Pilot manufacturing facilities for the development and manufacture of bio-pharmaceutical products

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11.1 Introduction

Biotechnology, 'the application of biological systems and organisms, to technical and industrial processes and products' is not a new discipline. The fermentation of grain using yeast to produce alcohol has been taking place for centuries in most cultures throughout the world. However, advances over the past 20 or so years in the field of molecular biology and hybridoma technology have provided us with many new opportunities for improved processes and products. Human healthcare in particular is now beginning to benefit from these rapid advances in modern biotechnology, proving that it offers much more than just the promise of new drugs to solve many of the serious health issues facing mankind. The first bio-pharmaceuticals reached the market nearly a decade ago and are making a significant contribution not only to health care around the world, but also to the finances of the companies manufacturing them.

Bio-pharmaceuticals, which generally include vaccines, blood and blood products, allergenic extracts, and biological therapeutics, are regulated under a whole range of guidelines from a variety of regulatory authorities. These authorities require that bio-pharmaceuticals be manufactured and prepared at a facility holding an unsuspended and unrevoked licence. Lack of clarity about licensing requirements can lead one to make major investments in large-scale manufacturing facilities before initiating the clinical trial(s) necessary to demonstrate the safety and effectiveness of the products. Such investments can result in significant financial loss if the product is not ultimately brought to market. This chapter will attempt to clarify the regulatory requirements for the use of small-scale and pilot facilities. For details of regulatory aspects see Chapters 2, 3 and 4.

The principals that apply to small-scale and pilot plant facilities equally apply to manufacturing facilities.

11.2 Regulatory, design and operating considerations

11.2.1 Regulatory considerations

The development of important new biological products is expensive and time-consuming and companies must be able to forecast and evaluate their expenditures for this process. Constructing a new large-scale facility to manufacture a product that has not been fully tested in clinical trials could result in a major financial loss, with the company being unable to recover a major capital expenditure if the product is not ultimately brought to market. For some companies the best financial option may be the use of a pilot facility where the product may be manufactured at a smaller scale than would be for an approved product. While regulatory authorities do not object to the use of pilot production facilities for the manufacture of clinical material, provided such manufacture is in compliance with the requirements applicable to investigational drugs, many companies are concerned that these facilities and the products manufactured in them would not be eligible for establishment licensure.

Although the advances in the technology have been staggering, it must be recognized that the same basic regulations and requirements are still applicable to the manufacture and control of bio-pharmaceuticals as for 'conventional' pharmaceuticals. The regulatory requirements for taking a conventional pharmaceutical through clinical trials to the market, however, emphasize the physico-chemical analysis of the 'final dosage form', which is then correlated with a suitable bio-assay to provide assurances of product uniformity. On the other hand, with a bio-pharmaceutical which cannot be totally defined by simple analyses of its physico-chemical characteristics and biological activity, most of the complexities occur during the bulk manufacturing process, while the preparation of the final dosage form for most part is rather 'uncomplicated'.

For this reason, the bio-pharmaceutical industry, together with the regulatory authorities, decided to focus upon the entire manufacturing process and not simply on the monitoring and analysis of the final dosage form. This is important as the quality, safety, and efficacy attributes of a bio-pharmaceutical for which end-product controls alone are inadequate, can only be assured by having comprehensive controls over the entire manufacturing

process. Therefore, as well as validating the consistency of manufacture and characterizing the final product, constant monitoring throughout processing is also stressed. This results in much work needing to be completed even before the clinical trials could commence. For example, over 750 different separate 'in-process tests' are carried out in the manufacture of a recombinant human growth hormone, whereas only about 60 tests are required in the chemical synthesis of a conventional peptide hormone such as the thyroid hormone.

In order to further streamline the approval process, the regulatory authorities have recently changed their procedures to eliminate the requirement for a separate establishment licence for certain 'well-defined' classes of biological products. Recent scientific advances, both in methods of manufacture and analysis, means that some products developed through biotechnology can be characterized in ways not historically considered possible, thereby enabling the authorities to allow well-characterized biological products to be regulated under a single application.

The guiding principle is that an application for establishment licensure can be made for any facility (regardless of the scale of manufacture) which has been fully qualified, validated, operates in accordance with current good manufacturing practices (cGMPs) and which also complies with applicable local laws and regulations. These facilities should be distinguished from facilities used in research and development that may not operate under appropriate current good manufacturing practices (cGMPs). When manufacture of a product is transferred from a pilot to a different facility, a demonstration of product consistency, as well as data comparing the two products, together with the relevant process validation data should be submitted to the regulatory authorities. This should include a description of the manufacturing changes that have occurred, a protocol for comparing the products made in each facility, and the data generated using this protocol, as well as documentation on process validation and all stability data for the product manufactured in the new facility. It would be expected that the methods of cell expansion, harvest, and product purification would be identical except for the scale of production. For each manufacturing location, a floor diagram should be included that indicates the general production facility layout, as well as information on product, personnel, equipment, waste and air flow for production areas; an illustration or indication of which areas are served by each air handling unit; and air pressure differentials between adjacent areas.

It is, therefore, quite obvious how important it is that the manufacturer discusses with the regulatory authorities what data are necessary to compare products, as such data may range from simple analytical testing to full clinical

trials, and could well be required even before the product made using the new facility or process is allowed to be included in any further clinical trials.

11.2.2 Design considerations

The cost of building facilities that are fully validated and in compliance with cGMP can be overwhelming to biotechnology companies with limited finances. The basic design and construction costs are driven higher by the various regulatory, containment, process utilities and waste treatment requirements. In addition, companies also demand increased value from their clinical production facilities. The facility design must, therefore, allow for flexibility of operations, diverse process utility requirements, as well as for campaigning different products in the same facilities.

It is possible to build such facilities in a cost effective, flexible manner, while satisfying the regulatory requirements as well as ensuring that the completed facility will provide all the functions intended. The most effective techniques used to manage such a project would be the use of the concept of 'total project management'. 'Total project management' means integrating regulatory requirements, design and engineering, validation, as well as construction requirements on one single schedule, to determine the critical path (least time to completion). This leads to more effective management, permitting 'what if' scenarios that can result in substantial savings in time and cost, especially if cost estimation is implemented early in the design phase.

The key element is to begin with the careful analysis of manufacturing process needs and to define the facility requirements specifically. Careful site selection is important to eliminate any costly surprises. It is also important to avoid over-specifying very expensive process utilities. This would be followed by the implementation of modular facilities design and construction. A well thought-out facility design using pre-engineered, self-contained elements can in many cases be the most cost effective, flexible solution to clinical production. The application of modular clean rooms to create the cGMP facilities for different products can therefore be achieved.

Buildings and facilities used in the manufacture, processing, packing, or holding of bio-pharmaceuticals should be of suitable design, size, construction and location to facilitate cleaning, maintenance and proper operations. Adequate space should be provided for the orderly placement of equipment and materials, to prevent mix-ups and contamination among different raw materials, intermediates, or the final product. The flow of raw materials, intermediates and the product through the building or buildings, should be designed to prevent mix-ups and contamination. To prevent mix-ups and contamination, there should be defined areas and/or other control systems

for all the important activities. Also, facility design must be integrated in support of the process in order to comply with cGMP and other regulatory requirements such as:

- flow of personnel, materials, product, equipment or glassware, and waste flows;
- product separation and/or segregation;
- aseptic and/or sterile processing;
- sanitary design — cleaning and decontamination and spill containment;
- bio-hazard containment and/or isolation;
- special clean utilities;
- solvent recovery, handling, and storage;
- HVAC zoning, pressurization, and filtration;
- drain and exhaust systems.

11.2.3 Operating considerations

Implementing cGMP

The current Good Manufacturing Practices (cGMPs) mentioned above are those practices designed to demonstrate that the control over the process, the facility, and the procedures used in the manufacture, maintains the desired quality of the product, be it a conventional drug or a bio-pharmaceutical, and consequently protects the product's integrity and purity. The implementation of cGMP is now a legal requirement and certainly makes for better quality products and sound economic sense.

As technology and scientific knowledge evolve, so does understanding of critical material, equipment and process variables that must be defined and controlled to ensure end product homogeneity and conformity with appropriate specifications. The cGMP regulations would not achieve their statutory mandated purposes if they were not periodically reassessed to identify and eliminate obsolete provisions or to modify provisions that no longer reflect the level of quality control that current technology dictates and that the majority of manufacturers have adopted. cGMP regulations are based on the fundamental concepts of quality assurance:

- quality, safety, and effectiveness must be designed and built into a product;
- quality cannot be inspected or tested into a finished product;
- each step of the manufacturing process must be controlled to maximize the likelihood that the finished product will be acceptable.

Even though cGMPs have been known and have been evolving for over 20 years, many pharmaceutical and biotechnology companies (both established companies and those just starting operations) still need to achieve a sound basic understanding and implementation of the fundamental rationale and requirements of cGMP. There is still a persistent lack of understanding among a limited number of manufacturers with respect to certain of the cGMP regulations. Some pharmaceutical firms have not subjected their procedures to sufficient scrutiny, while others have failed to update such procedures to accommodate changes or advances in the manufacturing process. In some cases, manufacturers may be relying on methods and procedures that were acceptable at some time in the past, but that are not acceptable in light of current standards. The regulatory authorities have also encountered serious deficiencies particularly with validation procedures designed to ensure the quality of the manufacturing process.

Those implementing cGMPs in the design of bio-pharmaceutical facilities must recognize the inherent variability in the manufacturing processes. A distinction can be drawn between the application of cGMPs to well-characterized operations, such as filling and finishing, and the nature of the early stages of biotech product manufacturing typified by the attributes below:

- raw material variances;
- product yields;
- non-linear process flow, reprocessing;
- process complexity.

The role of process validation

cGMP regulations specify the nature and extent of validation that is necessary to ensure that the resulting products have the identity, strength, quality and purity characteristics that they purport to possess. The term validation is used for those elements of the manufacturing process under the control of the manufacturer, while the term qualification is used for those items produced by a person other than the manufacturer, or otherwise not under the control of the manufacturer. Process validation is the establishment of documentary evidence to provide a high degree of assurance that a specifically defined process, using specified equipment and systems, which when in control, will consistently and reliably yield a product meeting its pre-determined specifications and quality attributes or characteristics.

So what does validation actually mean to the ordinary scientist responsible for putting together a process for the manufacture of a bio-pharmaceutical. Validation is simply the formal process of establishing with a high degree of

assurance, and demonstrating to the relevant authorities, through a programme of documented tests, challenges, and results, that an item of equipment, system, or process actually and consistently does what it claims to do. Because it guarantees the ability to achieve and routinely maintain a product of a quality which meets all its pre-determined specification, it provides for a better understanding of how the equipment, system, or the process works, as it highlights potential weaknesses and enables corrective action to be taken. Also, by demonstrating reliable and consistent performance, validation also ensures profitability, because a validated process should be under control to such an extent that any deviation could be detected and enable corrective action to be taken.

So how and when do the regulatory authorities recommend that process validation be carried out? The validation programme should begin with the raw material in the warehouse or stores, and finish when the final product is fully packaged and ready for use. When any new manufacturing formula or method of preparation is adopted, steps should be taken to demonstrate its suitability for routine processing. However, validation is required not just when a totally new and untried item of equipment or system is adopted, but on every occasion that any of the above is substantially amended, as product quality and/or the reproducibility of the process may be affected. Also, processes and procedures should undergo periodic critical re-validation to ensure that they remain capable of achieving the intended results.

Experience has shown that a simple, logical, well-planned approach is the key to achieving success with process validation. Not only will this minimize the mountain of documentation required, but will also provide the training for process, plant and maintenance personnel, as well as providing the basis for any calibration and preventative or routine engineering maintenance programmes required. Also, if validation is planned, interfaced and integrated with the design and construction phase of the operation, then user requirements can be addressed, enabling the overall timelines to completion to be shortened. This in turn will minimize expensive duplication of effort, by identifying and enabling correction of potential design mistakes or omissions.

Validation strategy

The validation programme should embrace steps in the process that are critical to the quality and purity of the final product and should include all associated facilities, operating utilities and equipment. All critical process operations and facilities are required to be systematically investigated to ensure that the product can be manufactured reliably and reproducibly using all the pre-defined production and control methods. It is important to remember, however,

that the level of validation should be appropriate to the end use of the product. The requirements become less stringent, but no less important, further away from the final process step. A final dosage filling facility for a parenteral will require a much higher degree of validation than an intermediate bulk production facility.

Validation begins with the development of the Master Validation Plan. It is important to combine the MVP with the construction schedule to ensure that validation is a focus of the total effort and that validation documentation is available as necessary and prepared concurrently with construction, and to ensure that the overall time to complete validation is minimized. The VMP should include and cover the following:

- a summary of the validation philosophy, its approach and rationale;
- a definition of the product in terms of its critical quality attributes, including purity, qualitative and quantitative impurity profiles, physical characteristics such as particle size, density, polymorphic forms, moisture and solvent content, if appropriate, homogeneity, and whether the product is susceptible to microbial contamination;
- a summary of the methodologies and techniques to be used;
- identification of process steps and parameters that could affect the critical quality attributes of the product, and the range for each critical process parameter expected to be used during routine manufacturing and process control. These should be determined by scientific judgment, and typically be based on knowledge derived from research and scale-up batches, unless a specific parameter can only be determined from manufacturing experiences gained from a production-scale batch;
- validation planning worksheet identifying individual tasks;
- list of available resources — both internal and external; and resource levelling to establish the time required for the project based on the available resources.

The documentation related to the validation programme is as important as the execution of the programme itself, if not more so. The design and implementation of the documentation system involves the preparation, review (audit), and authorization of all required validation protocols for the standard operating procedures (SOPs), and manufacturing instructions, including calibration methods (metrology programmes), acceptance and certification criteria, as well as the assignment of responsibility. The validation protocol is the blueprint of the validation process for a particular drug product. It is the written plan describing the process to be validated, including the equipment used, and how validation will be conducted. The protocol should specify a

sufficient number of replicate process runs to demonstrate reproducibility, and provide an accurate measure of variability among successive runs.

Execution of validation field activities

This begins with installation qualification (IQ), followed by operational qualification (OQ) and finishes with performance qualification (PQ), which covers both equipment (or system) validation, and process qualification, including establishing critical circumstances for re-validation.

Installation qualification (IQ) is the formal process of verifying and establishing confidence that an item of equipment or system was received and installed, meets the specification as ordered and intended, that the proper utilities are available and supplied, that it is installed as recommended by the manufacturer, any local or state codes, standards and cGMP, and is capable of consistently operating within established limits and tolerances.

It is clear, therefore, that the 'as-built' drawings and other documents supplied by the manufacturer are essential to successfully carry out installation qualification (IQ).

Operational qualification (OQ) is the formal process of verifying and establishing that such an item of equipment or system, once installed, is capable of satisfactory operation as specified and intended, over the entire range of operational parameters such as pressures, temperatures, etc. It involves water commissioning to check the various ancillaries such as motors and valves, and usually follows installation qualification (IQ), but can also be carried out concurrently.

Performance qualification (PQ) is the formal process of verifying and demonstrating confidence by rigorous challenges and testing, that this item of equipment or system, once installed and operationally qualified, is capable of operating effectively and reproducibly in the process step for which it is intended. This is normally carried out in two parts:

- equipment (or system) validation;
- process qualification.

Equipment (or system) validation involves the following as appropriate:

- sterilization validation by using temperature mapping techniques, followed by the verification of asepsis, or sterility testing;
- containment validation, using the host organism or another 'safe' organism;
- calibration of instruments and certification;
- validation of computer hardware and software used in the process;
- cleaning validation, particularly important in multi-product facilities.

Next comes process qualification. Process qualification is the major component of the whole validation effort, as it relates directly to the changes the raw material undergoes during its transformation to the final product. Process qualification is where each critical process step in the manufacture is defined with sufficient specificity and each such step is suitably challenged and tested to determine its adequacy and capability. It is essential that the validation runs are as representative as possible to routine manufacturing steps in terms of activities, conditions and characteristics, to ensure that the results obtained are relevant to routine production. The performance of the various challenges and the compilation of the results must confirm conclusively that the equipment or system involved in the process step is capable of providing the pre-described confidence levels. Manufacturers are also expected to have validation reports for the various key process steps. For example, if an ion-exchange column is used to remove endotoxins, there should be data documenting that this process is consistently effective. By determining endotoxin levels before and after processing, a manufacturer should be able to demonstrate the validity of this process. It is important to monitor the process before, during and after to determine the efficiency of each key purification step. Spiking the preparation with a known amount of a contaminant to demonstrate its removal is a useful method to validate such a procedure.

Prospective, concurrent and retrospective validation

Prospective validation covers activities that should be conducted prior to the commercial distribution of the product manufactured by either a new or substantially modified process. When carrying out prospective validation, data from laboratory and/or pilot-scale batches should identify critical quality attributes and specifications, critical process steps, control ranges, and in-process tests. Scale-up batches can be used to generate data to confirm or refine earlier work, however production-scale batches are needed to provide data showing consistency of the process, using validated analytical methods. The number of consistent process runs would depend on the complexity of the process or the magnitude of the process change being considered. Although three consecutive, successful production batches should be used as a guide, there may be situations where additional process runs are warranted to prove consistency of the process, for example, for products with complex processes such as a recombinant cell fermentation, or for processes with prolonged completion times, such as with an animal cell culture.

