



MANUAL OF ASEAN STANDARDS FOR GOOD MANUFACTURING PRACTICES (GMP) FOR ANIMAL VACCINES

I. INTRODUCTION

In manufacturing veterinary vaccine, overall control is essential to ensure that consumers receive vaccine of high quality. Hazard operations in the manufacture of substances that may be necessary to save lives or to restore or preserve health cannot be permitted.

The quality of veterinary vaccines depends on the starting materials, manufacturing process, building/ facilities, equipment and also personnel involved. All veterinary vaccines should be manufactured under carefully controlled and monitored conditions, and sole reliance should be placed on any test for assurance of the quality of the finished product.

Veterinary vaccines may be prepared as live or inactivated (killed) products. Both live and inactivated vaccines may be formulated with adjuvants to enhance their efficacy. When vaccines are used, successful performance requires that they be produced in a manner that ensures a uniform and consistent product of high quality.

The implementation of GMP Guidelines will ensure the quality of products in the market that will protect the public against risk of buying and using substandard products. It will also encourage all parties involved in the manufacture of animal vaccines to enhance and apply quality standards.

To reliably achieve the quality objectives there must be comprehensively designed and correctly implemented systems of Quality Assurance incorporating GMP, Quality Control and Quality Risk Management. These systems should be fully documented and their effectiveness monitored.

II. DEFINITION

The following definitions are adopted:

1. **Animal Facilities**

Quarters for animals used in production and control of biological (vaccine) products (used for quality control and safety testing) should be separated from production and control areas and be suitably designed.

2. **Audit (Internal and External)**

Auditing is a systematic process of objectively obtaining and evaluating evidence regarding personnel, premises, equipment, documentation, production, quality control, distribution of the medicinal products, arrangements for dealing with complaints and recalls, inspection of facilities, functions, or records.

3. **Authorized person**

The person recognized by the national regulatory authority as having the responsibility for ensuring that each batch of finished product has been

manufactured, tested and approved for release in compliance with the laws and regulations.

4. **Batch (Lot)**

A defined quantity of starting material, packaging material or product processed in one process or series of processes so that it could be expected to be homogeneous.

Note: To complete certain stages of manufacture, it may be necessary to divide a batch into a number of sub-batches, which are later brought together to form a final homogeneous batch. In the case of continuous manufacture, the batch must correspond to a defined fraction of the production, characterised by its intended homogeneity.

For control of the finished product, a batch of a proprietary medicinal product comprises all the units of a pharmaceutical form which are made from the same initial mass of material and have undergone a single series of manufacturing operations or a single sterilisation operation or, in the case of a continuous production process, all the units manufactured in a given period of time.

5. **Batch Number (Lot Number)**

A distinctive combination of numbers and/or letters that specifically identifies a batch on the labels, its batch records and corresponding certificates of analysis, etc.

6. **Batch records**

All documents associated with the manufacture of a batch of bulk product or finished product. They provide a history of each batch of product and of all circumstances pertinent to the quality of the final product.

7. **Veterinary Biological Products**

A medicinal product such as a vaccine, blood or blood component, allergenic, somatic cell, gene therapy, tissue, recombinant therapeutic protein, or living cells that are used as therapeutics to treat diseases.

8. **Bulk Product**

Any product that has completed all processing stages up to, but not including, final packaging

9. **Calibration**

The set of operations that establish, under specified conditions, the relationship between values indicated by an instrument or system for measuring, recording, and controlling, or the values represented by a material measure, and the corresponding known values of a reference standard. Limits for acceptance of the results of measuring should be established.

10. **Contamination**

The undesired introduction of impurities of a chemical or microbiological nature, or of foreign matter, into or on to a starting material or intermediate during production, sampling, packaging or repackaging, storage or transport.

11. **Cross-contamination**

Contamination of a starting material, intermediate product or finished product with another starting material or product during production.

12. **Documentation**

All written data on suppliers, producers, instructions and records involved in the manufacture of veterinary vaccines.

13. **Final Product (Finished Product)**
A medicinal product that has undergone all stages of production, including packaging in its final container and labelling.
14. **In Process Control**
Checks performed during production in order to monitor and, if necessary, to adjust the process to ensure that the product conforms to specification. The control of the environment or equipment may also be regarded as a part of in-process control.
15. **Intermediate product**
Partly processed product must undergo further manufacturing steps before it becomes a bulk product.
16. **Manufacture**
All operations of purchase of materials and products, production, quality control, release, storage and distribution of vaccines, and the related controls.
17. **Manufacturer**
A company that carries out operations such as production, packaging, re-packaging, labeling and re-labelling of vaccines
18. **Packaging**
All operations, including filling and labeling, that a bulk product has to undergo in order to become a finished product.
19. **Packaging material**
Any material, including printed material, employed in the packaging of a vaccine, but excluding any outer packaging used for transportation or shipment.
20. **Production**
All operations involved in the preparation of a vaccine product, from receipt of materials, through processing, packaging and repackaging, labeling and relabeling, to completion of the finished product.
21. **Quarantine**
The status of starting or packaging materials, intermediate, bulk or finished products isolated physically or by other effective means whilst awaiting a decision on their release, refusal or re-processing.
22. **Quality Assurance**
The sum total of the organized arrangements made with the objective of ensuring that products will be of the quality which meets the required standard of specification.
23. **Quality Controls**
Checks and tests instituted by the manufacturer which are carried out in the course of the manufacture of veterinary vaccines on raw materials, during the process and on final product.
All measure to be taken during manufacturing designed to ensure that uniform outputs of veterinary vaccines conforming to established specifications of identity, strength, purity and other characteristics such as potency, safety, and toxicity.
24. **Quality Risk Management**
Is a systematic process for the assessment, control and review of risks to the quality of veterinary vaccine or products.
25. **Raw Materials**

