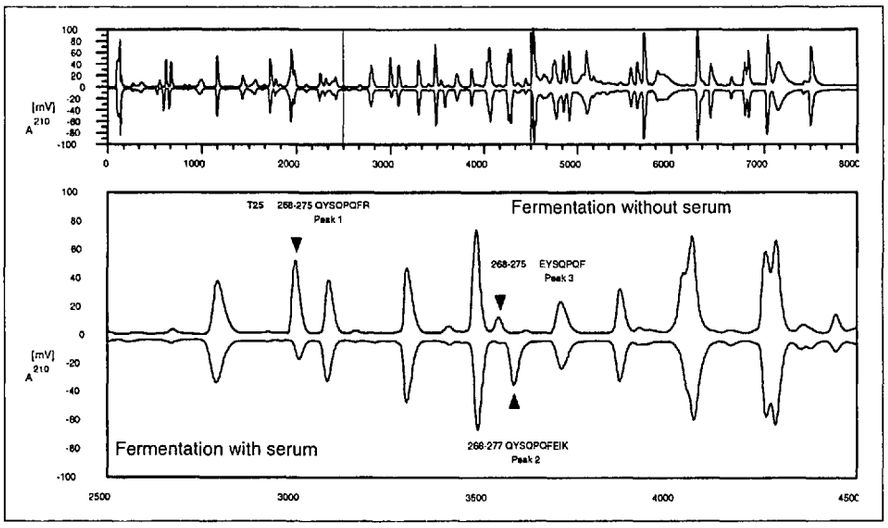

Fig. 3. *Tryptic peptide map of tissue plasminogen activator produced in the presence and absence of serum*

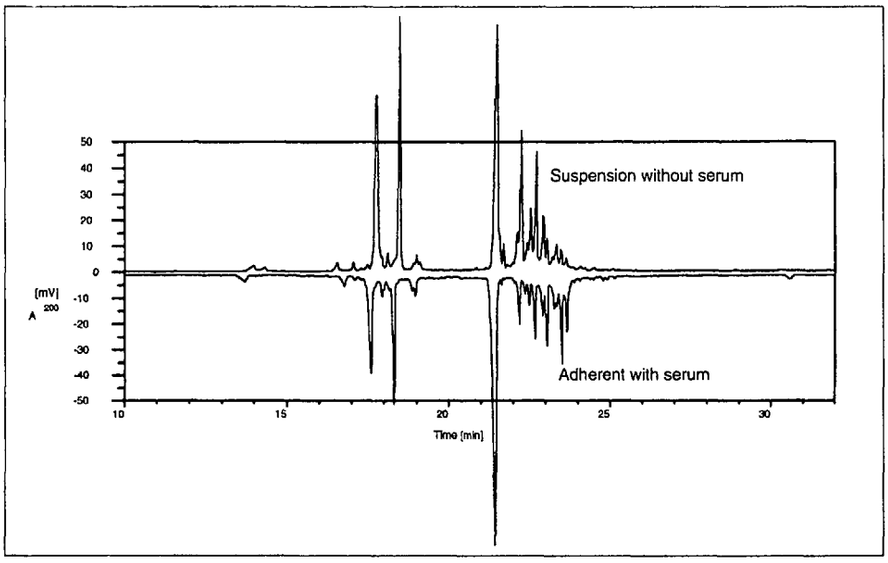

Fig. 4. *Changes of the microheterogeneity of glycopeptide 2 of tissue plasminogen activator produced by CHO cells grown adherent or in suspension*

