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**Draft Annex 2:**  
**Manufacture of Biological Medicinal Products for Human Use**

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**Explanatory Note:**

Annex 2 of the GMP Guide has been revised for the following reasons:

1. As a consequence of the restructuring of the GMP Guide and the introduction of GMP for active substances used as starting materials Annex 2 had to be revised to complement these new requirements.
2. The breadth of biological products has increased and several new product types need to be included in Annex 2.
3. In addition, with the agreement on the new regulation on advanced therapies (publication pending) the Commission is asked to draw up specific GMP guidelines for advanced therapy medicinal products, including gene therapy, somatic cell therapy medicinal products and tissue engineered products as defined in the regulation.

It should be noted that for industrially manufactured products (such as pharmaceuticals), Directive 2004/23/EC on human tissue and cells covers only the donation, procurement and testing of the tissues and cells which become the 'active substances' for many new biological medicinal products.

A concept paper was published in December 2003 on GMP for Gene Therapy and Somatic Cell Therapy products and this work is now incorporated into Annex 2 as mentioned in a further Concept Paper published in May 2005.

Input from organisations with particular experience in advanced therapies is expected though this public consultation. The relevant section of Annex 2 on tissue engineered products will be developed subsequently.

Attention is also drawn to section 36 of this annex with respect to the appropriate environmental conditions for the preparation of solutions, as this cross references to GMP Annex 1, currently under revision.

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## **MANUFACTURE OF BIOLOGICAL MEDICINAL PRODUCTS FOR HUMAN USE**

### **Scope**

The methods employed in the manufacture of biological medicinal products are a critical factor in shaping the appropriate regulatory control. Biological medicinal products can be defined therefore largely by reference to their method of manufacture. Biological medicinal products obtained from the sources and prepared by the methods listed in Table 1 will fall under the scope of this annex.

General guidance on biological medicinal products manufacture, from control over master banks through to finishing activities, and testing is contained in Part A. The finishing stage (formulation, filling and packaging) for all product types and sources are subject to the Chapters in Part I and other relevant annexes.

For biological substances, the appropriate section of this Annex should be read in conjunction with that given in Part II of the GMP Guide. Blood derived products are covered in Annex 14 and non-transgenic herbal medicinal products are covered in Annex 7.

Further guidance on specific types of biological medicinal products is contained in Part B. Additional detailed requirements may be obtained from guidelines issued by the Committee for Medicinal Products for Human Use (CHMP) and in "The rules governing medicinal product in the European Community", Volume III.

Biological medicinal products which incorporate human tissues or cells (e.g. gene therapy and somatic cell therapy products) must comply with the requirements of Directive 2004/23/EC for donation, procurement, testing. Arrangements to ensure traceability from starting material through to finished products are required.

Biological medicinal products must comply with the latest version of the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products.

Establishments involved with genetically modified organisms (e.g. B6, B8, and B9) must comply with the appropriate directive or any subsequent amendments on their contained use (98/81/EC) or deliberate release (2001/18/EC). Appropriate authorisations should be in place from the national competent authorities. Routine operations and maintenance procedures should comply with these and GMP requirements, where there should be no conflict.

**Table 1. Manufacturing activities within the scope of Annex 2.**

Type and source of material	Example product	Application of this guide to steps (shown in grey) used in this type of manufacturing			
Animal sources: non-transgenic	Heparin, insulin, enzymes, proteins, allergen extract, somatic cell therapy, tissue engineered products	Collection of organ, fluid, or tissue	Cutting, mixing, and / or initial processing	Isolation and purification	
Animal sources: transgenic	Recombinant proteins, somatic cell therapy, tissue engineered products	Master and working transgenic bank	Collection, cutting, mixing, and / or initial processing	Isolation, purification and modification	
Human sources	Somatic cell therapy, stem cell therapy, gene therapy, gene silencing, tissue engineered products	Donation, procurement and testing of cells / tissues <sup>1</sup>	Establish MCB, WCB or cell pool	Isolation, purification, ex-vivo manipulation integration into vector incorporate into matrix	
Plant sources: transgenic	Mammalian proteins, vaccines, allergen extract	Master and working transgenic bank	Growing, harvesting, fermentation (if applicable)	Initial extraction	Isolation, purification, modification
Plant sources: non-transgenic	Allergens	Collection of plants and/or pollens <sup>2</sup>	Initial isolation and purification	Purification and modification	
Fermentation	Microbial vaccines: BCG, tetanus, diphtheria, enzymes, proteins	Establishment of MCB and WCB	Cell culture and/or fermentation	Isolation, purification, inactivation	
Virus bioreactors / cell culture	Virus vaccines MMR, smallpox, influenza	Establishment of MCB, WCB, MSL, WSL	Cell culture and/or fermentation	Inactivation, isolation and purification	
Biotechnology: fermentation/ cell culture	Recombinant products: EPO insulin, MAb, allergens, sub-unit vaccines	Establishment of MCB and WCB	Cell culture and/or fermentation	Isolation, purification, modification	

<sup>1</sup> Must comply with Directive 2004/23/EC

<sup>2</sup> The agricultural practices and Quality Systems should take EMEA and WHO Good Agricultural and Collection Practice (GACP) into account.