Regulatory authorities consider concurrent validation to be a sub-set of prospective validation. They recognize that in a limited number of cases it may not be possible to complete validation of a process in a timely manner before

distribution of the product, when data from replicate production runs are unavailable, possibly because only a limited number of batches intended for clinical or orphan drug products have been produced. In such cases, the manufacturer should do all the following:

- perform all the elements of prospective validation, exclusive of replicate production run testing, before releasing any batch for distribution;
- document the reasons for not completing process validation;
- batch production records, in-process controls, and analytical data from each process run should be evaluated thoroughly to determine whether or not each batch should be released.

This approach should not be viewed as a viable alternative if the number and frequency of production batches permit timely completion of process validation prior to product distribution. Also, if analysis of the data shows that the process used to manufacture the distributed batches was not, in fact, validated, no additional batches should be distributed until corrections have been implemented and the process is deemed to be validated.

Retrospective validation may be conducted for a well-established process that has been used without significant changes, such as changes in raw materials, equipment, systems, facilities, or in the production process, that affect the critical quality attributes of the product. This validation approach should only be used when there is sufficient history on past production batches to demonstrate that the process consistently produces acceptable products, and where:

- critical quality attributes and critical process parameters have been identified and documented;
- appropriate in-process specifications and controls have been established and documented;
- there have not been excessive process or product failures attributable to causes other than operator error or equipment failure unrelated to equipment suitability;
- impurity profiles have been established for the existing product.

The number of batches to review will depend on the process, but, in general, data from 10 to 30 consecutive batches should be examined to assess process consistency. All batches within the selected review period should have been manufactured by the same process and have the same documented history of controls and tests as the current products.

Cost of validation

So why does validation cost so much, take so long, and what can be done about it? Validation of a bio-pharmaceutical facility is based on the time-consuming accumulation of details and sometimes the cost of validation can exceed the total cost of a project's architecture and engineering fees. Precious validation time could be spent trying to obtain information from designers, engineers, contractors and manufacturers, which could have been specified and provided if it were considered an integral part of the project. Additionally, most project managers are more concerned with completing the facility than with completing validation. The key is, therefore, to make validation an integral part of the project and include the validation master plan, preparation of protocols and SOPs, and their execution, as a series of tasks on the critical path in the total project schedule.

In conclusion

It is clear that process validation represents a sizeable investment in time and resources, usually taking place during a time period when the scientist and plant personnel are already heavily involved in start-up related activities. The resulting time constraints can often affect the quality of the work needed, so it is important to identify the pitfalls normally encountered during the process of validation so that they can be avoided.

Under-estimating or under-resourcing the amount of work required is the most common problem; a simple, well planned, and logical approach to validation is the key to overcoming this problem.

Surprisingly, too much validation can also be a problem; however, by identifying the critical conditions for each step in the process, it should be possible to avoid this pitfall and save valuable resource and effort.

Re-validation and change control

Once the validation and certification procedure is completed, the equipment, system or process is considered acceptable for use, but only under those conditions and functions specified in the validation protocol. To preserve the validated status of a process, measures must, therefore, be taken that will allow any significant process changes to be recognized and addressed promptly. For example, a slight change in the physical characteristics of an ingredient, or in the order of adding ingredients, may alter the specification of a product. Because of such effects, re-validation is necessary after any change in process or product characteristics or control procedures. Such a change control programme should provide for a classification procedure to evaluate changes in raw materials, manufacturing sites, scale of manufacturing, manufacturing

equipment and production processes. Regulatory authorities categorize changes to an approved application as major, moderate, or minor, depending on the nature and extent of the changes, and on their potential to have an adverse effect on the identity, strength or concentration, quality, purity, or the potency of the product, and on the process, as they may relate to the safety or effectiveness of the product.

A major change is defined as one that could significantly affect the critical quality attributes of the product. Such changes that have a substantial potential to have an adverse effect on the product and require submission of a supplement for approval by the regulatory authorities prior to the distribution of the product made using the change, should be justified by additional testing and if appropriate, re-validation. Some examples include:

- process-related changes, such as the extension of culture growth time leading to a significant increase in the number of cell doublings beyond validated parameters; new or revised recovery procedures; new or revised purification process, including a change in a column; a change in the chemistry or formulation of solutions used in processing; a change in the sequence of processing steps, or addition, deletion, or substitution of a process step; reprocessing of a product without a previously approved reprocessing protocol;
- changes relating to the manufacturing processes or analytical methods that results in changes of specification limits or modifications in potency, sensitivity, specificity, or purity; establishes a new analytical method; deletes a specification or an analytical method; eliminates tests from the stability protocol; or alters the acceptance criteria of the stability protocol;
- scale-up requiring a larger fermenter, bioreactor or purification equipment (applies to production stages up to the final purified bulk);
- changes in the composition or the final dosage form of the biological product or even of ancillary components, such as new or different excipients, carriers, or buffers;
- new or different lot of, or source for, in-house reference standard or reference panel, resulting in the modification of reference specifications and/or an alternative test method;
- extension of the expiration dating period and/or a change in storage temperature, container/closure composition, or other conditions, other than changes based on real time data in accordance with a stability protocol in the approved licence application;
- installation of a new Water for Injection (WFI) system, or modifications to an existing WFI system that would have a significant potential to stress or

challenge the system, such as lengthy or complicated distribution system extensions to service new or remote production areas, use of components of lesser quality or function, expansions of ambient temperature water distribution loops, or conversion from hot loop to ambient loop;

- change of the sites at which manufacturing, other than testing, is performed; addition of a new location; contracting of a manufacturing step in the approved licence to be performed at a separate facility;
- conversion of production and related areas from single into multiple product manufacturing areas, especially as there may be changes to the approved and validated cleaning procedures as well as additional containment requirements;
- changes in the location (room, building, etc.) of steps in the production process, which could affect contamination or cross-contamination precautions;
- major construction, or changes in location, involving or affecting environmentally controlled manufacturing or related support areas such as new buildings; new production areas or rooms in existing build-in-support systems with significant potential to affect air, water, or steam quality; installation of a new HVAC system involving or affecting environmentally controlled manufacturing or related support areas; modifications to an existing HVAC system that supplies aseptic processing areas.

Moderate changes have a moderate potential to adversely affect the product and require a supplementary submission to the regulatory authorities at least 30 days prior to distribution of the product made using the change. Some examples include:

- automation of one or more process steps without a change in process methodology;
- addition of duplicated process chain or unit process, such as a fermentation process or duplicated purification columns, with no changes to the in-process parameters;
- addition or reduction in number of pieces of equipment (e.g., centrifuges, filtration devices, blending vessels, columns) to achieve a change in purification scale not associated with a process change;
- change in the fill volume (per vial or syringe) from an approved production batch size and/or scale, excluding those that involve going from a single dose to a multi-dose vial, or changes in product concentration, both of which should be submitted as a supplement requiring prior approval;
- changes in responsible individuals specified in the approved application, including manufacturers' representatives, responsible experts and other individuals designated to communicate with the authorities;

- modification of an approved manufacturing facility or room that is not likely to have an adverse effect on safety, sterility assurance, purity or potency of product, such as adding new interior partitions or walls to increase control over the environment;
- manufacture of an additional product in a previously approved multiple-product manufacturing area using the same equipment and/or personnel, if there have been no changes to the approved and validated cleaning procedures and there are no additional containment requirements;
- change in the site of testing from one facility to another, such as from a contract laboratory to the licence holder, from an existing contract laboratory to a new contract laboratory, or from the licence holder to a new contract laboratory;
- change in the structure of a legal entity that would require issuance of new licences, or a change in name of the legal entity or location;
- addition of release tests and/or specifications, or tightening of specifications for intermediates;
- minor changes in fermentation batch size using the specifications of the bulk or final product;
- modifications to an existing HVAC system involving or affecting environmentally controlled manufacturing or related support areas, but not aseptic processing areas, with no change in air quality.

Minor changes are those that are unlikely to have a detectable impact on the critical attributes of the product. Such changes would not shift the process in any discernible manner and might be implemented with minimal testing and revalidation. For example, like-for-like equipment replacements where identical or similar equipment is introduced into the process, is unlikely to affect the process if adequately installed and qualified. Such changes should be described and reported by the manufacturer on an annual basis. Examples would include:

- addition of equipment for manufacturing processes which is identical to the primary system and serves as an alternate resource within an approved production room or area;
- upgrade or minor corrective change to production air handling, water, or steam supply systems using equipment of the same or similar materials of construction, design and operating parameters, and not affecting established specifications; such as the removal of dead legs in the WFI system. This, however, does not include replacement of parts or routine repair and maintenance, which would not be changes to an approved application and would not need to be reported;
- relocation of analytical testing laboratories between areas specified in the licence;

- room upgrades, such as the installation of improved finishes on floors/walls;
- installation of non-process-related equipment or rooms to improve the facility, such as warehousing refrigerators or freezers;
- modifications in analytical procedures with no change in the basic test methodology or existing release specifications provided the change is supported by validation data;
- change in harvesting and/or pooling procedures, which does not affect the method of manufacture, recovery, storage conditions, sensitivity of detection of adventitious agents or production scale;
- replacement of an in-house reference standard or reference panel (or panel member) according to SOPs and specifications in an approved licence application;
- tightening of specifications for existing reference standards to provide greater assurance of product purity, identity and potency;
- establishment of an alternative test method for reference standards, release panels or product intermediates, except for release testing of intermediates licensed for further manufacture;
- establishment of a new Working Cell Bank (WCB) derived from a previously approved Master Cell Bank (MCB) according to a SOP on file in the approved licence application;
- change in the storage conditions of in-process intermediates based on data from a stability protocol in an approved licence application, which does not affect labelling, except for changes in storage conditions, which are specified by regulation;
- change in shipping conditions, such as temperature, packaging or custody, based on data derived from studies following a protocol in the approved licence application;
- a change in the stability test protocol to include more stringent parameters, such as additional assays or tightened specifications;
- addition of time points to the stability protocol;
- replacement of equipment with that of identical design and operating principle involving no change in process parameters;
- upgrade in air quality, material, or personnel flow where product specifications remain unchanged. Involves no change in equipment or physical structure of production rooms;
- relocation of equipment within an approved operating room, rearrangement of the operating area or rooms where production is performed or relocation of equipment to another approved area to improve product/personnel/raw material flow and improve segregation of materials with no change in room air classification;

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- modifications to the pre-treatment stages of a WFI system, including purified water systems used solely for pre-treatment in WFI production;
- change in the simple floor plan that does not affect production process or contamination precautions;
- trend analyses of release specification testing results for bulk drug substances and drug products obtained since the last annual report.

Change control procedures

No change that could affect performance in any way should be allowed without the written approval of at least the production, QA and engineering departments. Such changes should only be handled through a change control procedure with protocols for initiating and proving the change, together with procedures for re-validation. Such change control measures may apply to equipment, SOPs, manufacturing instructions, environmental conditions, or any other aspect of the process or system that has an effect on its state of control and, therefore, on the state of validation and should include procedures to:

- prevent unauthorized modifications to a validated system;
- evaluate proposed changes against development and technology transfer documents;
- identify and evaluate all proposed changes to assess their potential effects on the process and determine if, and to what extent, re-validation is needed;
- ensure that all documents affected by changes are promptly revised;
- determine the impact of changes on the critical chemical and physical attributes of the product, such as its impurity profiles, stability, etc.

Changes implemented to improve process yields should be evaluated carefully to determine if they result in new or higher levels of impurities; impurity profiles of resulting batches should be comparable to the batches used in drug safety and clinical testing, and evaluated to ensure that these do not have an adverse effect on analytical methods, due to increased interference caused by new or higher levels of impurities and by-products; and analytical methods should be modified as necessary to ensure that they are capable of detecting and quantifying impurities.

11.3 Primary production

The manufacture of bio-pharmaceuticals involves certain specific considerations arising from the nature of the products and the processes. Unlike conventional pharmaceuticals, which can be manufactured, analysed and characterized using

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- ensure that all documents affected by changes are promptly revised;
- determine the impact of changes on the critical chemical and physical attributes of the product, such as its impurity profiles, stability, etc.

Changes implemented to improve process yields should be evaluated carefully to determine if they result in new or higher levels of impurities; impurity profiles of resulting batches should be comparable to the batches used in drug safety and clinical testing, and evaluated to ensure that these do not have an adverse effect on analytical methods, due to increased interference caused by new or higher levels of impurities and by-products; and analytical methods should be modified as necessary to ensure that they are capable of detecting and quantifying impurities.

11.3 Primary production

The manufacture of bio-pharmaceuticals involves certain specific considerations arising from the nature of the products and the processes. Unlike conventional pharmaceuticals, which can be manufactured, analysed and characterized using

chemical and physical techniques capable of a high degree of consistency, the production of bio-pharmaceuticals involves processes and materials that display an inherent variability, resulting in variability in the range and nature of the by-products. Moreover, the control and characterization of bio-pharmaceuticals usually involves bio-analytical techniques, which have a greater variability than the usual physico-chemical determinations. In addition, genetically modified cells, although providing special opportunities for producing novel protein sequences that exhibit improved activity compared to that of the natural molecule, necessitate special considerations of process design and operation.

Therefore, the methods used in the manufacture, control, and use of these bio-pharmaceuticals make certain precautions necessary, and are a critical factor in shaping the appropriate regulatory control. Bio-pharmaceuticals manufactured by such methods include vaccines, immune-sera, antigens, hormones, cytokines, enzymes and other products of fermentation, including monoclonal antibodies and products derived from r-DNA, and can be largely defined by reference to their method of manufacture:

- microbial cultures, excluding those resulting from r-DNA techniques;
- microbial and cell cultures, including those resulting from recombinant DNA or hybridoma techniques;
- extraction from biological tissues;
- the propagation of live agents in embryos or animals.

This chapter applies to the production, extraction, purification and control of such bio-pharmaceuticals manufactured for use in clinical trials or for marketing, as human or veterinary medicines, and applies to the point where the product is rendered sterile — i.e. the bulk active substance.

11.3.1 Starting materials

The source, origin and suitability of starting materials should be clearly defined. In instances, where the necessary tests take a long time, it may be permissible to process starting materials before the results of the tests are available. In such cases, release of a finished product is conditional on satisfactory results of these tests. Where sterilization of starting materials is required, it should be carried out where possible by heat, although other appropriate methods may also be used for inactivation of biological materials, such as gamma-irradiation for serum supplements used in the culture of animal cells.

Control of raw materials

Many of the raw materials used in fermentation processes can have significant impact on the subsequent recovery. As they are usually derived from animal

sources (such as serum, transferrin, etc.), they represent potentially variable sources of contaminants such as viruses, mycoplasma, or even hydrolytic enzymes. Pre-treatment of these raw materials by heating, acidification or sterile filtration is often necessary to avoid contaminating the production cells as well as the product. For example, contamination of the seed train by serum borne mycoplasma or virus may irreversibly repress cell growth and product titre; and once the cells are contaminated, they will produce poorly, and the harvest fluid will most likely contain degradative enzymes that decrease the quality of the purified product.

A monoclonal antibody (mAb) may also be a raw material when used for purification of the product. In such cases, the standards for their production should be at least as rigorous as those for the product it is used to purify. The manufacturer must fully characterize the mAb-producing cell line, establish that it is free from adventitious agents, assess the purity of the mAb and validate its purification process for the removal of nucleic acids and viruses, as well as minimize residual levels of the mAb in the product of interest.

Raw materials should be handled and stored in a manner to prevent contamination and cross-contamination. Identifying labels should remain legible, and containers should be appropriately cleaned before opening to prevent contamination. Written procedures should be established describing the purchase, receipt, identification, quarantine, storage, handling, sampling, testing and approval or rejection of raw materials, and such procedures should be followed. Bagged and boxed raw materials should be stored off the floor and suitably spaced to allow cleaning and inspection, and those stored outdoors should be in suitable containers. For solvents or reagents delivered in bulk vessels, such as in tanker trucks, a procedural or physical system, such as valve locking or unique couplings, should be used to prevent accidental discharge of the solvent into the wrong storage tank. Each container or grouping of containers of raw materials should be assigned and identified with a distinctive code, lot or receipt number with a system in place to identify each lot's status. Large containers, such as tanks or silos, which are used for storing raw materials, including their attendant manifolds, filling and discharge lines, should also be appropriately identified.

Receipt, sampling, testing, and approval of raw materials

Upon receipt and before acceptance, each container or grouping of containers of raw materials should be examined visually for appropriate labelling, container damage, seal integrity (where appropriate) and contamination. Raw materials should be held under quarantine until they have been sampled, tested or examined as appropriate and released for use. Representative samples of

each shipment of each lot should be collected for testing or examination in accordance with an established procedure. The number of containers to sample and the sample size should be based upon appropriate criteria, such as the quantity needed for analysis, sample variability, degree of precision desired, and past quality history of the supplier, and the sample containers properly identified.

At least one test should be conducted to verify the identity of each raw material. A supplier's certificate of analysis may be used in lieu of performing other testing, provided the manufacturer has a system in place to evaluate vendors (vendor audits) and establishes the reliability of the supplier's test results at appropriately regular intervals. For hazardous or highly toxic raw materials, where on-site testing may be impractical, suppliers' certificates of analysis should be obtained showing that the raw materials conform to specifications. However, the identity of these raw materials must be confirmed by examination of containers and labels, and the lack of on-site testing for these hazardous raw materials should be documented.

Use and re-evaluation of approved raw materials

Approved raw materials should be stored under suitable conditions and, where appropriate, rotated so that the oldest stock is used first. Raw materials should be re-evaluated, as necessary, to determine their suitability for use, for example, after prolonged storage, or after exposure to heat or high humidity.

Rejected raw materials

Rejected raw materials should be identified and controlled under a quarantine system designed to prevent their use in manufacturing or processing operations for which they are unsuitable, and if necessary discarded by appropriate methods.

