

ANNEX 5

MANUFACTURE OF IMMUNOLOGICAL VETERINARY MEDICINAL PRODUCTS

Principle

The manufacture of immunological veterinary medicinal products has special characteristics which should be taken into consideration when implementing and assessing the quality assurance system.

Due to the large number of animal species and related pathogenic agents, the variety of products manufactured is very wide and the volume of manufacture is often low; hence, work on a campaign basis is common. Moreover, because of the very nature of this manufacture (cultivation steps, lack of terminal sterilisation, etc.), the products must be particularly well-protected against contamination and cross-contamination. The environment also must be protected especially when the manufacture involves the use of pathogenic or exotic biological agents and the worker must be particularly well-protected when the manufacture involves the use of biological agents pathogenic to man.

These factors, together with the inherent variability of immunological products and the relative inefficiency in particular of final product quality control tests in providing adequate information about products, means that the role of the quality assurance system is of the utmost importance. The need to maintain control over all of the following aspects of GMP, as well as those outlined in this Guide, cannot be overemphasised. In particular, it is important that the data generated by the monitoring of the various aspects of GMP (equipment, premises, product etc.) are rigorously assessed and informed decisions, leading to appropriate action, are made and recorded.

Personnel

1. All personnel (including those concerned with cleaning and maintenance) employed in areas where immunological products are manufactured should be given training in and information on hygiene and microbiology. They should receive additional training specific to the products with which they work.
2. Responsible personnel should be formally trained in some or all of the following fields: bacteriology, biology, biometry, chemistry, immunology, medicine, parasitology, pharmacy, pharmacology, virology and veterinary medicine and should also have an adequate knowledge of environmental protection measures.
3. Personnel should be protected against possible infection with the biological agents used in manufacture. In the case of biological agents known to cause disease in humans, adequate measures should be taken to prevent infection of personnel working with the agent or with experimental animals.

Where relevant, the personnel should be vaccinated and subject to medical examination.

4. Adequate measures should be taken to prevent biological agents being taken outside the manufacturing plant by personnel acting as a carrier. Dependent on the type of biological agent, such measures may include complete change of clothes and compulsory showering before leaving the production area.
5. For immunological products, the risk of contamination or cross-contamination by personnel is particularly important.

Prevention of *contamination* by personnel should be achieved by a set of measures and procedures to ensure that appropriate protective clothing is used during the different stages of the production process.

Prevention of *cross-contamination* by personnel involved in production should be achieved by a set of measures and procedures to ensure that they do not pass from one area to another unless they have taken appropriate measures to eliminate the risk of contamination. In the course of a working day, personnel should not pass from areas where contamination with live micro-organisms is likely or where animals are housed to premises where other products or organisms are handled. If such passage is unavoidable, clearly defined decontamination procedures, including change of clothing and shoes, and, where necessary, showering, should be followed by staff involved in any such production.

Personnel entering a contained area where organisms had not been handled in open circuit operations in the previous twelve hours to check on cultures in sealed, surface decontaminated flasks would not be regarded as being at risk of contamination, unless the organism involved was an exotic.

Premises

6. Premises should be designed in such a way as to control both the risk to the product and to the environment.
This can be achieved by the use of containment, clean, clean/contained or controlled areas.
7. Live biological agents should be handled in contained areas. The level of containment should depend on the pathogenicity of the micro-organism and whether it has been classified as exotic. (Other relevant legislation, such as Directives 90/219/EEC¹ and 90/220/EEC², also applies).
8. Inactivated biological agents should be handled in clean areas. Clean areas should also be used when handling non-infected cells isolated from multicellular organisms and, in some cases, filtration-sterilised media.
9. Open circuit operations involving products or components not subsequently sterilised should be carried out within a laminar air flow work station (grade A) in a grade B area.
10. Other operations where live biological agents are handled (quality control, research and diagnostic services, etc.) should be appropriately contained and separated if production operations are carried out in the same building. The level of containment should depend on the pathogenicity of the biological agent and whether they have been classified as exotic. Whenever diagnostic activities are carried out, there is the risk of introducing highly pathogenic organisms. Therefore, the level of containment should be adequate to cope with all such risks. Containment may also be required if quality control or other activities are carried out in buildings in close proximity to those used for production.
11. Containment premises should be easily disinfected and should have the following characteristics:
 - a) the absence of direct venting to the outside;

¹ Council Directive 98/81/EC of 26 October 1998 amending Directive 90/219/EEC on the contained use of genetically modified micro-organisms (OJ L 330, 05.12.1998, p. 13-31)

² Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC – Commission Declaration (OJ L 106, 17.04.2001, p. 01-39)