## **Principle**

The manufacture of biological medicinal products involves certain specific considerations arising from the nature of the products and the processes. The ways in which biological medicinal products are produced, controlled and administered make some particular precautions necessary.

Unlike conventional medicinal products, which are produced using chemical and physical techniques capable of a high degree of consistency, the production of biological medicinal products involves biological processes and materials, such as cultivation of cells or extraction of material from living organisms. These biological processes may display inherent variability, so that the range and nature of by-products may be variable. In order to minimise variability (and reduce the opportunity for cross-contamination) steps such as dedicating equipment to product, should be considered.

Moreover, the materials and processing conditions used in these cultivation processes are generally designed to provide conditions for the growth of specific cells and microorganisms; this provides extraneous microbial contaminants the opportunity to grow. Most biological medicinal products cannot be terminally sterilised and processing must therefore be conducted aseptically. In addition, many products are limited in their ability to withstand a wide range of purification techniques particularly those designed to inactivate or remove adventitious viral contaminants. Great care must therefore be taken in the design of the processes, equipment, facilities, utilities, the conditions of preparation and addition of buffers and reagents, and in the training of the operators to minimise contamination.

Control of biological medicinal products usually involves biological analytical techniques, which have a greater variability than physico-chemical determinations. A robust manufacturing process is therefore crucial and in-process controls take on a greater importance in the manufacture of biological medicinal products.

## **PART A. GENERAL GUIDANCE**

### **Personnel**

1. All personnel (including those concerned with cleaning, maintenance or quality control) employed in areas where biological medicinal products are manufactured and tested should receive additional training, and periodic retraining, specific to the products manufactured and to their work. Personnel should be given relevant information and training in hygiene and microbiology and in general security measures to protect personnel and the environment. Staff employed in such areas must be provided with special clothing and changing facilities
2. Persons responsible for production and quality control should have an adequate background in relevant scientific disciplines, such as bacteriology,

biology, chemistry, medicine, pharmacy, pharmacology, virology, immunology and veterinary medicine, together with sufficient practical experience to enable them to exercise their management function for the process in which they are involved.

3. The immunological status of personnel may have to be taken into consideration for product safety. All personnel engaged in production, maintenance, testing and animal care (and inspections) should be vaccinated where necessary with appropriate specific vaccines and have regular health checks. Apart from the risk of exposure of staff to infectious agents, potent toxins or allergens, it is necessary to avoid the risk of contamination of a production batch with infectious agents. Visitors should generally be excluded from production areas.
4. Any changes in the immunological status of personnel, which could adversely affect the quality of the product, should preclude work in the production area. Production of BCG vaccine and tuberculin products should be restricted to staff who are carefully monitored by regular checks of immunological status or chest X-ray. Advice should be sought for personnel involved with live and genetically modified organisms.
5. In the course of a working day, personnel should not pass from areas where exposure to live organisms, genetically modified organisms, toxins or animals is possible to areas where other products, dead or inactivated products or different organisms are handled. If such passage is unavoidable, clearly defined decontamination measures should be followed by staff, including change of clothing and shoes and, where necessary, showering. The restrictions on the movement of all personnel (including QC, maintenance and cleaning staff) should be controlled on the basis of a documented risk assessment.

### **Premises and Equipment**

6. Given the variability inherent in most biological processes, steps to reduce variability and enhance reproducibility, such as dedicating equipment to products, should be considered.
7. The degree of environmental control of particulate and microbial contamination of the production premises should 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. The environmental monitoring programme should be supplemented by the inclusion of methods to detect the presence of specific organisms.
8. The measures and procedures necessary for a containment facility should not conflict with GMP. Advice should be obtained in order to establish and maintain the appropriate Biological Safety Levels.

9. Manufacturing processes and environmental classifications should be designed to prevent extraneous contamination. Although contamination is likely to become evident during processes such as fermentation, prevention is more appropriate than detection. Where aseptic processes are used (e.g. inoculation), control measures should be put in place following a documented risk-assessment, utilising the principles in Annexe 1.
10. The risk of cross-contamination between biological medicinal products, especially during those stages of the manufacturing process in which live organisms, including human and animal cells, 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. The nature of the product as well as the equipment used will determine the level of segregation (and therefore the level of dedication) needed to avoid cross-contamination. Measures should be put in place to minimise this potential and should be based on documented risk management and documented risk assessment. Further guidance can be found in Chapters 3 and 5 of Part II of the Guide.
11. Special considerations are required where there are live organisms or cells in the finished product and additional controls should be put in place when using certain monitoring equipment, e.g. particle monitoring equipment.
12. Processing steps after harvesting may be carried out simultaneously in the same production area provided that adequate precautions are taken to prevent cross contamination, such as the use of closed systems, based on documented risk management and documented risk assessment. For killed vaccines and toxoids, such parallel processing should only be performed after inactivation of the culture or after detoxification. Precautions should be in place to prevent contamination of inactivated products with non-inactivated products.
13. Positive pressure areas should be used to process sterile products but negative pressure in specific areas at point of exposure of pathogens is acceptable for containment reasons.
14. Where negative pressure areas or safety cabinets are used for aseptic processing of pathogens, they should be surrounded by a positive pressure clean zone of appropriate grade.
15. Air filtration units should be specific to the processing area concerned and recirculation of air should not occur from areas handling live pathogenic organisms or genetically modified cells and organisms.
16. The layout and design of production areas and equipment should permit effective cleaning and decontamination (e.g. by fumigation). The adequacy of cleaning and decontamination procedures should be validated.