11.3.2 Cell culture, fermentation and process control

Cell bank system and cell culture

The starting material for manufacturing a bio-pharmaceutical includes bacterial, yeast, insect or mammalian cell culture which expresses the protein product or monoclonal antibody (mAb) of interest. In order to prevent the unwanted drift of characteristics which might ensue from the repeated subcultures or multiple generations, the production of biological medicinal products obtained by microbial or animal cell culture should be based on a system of master and working cell banks (MCB, WCB) consisting of aliquots of a single

culture. Also known as seed lots, such cell bank systems are used by manufacturers to assure the identity and purity of the starting raw material.

The MCB is derived from a single colony of prokaryotic (bacteria, yeast), or a single eukaryotic (mammalian, insect) cell stored cryogenically, and is composed of sufficient ampoules of culture to provide source material for the WCB. The WCB is defined as a quantity of cells derived from one or more ampoules of the MCB, stored cryogenically, and used to initiate a single production batch. Both the MCB and the WCB must be stored in conditions that assure genetic stability. Generally, cells stored in liquid nitrogen or its vapour phase are stable longer than cells stored at $-70°C$.

Establishment of cell banks should be performed in a suitably controlled environment to protect the cells and, where applicable, the personnel handling them. During the establishment of the cell banks, no other living or infectious material such as viruses, other cell lines or cell strains, should be handled simultaneously in the same area or by the same persons. Only authorized personnel should be allowed to handle the material, and this handling should be done under the supervision of a responsible person. It is desirable to split the cell banks and to store the parts in more than one location so as to minimize the risks of total loss. All ampoules containing the cell banks should be treated identically during storage.

Cell banks should be established, stored and used in such a way as to minimize the risks of contamination or alteration. They should be adequately characterized and tested for contaminants and shown to be free of adventitious agents such as fungi, bacteria, mycoplasma, and exogenous viruses; tested for tumourigenicity; and probed for the expression of any endogenous retroviral sequences by using conditions known to cause their induction; and their suitability for use demonstrated by the consistency of the characteristics, and quality of the successive batches of product. The number of generations (or doublings or passages) between the cell bank and the finished product should be as low as is practicable.

Inoculation and aseptic transfer

Inoculation of the seed culture into the fermenter or bioreactor, as well as all transfer and harvesting operations must be done using validated aseptic techniques. Additions or withdrawals from fermenter or bioreactors are generally done through steam sterilized lines and steam-lock assemblies. Steam may be left on in situations where the heating of the line or the vessel wall would not be harmful to the culture. If possible, the media used should be sterilized in-situ, using a Sterilization in Place (SIP) or a continuous sterilization system (CSS), and any nutrients or chemical added

beyond this point must be sterile. Additions of materials or cultures, and the taking of samples, should be carried out under carefully controlled conditions to ensure that the absence of contamination is maintained. Care should be taken to ensure that vessels are correctly connected when additions or samplings take place. In-line sterilizing filters should be used where possible for the routine addition of air and other gases, media, acids or alkalis, and defoaming agents, to the fermenter or bioreactor.

Process monitoring and control

It is important for a fermenter or bioreactor to be closely monitored and tightly controlled to achieve the proper and efficient expression of the desired product. The parameters for the fermentation process, including information on growth rate, pH, waste by-product levels, addition of chemicals, viscosity, density, mixing, aeration, and foaming, must, therefore, be specified and monitored. Other factors that may affect the finished product, such as shear forces, process-generated heat, should also be considered. Many growth parameters can influence protein production. Although nutrient-deficient media are used as a selection mechanism in certain cases, media deficient in certain amino acids may cause substitutions. The presence of such closely related products may cause difficulties later on during the separation and purification stages, and may have implications both for the application of release specifications and the effectiveness of the product purification process.

Containment considerations

Bioreactor systems designed for recombinant microorganisms require not only that a pure culture is maintained, but also that the culture be contained within that system. Such containment can be achieved by the proper choice of a host-vector system that is less capable of surviving outside a laboratory environment, as well as by physical means, when this is considered necessary. For the cultivation of recombinant cell lines, there are defined and established physical containment levels. Good Large-Scale Practice (GLSP) level of physical containment is recommended for large-scale production involving viable, non-pathogenic and non-potent recombinant strains derived from host organisms that have an extended history of safe large-scale use, and for organisms that have built-in environmental limitations that, although allowing optimum growth in the fermenter, have limited survival outside in the environment. Biosafety level 1 (BL1) level of physical containment is recommended for large-scale production of viable recombinant organisms that require BL1 containment at the laboratory scale. Similar recommendations exist for BL2 and BL3. No provisions are made for the large-scale research or production of

viable recombinant organisms that require BL4 containment at the laboratory scale.

Personnel considerations

The immunological status of personnel should be taken into consideration for product safety. All personnel engaged in the production, maintenance and testing should be vaccinated where necessary with appropriate specific vaccines and have regular health checks. Apart from the obvious problem of staff exposure to infectious agents, potent toxins, or allergens, it is necessary to avoid the risk of contaminating a production batch with infectious agents. Therefore, visitors are generally excluded from production areas. Furthermore, in the course of a working day, personnel should not pass from areas where exposure to live organisms or animals is possible to areas where other products or different organisms are handled. If such passage is unavoidable, clearly defined decontamination measures including change of clothing and shoes and, where necessary, showering should be followed by staff involved in any such production.

11.3.3 Product recovery and purification

Once the fermentation process is completed, the desired product is extracted, isolated, separated and, if necessary, refolded to restore configurational integrity, and then purified. Whether the product is intra-cellular or extra-cellular, soluble, insoluble or membrane bound or located in a subcellular organelle will influence the choice of extraction method and buffer components used. Typically, manufacturers develop downstream processes on a small scale and determine the effectiveness and limitations of each particular processing step. Allowances must, therefore, be made for several differences when the process is scaled-up. Longer processing times can adversely affect product quality since the product is exposed to various reaction conditions, such as pH and temperature, for longer periods. Product stability under such varying purification conditions must, therefore, be carefully defined.

Product recovery

Determining the optimal time of harvest is an important area of interaction between fermentation and recovery. Often, allowing a culture to run longer results in an increase in titre, but with a concomitant increase in cellular debris and degraded forms of the product. Although it may be simple to overcome the effect of increased cell debris by increasing the capacity of the downstream equipment, it is much more difficult to purify away the slightly altered or degraded forms of the product.

With extra-cellular products, it is possible to achieve a high degree of purification by simply removing the cells. For the recovery of extra-cellular proteins, the primary separation of product from producing organisms is accomplished by centrifugation or membrane filtration. Ultra filtration is commonly used to remove the desired product from the cell debris. The porosity of the membrane filter is calibrated to a specific molecular weight, allowing molecules below that weight to pass through while retaining molecules above that weight. Centrifugation can be open or closed, although the adequacy of the environment must be evaluated for open centrifugation. Following centrifugation, other separation methods, such as ammonium sulphate precipitation and aqueous two-phase separation, can also be employed to concentrate the product.

With extra-cellular products, cell breakage is unnecessary and undesirable. Cell disintegration not only releases membrane fragments that can foul process equipment, but also undesirable impurities derived from the cell cytoplasm, particularly host cell proteins and DNA. The harvest/cell separation operation is more difficult with mammalian and other animal cells, as they are much more fragile than bacterial or yeast cells. Consequently, high-speed centrifuges may not be appropriate and these cells must be harvested with special low shear, low centrifugal field centrifuges. Harvesting can also be carried out effectively and efficiently using depth or tangential flow filtration. The advantage of filtration is its ability to achieve quantitative increases in product yield by washing (diafiltration) the cells.

Intra-cellular or membrane-bound products will require detergents or organic solvents to solubilize them. For the recovery of completely intracellular products, the cells must be disrupted after fermentation, which can be achieved by chemical, enzymatic or physical methods. Following disruption, the cellular debris is removed either by centrifugation or filtration.

Purification

Further purification steps primarily involve a variety of chromatographic methods to remove impurities and to bring the product closer to final specifications. One or more of the following column chromatography techniques usually achieves this:

- affinity chromatography;
- ion-exchange chromatography (IEC);
- gel filtration or size-exclusion chromatography (SEC);
- hydrophobic interaction chromatography (HIC);
- reverse-phase HPLC (RP-HPLC).

A prior knowledge of the protein stability and its sensitivity to temperature, extremes of pH, proteases, air and metal ions will also aid the design of a purification procedure. If the product to be purified is an enzyme or receptor it may be possible to exploit its activity by affinity purification on a substrate or ligand, or an analogue. Knowledge of the size and pH of the protein will indicate suitable matrices and conditions for gel filtration and ion-exchange chromatography. The final use of the product will define how much of the purified protein is required, whether loss of activity can be tolerated, how pure it should be, and the time and cost of purifying it. If it is for research use, the quantities required are reasonably small, whilst in terms of purity the removal of interfering activities becomes essential. In contrast, for therapeutic applications, purity is of the utmost importance and quantities required are relatively small.

Selection and sequence of the downstream processing steps

Each protein has a unique combination of properties that can be exploited for purification. Thus by combining a series of steps that exploit several of these properties, the protein can be purified from a mixture. Each technique should be evaluated for its capacity, resolving power, probable product yield and cost, and would use a different property of the product, such as charge or hydrophobicity, to effect adsorption and separation. These factors must be balanced against one another and the requirement for each stage of the purification. Moreover, the number of steps in a purification process should be limited by ensuring that the product from one technique can be applied directly onto the next step without further manipulations.

The capacity of the technique is defined as the amount of sample (in terms of volume and protein concentration) that can be handled. A key requirement early in the purification is often to reduce the volume when high capacity techniques such as precipitation methods, which can handle the large initial volumes and protein concentrations, are often used first. Of the chromatography steps, those involving adsorption have the highest capacity. Gel filtration or size exclusion chromatography has a low capacity and is, therefore, usually inappropriate for early stages and is mostly used as a final clean up.

The resolution of a technique determines how efficiently it separates proteins from one another. Precipitation steps have low resolution, whilst chromatography steps are more highly resolving. Affinity chromatography often shows extremely high resolution and it is possible to frequently achieve purification factors of greater than 1000 fold.

Due to the nature of the various interactions and the conditions used, each technique will show a range of average yields. Precipitation with ammonium

sulphate and aqueous two-phase extraction usually gives yield of more than 80%, whilst affinity methods often result in lower yield (~60%) due to the harsh conditions required for the elution of the product.

With respect to cost, affinity techniques are usually expensive and so not often used as an initial purification step. A cheaper technique such as ion exchange chromatography is usually used first to remove the bulk of the contaminants such as particulate matter, lipids and DNA.

Integration with upstream operations

In the narrowest definition, downstream processing is the purification of proteins from conditioned media or broths. However, many controllable factors that influence purification occur early in the production process. The integration of downstream processing with upstream operations such as molecular biology and fermentation can, therefore, provide significant downstream opportunities.

The interaction between molecular biology and recovery can take several forms. With recombinant DNA products, purification can be influenced before the starting material is even available. Given the gene sequence, it is possible to predict how the product will behave on size separation media and ion exchange resins, although the actual ionic properties of the protein may be influenced by its tertiary structure. Leading or tail sequences can be added to impart properties that will make the protein easier to purify. It is common practice in bacterial systems to employ fusion proteins to enhance expression, secretion or the subsequent recovery. In mammalian and animal cell systems, the tools of molecular biology are used to enhance expression levels and to alter the biological properties of the final product. Higher titres provide a direct benefit to the recovery process by increasing the ratio of product to contaminant, thereby reducing the fold purification that is ultimately required and also by enabling reductions in the operation volumes of early steps.

Perhaps the most important examples of process integration occur in the interaction between recovery and fermentation. One of the primary areas of interaction between these disciplines is the development of suitable media for cell growth. In cases where the expression system uses an amplified selectable marker, it may be necessary to maintain selective pressure during some or all stages of cell culture. The use of media supplements such as serum may release this selection pressure, resulting in a decrease in expression level, as well as adversely affecting the overall recoverability by leading to complex formation and product degradation. This problem can be overcome by the use of low serum, fractionated serum, or even serum-free medium.

11.3.4 Primary production facilities

The risk of cross-contamination between biological medicinal products, especially during those stages of the manufacturing process in which live organisms are used, may require additional precautions with respect to facilities and equipment, such as the use of dedicated facilities and equipment, production on a campaign basis and the use of closed systems, until the inactivation process is accomplished. The degree of environmental control of particulate and microbial contamination of production premises should, therefore, be adapted to the product and the production step, bearing in mind the level of contamination of the starting materials and the risk to the finished product.

Production on a campaign basis may be acceptable for spore-forming organisms provided that the facilities are dedicated to this group of products, and not more than one product is processed at any one time. Simultaneous production in the same area using closed systems such as fermenters may be acceptable for products such as monoclonal antibodies and products prepared by recombinant DNA techniques. Processing steps after harvesting may be carried out simultaneously in the same production area provided that adequate precautions are taken to prevent cross-contamination. For killed vaccines and toxoids, such parallel processing should only be performed after inactivation of the culture or after detoxification. Equipment used during the handling of live organisms should be designed to maintain cultures in a pure state and uncontaminated by external sources during processing.

Positive pressure areas should always be used to process sterile products, but negative pressure in specific areas at point of exposure of pathogens is acceptable for containment reasons. Where negative pressure areas or safety cabinets are used for aseptic processing of pathogens, they should be surrounded by a positive pressure sterile zone. Air filtration HVAC units should be specific to the processing area concerned and recirculation of air should not occur from areas handling live pathogenic organisms. The layout and design of production areas and equipment should allow effective cleaning and decontamination. The adequacy of cleaning and decontamination procedures should be validated. Pipework systems, valves and vent filters should be properly designed to facilitate cleaning and sterilization. The use of CIP and SIP systems should be encouraged. Primary containment should be designed and tested to demonstrate freedom from leakage risk. Effluents that may contain pathogenic microorganisms should be effectively decontaminated.

Genetically engineered organisms

When handling genetically engineered materials, the biosafety controls required should include testing facilities that adequately provide a controlled

environment and separation of test systems, as well as adequate and appropriate areas for receipt and storage of both the host organism and test substance, as well as for any other materials, such as any stocks of plants, feed and soils used in the study, as well as facilities for waste disposal. Both the laboratory facilities and any separate outdoor testing facilities, such as greenhouses and field sites, that are used for testing the genetically engineered substance should be of sufficient design (layout, size and location) to provide the necessary containment of appropriate biosafety level to protect personnel and the environment. They should be designed to provide a barrier to the unintended release of any organisms if a spill or application accident were to occur, and the decontamination facilities should be separated from the other areas of the facility. The laboratory should have decontamination procedures for containing or killing genetically engineered organisms and host organisms. Moreover, the facility should have proper ventilation, so that air flows from areas of low contamination to areas of higher contamination, and complete air containment and decontamination should be provided. Environmental conditions such as temperature, humidity and ventilation should be monitored using appropriate instruments, and recorded and specified in the protocol for the ongoing study.

Animal quarters and care

Animals are used for the manufacture of a number of biological products, for example polio vaccine (monkeys), snake anti-venoms (horses and goats), rabies vaccine (rabbits, mice and hamsters) and serum gonadotropin (horses). Animals may also be used in the quality control of most sera and vaccines, such as for pertussis vaccine (mice), pyrogenicity (rabbits), BCG vaccine (guinea-pigs). Quarters for animals used in the production and control of biological products should be separated from the production and control areas. The health status of animals from which some starting materials are derived, and of those used for quality control and safety testing, should be routinely monitored and recorded. Staff employed in such areas must be provided with special clothing and changing facilities.

11.3.5 Safety issues

The presence of process-related contaminants in a bio-pharmaceutical is chiefly a safety issue. The sources of contaminants are primarily the cell substrate (DNA, host cell proteins and other cellular constituents, viruses), the media (proteins, sera and additives) and the purification process (process-related chemicals and product-related impurities).

Residual host cells

In the early days, there were concerns about the safety of immortal transformed cell lines since, by definition, they were thought to contain oncogenic DNA or proteins. In addition to the issues arising from the transformed nature of these cells, there were also concerns regarding the contamination of these cell lines by adventitious agents such as viruses, fungi and mycoplasma. Furthermore, there were also concerns about the immunogenicity resulting from residual host cell proteins, in patients who received drugs that were purified from recombinant sources. There are various regulatory guidelines for the characterization of the cells used in the manufacture of bio-pharmaceuticals. The exhaustive characterization of the cell banks by diverse methods provides at least an initial degree of confidence that the resultant products can be safely injected into humans. Concerns over the presence of residual host cell proteins have largely been put to rest by relying on well-established techniques for sterile filtration, as well as in advances in analytical method development.

Residual contaminating proteins

Due to the concern about the safety of proteins from non-human sources with respect to the generation of immune responses, recombinant proteins are generally being brought to unprecedented levels of purity. It can be as difficult to quantitate and prove the levels of purity as it is to achieve them. For example, whereas the purity of albumin preparations is commonly about 95–99%, the purity of recombinant products such as human growth hormone, human insulin or even hepatitis B vaccine is greater than 99.99% with respect to host proteins. In order to measure impurities at this level, two major analytical strategies have been developed. The first method, which is uniquely applicable to all recombinant products, is the use of a blank run. This involves fermentation and recovery using a host cell containing the selectable marker but lacking the gene for the product, thereby enabling the manufacturer to specifically prepare and quantitate the host cell derived impurities. The second approach is the direct measurement of the impurities. The most general method uses an immuno-assay based on antibodies to the host cell proteins. Although this type of assay is complex both in its development and composition, it provides an extremely sensitive way to quantitate protein impurities in each batch of product.