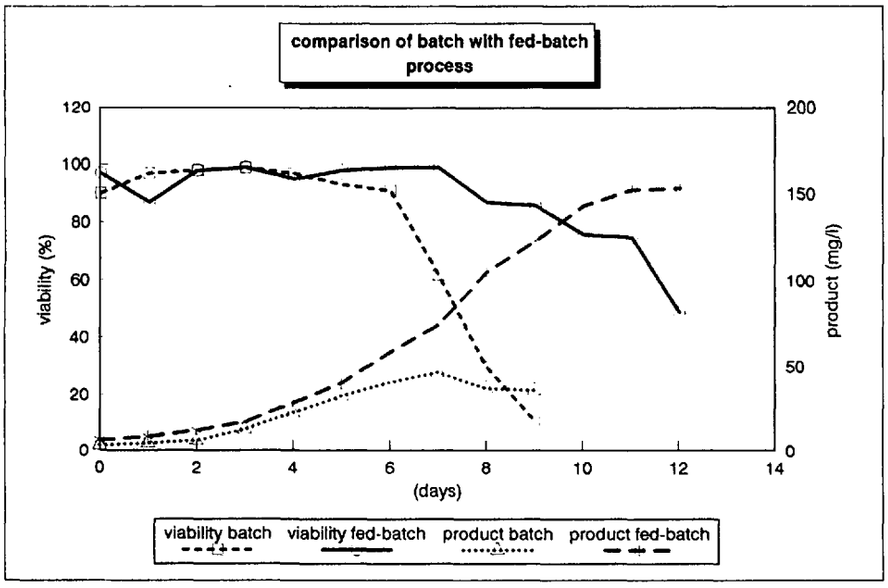

Fig. 5. *Comparison of batch with fed-batch process*

3. Changes in the Fermentation Process

Changes in fermentation to optimize the production titer can be achieved, *e.g.* by changing from a batch into a fed-batch process, where nutritions are replenished in order to increase cell viability, fermentation time and productivity or by changing to a continuous process in a perfusion system with cell retention to increase cell number and thereby productivity.

Applying defined media, feeding of essential nutrients can improve cell density, viability, and productivity. As long as no biological raw materials are used, it can be assumed that such process changes do not directly influence product quality.

By the optimization of a feeding strategy for improving productivity, the yield of a cell culture process can be increased up to 3-fold (*Fig. 5*). If tissue plasminogen activator from a batch and fed batch process is analyzed by tryptic peptide mapping no differences can be detected as to whether the product is produced at a low or high titer (*Fig. 6*).

Further, if mapping is done of the desialylated oligosaccharides by HPAE (high pH anion exchange) chromatography no significant differences in the carbohydrate moiety of the molecule can be detected. In addition, stability data concerning proteolytic processing and biological activity, do not present any differences between a high and low titer process in serum free media (*Fig. 7*).

4. Effect of Scale on Product Quality

Scale itself usually has no impact on product quality as long as identical process parameters are used, the cell line is genetically stable beyond the fermentation period and the product is stable during prolonged process times and during the downstream processing.

5. Changes in the Downstream Process

Causes for process changes in the recovery and purification of proteins result from increasing the yield by decreasing membrane and surface adsorption, increasing the number of life cycles of process steps, reducing bleeding of immunoaffinity columns or replacing immunoaffinity columns and reduction of aggregate formation.

From the perspective of product quality causes for improvements in the down-

stream process result from the need to reduce the impurity spectrum, the removal of homologous proteins or protein variants resulting from misfolding of the proteins as well as further reduction of DNA and viral contaminants.

Such challenges lead to addition, rearrangement, or substitution of purification steps, and altering different aspects of separation such as column size, column loading strategy, buffer composition, pH value or gradient, ionic strength, peak selection, and pooling strategy, buffer flow rates and column regeneration conditions.

In order to avoid changes in product quality by changes of the downstream process, the quality of the product from the new process has to be compared with that of the product derived from the previous process. Adequate analytical assays are peptide mapping, reversed phase HPLC, carbohydrate mapping, capillary electrophoresis, SDS-PAGE, isoelectric focusing, immunoassays to quantify contaminants and in addition specially designed *in vitro* potency assays for determination of the specific activity.