17. Equipment used during handling of live organisms should be designed to maintain cultures in a pure state and uncontaminated by external sources during processing.
18. Pipe work systems, valves and vent filters should be properly designed to facilitate cleaning and sterilization. The use of 'clean in place' and 'sterilize in place' systems should be encouraged. Valves on fermentation vessels should be completely steam sterilisable. Air vent filters should be hydrophobic and validated for their scheduled life span.
19. Primary containment should be designed and tested to demonstrate freedom from the risk of leakage.
20. Effluents that may contain pathogenic microorganisms or genetically modified organisms should be effectively decontaminated.
21. Due to the variability of biological products or processes, some additives or ingredients have to be measured or weighed during the production process (e.g. buffers). In these cases, small stocks of these substances may be kept in the production area.
22. Additional measures, dependent on the particular needs of the product, may be required during finishing operations such as formulation, filling and packaging. These may include the sequence of additions, mixing speeds, time and temperature controls, limits on exposure to light and cleaning procedures in the event of spillages.

### **Animals**

A wide range of animal species are used for the manufacture of a number of biological medicinal products, for example polio vaccine (monkeys), snake antivenoms (horses, sheep and goats), tetanus antisera (horses), allergens (cats), rabies vaccine (rabbits, mice and hamsters), transgenic products (goats, cattle). Animal tissues and cells may also be used in (i.e. xenogeneic cells) or to support (e.g. feeder cells) the manufacture of somatic cell medicinal products and gene therapy medicinal products.

In addition, animals may also be used in the quality control of most sera and vaccines, e.g. pertussis vaccine (mice), pyrogenicity (rabbits), BCG vaccine (guinea-pigs).

23. In addition to compliance with TSE regulations, other adventitious agents that are of concern (zoonotic diseases, diseases of source animals) should be monitored by an ongoing flock/herd health programme. Specialist advice should be obtained in establishing such programmes. Instances of ill-health occurring in the source animals should be investigated with respect to their suitability for continued manufacture and possible effects on other batches of

material, the decisions must be documented. A look-back procedure should also be in place for conditions that are not apparent at the time of harvest. The withdrawal period of therapeutic agents used to treat source animals must be documented and used to determine the removal of those animals from the programme for defined periods.

24. Particular care should be taken to prevent and monitor infections in the source / donor animals. Measures should include the sourcing, facilities, husbandry, testing regimes, control of starting and raw materials (including feed materials).
25. For products manufactured from transgenic animals, traceability should be maintained in the creation of such animals from the source animals.
26. General requirements for animal quarters, care and quarantine are laid down in Directive 86/609/EEC. Quarters for animals used in production should be separated from those used in quality control. The health status of animals from which some starting materials are derived and of those used for quality control and safety testing should be monitored and recorded.
27. Where monkeys are used for the production or quality control of biological medicinal products, special consideration is required as laid down in the current WHO Requirements for Biological Substances.
28. Key criteria should be defined, monitored, and recorded. These may include age, weight and health status of the animals. Housing and monitoring may be defined for some categories of animals in specific monographs (e.g. Specific Pathogens Free flocks) or in other guidance documents (e.g. healthy flocks or herds).
29. Animals, biological agents, and tests carried out should be the subject of an identification system so as to prevent any risk of confusion and to control all possible hazards.

### **Documentation**

30. Specifications for biological starting materials may need additional documentation on the source, origin, distribution chain, method of manufacture, and controls applied, particularly microbiological controls.
31. Specifications are routinely required for intermediate and bulk biological medicinal products.
32. A clear definition of what materials constitute a 'batch' should be made, the system must allow full traceability from source(s) to recipient(s) whilst maintaining the privacy of individuals and confidentiality of health related information.

## **Production**

33. Since cultivation conditions, media and reagents are designed to permit the growth of mammalian cells or microbial organisms, specific measures should be put in place to prevent or minimise unwanted bio burden and associated components, e.g. bacterial enzymes, endotoxins etc. Appropriate limits should be set dependent on the requirements of the product and the capability of the process to remove or inactivate contaminants.

## **Starting materials**

34. The source, origin and suitability of biological starting materials should be clearly defined. 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 subsequent tests. The identification of all starting materials should be in compliance with the requirements of Annex 8.
35. The risk of contamination of starting materials during their passage along the supply chain must be assessed, with particular emphasis on TSE. Materials that come into direct contact with manufacturing equipment or the product (such as media used in media fill experiments and lubricants) must also be considered.
36. Where sterilization of starting materials is required, it should be carried out where possible by heat. Where necessary, other appropriate methods may also be used for inactivation of biological materials (e.g. irradiation and filtration). The integrity of any filters used in such sterilization steps must be assured. Given that the risk and consequences of contamination to the product is the same irrespective of the stage of manufacture, the preparation of solutions and buffers should comply with the requirements of Annex 1.

## **Seed lot and cell bank system**

37. In order to prevent the unwanted drift of properties which might ensue from repeated subcultures or multiple generations, the production of biological medicinal products obtained by microbial culture, cell culture or propagation in embryos and animals should be based on a system of master and working seed lots and/or cell banks.
38. The number of generations (doublings, passages) between the seed lot or cell bank and the finished product should be consistent with the marketing authorization dossier.