Residual nucleic acids

When immortalized mammalian cells were first considered as host systems for recombinant protein, there was substantial theoretical concern about the possibility of DNA from recombinant immortal cell lines causing oncogenic events in patients receiving products from these cell substrates. However, various scientists have shown that DNA does not induce any oncogenic events when injected into immuno-suppressed rodents, even at levels at least eight orders of magnitude greater than that expected in a dose of human therapeutic protein such as t-PA. It is most likely the naked DNA is degraded very quickly to inactive fragments and nucleotides by circulating nucleases. With current technology it is possible to directly measure the DNA content of clarified cell culture fluid and the early processing steps with a DNA dot blot assay using 32P labelled DNA derived from the host cell line. For some products, especially those that are administered in multi-milligram quantities, it is necessary to demonstrate a reduction to assure a level of DNA of less than 10 pg per human dose. This can be further validated by spiking 32P labelled DNA into aliquots of process fluid and then purifying the samples on representative scaled-down versions of the recovery process operations.

Viruses

The presence of retro-viruses in continuous mammalian and other animal cell lines has received a great deal of attention because of concern that these particles can potentially cause oncogenic events in man. However, the approach of demonstration of freedom from functional retro-viruses in the culture is not usually sufficient to answer regulatory concerns, because it is always possible that there might be levels of retro-virus just below the sensitivity limit of these assays or that the specificity of the retro-virus assays might not be broad enough to pick up some unusual potential contaminant. To address this issue, the authorities require the testing of the harvested culture fluid directly for the presence of retro-viruses, or following concentration by ultracentrifugation before analysis, to increase the sensitivity of electron microscopy past the estimated detection limit of 106 particles per ml. These direct measurements can be supplemented by validated process procedures for removal and/or inactivation of putative retro-viruses. Only with steps that are truly independent is it legitimate to determine the total clearance as a result of the clearances from the individual steps. Moreover, the use of more than one model virus and the assay of the virus by more than one technique would also serve to strengthen the believability and validity of this approach.

Pyrogen and endotoxins

In contrast to bacterial fermentations, especially of gram-negative bacteria such as *E.coli*, mammalian and other animal cell fermentations should contain little or no pyrogen, and the recovery process should not need to incorporate steps to remove pyrogens. The process strategy thus becomes oriented more towards keeping pyrogens out rather reducing their levels, and it is much more important to keep raw materials and equipment pyrogen-free.

11.4 Secondary production

One of the more difficult processes to regulate, and one which has presented considerable problems over the years, is that of the manufacture of sterile bio-pharmaceuticals. During the past few years, a number of sterile batches from different manufacturers have been reported to have exhibited microbiological contamination. One manufacturer had approximately 100 batches contaminated in a six month time period, whilst another had approximately 25 batches contaminated in a similar period; other manufacturers have had recalls due to the lack of assurance of sterility. Not surprisingly, the manufacture of sterile bio-pharmaceuticals is subjected to special requirements relating to the minimizing of risks of microbiological, as well as of particulate and pyrogen contamination.

The manufacture of a sterile pharmaceutical must be performed in closed systems with minimal operator handling, although much of this depends on the skills, training and attitudes of the personnel involved. Quality assurance is particularly important and this type of manufacture must strictly follow carefully established and validated methods of preparation and procedure. Most bio-pharmaceuticals cannot be terminally sterilized and must, therefore, be manufactured by aseptic processing. Thus, it is important to recognize that as there is no further processing to remove contaminants or impurities such as particulates, endotoxins and degradants, sole reliance for sterility or other quality aspects, must not be placed on any terminal process or finished product test.

11.4.1 Starting materials

The manufacture of a sterile bio-pharmaceutical should be performed and supervised by competent people. The purchase of starting materials is an important operation, which should involve staff who have a thorough knowledge of the suppliers and who should only purchase from approved suppliers named in the relevant specification. The source, origin and suitability of starting materials should be clearly defined; the various components, contain-

ers and closures that are received, identified, stored, handled, sampled, tested and approved or rejected should be regularly inspected, and the system should be challenged to test if it is functioning correctly. There must be written procedures describing how these operations are done and if the handling and storage of components are computer controlled, the programme must be validated.

Control of raw materials

Written procedures should be established describing the purchase, receipt, identification, quarantine, storage, handling, sampling, testing and approval or rejection of raw materials, and such procedures should be followed. In fact, it is beneficial for all aspects of the manufacture and control of the starting material in question, including handling, labelling and packaging requirements, as well as complaints and rejection procedures to be discussed with the supplier. All materials and products should be handled and stored under the appropriate conditions established by the manufacturer, in an orderly fashion to permit batch segregation and stock rotation, as well prevent contamination or cross-contamination. The manufacturer must be able to show that the containers and closures are compatible with the product, will provide adequate protection for the product against deterioration or contamination, are not additive or absorptive, and are suitable for use.

Receipt, sampling, testing and approval of raw materials

Incoming materials should be physically or administratively quarantined immediately on receipt, until they have been sampled, tested or examined as appropriate and released for use or distribution. They should be checked to ensure that the consignment corresponds to the order, and examined visually for integrity of package and seal, for correspondence between the delivery note and the supplier's labels, for damage to containers and any other problem that might adversely affect the quality of a material. The receiving records must be traceable to the component manufacturer and supplier and should contain the name of the component, manufacturer, manufacturer's lot number, supplier if different from the manufacturer, and carrier. All handling of starting materials, such as receipt and quarantine, sampling, storage, labelling, dispensing, processing, packaging and distribution should be done in accordance with written procedures or instructions and, where necessary, recorded.

The number of containers to sample and the sample size should be based upon appropriate criteria, such as the quantity needed for analysis, sample variability, degree of precision desired and past quality history of the supplier, and the sample containers properly identified. At least one test should be conducted to verify the identity of each raw material. A supplier's certificate of

analysis may be used instead of performing other testing, provided the manufacturer has a system in place to evaluate vendors (vendor audits) and establishes the reliability of the supplier's test results at appropriately regular intervals. For hazardous or highly toxic raw materials, where on-site testing may be impractical, suppliers' certificates of analysis should be obtained showing that the raw materials conform to specifications. However, the identity of these raw materials must be confirmed by examination of containers and labels, and the lack of on-site testing for these hazardous raw materials should be documented. Intermediate and bulk products purchased as such should also be handled as though they were starting materials.

Starting materials in the storage area should be appropriately labelled and should only be dispensed by designated persons, following a written procedure, to ensure that the correct materials are accurately weighed or measured into clean and properly labelled containers. Materials dispensed for each batch should be kept together and conspicuously labelled as such. Information on the labels should provide traceability from the component manufacturer to its use in the finished product, and should bear at least the following information:

- the designated name of the product and the internal code reference where applicable;
- a batch number given at receipt;
- the status of the contents (e.g. in quarantine, on test, released, rejected) where applicable;
- an expiry date or a date beyond which re-testing is necessary, if appropriate.

When fully computerized storage systems are used, all the above information need not necessarily be in a legible form on the label.

Use and re-evaluation of approved raw materials

Approved raw materials should be stored under suitable conditions and, where appropriate, rotated so that the oldest stock is used first. Raw materials should be re-evaluated as necessary to determine their suitability for use, for example, after prolonged storage or after exposure to heat or high humidity. Sanitary conditions in the storage area, stock rotation practices, re-test dates and special storage conditions, such as protection from light, moisture, temperature and air, should be checked regularly.

Rejected raw materials

Rejected raw materials should be identified and controlled under a quarantine system designed to prevent their use in manufacturing or processing operations for which they are unsuitable.

11.4.2 Final processing operations

Sterile products are usually produced by dissolving the non-sterile bulk active substance in a solvent and then filtering the solution through a sterilizing filter. After filtration, the sterile bulk material is separated from the solvent by crystallization, precipitation and spray-drying or lyophilization. During these final processing operations, all necessary in-process controls and environmental controls should be carried out and recorded, and any significant deviation from the expected yield should be recorded and investigated.

Critical manufacturing steps

Each critical step in the manufacturing process should be done by a responsible individual and checked by a second responsible individual. If such steps in the processing are controlled by automatic mechanical or electronic equipment, its performance should be verified. Critical manufacturing steps not only include the selection, weighing, measuring and identifying of components, and addition of components during processing, but also the recording of deviations in the manufacturing record, testing of in-process material and the determination of actual yield and percent of theoretical yield. These critical manufacturing steps should be fully validated and documented when done. At all times during processing, all materials, bulk containers, major items of equipment and, where appropriate, the rooms used, should be labelled or otherwise identified with an indication of the product or material being processed, its strength (where applicable), batch number and the stage of production. Labels applied to containers, equipment or premises should be clear, unambiguous and in the company's agreed format. It is often helpful in addition to the wording on the labels to use colours to indicate status, such as quarantined, accepted, rejected and clean.

Preparation

Before any processing operation is started, steps should be taken to ensure that the work area and equipment are clean and free from any starting materials, products, product residues or documents that are not required for the operation being planned. Intermediate and bulk products, and all starting materials should be kept under appropriate conditions. Checks should be carried out to ensure that pipelines and other pieces of equipment used for the transportation of products from one area to another are connected in a correct manner. Non-combustible gases, and all solutions, in particular large volume infusion fluids, should be passed through a microorganism retaining filter if possible, immediately prior to filling. Any components, containers, equipment and any other article required in the clean area where aseptic work takes place should be

sterilized and passed into the area through double-ended sterilizers sealed into the wall, or by a procedure which achieves the same objective of not introducing contamination. Bioburden and contamination levels should be monitored before sterilization and where appropriate, the absence of pyrogens should also be monitored. The interval between the washing, drying and the sterilization of components, containers and equipment, as well as between their sterilization and use should be minimized and subject to a time-limit appropriate to the storage conditions.

Batching

Many of these bio-pharmaceutical products lack preservatives, inherent bacteriostatic or fungistatic activity. Obviously, the batching or compounding of bulk solutions should, therefore, be controlled in order to prevent any potential increase in microbiological levels that may occur up to the time that the bulk solutions are filter sterilized. One concern with any microbiological level is the possible increase in endotoxins that may develop. Good practice would, therefore, include working in a controlled environment, and in sealed tanks to control accessibility, particularly if the non-sterile product solutions are to be stored for any period prior to sterilization.

Filling

The filling of bio-pharmaceuticals into ampoules or vials presents many of the same problems as the processing of conventional pharmaceuticals. The batch size of a bio-pharmaceutical is likely to be small and the validation of aseptic processes presents special problems when the batch size is small. In these cases, the number of units filled may be the maximum number filled in production and because of the small batch size, filling lines may not be as automated as for other products typically filled in larger quantities. Moreover, filling and sealing will often be a hand operation, presenting great challenges to sterility; and with more involvement of people filling these products, more attention should be given to environmental monitoring. Typically, vials to be lyophilized are partially stoppered by machine. However, some filling lines have even been observed using an operator to place each stopper on top of the vial by hand. The immediate concern in this case is the avenue of contamination offered by the operator. Due to the active involvement of people in filling and aseptic manipulations, the number of persons involved in these operations should be kept to a minimum, and the environmental programme should include an evaluation of microbiological samples taken from people working in such aseptic processing areas. Some of the problems that are routinely identified

during filling include inadequate attire, deficient environmental monitoring programmes and failure to validate some of the basic sterilization processes.

One major concern is the use of inert gas to displace oxygen during both the processing and filling of the solution, and therefore, limits for dissolved oxygen levels for the solution must be established for products that may be sensitive to oxidation, and parameters such as line speed and the location of the filling syringes with respect to their closures should be defined. In the absence of inert gas displacement, the manufacturer should be able to demonstrate that the product is not affected by oxygen. Another major concern with the filling operation of a lyophilized product is the assurance of fill volumes. Obviously, a low-fill would represent a sub-potency in the vial. Unlike a powder or large volume liquid fill, a low-fill would not be readily apparent after lyophilization, particularly for a product where the active ingredient may be only a milligram. Due to its clinical significance, sub-potency in a vial can potentially be very serious.

Lyophilization (freeze drying) or spray drying

Many bio-pharmaceuticals are lyophilized because of stability concerns. Unfortunately, the cGMP aspects of the design of lyophilizers have lagged behind the sterilization and control technology employed for other processing equipment. It is not surprising that many problems with the lyophilization process have been identified. These problems are not limited to bio-pharmaceuticals, but generally pertain to lyophilization of all products including bio-pharmaceuticals. With regard to bulk lyophilization, concerns include air classification, aseptic barriers for loading and unloading the unit, partial meltback, uneven freezing and heat transfer throughout the powder bed, and the additional aseptic manipulations required to break up the large cake. For bulk lyophilization, unlike other sterile bulk operations, media challenges can be performed, and hence suitable validation studies must be carried out.

There are also concerns over the spray drying of sterile bio-pharmaceuticals, including the sterilization of the spray dryer, the source of air and its quality, the chamber temperatures, and the particle residence or contact time. In some cases, charring and product degradation have been found for small portions of a batch. These should all be assessed during process validation.

Sterile filtration of products which cannot be sterilized in their final container

If the product cannot be sterilized in the final container, then solutions or liquids must be filtered through a sterile filter of nominal pore size of 0.22 micron (or less), or with at least equivalent microorganism retaining properties, into a previously sterilized container. Such filters can remove most bacteria and

moulds, but not all viruses or mycoplasmas, so consideration should be given to complementing the filtration process with some degree of heat treatment. Moreover, if other means of sterilization in the final container were possible, then final sterile filtration alone is not considered sufficient. The specification for the filters should include information such as its fibre shedding characteristics, the criteria used for the selection of the filter, as well as the procedures used for integrity testing of the filters. The integrity of the sterilized filter should be verified before use, and should be confirmed immediately after use by an appropriate method such as a bubble point, diffusive flow, or the pressure hold test. The time taken to filter a known volume of bulk solution, the maximum filtration pressures and the pressure differential across the filter should also be determined during validation, and any significant differences from this should be noted and investigated. The same filter should never be used for more than one working day unless such use has been validated. If filters were not changed after each batch is sterilized, there should be data to justify the integrity of the filters for the time periods utilized and prove that grow-through has not occurred.

Terminally sterilized products

Steam sterilization is the preferred method of those currently available. However, before any sterilization process is adopted, its suitability for the product and its efficacy in achieving the desired sterilizing conditions in all parts of each type of load to be processed should be demonstrated by physical measurements and by the use of biological indicators where appropriate. There should also be a clear means of differentiating products which have not been sterilized from those which have, with each basket, tray or other carrier of products or components clearly labelled with the name of the product, its batch number, and an indication of whether or not it has been sterilized. Typically, a sterile pharmaceutical contains no viable micro-organisms and is non-pyrogenic. Parenteral drugs in particular must be non-pyrogenic because the presence of pyrogens can cause a febrile reaction in human beings. Pyrogens are the products of the growth of microorganisms, so any condition that allows microbial growth should be avoided in the manufacturing process. Pyrogens may develop in water located in storage tanks, dead legs and pipework, or from surface contamination of containers, closures or other equipment, and may also contain chemical contaminants that could produce a pyretic response in humans or animals even though there may be no pyrogens present.

Therefore, the procedures used to minimize the hazard of contamination with microorganisms and particulates of sterile bio-pharmaceuticals become extremely important. Preparation of components and other materials should be

done in at least a grade D environment in order to give low risk of microbial and particulate contamination, suitable for filtration and sterilization. Where the bio-pharmaceutical is at a higher than usual or an unusual risk of microbial contamination; for example, because the product actively supports microbial growth, or must be held for a long period before sterilization, or needs to be processed in other than closed vessels, then all the preparation should be carried out in a grade C environment. Filling of a bio-pharmaceutical for terminal sterilization should be carried out in at least a grade C environment. Where the product is at an unusual risk of contamination from the environment because, for example, the filling operation is slow or the containers are wide-necked or is necessarily exposed for more than a few seconds before sealing, the filling should be done in a grade A zone, with at least a grade C background.

Finishing of sterile products

Filled containers of bio-pharmaceuticals should be closed by appropriately validated methods. Containers closed by fusion, for example, glass or plastic ampoules, should be subject to 100% integrity testing, while those closed by other means should be checked for integrity according to appropriate procedures. Containers sealed under vacuum should be tested for maintenance of that vacuum after an appropriate, pre-determined period. Filled containers should be inspected individually for extraneous contamination or other defects, and if inspection is done visually, it should be done under suitable and controlled conditions of illumination and background. Where other methods of inspection are used, the process should be validated and the performance of the equipment checked at intervals with the results recorded.

Some sterile bio-pharmaceuticals may be filled into different types of containers, such as sterile plastic bags. For sterile bags, sterilization by irradiation is the method of choice because it leaves no residues, although some manufacturers use formaldehyde. A major disadvantage is that formaldehyde residues may, and frequently do appear in the sterile product. If multiple sterile bags are used, operations should be performed in an aseptic processing area. Since all the inner bags have to be sterile, outer bags should also be applied over the primary bag containing the sterile product in the aseptic processing area. One manufacturer was found to apply only the primary bag in the aseptic processing area, resulting in the outer portion of this primary bag being contaminated when the other bags were applied over this bag in non-sterile processing areas! Important validation aspects of the sterile bag system include measurement of residues, testing for pinholes, foreign matter (particulates), as well as for sterility and endotoxins.