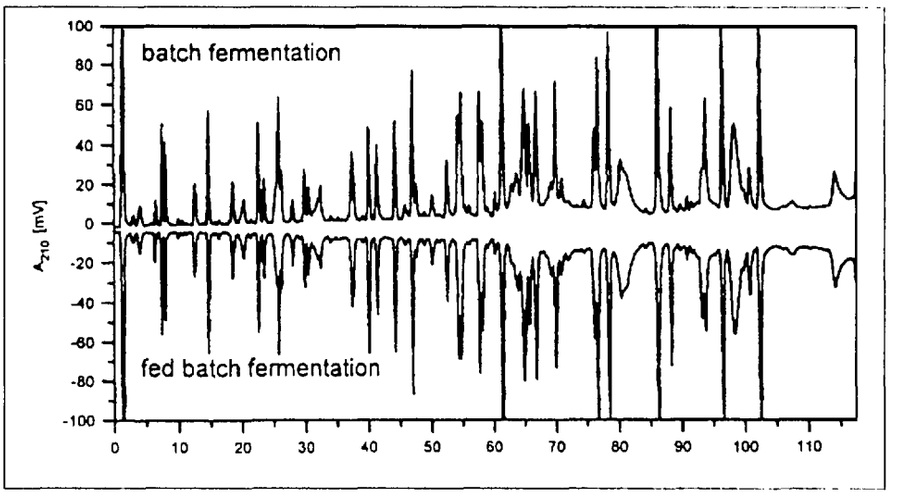

Fig. 6. *Comparison of tryptic peptide maps from batch and fed-batch fermentation*

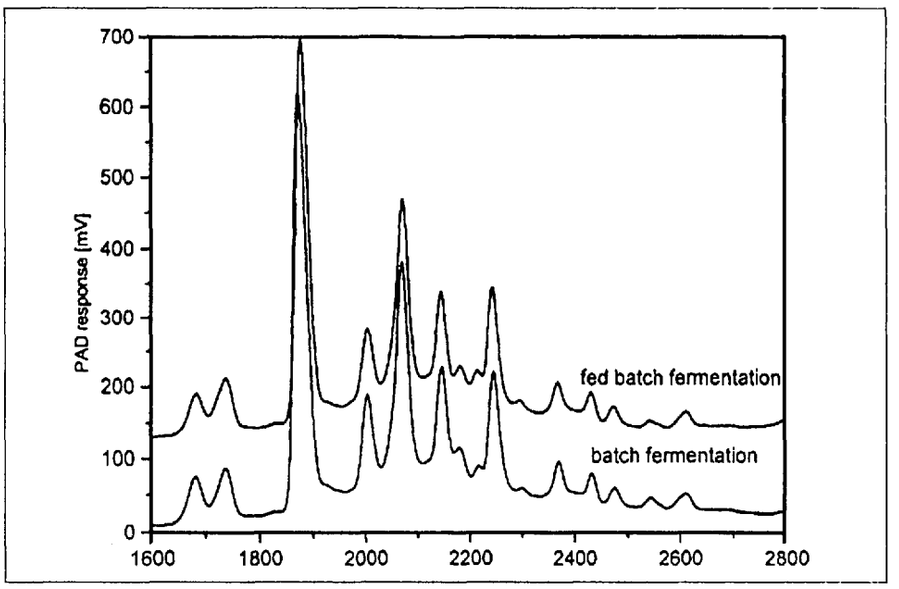

Fig. 7. *Comparison of the desialylated oligosaccharides from t-PA derived from batch and fed-batch fermentation*

6. Change of the Manufacturing Site

As long as the equivalent equipment with regard to dimension and qualification is employed the identical host vector system is used and the same standard operating and test procedures are applied, biopharmaceuticals can be manufactured at different sites with the same product quality and within the same range of set specifications.

An excellent example of this is tissue plasminogen activator manufactured at *Genentech Inc.* at San Francisco and at *Thomae GmbH* at Biberach. At both sites the biopharmaceutical is manufactured in identical bioreactors, identical downstream equipment using the same MCB (Master Cell Bank), similar standard operation and test procedures, yielding tissue plasminogen activator identical in product quality, purity, and potency.

7. Process Changes and Their Consequences

Various process changes with potential influence on product quality can be monitored by a broad spectrum of protein analytical methods, which are suitable for detecting differences in protein structure and activity. These analytical tools can be used to test the identity of a product derived from different processes.

Therefore, the emphasis with regard to a process change should not be on the technical manufacturing changes but on potential changes in product quality and its potential impact on potency, safety, and pharmacokinetic behavior.

For any significant process change, the regulatory authorities require a notification 30 days in advance.

If process changes during phase I/II clinical trials are performed, an extensive protein analytical characterization should be carried out, demonstrating identical product quality.

During phase III clinical studies, only materials from the commercial production method and scale should be used which are also intended for the market. Therefore, if for any reason process changes have to be carried out during this development phase of the product, a bioequivalence study is demanded in addition to extensive protein analytical characterization. Differences in potency or pharmacokinetic behavior have to be adjusted in the dose regiment of the ongoing phase III study.

Process changes after approval of product license application (PLA) require extensive protein analytical characterization of the biopharmaceutical to show identity of the product or at least to demonstrate that minor changes such as slight changes in the oligosaccharides of the carbohydrate structure do not negatively influence potency and pharmacokinetic behavior.