39. Seed lots and cell banks should be adequately characterized and tested for contaminants under laboratory conditions. Their suitability for use should be further demonstrated by the consistency of the characteristics and quality of the successive batches of product. Seed lots and cell banks should be established, stored and used in such a way as to minimize the risks of contamination (e.g. stored in the vapour phase of liquid nitrogen) or alteration.
40. Establishment of the seed lot and cell bank should be performed in a suitably controlled environment to protect the seed lot and the cell bank and, if applicable, the personnel handling it. During the establishment of the seed lot and cell bank, no other living or infectious material (e.g. virus, cell lines or cell strains) should be handled simultaneously in the same area or by the same persons.
41. Evidence of the stability and recovery of the seeds and banks should be documented, it is recommended that records are kept in a manner permitting trend evaluation Storage containers should be hermetically sealed, clearly labelled and kept at an appropriate temperature. An inventory should be meticulously kept. Storage temperature should be recorded continuously for freezers and properly monitored for liquid nitrogen. Any deviation from set limits and any corrective action taken should be recorded.
42. Only authorized personnel should be allowed to handle the material and this handling should be done under the supervision of a responsible person. Access to stored material should be controlled. Different seed lots or cell banks should be stored in such a way to avoid confusion or cross-contamination. It is desirable to split the seed lots and cell banks and to store the split stocks at different locations so as to minimize the risks of total loss.
43. All containers of master or working cell banks and seed lots should be treated identically during storage. Once removed from storage, the containers should not be returned to the stock.

### **Operating principles**

44. In view of the wide variety of products, the large number of stages typically involved in the manufacture of biological medicinal products and the nature of the biological processes, validated operating ranges must be adhered to with frequent monitoring through all production stages and to in-process controls and in-process storage conditions.
45. Critical process steps, process conditions or other input parameters which affect product safety and / or efficacy, particularly where set-points are unavoidably close to the edge of failure, must be identified, validated, documented and have in-process tests conducted to verify compliance with requirements.
46. Articles and materials, including documentation, entering a production room should be carefully controlled to ensure that only articles and materials

concerned with production are introduced. There should be a system, which ensures that articles and materials entering a room are reconciled with those leaving so that their accumulation within the room does not occur.

47. Heat stable articles and materials entering a clean area or clean/contained area should do so through a double-ended autoclave or oven. Heat labile articles and materials should enter through an air lock with interlocked doors where they are disinfected. Sterilisation of articles and materials elsewhere is acceptable provided that they are double wrapped and enter through an airlock with the appropriate precautions.
48. The growth promoting properties of culture media should be demonstrated.
49. If possible, media should be sterilized in situ. In-line sterilizing filters for routine addition of gases, media, acids or alkalis, defoaming agents etc. to fermenters should be used where possible.
50. Addition of materials or cultures to fermenters and other vessels and sampling should be carried out under carefully controlled conditions to ensure that absence of contamination is maintained. Care should be taken to ensure that vessels are correctly connected when addition or sampling takes place.
51. Centrifugation and blending of products can lead to aerosol formation and containment of such activities to prevent transfer of live microorganisms is necessary.
52. Accidental spillages, especially of live organisms, must be dealt with quickly and safely. Validated decontamination measures should be available for each organism. Where different strains of single bacteria species or very similar viruses are involved, the process may be validated against only one of them, unless there is reason to believe that they may vary significantly in their resistance to the agent(s) involved.
53. If obviously contaminated, such as by spills or aerosols, or if an potentially hazardous organism is involved, the paperwork must be adequately disinfected through an equipment pass, or the information transferred out by such means as photocopy or fax.
54. Careful consideration should be given to the validation of any methods for sterilisation, disinfection, virus removal or inactivation undertaken (see CHMP notes for guidance).
55. In cases where a virus inactivation or removal process is performed during manufacture, measures should be taken to avoid the risk of recontamination of treated products by non-treated products.
56. Products that are inactivated by the addition of a reagent should be thoroughly mixed. The mixture should then be transferred to a second sterile vessel unless

the original container is of such size and shape as to be easily inverted and shaken so as to wet all internal surfaces with the culture/inactivant mixture.

57. Vessels containing inactivated product should not be opened or sampled in areas containing live biological agents. All subsequent processing of inactivated products should take place in areas dedicated to inactivated products.
58. There should be a system to assure the integrity and closure of containers after filling with either intermediate or final products.
59. A wide variety of equipment is used for chromatography, and in general such equipment should be dedicated to the purification of one product and should be sterilized or sanitized between batches. The use of the same equipment at different stages of processing should be discouraged. Acceptance criteria, operating conditions, life span and sanitization or sterilization method of columns should be defined.
60. There should be documented procedures for the use of irradiation equipment. These should comply with the relevant ISO standards. Factors such as restricted access, periodic maintenance and re-qualification of the source, dose mapping of the irradiation container should be included. Annex 12 should be consulted for further guidance.
61. The capping of vials containing live biological agents must be performed in such a way that ensures that contamination of other products or escape of the live agents into other areas or the external environment does not occur.