11.4.3 Secondary (sterile) production facility

Manufacturing operations are divided into two categories — those where the product is terminally sterilized and those which are conducted aseptically at some or all stages. The design, validation and effective operation of clean rooms for the manufacture and testing of pharmaceuticals, biotechnology and medical device products is among the most exacting and challenging activities. Patient's lives, product integrity, company profitability and regulatory compliance all factor into the risks inherent if the clean room is not built right and does not function right. The manufacture of sterile products should be carried out in clean areas, entry to which should be through airlocks for personnel and/or for equipment and materials, and maintained to an appropriate standard of cleanliness, and supplied with air that has passed through filters of an appropriate efficiency. Adequate space must be provided for the placement of equipment and materials to prevent mix-ups for operations such as the receiving, sampling, and storage of raw materials; manufacturing, processing, packaging and labelling; storage for containers, packaging materials, labelling and finished products; as well as for production and control laboratories. Facility design features for the aseptic processing of sterile bulk active products should include temperature, humidity and pressure control, and there must be adequate lighting, ventilation, screening and proper physical barriers for all operations including dust, temperature, humidity and microbiological controls, with the various operations of component preparation, product preparation and filling carried out in separate areas within the clean area.

Area classification and monitoring of controlled environments

Clean areas for the manufacture of sterile products are classified according to the required characteristics of the environment. Each manufacturing operation requires an appropriate level of cleanliness in the operational state, in order to minimize the risks of particulate or microbial contamination of the product or materials being handled. In order to meet in-operation conditions, these areas should be designed to reach certain specified air-cleanliness levels in the at-rest occupancy state. The at-rest state is the condition where the installation is installed and operating, and is complete with production equipment, but has no operating personnel present. The in-operation state is the condition where the installation is functioning in the defined operating mode with the specified number of personnel.

For the manufacture of sterile medicinal products there are normally four grades of clean areas. The requirement and limit for these areas depend on the nature of the operations carried out. Grade A is for the aseptic preparation and filling of products, and the local zone for high risk operations such as the filling

zone, stopper bowls, open ampoules and vials, making aseptic connections. Normally such conditions are provided by a laminar airflow workstation, which should provide a homogeneous air speed of $0.45\,m\,s^{-1} \pm 20\%$ (guidance value) at the working position. Grade B is for aseptic preparation and filling, and the background environment for grade A zone. Grade C is for the preparation of solutions to be filtered and the filling of products that are at high risk. Grade D is a clean area for carrying out less critical stages in the manufacture of sterile products, for the handling of components after washing, and for the preparation of solutions and components for subsequent filling.

11.4.4 Safety issues

Contamination control

Manufacturing on a campaign basis is typical in the bio-pharmaceuticals industry. Whilst this may be efficient with regard to system usage, it can present problems when it is discovered in the middle of a campaign that a batch is contaminated. Frequently, all the batches processed in a campaign in which a contaminated batch is identified are suspect. Such failures should be investigated and reported, and the release of any other batches in the campaign should be justified. Some of the more significant product recalls have occurred because of the failure of a manufacturer to conclusively identify and isolate the source of a contaminant.

When working with dry materials and products, special precautions should be taken to prevent the generation and dissemination of dust. This could result in the risk of accidental cross-contamination arising from such uncontrolled release of dust, gases, vapours, sprays or organisms from materials and products in process, from residues on equipment and from operators' clothing. The significance of this risk varies with the type of contaminant, and the product being contaminated. Amongst the most hazardous contaminants are highly sensitizing materials, biological preparations containing living organisms, certain hormones, cytotoxics, and other highly active materials. Products in which contamination is likely to be most significant are those administered by injection and those given in large doses and/or over a long time.

Environmental control

Containers and materials liable to generate fibres should be minimized in clean areas. All components, containers and equipment should be handled after the final cleaning process in such a way that they are not re-contaminated. After washing, all components should be handled in at least a grade D environment.

The handling of sterile starting materials and components, unless subjected to sterilization or filtration through a micro-organism-retaining filter later in the process, should be done in a grade A environment with grade B background. However, the handling and filling of aseptically prepared products should be done in a grade A environment with a grade B background. The preparation of solutions that are to be sterile filtered during the process should be done in a grade C environment; however, if not filtered, the preparation of materials and products should be done in a grade A environment with a grade B background. The preparation and filling of sterile suspensions should be done in a grade A environment with a grade B background if the product is exposed and is not subsequently filtered. Prior to the completion of stoppering, the transfer of partially closed containers, as used in lyophilization (freeze drying) should be carried out either in a grade A environment with grade B background, or in sealed transfer trays in a grade B environment.

Prevention of cross-contamination

In clean areas, and especially when aseptic operations are in progress, all activities should be kept to a minimum, and the movement of personnel should be controlled and methodical to avoid excessive shedding of particles and organisms due to over-vigorous activity. The production of non-medicinal products should not be carried out in areas or with equipment destined for the final processing of bio-pharmaceuticals. Certainly, operations on different products should not be carried out simultaneously, or consecutively in the same room, unless there is no risk of mix-up or cross-contamination, and preparations of microbiological origin should not be made or filled in areas used for the processing of other sterile medicinal products; however, vaccines of dead organisms or of bacterial extracts may be filled, after inactivation, in the same premises as other sterile medicinal products. Manufacture in segregated areas is required for products such as penicillins, live vaccines, live bacterial preparations and certain other specified biologicals, or manufacture by campaign (separation in time) followed by appropriate cleaning. Precautions to minimize contamination should be taken during all processing stages including the stages before sterilization. These include using closed systems of manufacture, as well as appropriate air-locks and air extraction; using cleaning and decontamination procedures of known effectiveness, as ineffective cleaning of equipment is a common source of cross-contamination; as well as keeping protective clothing inside areas where products with special risk of cross-contamination are processed.

Control of sterility

Manufacturers are expected to validate all critical aseptic processing steps in the manufacture of bio-pharmaceuticals with at least three consecutive validation runs. Such validation must encompass all parts, phases, steps and activities of any process where components, fluid pathways or in-process fluids are expected to remain sterile. Furthermore, such validation must include all probable potentials for loss of sterility as a result of processing and account for all potential avenues of microbial ingress associated with the routine use of the process.

Sterility testing

The sterility test applied to the finished product should only be regarded as the last in a series of control measures by which sterility is assured. The test should be fully validated for the product(s) concerned with any examples of initial sterility test failures thoroughly investigated. In those cases where parametric release has been authorized, special attention should be paid to the validation and the monitoring of the entire manufacturing process. Samples taken for sterility testing should be representative of the whole of the batch, but should in particular include samples taken from parts of the batch considered to be most at risk of contamination. For example, for products that have been filled aseptically, samples should include containers filled at the beginning and at the end of the batch, and after any significant intervention. For products that have been heat sterilized in their final containers, consideration should be given to taking samples from the potentially coolest part of the load.

Media fill validation

Validation of aseptic processing should include simulating the process using a nutrient medium, the form of which is equivalent to the dosage form of the product, although suitable microbiologically-inert non-media alternatives would also be acceptable. This process simulation test should imitate as closely as possible the routine aseptic manufacturing process and include all the critical subsequent manufacturing steps, and should be repeated at defined intervals and after any significant modification to the equipment and process. The number of containers used for a medium fill should be sufficient to enable a valid evaluation. For small batches, the number of containers for the medium fill should at least equal the size of the product batch. The contamination rate should be less than 0.1% with 95% confidence level, and care should be taken that any validation does not compromise the processes, although the limitations of 0.1% media fill contamination rate should be recognized for the validation of aseptic processing of a non-preserved single dose bio-pharmaceutical, stored at

room temperature as a solution. Any alternative proposals for the validation of the aseptic processing of bio-pharmaceuticals may be considered by the regulatory authorities, but only on a case-by-case basis. For example, it may be acceptable to exclude from the aseptic processing validation procedure certain stages of the post-sterilization bulk process that take place in a totally closed system. Such closed systems should, however, be Sterilized in Place by a validated procedure, integrity tested for each lot, and should not be subject to any intrusions whereby there may be the likelihood of microbial ingress. Suitable continuous system pressurization would be considered an appropriate means for ensuring system integrity.

Control of pyrogens and endotoxins

Typically, a sterile pharmaceutical contains no viable microorganisms and is non-pyrogenic. Parenteral drugs must be non-pyrogenic because the presence of pyrogens can cause a febrile reaction in human beings. As pyrogens are the products of the growth of microorganisms, any condition that allows microbial growth should be avoided. Parenterals may also contain chemical contaminants that could produce a pyretic response in humans or animals, even if there are no pyrogens present. Moreover, in addition to pyrogens, microorganisms could contaminate the process stream with by-products such as glycosidases and proteases, which irreversibly alter or inactivate the product and as a result could adversely affect product stability.

The manufacturing process strategy, therefore, should be oriented more towards keeping endotoxins and pyrogens out as well as trying to reduce their levels. In some instances, where pipework systems for aqueous solutions have been shown to be the source of endotoxin contamination in sterile products, the manufacturer should be able to give assurance that there are no 'dead legs' in the system. In addition, water sources, water treatment equipment and treated water should be monitored regularly for such chemical and biological contamination and, as appropriate, for endotoxins.

Some manufacturers have argued that if an organic solvent is used in the manufacture of a sterile product, then the endotoxins levels are reduced at this stage. As with any operation, this may or may not be correct, and should be proven. For example, one manufacturer who conducted extensive studies using organic solvents for the crystallization of a non-sterile pharmaceutical to the sterile product observed no change from the initial endotoxin levels. In the validating the reduction or removal of endotoxins, challenge studies can be carried out on a laboratory or pilot scale to determine the efficiency of the step. However, since endotoxins may not be uniformly distributed, it is also important to monitor the bioburden of the non-sterile product(s) being sterilized. For

example, gram negative contaminates in a non-sterile bulk drug product prior to sterilization are of concern, particularly if the sterilization (filtration) and crystallization steps do not reduce the endotoxins to acceptable levels.

11.4.5 Out of specification

Regulatory authorities require that suitable process controls be established using scientifically sound and appropriate specifications, standards, sampling and re-sampling, testing and re-testing. These should be designed to ensure that all materials relating to the bio-pharmaceutical manufacture, such as components, containers, closures, in-process materials, labelling, including the product conform to appropriate standards of identity, strength, quality and purity. These controls should be used for the determination of conformity to applicable specification, for the acceptance of each batch (or lot) of material relating to manufacture, processing, packing, or the holding of the pharmaceutical. 'Out of specification' is defined as an examination, measurement, or test result that does not comply with such pre-established criteria. cGMP guidelines require written procedures to be in place to determine the cause of any apparent failure, discrepancy, or out of specification result. Out of specification results can be caused by laboratory error, non-process or operator error, or by process-related error, such as personnel or equipment failures. If, however, the result could not be clearly attributed to sampling or laboratory error, then there should be scientifically sound procedures and criteria for the exclusion of any test data found to be invalid and, if necessary, for any additional sampling and testing.

Re-testing

Although re-testing may be an appropriate part of the investigation, an investigation consisting solely of repeated re-testing is clearly inadequate. If quality is not built into a product, re-testing cannot make it conform to specifications. The number of re-tests performed before it can be concluded that an unexplained out of specification laboratory result is invalid, or that a product is unacceptable, is a matter of scientific judgment. There are no regulations on specific re-testing procedures, although manufacturers are expected to have written investigation and re-testing procedures, applying scientifically sound criteria. A variety of written and unwritten practices and procedures have been observed, under which manufacturers have disregarded out of specification laboratory results after minimal re-testing, re-sampling, inappropriate averaging of results or inappropriate testing. Some manufacturers then proceeded to release a product without a thorough investigation or an adequate justification for disregarding an out of specification result. Regulatory

authorities recognize the distinction between the limited investigation that may be necessary to identify a laboratory error and the more extensive investigation and testing necessary when out of specification results may be attributed to another cause. The manufacturer may impose additional criteria beyond those required to ensure identity, strength, quality and purity under cGMP regulations or as required for licensure. Although such internal controls are encouraged, under some circumstances it is possible to have test results that violate the internal standards, without being out of specification, as defined by regulations. The investigation should extend to other batches of the same product, and other products that may have been associated with the specific failure or discrepancy.

Re-testing for pyrogens and endotoxins

As with sterility, re-testing for pyrogens or endotoxins can be performed and is only acceptable if it is known that the test system was compromized and the cause of the initial failure is known, thereby invalidating the original results. It cannot be assumed that the initial failure is a false positive without sufficient documented justification. Again, any pyrogen or endotoxin test failures, the incidence, procedure for handling, and final disposition of the batches involved, should be investigated thoroughly, and the reasons for re-testing fully justified.

Sterility re-testing

The release of a batch, particularly of a sterile bio-pharmaceutical, which fails an initial sterility test and passes a re-test is very difficult to justify. Sterility re-testing is only acceptable if the cause of the initial non-sterility is known, and thereby invalidates the original results. It cannot be assumed that the initial sterility test failure is a false positive. This conclusion must be justified by sufficient documented investigation, and repeated sampling and testing may not identify any low level contamination. Sterility test failures, the incidence, procedures for handling, and final disposition of the batches involved should be routinely reviewed.

Reprocessing

The term reprocessing describes steps taken to ensure that the reprocessed batches will conform to all established standards, specifications and characteristics, and relates to steps in the manufacturing process that are out of the normal manufacturing processing sequence or that are not specifically provided for in the manufacturing process. As with the principal manufacturing process, reprocessing procedures should be validated. All the data pertaining to the reprocessed batches, as well as the data used to validate the process, should be

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reviewed and detailed investigation reports, including the description, cause and corrective action taken, should be available for the batch. The number and frequency of process changes made to a specific process or step can be an indicator of a problem experienced in a number of batches. For example, a number of changes in a short period of time can be an indicator that that particular process step is experiencing problems.

Rejection

The demonstration of the adequacy of the process to control other physico-chemical aspects is an important aspect of validation. Depending upon the particular bio-pharmaceutical, these include potency, impurities, particulate matter, particle size, solvent residues, moisture content and blend uniformity. For example, if the product is a blend of two active products or an active product and an excipient, then there should be some discussion and evaluation of the process for assuring uniformity. The process validation report for such a blend should include documentation for the evaluation and assurance of uniformity. Manufacturers occasionally reject the product following the purification process or after final processing. As with all pharmaceutical products, it is expected that any batch failing specifications is investigated thoroughly, and reports of these investigations are complete. For example, during one production campaign it was noted that approximately six batches of a bio-pharmaceutical product were rejected because of low potency and high levels of impurities. The problem was finally attributed to a defective column and, as a result, all the batches processed on that particular column were rejected.

11.5 Design of facilities and equipment

11.5.1 Facility design

When designing facilities for bio-pharmaceutical manufacture, the following activities should be considered as areas to control contamination:

- the receipt, identification, storage and withholding from use of raw materials or process intermediates, pending release for use in manufacturing; as well as the quarantine storage of intermediates and final products pending release for distribution;
- the holding of rejected raw materials, intermediates and final products before final disposition;
- the storage of released raw materials, intermediates and final products;
- manufacturing and processing operations;

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- the holding of rejected raw materials, intermediates and final products before final disposition;
- the storage of released raw materials, intermediates and final products;
- manufacturing and processing operations;

- packaging and labelling operations;
- all laboratory operations.

Control of microbiological, physical, and chemical contamination

The regulatory authorities require the establishment of, and adherence to, written procedures designed to prevent microbiological contamination of pharmaceuticals purporting to be sterile. These requirements also cover such procedures as the validation of any sterilization process, and are intended to reflect the fact that whether aseptic processing techniques or terminal sterilization methods are used, either technique must be validated. Where microbiological specifications have been established for the product, then facilities should also be designed to limit objectionable microbiological contamination, especially if different bio-pharmaceuticals are handled in the same premises and at the same time. For the production of the same products, campaign working may be acceptable in place of dedicated and self-contained facilities.

Products can become contaminated with physical or chemical contaminants in a variety of ways. For example, ineffective cleaning procedures may leave residues of the product or cleaning agents in the equipment; production workers may fail to take proper precautions while transporting a substance from one area to another thereby introducing a contaminant to the second production area; or particles may become airborne and travel to production areas throughout the facility. A number of substances such as dust, dirt, debris, toxic products, infectious agents, or residue of other drugs or drug components can also contaminate products.

Experience indicates that the potential dangers of contamination are more extensive and varied than once believed. For example, adulteration of the sterile product with sensitizing substances (such as penicillin, cephalosporins), substances having high pharmacological activity or potency (such as steroids, cytotoxic anti-cancer agents), infectious agents (such as spore-bearing organisms), and products that require viral inactivation or reduction (such as live viruses, products from animal cells), may pose health risks to humans or animals, even at minimal levels of exposure. Preventing cross-contamination of such potentially active substances is the goal and manufacturers are expected to identify any such substances posing a serious threat of contamination and to control it through dedicated production processes. Moreover, because the identity or even the presence of some of these contaminants may not be known, health care professionals providing care to a patient suffering from such an adverse effect may be unable to provide appropriate medical intervention.

Most contamination, however, can be controlled to an acceptable level through measures such as proper planning and implementation of cleaning and sanitation processes, employee training, gowning, and air filtration. cGMP guidelines require that manufacturers set contamination limits on a substance-by-substance basis, according to both the potency of the substance and the overall level of sensitivity to that substance, and prohibit the release of the product for distribution if these limits were exceeded. Depending on the product, a variety of measures may be acceptable to eliminate cross-contamination; there may, however, be situations where nothing short of dedicated facilities, air handling and process equipment would be sufficient, especially if there are no reasonable methods for the cleaning and removal of a substance or compound residues from buildings, facilities and equipment. For example, a manufacturer might develop a hypothetical product of high therapeutic potential that also poses a high risk of contamination and if it posed a special danger to human health, dedicated facilities would be required. If, however, experience demonstrated that the product did not pose such a risk, or if changes in manufacturing technology greatly reduced the risk, then dedicated facilities might no longer be required.