- b) a ventilation with air at negative pressure. Air should be extracted through HEPA filters and not be re-circulated except to the same area, and provided further HEPA filtration is used (normally this condition would be met by routing the re-circulated air through the normal supply HEPA's for that area). However, recycling of air between areas may be permissible provided that it passes through two exhaust HEPA's, the first of which is continuously monitored for integrity, and there are adequate measures for safe venting of exhaust air should this filter fail;
 - c) air from manufacturing areas used for the handling of exotic organisms should be vented through 2 sets of HEPA filters in series, and that from production areas not re-circulated;
 - d) a system for the collection and disinfection of liquid effluents including contaminated condensate from sterilizers, biogenerators, etc. Solid wastes, including animal carcasses, should be disinfected, sterilized or incinerated as appropriate. Contaminated filters should be removed using a safe method;
 - e) changing rooms designed and used as air locks, and equipped with washing and showering facilities if appropriate. Air pressure differentials should be such that there is no flow of air between the work area and the external environment or risk of contamination of outer clothing worn outside the area;
 - f) an air lock system for the passage of equipment, which is constructed so that there is no flow of contaminated air between the work area and the external environment or risk of contamination of equipment within the lock. The air lock should be of a size which enables the effective surface decontamination of materials being passed through it. Consideration should be given to having a timing device on the door interlock to allow sufficient time for the decontamination process to be effective.
 - g) in many instances, a barrier double-door autoclave for the secure removal of waste materials and introduction of sterile items.
12. Equipment passes and changing rooms should have an interlock mechanism or other appropriate system to prevent the opening of more than one door at a time. Changing rooms should be supplied with air filtered to the same standard as that for the work area, and equipped with air extraction facilities to produce an adequate air circulation independent of that of the work area. Equipment passes should normally be ventilated in the same way, but unventilated passes, or those equipped with supply air only, may be acceptable.
 13. Production operations such as cell maintenance, media preparation, virus culture, etc. likely to cause contamination should be performed in separate areas. Animals and animal products should be handled with appropriate precautions.
 14. Production areas where biological agents particularly resistant to disinfection (e.g. spore-forming bacteria) are handled should be separated and dedicated to that particular purpose until the biological agents have been inactivated.
 15. With the exception of blending and subsequent filling operations, one biological agent only should be handled at a time within an area.
 16. Production areas should be designed to permit disinfection between campaigns, using validated methods.
 17. Production of biological agents may take place in controlled areas provided it is carried out in totally enclosed and heat sterilised equipment, all connections being also heat sterilised after making and before breaking. It may be acceptable for connections to be made under local laminar air flow provided these are few in number and proper aseptic techniques are used and there is no risk of leakage. The sterilisation parameters used before breaking the connections must be validated for the organisms being used. Different products may be placed in different biogenerators, within the same area, provided that there is no risk of accidental cross-contamination. However, organisms generally subject to special requirements for containment should be in areas dedicated to such products.
 18. Animal houses where animals intended or used for production are accommodated, should be provided with the appropriate containment and/or clean area measures, and should be separate from other animal accommodation.

Animal houses where animals used for quality control, involving the use of pathogenic biological agents, are accommodated, should be adequately contained.

19. Access to manufacturing areas should be restricted to authorised personnel. Clear and concise written procedures should be posted as appropriate.
20. Documentation relating to the premises should be readily available in a plant master file.

The manufacturing site and buildings should be described in sufficient detail (by means of plans and written explanations) so that the designation and conditions of use of all the rooms are correctly identified as well as the biological agents which are handled in them. The flow of people and product should also be clearly marked.

The animal species accommodated in the animal houses or otherwise on the site should be identified.

The activities carried out in the vicinity of the site should also be indicated.

Plans of contained and/or clean area premises, should describe the ventilation system indicating inlets and outlets, filters and their specifications, the number of air changes per hour, and pressure gradients. They should indicate which pressure gradients are monitored by pressure indicator.

Equipment

21. The equipment used should be designed and constructed so that it meets the particular requirements for the manufacture of each product.

Before being put into operation the equipment should be qualified and validated and subsequently be regularly maintained and validated.

22. Where appropriate, the equipment should ensure satisfactory primary containment of the biological agents.

Where appropriate, the equipment should be designed and constructed as to allow easy and effective decontamination and/or sterilisation.

23. Closed equipment used for the primary containment of the biological agents should be designed and constructed as to prevent any leakage or the formation of droplets and aerosols.

Inlets and outlets for gases should be protected so as to achieve adequate containment e.g. by the use of sterilising hydrophobic filters.

The introduction or removal of material should take place using a sterilisable closed system, or possibly in an appropriate laminar air flow.