### **Quality control**

62. In-process controls play an especially important role in ensuring the consistency of the quality of biological medicinal products. Those conditions that are crucial for quality (e.g. virus removal, residual DNA content) but which cannot be carried out on the finished product, should be performed at an appropriate stage of production.
63. It may be necessary to retain samples of intermediate products in sufficient quantities and under appropriate storage conditions to allow the repetition or confirmation of a batch control.
64. Certain types of cells (e.g. autologous) may be available in limited quantities and a modified testing strategy may be developed, this strategy should be documented.
65. Continuous monitoring of certain production processes is necessary, for example fermentation. Such data should form part of the batch record.

66. Where continuous culture is used, special consideration should be given to the quality control requirements arising from this type of production method.

## **B. SPECIFIC GUIDANCE ON SELECTED PRODUCT TYPES**

### **B1. ALLERGEN PRODUCTS**

Attention is drawn to other texts that provide requirements and guidance such as the European Pharmacopoeia Monograph on Allergen Products and CHMP documents. For guidance on recombinant products, see section B4.

1. Source materials should be described by their origin, nature, contaminant limits, method of collection or production and pre-treatment. Those derived from animals should be from healthy sources. Allergen should be stored under defined conditions to minimise deterioration.
2. For allergen products that are modified chemically or adsorbed onto carriers, the process should be conducted within validated limits. Batch to batch consistency should be demonstrated by following validated procedures and by reference to suitable reference preparations.

### **B2. ANIMAL IMMUNOSERA PRODUCTS**

1. Particular care should be exercised on the control of antigens of biological origin to assure their quality and consistency. The preparation of materials used to immunise the source animals (e.g. antigens, hapten carriers, adjuvants, stabilising agents), the storage of such material immediately prior to immunisation should be in accordance with documented procedures.
2. The immunisation, test bleed and harvest bleed schedule should be defined in written procedures.
3. The manufacturing conditions for the preparation of antibody sub-fragments (e.g. Fab or F(ab')<sub>2</sub>) and any further modifications must be in accordance with validated parameters. Where such enzymes are made up of several components, their consistency should be assured.

### **B3. VACCINES**

1. The concept of a Vaccine Antigen Master File was introduced in Commission Directive 2003/63/EC (amending Directive 2001/83/EC). Where such

Certification of Compliance has been granted, the manufacturing and test methods in use must be demonstrably consistent with those conditions in the VAMF and the Marketing Authorisation.

2. Where eggs are used, the health status of all source flocks used in the production of eggs (whether specified pathogen free or healthy flocks) should be assured. The suitability of intermediate storage location(s) and the conditions for sanitisation, segregation, traceability, cold storage and initial incubation should be regularly reviewed as part of the self-inspection programme.
3. The integrity of containers used to store intermediate components and the hold times must be validated.
4. The sequence of addition of active ingredients and excipients during the formulation of an intermediate or final product must be in compliance with the documented process.
5. The cumulative effect of each minor change on the efficacy of vaccines should be considered.
6. When stability studies indicate decreasing potency of intermediates and/or final products during storage, cumulative “worst case” stability studies should be performed for final products.
7. Where organisms with a higher biological safety level (e.g. smallpox and pandemic vaccine strains) are to be used in manufacture or testing, appropriate containment arrangements must be in place. The approval of such arrangements should be obtained from the appropriate authority (ies) and the approval documents should be available for verification.

#### **B4. RECOMBINANT PRODUCTS**

1. Process condition and in-process controls during cell growth, protein expression and purification must be maintained within validated parameters to assure a consistent product with a defined range of impurities that is within the capability of the process to reduce to acceptable levels. The type of cell used in production may require increased measures to be taken to assure freedom from viruses. For multiple harvest production, the period of continuous cultivation should be defined.
2. The capability of the purification processes to remove unwanted host cell proteins, nucleic acids, carbohydrates, viruses and other impurities should be defined and validated.

3. Stability of the expression construct should be monitored. The parameters selected should be relevant to the risks inherent in the particular construct (e.g. expression vector structure, gene copy number, expressed protein).

## **B5. MONOCLONAL ANTIBODY PRODUCTS**

1. Monoclonal antibodies may be manufactured from murine hybridomas, human hybridomas or by recombinant DNA technology. Control measures appropriate to the different source cells (including feeder cells if used) and materials used to establish the hybridoma / cell line should be in place to assure the safety and quality of the product. Freedom from viruses should be given particular emphasis.
2. Stability of the cell / hybridoma lines should be monitored. The parameters selected should be relevant to the risks inherent to cell / hybridoma line (e.g. oncogenicity, gene copy number). Appropriate characteristics of the antibody (e.g. binding specificity) should be monitored.
3. The maximum permitted generation number for production should be defined and should be based on the stability of the cell / hybridoma line. Criteria to be monitored at the end of a production cycle and for early termination of production cycle should be defined.
4. The manufacturing conditions for the preparation of antibody sub-fragments (e.g. Fab, F (ab')<sub>2</sub>, and scFv) and any further modifications (e.g. radio labelling, conjugation, and chemical linking) must be in accordance with validated parameters.

## **B6. Gene Therapy Products**

Gene therapy (GT) is an evolving area and guidance is likely to be modified in the light of experience and further developments. Part IV (1) of Directive 2003/63/EC (modifying Directive 2001/83/EC) contains a definition of gene therapy medicinal products.