Sanitation

The sanitation of clean areas is particularly important. Any building used in the manufacture, processing, packing or holding of bio-pharmaceuticals and their intermediates should be maintained in a clean and sanitary condition. Sanitation procedures should apply to work performed by contractors or temporary employees as well as work performed by full-time employees during the ordinary course of operations. Written procedures should, therefore, be established, assigning responsibility for sanitation, and describing the cleaning schedules, methods, equipment and materials to be used in cleaning buildings and facilities, and for the use of suitable rodenticides, insecticides, fungicides, fumigating agents, or other cleaning and sanitizing agents to prevent the contamination of equipment, raw materials, packaging and labelling materials, as well as the final product. Where disinfectants are used, more than one type should be employed, and monitoring should be undertaken regularly to detect the development of resistant strains. Disinfectants and detergents should be monitored for microbial contamination, and those used in grades A and B areas especially should be sterile prior to use.

Monitoring programmes in controlled environments

It is the responsibility of the manufacturer to develop, initiate and implement an environmental monitoring programme tailored to specific facilities and condi-

tions and capable of detecting any adverse drift in microbiological conditions in a timely manner, allowing meaningful and effective corrective action. Such microbiological monitoring programmes should be utilized to assess the effectiveness of cleaning and sanitization practices and of personnel that could have an impact on the bioburden of the controlled environment. Routine microbial monitoring, regardless of how sophisticated the system may be, will not and need not identify and quantify all microbial contaminants present in the controlled environment. It can only provide information to demonstrate that the environmental control systems are operating as intended. The objective of microbial monitoring is, therefore, to obtain representative estimates of bioburden in the environment.

The environmental monitoring programme for the manufacture of sterile bio-pharmaceuticals should include the daily use of surface plates and the monitoring of personnel, with alert or action limits established, and appropriate follow-up corrective action taken when they are reached. Where critical aseptic operations are performed, monitoring should be frequent using methods such as settle plates, volumetric air and surface sampling (such as swabs and contact plates). Additional microbiological monitoring is also required outside production operations, for example, after validation of systems, cleaning and sanitization. The particulate conditions for the at-rest state should be achieved in the unmanned state after a short clean-up period of about 15–20 minutes (guidance value) after completion of operations. The particulate conditions for grade A in operation should be maintained in the zone immediately surrounding the product, whenever the product or open container is exposed to the environment. It may not always be possible to demonstrate conformity with particulate standards at the point of fill when filling is in progress due to the generation of particles or droplets from the product itself.

Some manufacturers utilize UV lights in operating areas. Such lights are of limited value as they may mask a contaminant on a settle or aerobic plate or may even contribute to the generation of a resistant (flora) organism. Therefore, the use of surface contact plates is preferred, as they will provide more information on levels of contamination. There are some manufacturers that set alert/action levels on averages of plates. For the sampling of critical surfaces, such as operators' gloves, the average of results on plates is unacceptable. The primary concern is any incidence of objectionable levels of contamination that may result in a non-sterile product. Since processing is commonly carried out around the clock, monitoring of surfaces and personnel during the second and third shifts should also be routine.

In the management of a sterile operation, periodic (weekly/monthly/quarterly) summary reports of environmental monitoring should be generated. Trained personnel should evaluate any trends when data are compiled and analysed. While it is important to review environmental results on a daily basis, it is also critical to review results over extended periods to determine whether trends are present, as they may be related to decontamination procedures, housekeeping practices, personnel training, cross-contamination and the potential for microbial build up during production. A full investigation should, therefore, include a review of area maintenance documentation, sanitization documentation, the inherent physical or operational parameters, and the training status of personnel involved, while a limited investigation triggered by an isolated, small excursion might include only some of these areas. Based on the review of the investigation and testing results, the significance of the event and the acceptability of the operations or products processed under that condition can be ascertained. Any investigation and the rationale for the course of action should be documented and included as part of the overall quality management system.

11.5.2 Laboratory design

The design of a laboratory that handles any bio-pharmaceutical, which may include infectious agents, should provide secondary containment to protect the people as well as the environment outside the laboratory from exposure to any infectious materials. Laboratory design should take into account the nature of the material being handled, the process step or study being planned for investigation, and the degree of biosafety necessary. They must be sufficient to enable the proper conduct of the study and must provide appropriate space, environmental conditions, containment, decontamination areas and support systems, such as air and water, for the study being conducted.

There are three types of laboratory designs that provide four different levels of containment. They all consist of three elements: laboratory practices and techniques, safety equipment, and laboratory facilities. The first two elements are considered primary containment, since they provide protection within the laboratory to personnel and the immediate environment. The third element, the design of the laboratory itself, is considered secondary containment since it protects persons and the environment outside of the facility. Changes in vendor and/or the specifications of major equipment and reagents would require re-validation. Each laboratory should have documentation and schedules for the maintenance, calibration and monitoring of all laboratory equipment involved in the measurement, testing and storage of raw materials, product, samples, and reference reagents, and more importantly the laboratory personnel

should be adequately trained for the jobs they are performing. Important characteristics of each of the biosafety levels are summarized below.

Basic laboratory

These are appropriate for Biosafety levels 1 and 2. They are used for studies where there is a minimum level of hazard, the personnel are able to achieve sufficient protection from the implementation of standard laboratory practices, and the organisms used in the study are not associated with any diseases in healthy adults.

Biosafety level 1

The organisms involved are defined and characterized strains, which are of minimal hazard and are not known to cause disease in healthy human adults. Although access to the laboratory may be restricted, the facility is generally not closed off from the rest of the building. The laboratory is designed to facilitate cleaning, with space between equipment and cabinets, and bench tops that are impervious to water and resistant to solutions. Personnel should be knowledgeable in all laboratory procedures and supervised by a scientist trained in microbiology or a related science. Most work is conducted on open bench tops, with procedures performed in a manner that limits the creation of aerosols, and special containment equipment is not usually needed. Decontamination of work surfaces should be done daily and after spills, and all contaminated wastes should be decontaminated before disposal. Each laboratory has a hand-washing sink. Personal safety equipment, such as laboratory coats or uniforms, should be worn and hands washed before and after handling viable materials. Any contaminated materials that will be decontaminated at another location should be transported in a durable leak proof container that is sealed before removal from the area.

Biosafety level 2

Work done under Biosafety level 2 involves organisms of moderate potential hazard. Many of the characteristics of this level are the same as those for Biosafety level 1. However, for Biosafety level 2, laboratory access is limited while work is being conducted, and only persons informed of the potential hazards of the environment and who meet any other entry restrictions developed by the organization should be allowed entry. Biological safety cabinets (Class I or II) should be used for containment when procedures with a high potential for creating infectious aerosols such as centrifugation or blending are conducted or when high concentrations or large volumes of infectious agents are used. An autoclave should be available for use in decontaminating

infectious wastes. Personnel should be trained in handling pathogenic agents and be under the direction of skilled scientists. Before leaving the area, personnel should either remove any protective clothing and leave it in the laboratory, or cover it with a clean coat. Skin contamination with infectious materials should be avoided and gloves worn when such contact is unavoidable. Spills and accidents causing overt exposure to infectious materials should be reported promptly with appropriate treatment provided and records of the incident maintained. If warranted by the organisms at use in the laboratory, baseline serum samples for all at-risk personnel should be collected and stored.

Containment laboratory

Containment laboratories qualify as Biosafety level 3 facilities and are designed with protective features to allow for the handling of hazardous materials in a way that prevents harm to the laboratory personnel, as well as the surrounding persons and environment. These may be freestanding buildings or segregated portions of larger buildings, as long as they are separated from public areas by a controlled access zone. Containment laboratories also have a specialized ventilation system to regulate airflow.

Biosafety level 3

Work done under Biosafety level 3 conditions can occur in clinical, diagnostic, teaching, research or production facilities, and involves organisms that may cause serious or potentially lethal disease following exposure through inhalation. The laboratory is, therefore, segregated from general access areas of the building, and two sets of self-closing doors must be passed through to enter the laboratory from access hallways. Access should be limited to persons who must be present for programme or support functions, and the doors remain closed during experiments. Protective clothing should be worn in the laboratory and removed before exiting the facility, and all such clothing should be decontaminated before laundering. All work with infectious materials should be conducted in a biosafety cabinet (Class I, II or III) or other physical containment device, or by personnel wearing the necessary personal protection clothing. Upon completing work with infectious materials, all work surfaces should be decontaminated. Walls, ceilings and floors should be water-resistant to facilitate cleaning, and windows should be closed and sealed. The laboratory sinks should be operable by foot, elbow or automation, and be located near the exit of each laboratory area. Vacuum lines should be protected with high efficiency particulate air (HEPA) filters and liquid disinfectant traps. The HEPA-filtered exhaust air from Class I or II biosafety cabinets may be discharged directly to the outside, or through the building exhaust system, or

be recirculated within the laboratory if the cabinet is appropriately certified and tested.

Maximum containment laboratory

These laboratories are Biosafety level 4 facilities. Maximum containment laboratories are designed to provide a safe environment for carrying out studies involving infectious agents that pose an extreme hazard to laboratory personnel, or may cause serious epidemic disease. These facilities have secondary barriers, including sealed openings into the laboratory, air locks, a double door autoclave, a separate ventilation system, a biowaste treatment system, and a room for clothing change and showers that adjoins the laboratory.

Biosafety level 4

This safety level is necessary for work with organisms that present a high individual risk of life-threatening disease. These facilities are usually located in an independent building, or in a separate, isolated, completely segregated, controlled area of a larger building. Access to the facility should be controlled by the use of locked doors. All personnel entering should sign a logbook, must enter and leave the facility through the clothing change and shower rooms, and must shower before exiting. Any supplies or materials that do not enter through the shower and change rooms must enter through a double door autoclave, fumigation chamber, or airlock that is decontaminated between each use. All organisms classified as Biosafety level 4 should be handled in Class III biosafety cabinets, or in Class I or II biosafety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life support system. All biological materials removed from a Class III cabinet, or the maximum containment laboratory in a viable condition, should be placed in a non-breakable, sealed primary container and enclosed in a secondary container that is removed through a disinfectant dunk tank, fumigation chamber, or airlock. All other materials must be autoclaved or decontaminated before removal from the facility. Walls, floors and ceilings of the facility together should form a sealed internal shell, with any windows resistant to breakage. Most importantly, the facility should be available for the quarantine isolation and treatment of personnel with potential or known laboratory-related illnesses.

11.5.3 Equipment design

The types of equipment commonly used in a bio-pharmaceutical facility will vary based not only on the types of processes and organisms used, but also on whether the equipment is used during development, during testing, or during manufacture of material for clinical trials and marketing. Types of equipment

commonly used include bioreactors, air compressors, sterilization equipment, product recovery systems such as centrifuges and cell disrupters, waste recovery and decontamination equipment, sampling and analysis instruments, safety equipment such as biosafety cabinets and protective clothing, equipment for transporting biological materials such as sealed containers, and environmental control equipment.

Equipment capacity and location

As always, the equipment used in the manufacture, processing, packing or holding of the bio-pharmaceutical product or any of the process intermediates should be of appropriate design, adequate size and construction, and suitably located to facilitate operations for its intended use and for its cleaning and maintenance. Closed equipment should be used when feasible to provide adequate protection of the bulk-active and any intermediates, and always in the case of sterile products. When equipment is opened or open equipment is used, appropriate precautions should be taken to prevent contamination or cross-contamination of bulk active substance and intermediates. New equipment must be properly installed and operate as designed, and must be cleaned before use according to written procedures, with the cleaning procedures documented and validated.

Equipment construction and installation

Equipment should be constructed and installed, to enable easy cleaning, adjustments and maintenance. Equipment should be constructed so that surfaces that come into contact with raw materials, intermediates, bulk active substances or sterile products, are not reactive, additive, or absorptive, so as to alter the quality, purity, identity, or strength of the product beyond the established specifications. Similarly, any substances required for the operation of equipment, such as lubricants, heating fluids or coolants, should not contact raw materials, packaging materials, intermediates, or the bulk active, so as to alter its quality and purity beyond established specifications. If the equipment requires calibration, there must written procedures for calibrating the equipment and documenting the calibration. With filters, the type of filter, its purpose, how it is assembled, cleaned, and inspected for damage, and if a microbial retentive filter, methods used for integrity testing, should be specified. Qualification of equipment should ensure that it is installed according to approved design specifications, regulatory codes, and the equipment manufacturers' recommendations, and that it operates within the limits and tolerances established for the process.

Biosafety cabinets

These are common primary containment devices for work involving infectious organisms. Their primary function is to protect the laboratory worker and the immediate environment by containing any infectious aerosols produced during the manipulation of organisms within the cabinet. Biosafety cabinets are classified into three types (I, II and III) based on their performance characteristics. Class I and II cabinets are appropriate for use with moderate and high-risk micro-organisms. They have an inward face velocity of 75 linear feet per minute and their exhaust air is filtered by HEPA filters. They can be used with a full width open front, an installed front closure panel, or an installed front closure panel equipped with arm-length rubber gloves. The Class II cabinet is a vertical laminar-flow cabinet with an open front. In addition to the protection provided by the Class I cabinet, these cabinets also protect materials inside the cabinet from extraneous airborne contaminants since the HEPA filtered air is recirculated within the workspace. The Class III cabinet is a totally enclosed, ventilated, gas tight cabinet used for work with infectious organisms. Work in a Class III cabinet is conducted through connected rubber gloves. The cabinet is maintained under negative pressure with supply air drawn in through HEPA filters, and exhaust air filtered by two HEPA filters and discharged to outside the facility using an exhaust fan that is generally separate from the facility's overall exhaust fan. However, it is important to remember that each of the cabinet types is only protective if it is operated and maintained properly by trained personnel.

Organism preparation

Other commonly used laboratory equipment in a biotechnology laboratory or facility includes culture plates, roller bottles, shake flasks, and a seed fermenter. These are used to bring the organism or the cell line from its origination in the master cell bank through its preparation for growth and/or propagation.

Bioreactors or fermenters

Fermenters or bioreactors play a central role in biotechnological processes, with their main purpose being to grow and/or propagate a microorganism or a cell line in a controlled, aseptic environment. The most popular type is the mechanical fermenter, which uses mechanical stirrers to agitate the culture, and one of the most commonly used mechanical fermenters is the stirred tank reactor. In order to satisfy the metabolic requirements of the microorganism or the cell line, aeration must be adequate to provide sufficient oxygen, and those using agitation need to be designed to maintain a uniform environment within the bioreactor. Major attributes of a good bioreactor are that it should be economical, robust, of simple mechanical design, easy to operate under aseptic

conditions, of reasonably flexible design with respect to the various process requirements, with no dead zones giving good control to bulk flow, and have good heat and mass transfer.

The level of sophistication involved in the design of a fermenter is largely a function of the requirements of the process. Stainless steel is commonly chosen as the material of construction for the fermenter, as it can withstand repeated cycles of sterilization (121°C for at least 30 min) without breakage and has better heat transfer than glass. Other sterility considerations include smooth and crevice free welded joints; short, straight pipework with appropriate slopes to avoid accumulation of pockets of liquid during operation; all wetted internals polished to 180–200 grit finish, and all other materials used amenable to steam sterilization.

There should be adequate monitoring and control equipment to control the metabolic processes, by monitoring parameters such as pH, temperature, agitation, and aeration rates within the bioreactor. For off-line systems, a sample is taken from the bioreactor at specified intervals and chemically analysed using automated laboratory instruments — these can have a lengthy turnaround time for analytical results and do not provide a high level of containment. For on-line systems, sampling and analysis are done continuously, often requiring additional secondary containment. In-line or at-line systems, however, provide a continuous, non-invasive indication of bioreactor conditions, through the use of probes, sensors, and sampling devices that directly contact the material.

Temperature within the fermenter is maintained by circulating water at a controlled temperature through the jacket of the fermenter, which envelops the complete level of liquid in the shell. Baffle plates are provided inside the jacket for effective circulation of the cooling or heating medium in the jacket, with a drain port provided at the bottom for efficient removal of condensate at the end of sterilization, and a vent at the top of the jacket. Bioreactor aeration system is designed for supplying sterile moisture-free air rate at 0–3 vvm (volume of air per volume of liquid per minute), although an aeration rate of 0.2–0.3 vvm is commonly used. Medical air (compressed air) at 1.5 bar g, from which moisture and oil vapours are stripped, is supplied from an air compressor, passed through a pressure regulator, flowmeter and a steam sterilizable air filter to remove undesirable organisms and particles from the air. This sterile filtered air is sparged into the fermenter through the sparger, which usually consists of an open-ended stainless steel pipe discharging directly under the agitator. The fermenter requires a versatile agitation system to ensure optimal mixing at low shear. The agitator port is sealed, either with a double mechanical seal with a sterile condensate lubrication system, or a magnetically coupled seal system.

The seal assembly is selected primarily with consideration of the cell line used, the heavy wear and tear and the repeated sterilization cycle the system undergoes. The main elements of the agitation system consist of the baffles on the shell wall for breaking vortex during peak agitation and impellers with adjustable height on the vertical shaft.

Product recovery

A product recovery or purification system is required to separate and concentrate the desired product from the contents of the bioreactor. Such systems include centrifugation, cell disruption, broth conditioning, filtration, extraction, chromatography, and drying and freezing techniques — the type of equipment depending on the type(s) of product handled.