24. Equipment where necessary should be properly sterilised before use, preferably by pressurised dry steam. Other methods can be accepted if steam sterilisation cannot be used because of the nature of the equipment. It is important not to overlook such individual items as bench centrifuges and water baths.

Equipment used for purification, separation or concentration should be sterilised or disinfected at least between use for different products. The effect of the sterilisation methods on the effectiveness and validity of the equipment should be studied in order to determine the life span of the equipment.

All sterilisation procedures should be validated.

25. Equipment should be designed so as to prevent any mix-up between different organisms or products. Pipes, valves and filters should be identified as to their function.

Separate incubators should be used for infected and non infected containers and also generally for different organisms or cells. Incubators containing more than one organism or cell type will only be acceptable if adequate steps are taken to seal, surface decontaminate and segregate the containers. Culture vessels, etc. should be individually labelled. The cleaning and disinfection of the items can be particularly difficult and should receive special attention.

Equipment used for the storage of biological agents or products should be designed and used in such a manner as to prevent any possible mix-up. All stored items should be clearly and unambiguously labelled and in leak-proof containers. Items such as cells and organisms seed stock should be stored in dedicated equipment.

26. Relevant equipment, such as that requiring temperature control, should be fitted with recording and/or alarm systems.

To avoid breakdowns, a system of preventive maintenance, together with trend analysis of recorded data, should be implemented.

27. The loading of freeze dryers requires an appropriate clean/contained area.

Unloading freeze dryers contaminates the immediate environment. Therefore, for single-ended freeze dryers, the clean room should be decontaminated before a further manufacturing batch is introduced into the area, unless this contains the same organisms, and double door freeze dryers should be sterilised after each cycle unless opened in a clean area.

Sterilisation of freeze dryers should be done in accordance with item 24. In case of campaign working, they should at least be sterilised after each campaign.

Animals and animal houses

28. General requirements for animal quarters, care and quarantine are laid down in Directive 86/609/EEC³.
29. Animal houses should be separated from the other production premises and suitably designed.
30. The sanitary status of the animals used for production should be defined, monitored, and recorded. Some animals should be handled as defined in specific monographs (e.g. Specific Pathogens Free flocks).
31. 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.

Disinfection – Waste disposal

32. Disinfection and/or wastes and effluents disposal may be particularly important in the case of manufacture of immunological products. Careful consideration should therefore be given to procedures and equipment aiming at avoiding environmental contamination as well as to their validation or qualification.

Production

33. Because of the wide variety of products, the frequently large number of stages involved in the manufacture of immunological veterinary medicinal products and the nature of the biological processes, careful attention must be paid to adherence to validated operating procedures, to the constant monitoring of production at all stages and to in-process controls.

Additionally, special consideration should be given to starting materials, media and the use of a seed lot system.

Starting materials

34. The suitability of starting materials should be clearly defined in written specifications. These should include details of the supplier, the method of manufacture, the geographical origin and

³ Directive 2003/65/EC of the European Parliament and of the Council of 22 July 2003 amending Council Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes (OJ L 230 , 16.09.2003, p. 32-33)

the animal species from which the materials are derived. The controls to be applied to starting materials must be included. Microbiological controls are particularly important.

35. The results of tests on starting materials must comply with the specifications. Where the tests take a long time (e.g. eggs from SPF flocks) it may be necessary to process starting materials before the results of analytical controls are available. In such cases, the release of a finished product is conditional upon satisfactory results of the tests on starting materials.
36. Special attention should be paid to a knowledge of the supplier's quality assurance system in assessing the suitability of a source and the extent of quality control testing required.
37. Where possible, heat is the preferred method for sterilising starting materials. If necessary, other validated methods, such as irradiation, may be used.

Media

38. The ability of media to support the desired growth should be properly validated in advance.
39. Media should preferably be sterilised in situ or in line. Heat is the preferred method. Gases, media, acids, alkalis, defoaming agents and other materials introduced into sterile biogenerators should themselves be sterile.