Such products involve the use of vectors, which may be viral, or non-viral in origin. Advice should be obtained in order to establish and maintain the appropriate Biological Safety Level within viral vector facilities.

The procurement, donation and testing of human tissues and cells is regulated by Directive 2004/23/EC. Approvals from the national competent authority (ies) should

be verifiably in place for such establishments. There should be no regulatory 'gap' between Good Practice under 2004/23/EC and GMP.

1. Since the cells used in the manufacture of gene therapy products are obtained either from humans (autologous or allogeneic) or animals (xenogeneic), there is a potential risk of contamination by adventitious agents. The robustness of the control and test measures put in place for these source materials will have a significant impact on the safety of the medicinal products.
2. The manufacture and testing of gene therapy medicinal products raises specific issues regarding the safety and quality of the final product and safety issues for recipients and staff.
3. Factors such as the nature of the genetic material, type of (viral or non-viral) vector and type of cells have a bearing on the range of potential impurities and adventitious agents that should be considered as part of the development of an overall strategy to minimise risk. This strategy should be used as a basis for the design of the process, the manufacturing and storage facilities and equipment, cleaning and decontamination procedures, packaging, labelling and distribution.
4. Restriction of the movement of all personnel (including QC and maintenance staff) should be controlled on the basis of a documented risk assessment. This should take into account movement between areas containing different genetically modified organisms and areas containing non-genetically-modified organisms.
5. Any special cleaning and decontamination methods required for the range of organisms being handled should be considered in the design of facilities and equipment. Where possible, the environmental monitoring programme should be supplemented by the inclusion of methods to detect the presence of specific organisms being cultivated.
6. Production facilities for viral vectors should be separated from other areas. Dedicated facilities should be used for handling of live viral vectors and should be separated from other areas by specific measures. The arrangements for containment should be demonstrated to be effective. Closed systems should be used wherever possible, sample collection additions and transfers should prevent the release of material.
7. Ex-vivo manipulation of recipient cells should occur in separate facilities. Measures to minimise the potential for cross-contamination and mix-up between cells from different patients are required.
8. Where replication limited viruses are used, care must be taken to prevent the introduction of wild-type viruses, which may lead to the formation of replication competent recombinant viruses.

9. An emergency plan for dealing with accidental release of viable organisms should be in place. This should address methods and procedures for containment, protection of operators, cleaning, decontamination and safe return to use. An assessment of impact on the immediate products and any others in the affected area should also be made.
10. Special consideration should be given to starting materials, cryoprotectants, culture media and vectors obtained from other sources. Virus seed lots and cell banking systems should be used where relevant.
11. Description of cell collection methodology including location, type of tissue or cells, operating process, transportation, storage and traceability as well as controls carried out during the collection process should be documented. There should be a clear definition of a batch that allows traceability from donor(s) to recipient(s).
12. Concurrent production of different gene therapy vectors in the same area is not acceptable. Changeover procedures between campaigns should be demonstrated to be effective.
13. The production of vectors should be described in a sufficiently detailed flow chart ensuring the traceability of the products from the starting material (plasmids, gene of interest and regulatory sequences, cell banks, and viral or non viral vector stock) to the finished product.
14. For products that utilise non-biological means to transfer the gene, their physico-chemical properties should be documented and tested.
15. The production of genetically modified somatic cells should be conducted under appropriate containment conditions. A validated cleaning procedure should be used between the processing of different cell lines
16. Critical parameters and process steps (e.g. fermentation conditions, linkage to carriers, cell culture stability, nucleic acid sequences, gene expression, and characteristics of genetically modified somatic cells) should be identified, validated, documented and in-process tests conducted to verify compliance with requirements.
17. For viruses that remain cell or nucleus associated, validated release processes should be used. For viruses that form a large proportion of 'empty' virus particles, the purification process should minimise antigen exposure to recipients, factors to take into account include reducing the ratio of un-infectious to infectious particles to acceptable levels.

18. Particular care should be taken in the preparation, printing, storage and application of labels, including any specific text signifying genetic modification of the contents on the primary container and secondary packaging. External labelling should include the biosafety hazard symbol. The compatibility of the labels with ultra-low temperatures should be verified.
19. Arrangements for the collection, storage and testing of starting materials, in-process and final product samples and environmental monitoring samples should be in place. Such activities should not compromise the security or classification of any area or increase the risk of cross-contamination.
20. Distribution records should maintain the traceability of batches, confirmation of the receipt of the products should be obtained. These records should be maintained for at least 30 years by the manufacturer. Shipment of products containing and/or consisting of GMO should conform to appropriate legislation. The packaging should be designed to protect the product and to maintain critical products parameters. Instructions to follow in the event of package damage should be readily available.

## **B7. SOMATIC AND XENOGENEIC CELL THERAPY PRODUCTS**

Part IV (2) of Directive 2003/63/EC (modifying Directive 2001/83/EC) contains a definition of somatic cell therapy (SCT) medicinal products. The use of stem cells (whether embryonic, foetal or adult origin) as medicinal products involves their “substantial alteration” and is therefore considered within the remit of this section.

The procurement, donation and testing of human tissues and cells is regulated by Directive 2004/23/EC. Approvals from the national competent authority (ies) should be verifiably in place for such establishments. There should be no regulatory ‘gap’ between Good Practice under 2004/23/EC and GMP.