Centrifuges are used to separate viable cells from liquid culture broth and include batch-operated solid bowl machines, semi-continuous solids-discharging disc separators, or continuous decanter centrifuges. Batch centrifuges include the solid-bowl disc centrifuge, one-chamber centrifuges (used for protein fractionation from blood plasma), zonal centrifuges (used to separate intracellular and extra-cellular products such as in virus purification or cell constituent isolation), and tubular centrifuges (used to separate liquid phases). Biosafety cabinets must be used during solids removal from batch centrifuges. Semi-continuous solids-discharging machines generally provide the best containment and are the most widely used type for biotechnology applications. Filtration units are also used to separate cellular, intra-cellular or extra-cellular, solids from broth. Types of filtration units include continuous rotary drums, continuous rotary vacuum filters or tangential flow filtration systems using either microporous or ultrafiltration membrane filters. The type of filtration unit used depends on the type of product being recovered.

Cell disruption is used to recover intra-cellular products and can be performed using mechanical or non-mechanical methods. Mechanical methods include ball mills and high-speed homogenizers, whilst non-mechanical methods include chemical or enzymatic lysis, heat treatment, freeze-thaw or osmotic shock. Non-mechanical methods are easily contained and are most often used in biotechnology laboratories. Chromatography processes such as affinity or gel filtration are used to purify intra-cellular or extra-cellular products, using an eluting solvent in a packed column and collected in a fraction collector. If adequate containment is provided, such as a biological safety cabinet, product recovery using chromatography can be used to purify hazardous organisms. Other purification equipment includes centrifugal extractors (used for liquid–liquid extraction), spray packed, mechanically agitated, or pulsed columns. Either freezing or drying may be used to facilitate the handling

and storage of products. Organisms to be frozen are placed in vials and frozen. The most common types of dryers used are freeze dryers and vacuum tray dryers, and since freezing provides primary containment and produces less aerosols than dryers, it is more appropriate for product storage. If drying is performed, proper filtration and ventilation systems must be provided.

Isolator technology

The use of isolator technology to minimize human interventions in processing areas usually results in a significant decrease in the risk of microbiological contamination of aseptically manufactured products from the environment. There are many possible designs of isolators and transfer devices. The isolator and the background environment should be designed so that the required air quality for the respective zones can be realized. The air classification required for the background environment depends on the design of the isolator and its application and for aseptic processing it should be at least grade D. In general, the area inside the isolator is the local zone for high-risk manipulations, although it is recognized that laminar airflow may not exist in the working zone of all such devices. The transfer of materials into and out of the unit is one of the greatest potential sources of contamination. Such transfer devices may vary from a single door to double door designs to fully sealed systems incorporating sterilization mechanisms. Isolators should be introduced only after appropriate validation. Validation should take into account all critical factors of isolator technology, such as the quality of the air inside and outside (background) the isolator, sanitization of the isolator, the transfer process and isolator integrity. Isolators are constructed of various materials more or less prone to puncture and leakage. Monitoring should be carried out routinely and should include frequent leak testing of the isolator and glove/sleeve system.

Computer and related automatic and electronic systems

These are used in the control of critical manufacturing steps in bio-pharmaceutical manufacture. They should be appropriately qualified and validated to demonstrate the suitability of the hardware and software, to perform assigned tasks in a consistent and reproducible manner. The depth and scope of the validation programme would depend on the diversity, complexity and criticality of the system. All changes should be approved in advance and performed by authorized and competent personnel, and records kept of all changes, including modifications and enhancements to the hardware, software and any other critical components of the system, to demonstrate that the modified system is maintained in a validated state.

Appropriate controls over computer or related automatic and electronic systems should be exercised to ensure that only authorized personnel make changes in master production and control records. Procedures should be established to prevent unauthorized entries or changes to existing data. Systems should identify and document the persons entering or verifying critical data. Input to and output from the computer or related system should be checked for accuracy at appropriate intervals and where critical data are entered manually, there should be an additional check on the accuracy of the entry. This may be performed by a second operator, or by the system itself.

A back-up system should be available to respond to system breakdowns or failures that result in permanent loss of critical records. Back-ups may consist of hard copies or other forms, such as tapes or microfilm, that ensure back-up data are exact, complete and secure from alteration, inadvertent erasure or loss. The current regulations also require that a 'back-up file of data entered into the computer or related system shall be maintained except where certain data, such as calculations performed in connection with laboratory analysis, are eliminated by computerization or other automated processes'. If computerization or another automated process has eliminated such calculations 'then a written record of the programme shall be maintained along with data establishing proper performance' emphasizing that the manufacturer must actually establish proper performance.

Regulatory authorities require additional information to be available for pre-approval inspection. The information provided should include a brief description of procedures for changes to the computer system. For each of the systems, a list of the manufacturing steps that are computer-controlled should be provided, together with the identity of the system's developer (i.e. developed in-house or by an external contractor). The validation summary should include:

- a narrative description of the validation process (or protocol), including acceptance criteria;
- certification that IQ and OQ have been completed;
- an explanation of the parameters monitored and tests performed;
- a validation data summary;
- an explanation of all excursions or failures;
- deviation reports and results of investigations for all excursions or failures.

11.5.4 Sterilization methods

All the equipment used in the processing of bio-pharmaceuticals should be capable of being sterilized and maintaining sterility. Sanitization rather than

sterilization of critical equipment such as crystallizers, centrifuges, filters, spray and freeze dryers is totally unacceptable. All sterilization processes should be validated, with particular attention given when the adopted sterilization method is not described in the current edition of the Pharmacopoeia, or when it is used for a product that is not a simple aqueous or oily solution. Where possible, heat sterilization is the method of choice.

Biological indicators

If biological indicators are used, strict precautions should be taken to avoid transferring microbial contamination from them. In some cases, testing of biological indicators may become all or part of the sterility testing. Various types of indicators are used as an additional method for monitoring the sterilization and assuring sterility, including lag thermometers, peak controls, Steam Klox, test cultures and biological indicators. Biological indicators are of two forms, each of which incorporates a viable culture of a single species of microorganism. In one form, the culture is added to representative units of the lot to be sterilized, or to a simulated product that offers no less resistance to sterilization than the product to be sterilized. In the second form, the culture is added to disks or strips of filter paper, metal, glass or plastic beads, and used when the first form is not practical, as is the case with solids. If using indicators, there should be assurances that the organisms are handled so they do not contaminate the manufacturing area or the product, and they should be stored and used according to the manufacturer's instructions, and their quality checked by positive controls.

Sterilization by moist heat

The method of choice for the sterilization of equipment and transfer lines is saturated clean steam under pressure. In the validation of the sterilization of equipment and transfer systems, temperature sensors and biological indicators should be strategically located in cold spots where condensate may accumulate, such as the point of steam injection and steam discharge, and in low spots such as the exhaust line. Steam must expel all the air from the sterilizer chamber to eliminate cold spots, and from the drain lines connected to the sewer by means of an air break to prevent back siphoning. After the high temperature phase of a heat sterilization cycle, precautions should be taken against contamination of a sterilized load during cooling. There should be frequent leak tests on the chamber when a vacuum phase is part of the cycle. One manufacturer utilized a steam-in-place system, but only monitored the temperature at the point of discharge and not in low spots in the system where condensate accumulated and caused problems. Care should be taken to ensure that steam used for steriliza-

tion is of suitable quality and does not contain additives at a level that could cause contamination of product or equipment. Any cooling fluid or gas in contact with the product should be sterilized unless it can be shown that any leaking container would not be approved for use.

Both temperature and pressure should be used to monitor the process. Control instrumentation should normally be independent of monitoring instrumentation and recording charts. Where automated control and monitoring systems are used, they should be validated to ensure that critical process requirements are met. Each heat sterilization cycle should be recorded on a time/temperature chart with a sufficiently large scale, or by other appropriate equipment with suitable accuracy and precision. The position of the temperature probes used for controlling and recording should be determined during the validation, and where applicable checked against a second independent temperature probe located at the same position. Chemical or biological indicators may also be used, but should not take the place of physical measurements. The time required to heat the centre of the largest container to the desired temperature must be known, and sufficient time must be allowed for the whole of the load to reach the required temperature before measurement of the sterilizing time-period is commenced. Charts of time, temperature and pressure should be filed for each sterilizer load. The items to be sterilized, other than products in sealed containers, should be wrapped in a material which allows removal of air and penetration of steam but which prevents recontamination after sterilization.

Sterilization by dry heat

There are some manufacturers who sterilize processed bulk bio-pharmaceutical powders by the use of dry heat. As a primary means of sterilization, its usefulness is questionable because of the lack of assurance of penetration into the crystal core of a sterile powder, although some sterile bulk powders can withstand the lengthy times and high temperatures necessary for dry heat sterilization. Process validation should cover aspects of heat penetration and heat distribution, times, temperatures, stability (in relation to the amount of heat received) and particulates. Any air admitted to maintain a positive pressure within the chamber should be passed through a HEPA filter. Where this process is also intended to remove pyrogens, challenge tests using endotoxins should be used as part of the validation.

Sterilization by radiation

Radiation sterilization is used mainly for the sterilization of heat sensitive materials and products, although ultra-violet irradiation is not normally an

acceptable method of sterilization. Many medicinal products and some packaging materials are radiation-sensitive, so this method is permissible only when the absence of deleterious effects on the product has been confirmed experimentally. Validation procedures should ensure that the effects of variations in density of the packages are considered, and biological indicators may be used as an additional control. Materials handling procedures such as the use of radiation sensitive colour disks should also be used on each package to differentiate between irradiated and non-irradiated materials and prevent mix-ups. During the sterilization procedure the radiation dose should be measured, and the total radiation dose should be administered within a predetermined time span. For this purpose, dosimetry indicators that are independent of dose rate should be used, giving a quantitative measurement of the dose received by the product itself. These should be inserted in the load in sufficient numbers and close enough together to ensure that there is always a dosimeter in the irradiator. Where plastic dosimeters are used they should be used within the time limit of their calibration, and dosimeter absorbances should be read within a short period after exposure to radiation.

Sterilization with ethylene oxide

There are some manufacturers who still use ethylene oxide for the surface sterilization of powders as a precaution against potential microbiological contamination during aseptic handling, even though a substantial part of the sterile pharmaceutical industry has discontinued its use as a sterilizing agent. Its use is now in decline because of residual ethylene oxide in the product and the inability to validate ethylene oxide sterilization, as well as employee safety considerations. As a primary means of sterilization, its use is questionable because of the lack of assurance of penetration into the crystal core of a sterile powder, and therefore, this method should only be used when no other method is practicable. Process validation should show that there is no damaging effect on the product and that the conditions and time allowed for degassing are such as to reduce any residual gas and reaction products to acceptable limits for the type of product or material. The nature and quantity of packaging materials can significantly affect the process, so materials should be pre-conditioned by being brought into equilibrium with the humidity and temperature required by the process before exposure to the gas. The time required for this should be balanced against the opposing need to minimize the time before sterilization. For each sterilization cycle, records should be made of the time taken to complete the cycle, of the pressure, temperature and humidity within the chamber during the process, the gas concentration, and the total amount of gas used. After sterilization, the load should be stored in a controlled manner under

ventilated conditions to allow residual gas and reaction products to reduce to the defined level.

Sterilization with formaldehyde

The use of formaldehyde is a much less desirable method of equipment sterilization. A major problem with formaldehyde is its removal from pipework and surfaces and it is rarely used primarily because of residue levels in both the environment and the product. Since formaldehyde contamination in a system or in a product is not going to be uniform, merely testing the product as a means of demonstrating and validating the absence of formaldehyde levels is not acceptable; there should be some direct measure, or determination of the absence of formaldehyde. Key surfaces should be sampled directly for residual formaldehyde. One large pharmaceutical manufacturer had to reject the initial batches coming through the system because of formaldehyde contamination. Unfortunately, they relied on end product testing of the product, and not on direct sampling to determine the absence of formaldehyde residues on equipment.

Sterilization In Place (SIP)

SIP systems require considerable maintenance, and their malfunction has directly led to considerable product contamination and recall. One potential problem with SIP systems is condensate removal from the environment. Condensate and excessive moisture can result in increased humidity, and increases in levels of microorganisms on surfaces of equipment. Therefore, environmental monitoring after sterilization of the system is particularly important. Another potential problem is the corrosive nature of the sterilant, whether it is clean steam, formaldehyde, peroxide or ethylene oxide. In two recent cases, inadequate operating procedures have led to weld failures. Therefore, particular attention should be given to equipment maintenance logs, especially to non-scheduled equipment maintenance, and the possible impact on product quality. Suspect batches manufactured and released prior to the repair of the equipment should be identified.

11.5.5 Cleaning procedures and validation

Regulatory authorities requiring that all equipment and facilities be clean prior to use and be maintained in a clean and orderly manner, are nothing new. Of course, the main rationale for requiring clean equipment and facility is to prevent contamination or adulteration of medicinal products. Historically, authorities have looked for gross insanitation due to inadequate cleaning and maintenance of equipment and/or poor dust control systems, and were more

concerned about the contamination of non-penicillin drug products with penicillins, or the cross-contamination of drug products with potent steroids or hormones. Certainly, a number of products have been recalled over the past decade due to actual or potential penicillin cross-contamination.

Rationale and procedures

Cleaning, and its validation, including facility disinfection, personnel control and equipment cleaning, has recently come under increasing scrutiny. Numerous regulatory actions and comments have been issued, resulting in many questions regarding the selection, use, testing, documentation and validation of cGMP sanitation programmes. Regulatory authorities now expect manufacturers to have written procedures detailing the cleaning processes used for various pieces of equipment. If manufacturers have only one cleaning process for cleaning between different batches of the same product, and use a different process for cleaning between product changes, then the written procedures should address these different scenarios. Similarly, if manufacturers have one process for removing water-soluble residues and another process for non-water soluble residues, the written procedure should address both scenarios and make it clear when a given procedure would be followed. Some manufacturers may decide to dedicate certain equipment for certain process steps that produce residues that are difficult to remove from the equipment. Any residues from the cleaning process itself, such as detergents and solvents, also have to be removed from the equipment.

Equipment should be cleaned, held and, where necessary, sanitized at appropriate intervals to prevent contamination or cross-contamination that would alter the quality or purity of the product beyond the established specifications. Even dedicated equipment should be cleaned at appropriate intervals to prevent the build-up of objectionable material or microbial growth. As processing approaches the purified bulk active substance, it becomes important to ensure that incidental carry-over of contaminants or degradants between batches does not adversely impact the established impurity profile. However, this does not always apply to a bio-pharmaceutical, where many of the processing steps are accomplished aseptically, and where it is often necessary to clean and sterilize equipment between batches. Non-dedicated equipment should be thoroughly cleaned between different products and, if necessary, after each use. If cleaning a specific type of equipment is difficult, the equipment may need to be dedicated to a particular bulk active substance or intermediate. Moreover, because the potency of some of these materials may not be fully known, cleaning becomes particularly important.

The microbiological aspects of equipment cleaning consist largely of preventive measures rather than removal of contamination once it has occurred. There should be some evidence that routine cleaning and storage of equipment does not allow microbial proliferation. For example, equipment should be dried before storage, and under no circumstances should stagnant water be allowed to remain in equipment. Subsequent to the cleaning process, equipment should be sterilized or sanitized where such equipment is used for sterile processing, or for non-sterile processing where the products may support microbial growth. Thus, the control of the bioburden through adequate cleaning and storage of equipment is important to ensure that subsequent sterilization or sanitization procedures achieve the necessary assurance of sterility. This is also particularly important from the standpoint of the control of pyrogens in sterile processing, since equipment sterilization processes may not be adequate to achieve significant inactivation or removal of pyrogens.

In sterile secondary production areas, all the equipment, fittings and services, as far as is practicable, should be designed and installed so that operations, maintenance and repairs can be carried out outside the clean area. If sterilization is required, it should be carried out after complete re-assembly wherever possible. The practice of re-sterilizing equipment if sterility has been compromised is important. When equipment maintenance has been carried out within the clean area, the area should be cleaned, disinfected and/or sterilized where appropriate before processing recommences if the required standards of cleanliness and/or asepsis have not been maintained during the work. A conveyor belt should not pass through a partition between a grade A or B area and a processing area of lower air cleanliness unless the belt itself is continually sterilized (for example, in a sterilizing tunnel).

Equipment must be clearly identified as to its cleaning status and content. The cleaning and maintenance of the equipment should be documented in a logbook maintained in the immediate area. Establishing and controlling the maximum length of time between the completion of processing and each cleaning step is often critical in a cleaning process. This is especially important for operations where the drying of residues will directly affect the efficiency of a cleaning process. In all cases, the choice of cleaning methods, cleaning agents and levels of cleaning should be established and justified. When selecting cleaning agents, the following should be considered:

- the cleaning agent's ability to remove residues of raw materials, precursors, by-products, intermediates, or even the bulk active substance;
- whether the cleaning agent leaves a residue itself;
- compatibility with equipment construction materials.

Validation of cleaning methods

Validation of cleaning procedures has generated considerable discussion since the regulatory authorities started to address this issue. The first step is to focus on the objective of the validation process, and some manufacturers fail to develop such objectives. It is not unusual to see manufacturers use extensive sampling and testing programmes following the cleaning process without really evaluating the effectiveness of the steps used to clean the equipment. Several questions need to be addressed when evaluating the cleaning process. For example, at what point does a piece of equipment or system become clean? Does it have to be scrubbed by hand? What is accomplished by hand scrubbing rather than just a solvent wash? How variable are manual cleaning processes from batch to batch and product to product? What other methods for cleaning can be utilized — wipe clean, spray, fog, immersion, ultrasonic, re-circulating spray? Is the contamination viable or non-viable? Are there identifiable baseline bioburden and residue levels? The answers to these questions are obviously important to the inspection and evaluation of the cleaning process, and to determine the overall effectiveness of the process. They may also identify steps that can be eliminated for more effective measures and result in resource savings for the manufacturer.