If protein analytical identity cannot be demonstrated, especially when changes in the protein chemistry of the active protein influence potency or pharmacokinetic be-

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havior the process change has to be considered major. This may result in a PLA amendment, if in a limited clinical study the therapeutic dosage can be adjusted, or if not, in a new PLA with new clinical studies.

Influenced by these regulatory requirements, decisions for process changes are dominated by the concern for whether the process change might influence product identity or quality and by the economic value achieved by the process change. Economic value in turn is determined by the expenditure for process development on the one hand and the economic and/or quality improvement in comparison to the market potential of the biopharmaceutical on the other.

In conclusion, potent protein analytical methods are available and will be further improved for the detection of changes in product quality initiated by process changes. These analytical tools are suitable for guiding process development.

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Requirements and Validation of a Biotech Multipurpose Plant

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As biotechnical derived products become more and more available through the pipeline of the biopharmaceutical industry the need for biotechnical manufacturing plants in which more than one product could be handled is of utmost importance with regard to flexibility and process economics. However, to deal with living organisms and associated impurities thereof requires special attention concerning the prevention of potential crosscontamination in all involved areas such as inoculum, fermentation, harvest, downstream processing, formulation and aseptic filling/lyophilization. The requirements of a mammalian cell culture facility operating in a multipurpose mode with special emphasis to the validation strategy guided by the new FDA form 3210, Application for Establishment License for Manufacture of Biological Products, is given.

General Situation and Regulatory Requirements

The increasing number of Investigational New Drug Applications (IND's), Product License and Establishment License Applications (PLA's/ELA's) and finally the increasing number of approved biologics has put pressure both on the regulatory bodies and the biopharmaceutical industry to define the requirements for a biopharmaceutical plant operating in a multipurpose mode. What does make biotech manufacturing so unique if compared with other branches like the bulk pharmaceutical chemical manufacturing and the finished dosage form manufacturing where multiuse manufacturing is a given for decades? The fear from the selfreplicating mechanism of living organisms is the main reason for doubting that cell lines could be handled simultaneously in a manufacturing facility. In addition, host cell line derived impurities like mycoplasma and exogeneous and endogeneous viruses must be considered thoroughly. It is both the possibility of a biochemical crosscontamination and the possibility of a biological crosscontamination which requires special attention with regard to the cultivation of more than one cell line in an existing facility.

According to 21 CFR 600.11 spore bearing organisms must be handled in a separate facility [1]. This is also true for the fermentation of penicilline which is regulated in the 'Guide to Inspection of Bulk Pharmaceutical Chemicals' [2] and the Code of Federal Regulations 21 CFR 211.42 [3]. In contrast, the European 'Supplementary Guidelines for the Manufacture of Biological Medicinal Products' [4] state that 'parallel production using closed systems of biofermentors may be acceptable for production such as monoclonal antibodies and products prepared by rDNA techniques'. In the United States, a 'White Paper' named 'Multi-Use Manufacturing Facilities for Biologicals' [5] issued by the Pharmaceutical Manufacturer's Association (PMA) and the subsequent discussions led to the acceptance of biological multiuse manufacturing facilities indicated by the form 3210 issued by the FDA 'Application for Establishment License for Manufacture of Biological Products' in which a statement must be made 'whether this is a multiproduct facility' [6]. An accompanying document named 'Points to Consider on the Use of the revised ELA Format' will be issued soon and defines the requirements in detail [7].

Very often, the approach of 'campaign manufacturing' *vs.* 'concurrent manufacturing' is discussed as the two main manufacturing strategies in biotech multiuse facilities. However, in a given facility consisting of the four main areas inoculum, fermentation and harvest, purification and final formulation the strict requirement of campain manufacturing as being defined as 'Processing of more than one product in the same facility and/or equipment in a sequential manner. Only one product is present in the facility at a time' is of less importance because product changeover times between 40 and 70 days depending on the manufacturing scale and the used mammalian cell line are not economically feasable. Therefore, provisions must be taken into account allowing either the parallel cultivation and purification by the use of closed systems in common areas or by means of spatial separation in case of working with open systems. The layout of the manufacturing facility is, therefore, of utmost importance for the concurrent manufacturing of more than one product.

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