Traceability from the donor to the recipient(s), and vice versa, including in-contact products and materials, should be maintained. There should be a clear definition of a batch in the context of somatic cells from cell sourcing to final container.

1. Since somatic cells are obtained either from humans (autologous or allogeneic) or animals (xenogeneic), there is a potential risk of contamination by adventitious agents. Pooling of cells increases the risk and must be justified. The robustness of the control and test measures put in place for these source materials will have a significant impact on the safety of the medicinal products. Added importance is given to these measures due to the relative limitations of purification and virus removal steps available for these products.

2. Cell types and the health status of the donor(s), donation and procurement information, facility design, environmental standards and controls (Annex 1), cleaning regimes, changeover procedures, separation of work stages and flow path should take into account the particular risks arising from the type of cells being processed. Single use components and the use of closed systems should be used as much as possible to minimise the risk of cross-contamination.
3. Special consideration should be given to starting materials, cryoprotectants, feeder cells (murine or human), culture media, enzymes, cytokines and growth factors. Where applicable, matrices, biocompatible polymers and other ancillary materials should also be controlled. Description of cell collection methodology including location, type of tissue or cells collected, mobilisation or in vivo activation, processing, transportation, storage and traceability as well as controls carried out during the collection process should be documented. Where possible, cell-banking systems should be established.
4. For xenogeneic cells, the safety of materials should be demonstrated. Factors such as animal source, husbandry and care, genetic modification and health monitoring information should be taken into consideration.
5. The processing of SCT products should occur in dedicated facilities.
6. In-process sampling requirements for culture conditions and temperature and humidity controls should be established and monitored to assure the consistency of each batch. Critical process steps should be identified.
7. Particular care should be taken in the preparation, printing, storage and application of labels, including any specific text for genetic modification on the primary container and secondary packaging. The compatibility of the label with ultra-low storage temperatures should be verified where applicable.
8. Special attention should be paid to any cryopreservation stages including any specific requirements for the rate of temperature change (during freezing or thawing). Where liquid nitrogen is used, this should be pharmaceutical grade. The type of storage chamber, placement and retrieval process should minimise the risk of cross-contamination, maintain the quality of the products and facilitate their accurate retrieval. Documented procedures should be in place for the secure handling and storage of products with positive serological markers.
9. Procedures for batch certification and release should be established. Donor health information should also be taken into consideration.
10. The range of adventitious agents to be tested should be adapted to the source and type of cells and may include endogenous retroviruses. Where relevant, a stability-monitoring programme should be in place together with reference and retain samples in sufficient quantity to permit further examination. Additional

aspects should also be monitored in stem cells; these may include genetic stability, tumourigenicity and oncogenicity.

11. Distribution records should maintain the traceability of batches and confirmation of the receipt of the products should be obtained. These records should be maintained for at least 30 years by the manufacturer. Shipment of products containing and/or consisting of GMO should conform to appropriate legislation. The packaging should be designed to protect the product and to maintain critical products parameters. Instructions to follow in the event of package damage should be readily available.
12. Recall procedures should also take into account any donor health information not apparent at the time of donation.

## **B8. TRANSGENIC ANIMAL PRODUCTS**

Consistency of starting material from a transgenic source is likely to be more problematic than is normally the case for non-transgenic biotechnology sources. Consequently, there is an increased requirement to demonstrate batch-to-batch consistency of product in all respects.

1. A range of species may be used to produce biological medicinal products, which may be expressed into body fluids (e.g. milk) for collection and purification. Animals should be clearly and uniquely identified and backup arrangements should be put in place in the event of loss of the primary marker.
2. The arrangements for housing and care of the animals should be defined such that they minimise the exposure of the animals to pathogenic and zoonotic agents. Appropriate measures to protect the external environment should be established. A health-monitoring programme should be established and all results documented, any incident should be investigated and its impact on the continuation of the animal and on previous batches of product should be determined. Care should be taken to ensure that any therapeutic products used to treat the animals do not contaminate the product.
3. The genealogy of the founder animals through to production animals must be documented. Since a transgenic line will be derived from a single genetic founder animal, materials from different transgenic lines should not be mixed. The stability of the gene on breeding should be demonstrated. Variable expression of product and host related impurities in different animals and throughout the production period should be taken into account in order to ensure an acceptable and consistent yield of product.
4. The conditions under which the product is harvested should be defined and should minimise the opportunity for microbial contamination of the product.

The harvest schedule and conditions under which animals may be removed from production should be defined. Acceptance limits should be set for materials (e.g. host proteins) that may interfere with the purification process.

## **B9. TRANSGENIC PLANT PRODUCTS**

Consistency of starting material from a transgenic source is likely to be more problematic than is normally the case for non-transgenic biotechnology sources. Consequently, there is an increased requirement to demonstrate batch-to-batch consistency of product in all respects.