In general, cleaning validation efforts should be directed to situations or process step where contamination or incidental carry-over of degradants poses the greatest risk to the product's quality and safety. The manufacturer should have determined the degree of effectiveness of the cleaning procedure for each bio-pharmaceutical or intermediate used in that particular piece of equipment. In the early stages of the operation, it may be unnecessary to validate cleaning methods if it could be shown that subsequent purification steps can remove any remaining residues. It must be recognized that for cleaning, as with any other processes, there may be more than one way to validate the process. In the end, the test of any validation process is whether the scientific data shows that the system consistently does as expected and produces a result that consistently meets pre-determined specifications. Moreover, cleaning should also be shown to remove endotoxins, bacteria, active elements and contaminating proteins, while not adversely affecting the performance of the equipment. In cases where cleaning reagents are required for decontamination or inactivation, validation should also demonstrate the effectiveness of the decontamination/inactivating agent(s).

Validation of cleaning methods should, therefore, reflect the actual equipment use patterns. For example, if various bulk actives or intermediates are manufactured using the same equipment, and if the same process is used to clean the equipment, a worst-case bulk active or intermediate can be selected

for the purposes of cleaning validation. The worst-case selection should be based on a combination of potency, activity, solubility, stability and difficulty of cleaning. In addition, such cleaning and sanitization studies should address microbiological and endotoxin contamination for those processes intended or purported to reduce bioburden or endotoxins in the bulk active substance or other processes where such contamination may be of concern, for example with non-sterile substances used to manufacture parenteral products.

Documentation

Depending upon the complexity of the system and the cleaning process, and the ability and training of operators, the amount of detail and specificity in the documentation necessary for executing various cleaning steps or procedures will vary. Some manufacturers use general SOPs, while others use a batch record or log sheet system that requires some type of specific documentation for performing each step. When more complex cleaning procedures are required, it is important to document the critical cleaning steps, including specific documentation on the equipment itself and information about who cleaned it and when. However, for relatively simple cleaning operations, the mere documentation that the overall cleaning process was performed might be sufficient. Other factors such as history of cleaning, residue levels found after cleaning and variability of test results may also dictate the amount of documentation required. For example, when variable residue levels are detected following cleaning, particularly for a process that is believed to be acceptable, the manufacturer must establish the effectiveness of the process and operator performance.

Protocols

Cleaning validation protocols should have general procedures on how cleaning processes will be validated. It must describe the equipment to be cleaned; methods, materials and extent of cleaning; parameters to be monitored and controlled; and validated analytical methods to be used. The protocol should also indicate the type of samples (rinse, swabs) to be obtained, and how they are collected, labelled and transported to the analysing laboratory. Validation procedures should address who is responsible for performing and approving the validation study, the acceptance criteria and when re-validation will be required. Validation studies should be conducted in accordance with the protocols, and the results of the studies documented. There should be a detailed written equipment cleaning procedure that provides details of what should be done and the materials to be utilized. Some manufacturers list the specific solvent for each bio-pharmaceutical and intermediate. For stationary vessels,

Clean In Place (CIP) apparatus is often encountered. Diagrams, along with identification of specific valves, will be necessary for evaluating these systems.

Sampling

After cleaning, there should be some routine testing to assure that the surface has been cleaned to the validated level, and to ensure these procedures remain effective when used during routine production. Where feasible, equipment should be examined visually for cleanliness. This may allow detection of gross contamination concentrated in small areas that could go undetected by analytical verification methods. Sampling should include swabbing, rinsing, or alternative methods such as direct extraction, as appropriate, to detect both insoluble and soluble residues. The sampling methods used should be capable of quantitatively measuring levels of residues remaining on the equipment surfaces after cleaning. There are two general types of sampling that have been found acceptable — the most desirable is the direct method of sampling the equipment surface, and the other is the use of rinse solutions.

Direct surface sampling

The advantages of direct sampling are that areas hardest to clean, but which are reasonably accessible, can be evaluated, leading to the establishment of a level of contamination or residue per given surface area. Additionally, residues that are dried out, or are insoluble, can be sampled by physical removal. Swab sampling may be impractical when product contact surfaces are not easily accessible due to equipment design and/or process limitations, such as the inner surfaces of hoses, transfer pipes, reactor tanks with small ports or handling active materials, and small intricate equipment such as micronizers and micro-fluidizers. One major concern is the type of sampling material used and its impact on the test data, since the sampling material may interfere with the test. For example, the adhesive used in swabs has been found to interfere with the analysis of samples. Therefore, it is important to assure early in the validation programme that the sampling medium and the solvent used for extraction from the medium are satisfactory and can be readily used.

Rinse samples

This is the analysis of the final rinse water or solvent for the presence of the cleaning agents last used in that piece of equipment. Two advantages of using rinse samples are that a larger surface area may be sampled, and inaccessible systems or ones that cannot be routinely disassembled can be sampled and evaluated. However, the disadvantage of rinse samples is that the residue or contaminant may not be soluble or may be physically occluded in the

equipment. An analogy that can be used is the dirty pot — in the evaluation of cleaning of a dirty pot, particularly with dried out residue, one does not look at the rinse water to see that it is clean; one looks at the pot. A direct measurement of the residue or contaminant should be made for the rinse water when it is used to validate the cleaning process. For example, it is not acceptable to simply test rinse water for water quality (does it meet the compendia tests?), rather than test it for potential contaminates. In addition, indirect monitoring such as conductivity testing may be of some value for routine monitoring once the cleaning process has been validated. This would be particularly true, where reactors and centrifuges and pipework between such large equipment can only be sampled using rinse solution samples.

Analytical methods and establishment of limits

How do you evaluate and select analytical methods to measure cleaning and disinfection effectiveness in order to implement basic cleaning validation and to establish routine in-use controls. Regulatory authorities do not set acceptance specifications or methods for determining whether a cleaning process is validated because it is impractical for them to do so due to the wide variation in equipment and products used throughout the industry.

With advances in analytical technology, residues from the manufacturing and cleaning processes can be detected at very low levels. The sensitivity of some modern analytical apparatus has lowered some detection thresholds to below parts per million (ppm) down to parts per billion (ppb). Some limits that have been mentioned by industry representatives in literature or presentations, include analytical detection levels such as 10 ppm, biological activity levels such as 1/1000 of the normal therapeutic dose, and organoleptic levels such as no visible residue. The residue limits established for each piece of apparatus should, therefore, be practical, achievable and verifiable. If levels of contamination or residual are not detected, it does not mean that there is no residual contaminant present after cleaning; it only means that levels of contaminant greater than the sensitivity or detection limit of the analytical method are not present in the sample. The manufacturer's rationale for establishing specific residue limits should be logical, based on their knowledge of the materials involved, be practical, achievable and verifiable, have a scientifically sound basis, and be based on the most deleterious residue. Limits may, therefore, be established, based on the minimum known pharmacological or physiological activity of the product or its most deleterious component.

Another factor to consider is the possible non-uniform distribution of the residue on a piece of equipment. The actual average residue concentration may

be more than the level detected. It may not be possible to remove absolutely every trace of material, even with a reasonable number of cleaning cycles. The permissible residue level, generally expressed in parts per million (ppm), should be justified by the manufacturer. The manufacturer should also challenge the analytical method in combination with the sampling method(s) used, to show that the contaminants can be recovered from the equipment surface, and at what levels, i.e. 50% or 90% recovery. This is necessary before any conclusions can be made based on the sample results. A negative test may also be the result of poor sampling technique.

Clean In Place methods

Where feasible, Clean In Place (CIP) methods should be used to clean process equipment and storage vessels. CIP methods might include fill and soak/agitate systems, solvent refluxing, high-impact spray cleaning, spray cleaning by sheeting action, or turbulent flow systems. CIP systems should be subjected to cleaning validation studies to ensure that they provide consistent and reproducible results, and once they are validated, appropriate documentation should be maintained to show that critical parameters, such as time, temperature, turbulence, cleaning agent concentration, rinse cycles, are achieved with each cleaning cycle. However, the design of the equipment, particularly in facilities that employ semi-automatic or fully automatic Clean In Place (CIP) systems, can represent a significant concern. For example, sanitary type pipework without ball valves should be used, since non-sanitary ball valves make the cleaning process more difficult. Such difficult to clean systems should be properly identified and validated, and it is important that operators performing these cleaning operations are aware of potential problems and are specially trained in cleaning these systems and valves. Furthermore, with systems that employ long transfer lines or pipework, clearly written procedures together with flow charts and pipework diagrams for the identification of valves should be in place. Pipework and valves should be tagged and easily identifiable by the operator performing the cleaning function. Sometimes, inadequately identified valves, both on diagrams and physically, have led to incorrect cleaning practices. Equipment in CIP systems should be disassembled during cleaning validation where practical to facilitate inspection and sampling of inner product surfaces for residues or contamination, even though the equipment is not normally disassembled during routine use.

Test until clean

Some manufacturers are known to test, re-sample and re-test equipment or systems until an 'acceptable' residue level is attained. For the system or

equipment with a validated cleaning process, this practice of re-sampling should not be utilized and is only acceptable in rare cases. Constant re-testing and re-sampling can show that the cleaning process is not validated, since these re-tests actually document the presence of unacceptable residue and contaminants from an ineffective cleaning process. The level of testing and the re-test results should, therefore, be routinely evaluated.

Detergent

The manufacturer must consider and determine the difficulty that may arise when attempting to test for residues if a detergent or soap is used for cleaning. A common problem associated with detergent use is its composition — many detergent suppliers will not provide specific composition, making it difficult for the user to evaluate residues. As with product residues, it is important that the manufacturer evaluate the efficiency of the cleaning process for the removal of residues from the detergents. However, unlike product residues, it is expected that no (or for ultra sensitive analytical test methods — very low) detergent remains after cleaning. Detergents are not part of the manufacturing process and are only added to facilitate cleaning during the cleaning process, so they should be easily removable or a different detergent should be selected.

11.6 Process utilities and services

11.6.1 Water systems

Water is a very important component of bio-pharmaceutical processes. Water of suitable quality is required depending on the culture system used, the phase of manufacture and the intended use of the product. Tighter chemical and microbiological quality specifications are required during certain process steps such as cell culture, final crystallization and isolation, and during early process steps if impurities that affect product quality are present in the water and cannot be removed later. Where water is treated to achieve an established quality, the treatment process and associated distribution systems should be qualified, validated, maintained and routinely tested following established procedures to ensure water of the desired quality. The water used should meet the standards for potable water as a minimum for the production of bio-pharmaceuticals.

The potable water supply, regardless of source, should be assessed for chemicals that may affect the process, and information should be periodically sought from local authorities about potential contamination by pesticides or other hazardous chemicals. For example, if water is used for a final wash of a

filter cake, or if the bulk active substance is crystallized from an aqueous system, then the water should be suitably treated, such as by de-ionization, ultrafiltration, reverse osmosis or distillation, and tested to ensure routine compliance with appropriate chemical and microbiological specifications. If the water is used for final rinses during equipment cleaning, then the water should be of the same quality as that used in the manufacturing process. Water used in the final isolation and purification steps of non-sterile bulk actives intended for use in the preparation of parenteral products should be tested and controlled for bioburden and endotoxins.

The quality of water, therefore, depends on the intended use of the finished product. For example, only Water for Injection (WFI) quality water should be utilized as process water; this is because, even though water may not be a component of the final sterile product, water that comes in contact with the equipment or that enters into the bioreactor can be a source of impurities such as endotoxins. On the other hand, for in-vitro diagnostics purified water may suffice. For heat-sensitive products where processing such as formulation is carried out cold or at room temperature, only cold WFI will suffice, and the self-sanitization of a hot WFI system at 75° to 80°C is lost. As with other WFI systems, if cold WFI water is needed, point-of-use heat exchangers can be used; however, these cold systems are still prone to contamination, and should be fully validated and routinely monitored both for endotoxins and microorganisms.

Water treatment plants and distribution systems should be designed, constructed and maintained to ensure a reliable source of water of an appropriate quality. They should never be operated beyond their designed capacity. For economic reasons, some biotechnology companies manufacture WFI utilizing marginal systems, such as single pass reverse osmosis, rather than by distillation. Many such systems have been found to be contaminated, typically because they use plastic pipes and non-sealed storage tanks, which are difficult to sanitize. Although some of the systems employ a terminal sterilizing filter to minimize microbiological contamination, the primary concern is endotoxins which the terminal filter may merely serve to mask. Such systems are, therefore, totally unacceptable. Moreover, the limitations of relying on a 0.1 ml sample of WFI for endotoxins from a system should also be recognized.

New water quality requirements were brought into effect in 1996. These updated requirements provide major cost savings to those manufacturers who needed to produce and maintain pure water systems, and allowed for the continuous monitoring of water systems with a reliance on instrumentation rather than laboratory work, thereby reducing labour and operating costs.

Previous standards required a battery of expensive and labour intensive chemical, physical, and microbiological testing, many of which only provided qualitative information. Advances in technology and instrumentation mean that simple, cost effective replacements have become available. However, before changing to the new testing standards, manufacturers should evaluate their existing water system in terms of compliance with existing operations, reliability, maintenance and improved monitoring.

11.6.2 Medical air

Medical air is a natural or synthetic mixture of gases consisting largely of nitrogen and oxygen, containing no less than 19.5 percent and not more than 23.5 percent by volume of oxygen. Air supplied to a non-sterile preparation or formulation area, or for manufacturing solutions prior to sterilization, should be filtered at the point of use as necessary to control particulates. However, air supplied to product exposure areas, where sterile bio-pharmaceuticals are processed and handled, should be filtered under positive pressure through high efficiency particulate air (HEPA) filters. These HEPA filters should be certified and/or Dioctyl Phthalate tested. Tests for oil (none discernible by the mirror test), odour (no appreciable odour), carbon dioxide (not more than 0.05%), carbon monoxide (not more than 0.001%), nitric oxide and nitrogen dioxide (not more than 2.5 ppm), and for sulphur dioxide (not more than 5 ppm) should also be carried out. Medical air is packaged in cylinders or in a low pressure collecting tank. Containers used should not be treated with any active, sleep-inducing, or narcosis-producing compounds, and should not be treated with any compound that would be irritating to the respiratory tract. Where it is piped directly from the collecting tank to the point of use, each outlet should be labelled Medical Air.

11.6.3 Heating, ventilation and air conditioning (HVAC) systems

A bio-pharmaceutical facility should have proper ventilation, air filtration, air heating and cooling. Therefore, adequate ventilation should be provided where necessary, and equipment for the control and monitoring of air pressure, microorganisms, dust, humidity and temperature should be provided when appropriate. This is especially important in areas where the product is exposed to the environment or handled in the final state. Air filtration, dust collection and exhaust systems should be used when appropriate, and if the air is recirculated, appropriate measures should be taken to control contamination and cross-contamination. For example, air from pre-viral inactivation areas should not be recirculated to other areas used for the manufacture of the sterile

bio-pharmaceuticals. Regulatory authorities require the following information to be available for pre-approval inspection:

- A general description of the HVAC system(s) including the number and segregation of the air handling units, whether air is once-through or recirculated, containment features, and information on the number of air changes per hour;
- Validation summary for the system with a narrative description of the validation process (or protocol), including the acceptance criteria; the certification that IQ, OQ, and certification of filters has been completed; the length of the validation period; validation data should include Performance Qualification data accumulated during actual processing; and an explanation of all excursions or failures, including deviation reports and results of investigations;
- A narrative description of the routine monitoring programme including the tests performed and frequencies of testing for viable and non-viable particulate monitoring parameters; viable and non-viable particulate action and alert limits for production operations for each manufacturing area; and a summary of corrective actions taken when limits are exceeded.

11.6.4 Decontamination techniques and waste recovery

Air and gaseous waste streams

Filtration

The primary method of decontaminating exhaust gases mixed with liquid broth is through the use of filters. Before filtration, the mixture may be passed through a condenser, a coalescing filter and a heat exchanger. Filtration is accomplished either through pairs of high efficiency particulate air (HEPA) filters, or membrane filters used in series to decontaminate vent or exhaust gases.

Incineration

Another method of decontaminating air and gaseous waste streams is thermal destruction or incineration. Incineration may be used independently, or as a supplement to filtration, and is generally used for small volume gas streams. Automatic safety devices should be used with incinerators to protect against problems resulting from power failures and overheating.

Irradiation

Irradiation involves exposing the waste materials to x-rays, ultraviolet rays or other ionizing radiation to decontaminate them.

Liquid wastes

Liquid wastes can be decontaminated through chemical or heat treatment. When liquid wastes are of limited volume, chemical treatment is often used, whilst for large volumes of liquid wastes, heat treatment is generally preferred. Also, since proteins present in liquid wastes can deactivate the sterilant used in chemical treatment, thermal sterilization may be more appropriate for wastes involving bioengineered microorganisms.

Solid wastes

Solid wastes such as microbial cultures, cell debris, glassware, and protective clothing, are generally decontaminated by autoclaving, followed by incineration if necessary. To decontaminate laboratory devices exposed to genetically engineered products, the most common practice is the use of pressurized steam that contains an appropriate chemical. For heat-sensitive equipment, such as electronic instruments, decontamination is generally achieved through chemical sterilization or irradiation. Gaseous sterilants are applied by a steam ejector that sprays down from overhead. If decontamination by steam, liquid, or gas sterilization is not possible, ionizing or ultraviolet radiation is used. However, since irradiation methods do not always inactivate all types of microbes, steam or gaseous chemical sterilization should be used for devices contaminated with genetically engineered organisms.