Seed lot and cell bank system

40. In order to prevent the unwanted drift of properties which might ensue from repeated subcultures or multiple generations, the production of immunological veterinary medicinal products obtained by microbial, cell or tissue culture, or propagation in embryos and animals, should be based on a system of seed lots or cell banks.
41. The number of generations (doublings, passages) between the seed lot or cell bank and the finished product should be consistent with the dossier of authorisation for marketing.
42. Seed lots and cell banks should be adequately characterised and tested for contaminants. Acceptance criteria for new seed lots should be established. Seed lots and cell banks shall be established, stored and used in such a way as to minimise the risks of contamination, or any alteration. During the establishment of the seed lot and cell bank, no other living or infectious material (e.g. virus or cell lines) shall be handled simultaneously in the same area or by the same person.
43. Establishment of the seed lot and cell bank should be performed in a suitable environment to protect the seed lot and the cell bank and, if applicable, the personnel handling it and the external environment.
44. The origin, form and storage conditions of seed material should be described in full. Evidence of the stability and recovery of the seeds and cells should be provided. Storage containers should be hermetically sealed, clearly labelled and stored at an appropriate temperature. Storage conditions shall be properly monitored. An inventory should be kept and each container accounted for.
45. Only authorised personnel should be allowed to handle the material and this handling should be done under the supervision of a responsible person. Different seed lots or cell banks shall be stored in such a way to avoid confusion or cross-contamination errors. It is desirable to split the seed lots and cell banks and to store the parts at different locations so as to minimise the risk of total loss.

Operating principles

46. The formation of droplets and the production of foam should be avoided or minimised during manufacturing processes. Centrifugation and blending procedures which can lead to droplet formation should be carried out in appropriate contained or clean/contained areas to prevent transfer of live organisms.
47. 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 need 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.

48. Operations involving the transfer of materials such as sterile media, cultures or product should be carried out in pre-sterilised closed systems wherever possible. Where this is not possible, transfer operations must be protected by laminar airflow work stations.
49. Addition of media or cultures to biogenerators and other vessels should be carried out under carefully controlled conditions to ensure that contamination is not introduced. Care must be taken to ensure that vessels are correctly connected when addition of cultures takes place.
50. Where necessary, for instance when two or more fermenters are within a single area, sampling and addition ports, and connectors (after connection, before the flow of product, and again before disconnection) should be sterilised with steam. In other circumstances, chemical disinfection of ports and laminar air flow protection of connections may be acceptable.
51. Equipment, glassware, the external surfaces of product containers and other such materials must be disinfected before transfer from a contained area using a validated method (see 47 above). Batch documentation can be a particular problem. Only the absolute minimum required to allow operations to GMP standards should enter and leave the area. If obviously contaminated, such as by spills or aerosols, or if the organism involved is an exotic, the paperwork must be adequately disinfected through an equipment pass, or the information transferred out by such means as photocopy or fax.
52. Liquid or solid wastes such as the debris after harvesting eggs, disposable culture bottles, unwanted cultures or biological agents, are best sterilised or disinfected before transfer from a contained area. However, alternatives such as sealed containers or piping may be appropriate in some cases.
53. 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.
54. 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.
55. Precautions must be taken to avoid contamination or confusion during incubation. There should be a cleaning and disinfection procedure for incubators. Containers in incubators should be carefully and clearly labelled.
56. With the exception of blending and subsequent filling operations (or when totally enclosed systems are used) only one live biological agent may be handled within a production room at any given time. Production rooms must be effectively disinfected between the handling of different live biological agents.
57. Products should be inactivated by the addition of inactivant accompanied by sufficient agitation. The mixture should then be transferred to a second sterile vessel, unless the container is of such a size and shape as to be easily inverted and shaken so as to wet all internal surfaces with the final culture/inactivant mixture.
58. Vessels containing inactivated products should not be opened or sampled in areas containing live biological agents. All subsequent processing of inactivated products should take place in clean areas grade A-B or enclosed equipment dedicated to inactivated products.
59. Careful consideration should be given to the validation of methods for sterilisation, disinfection, virus removal and inactivation.
60. Filling should be carried out as soon as possible following production. Containers of bulk product prior to filling should be sealed, appropriately labelled and stored under specified conditions of temperature.
61. There should be a system to assure the integrity and closure of containers after filling.

62. 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.
63. For various reasons there may be a delay between the filling of final containers and their labelling and packaging. Procedures should be specified for the storage of unlabelled containers in order to prevent confusion and to ensure satisfactory storage conditions. Special attention should be paid to the storage of heat labile or photosensitive products. Storage temperatures should be specified.
64. For each stage of production, the yield of product should be reconciled with that expected from that process. Any significant discrepancies should be investigated.

Quality control

65. In-process controls play a specially important role in ensuring the consistency of the quality of biological medicinal products. Those controls which are crucial for the quality (e.g. virus removal) but which cannot be carried out on the finished product, should be performed at an appropriate stage of production.
66. It may be necessary to retain samples of intermediate products in sufficient amount and under appropriate storage conditions to allow repetition or confirmation of a batch control.
67. There may be a requirement for the continuous monitoring of data during a production process, for example monitoring of physical parameters during fermentation.
68. Continuous culture of biological products is a common practice and special consideration needs to be given to the quality control requirements arising from this type of production method.