1. Additional measures, over and above those given in Part A, may be required to prevent contamination of master and working transgenic banks by extraneous plant materials and relevant adventitious agents. The stability of the gene within defined generation numbers should be demonstrated.
2. Plants should be clearly and uniquely identified, the presence of key plant features, including health status, across the crop should be verified at defined intervals through the cultivation period to assure consistency of yield between crops.
3. Security arrangements for the protection of crops should be defined, wherever possible, such that they minimise the exposure to contamination by microbiological agents and cross-contamination with non-related plants. Measures should be in place to prevent materials such as pesticides and fertilisers from contaminating the product. A monitoring programme should be established and all results documented, any incident should be investigated and its impact on the continuation of the crop in the production programme should be determined.
4. Variable expression in different plants across the crop should be taken into account in order to ensure an acceptable and consistent yield and purity of product.
5. Conditions under which plants may be removed from production should be defined. Acceptance limits should be set for materials (e.g. host proteins) that may interfere with the purification process.
6. Environmental conditions (temperature, rain), which may affect the quality attributes and yield of the recombinant protein from time of planting, through cultivation to harvest should be documented, Good Agricultural and Collection Practice (GACP) should be taken into account. Criteria for harvesting, manipulation and interim storage of harvested materials should be defined.

**B10. TISSUE ENGINEERED PRODUCTS.**

[Guidance under development.]

## GLOSSARY

**Adjuvant.** A chemical or biological substance that enhances the immune response against an antigen.

**Antigens.** Substances (e.g. toxins, foreign proteins, bacteria, tissue cells) capable of inducing specific immune responses.

**Antibody.** Proteins produced by the B-lymphocytes that bind to specific antigens. Antibodies may be divided into 2 main types based on key differences in their method of manufacture:

**Monoclonal antibodies (MAb)** – homogenous antibody population obtained from a single clone of lymphocytes or by recombinant technology and which bind to a single epitope.

**Polyclonal antibodies** – derived from a range of lymphocyte clones, produced in human and animals in response to the epitopes on most ‘non-self’ molecules.

**Biological medicinal product:** is a medicinal product in which the active substance is a biological substance.

**Biological substance:** is a substance that is produced by or extracted from a biological source and that needs for its characterisation and the determination of its quality a combination of physicochemical-biological testing, together with the production process and its control.

**Biological safety level (BSL).** The containment conditions required to safely handle organisms of different hazards ranging from BSL1 (lowest risk, unlikely to cause human disease) to BSL4 (highest risk, cause severe disease, likely to spread and no effective prophylaxis or treatment available).

**Contained use.** An operation, in which genetically modified organisms are cultured, stored, used, transported, destroyed or disposed of and for which barriers (physical / chemical / biological) are used to limit their contact with the general population and the environment.

**Deliberate release.** The deliberate release into the environment of genetically modified organisms.

**Expression construct.** The gene(s) or nucleic acid sequence(s) along with the required regulatory sequences for its expression.

**Ex-vivo.** Where procedures are conducted on tissues or cells outside the living body and returned to the living body.

**Gene.** A sequence of DNA that codes for one (or more) protein(s).

**Gene therapy.** The deliberate introduction of nucleic acids into human somatic cells for therapeutic, prophylactic or diagnostic purposes. Cells may be modified ex-vivo for subsequent administration or modified in-vivo by gene therapy products given directly to the subject.

**Gene therapy product.** A product obtained through a set of manufacturing processes aimed at the transfer, to be performed either in vivo or ex vivo, of a prophylactic, diagnostic or therapeutic gene (i.e. a piece of nucleic acid) to human/animal somatic cells and its subsequent expression in-vivo.

**Gene transfer.** A process to transfer a gene in cells, involving an expression system contained in a delivery system known as a vector, which can be of viral, as well as non-viral origin. The vector can also be included in a human or animal cell. (Directive 2003/18/EC, Part IV)

**Hapten.** A low molecular weight molecule that is not in itself antigenic unless conjugated to a 'carrier' molecule.

**Hybridoma.** An immortalised cell line typically derived by fusing B-lymphocytes with a tumour cells that secrete desired (monoclonal) antibodies.

**In-vivo.** Procedures conducted in living organisms.

**Master cell bank (MCB)** – a homogeneous pool of micro-organisms or cells that are distributed uniformly into a number of containers that are stored in such a way to ensure stability normally used to produced working cell banks. **Master virus seed lot (MVL)** – as above, but in relation to viruses; **master transgenic bank** – as above but for transgenic plants or animals.

**Plasmid.** A small piece of circular DNA that exists inside a cell, separate from the cell's main DNA, and which can be transferred from one cell to another.

**Somatic cells.** Cells which make up the body of a human or animal. These cells may be autologous (from the patient), allogeneic (from another human being) or xenogeneic (from animals) somatic living cells, that have been manipulated or altered ex vivo, to be administered in humans to obtain a therapeutic, diagnostic or preventive effects.

**Starting material:** any substance of biological origin such as micro-organisms, organs, and tissues of either plant, or animal origin, cells or fluids of human or animal origin, and biotechnological cell constructs.

**Transgenic:** an organism that contains a foreign gene in its normal genetic component for the expression of biological pharmaceutical materials.

**Vector:** an agent of transmission, which transmits genetic information from one cell or organism to another, e.g. plasmids, liposomes, viruses.

**Viral vector:** a replication-deficient virus, in some cases a replication competent virus.

**Working cell bank (WCB)** – a homogeneous pool of micro-organisms or cells, that are distributed uniformly into a number of containers derived from a MCB that are stored in such a way to ensure stability and for use in production. **Working virus seed lot (WVL)** – as above but in relation to viruses, **working transgenic bank** – as above but for transgenic plants or animals.

**Zoonosis:** Animal diseases that can be transmitted to humans.