All substances that are employed in the production of veterinary vaccines.

26. Rejected

The status of product which is not permitted to be used for processing, packaging or distribution by the quality control department.

27. Released

The status of product which is permitted for distribution by the authorized person bearing the appropriate responsibilities.

28. Representative Sample

A representative sample consisting of a number of units that are drawn based on rational criteria such as random sampling and intended to assure that the sample accurately portrays the batch or total amount of materials being sampled.

29. Retained Sample

Sufficient representative samples of each batch of final products which are retained and stored in accordance with the guide. The container used for storage should be composed of the same material as the market primary container in which the product is marketed

30. Sanitation

Hygiene control on manufacturing and material handling (from starting materials to finished product).

31. Seed Management (Master and Working Seed)

Seed lot system: A seed lot system is a system according to which successive batches of a product are derived from the same master seed lot at a given passage level. For routine production, a working seed lot is prepared from the master seed lot. The final product is derived from the working seed lot and has not undergone more passages from the master seed lot than the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy. The origin and the passage history of the master seed lot and the working seed lot are recorded.

Master seed lot: A culture of a micro-organism distributed from a single bulk into containers in a single operation in such a manner as to ensure uniformity, to prevent contamination and to ensure stability. A master seed lot in liquid form is usually stored at or below -70°C. A freeze dried master seed lot is stored at a temperature known to ensure stability

Working seed lot: A culture of a micro-organism derived from the master seed lot and intended for use in production. Working seed lots are distributed into containers and stored as described above for master seed lots.

32. Standard operating procedure (SOP)

An authorized written procedure giving instructions for performing operations not necessarily specific to a given product or material.

33. Starting Material

Any substance used in the production of a medicinal product, but excluding packaging materials.

34. Toll Manufacturing (Contract manufacturer)

Production of veterinary vaccines or medical products by a manufacturer, under the label or brand by others company.

35. Vaccine (refer to OIE definition)

Include all products designed to stimulate active immunisation of animals against disease, without regard to the type of microorganism or microbial toxin from which they may be derived or that they contain.

36. Vaccine Manufacturing

In relation to any agricultural compound, 'manufacture' includes all the following aspects: acquiring materials, making up, preparing, producing or processing, and assessing the agricultural compound for release; it also includes the packing of an agricultural compound in a container for the purposes of sale.

37. Vaccine Production

All operations involved in the preparation of a medicinal product, from receipt of materials through processing and packing, to its completion as a finished product.

38. Vaccine Vigilance (Post Licensing Monitoring)

A reliable system to identify, at the earliest stage, any serious problems encountered from the use of veterinary vaccines and should be ongoing and an integral part of all regulatory programs for veterinary vaccines, especially live vaccines.

39. Validation

Action of proving, in accordance with the principles of Good Manufacturing Practice, that any procedure, process, equipment, material, activity or system actually leads to the expected results

III. ORGANIZATION STRUCTURE

1. Manufacturers of veterinary vaccine products must have in place a quality assurance system to ensure that finished products are fit for their intended use, comply with registration requirements and do not place treated animals or users at risk due to inadequate quality, safety or efficacy.

The basic concepts of Quality Assurance, Good Manufacturing Practices, Quality Control and Quality Risk Management are inter-related aspects of Quality Management.

The basic elements of the quality management are:

- a. An appropriate infrastructure or quality system encompassing the organizational structure, procedures, processes and resources; and
- b. Systematic actions necessary to ensure adequate confidence that products will satisfy given requirements for quality. The totality of these actions is termed Quality Assurance.

The system of quality assurance must ensure that:

- a. Vaccines are designed and developed in a way that takes account of requirements of GMP;
- b. Production and control operations are clearly specified and GMP adopted. Appropriate procedures are in place to ensure that relevant quality standards are met;
- c. Managerial responsibilities are clearly specified in job description;
- d. All materials involved in the manufacturing process comply with required quality standards before they are released for use in manufacture;

- e. Vaccines are not sold or supplied before the Quality Assurance Manager has certified that each production batch has been produced and are controlled in accordance with the requirements of the marketing authorization and any other regulations relevant to the production, control, release of vaccine final products;
- f. There is a procedure for audit which regularly appraises the effectiveness and applicability of the quality assurance systems.

The quality risk management should ensure that:

- a. The evaluation of the risk to quality is based on scientific knowledge, experience with the process and ultimately links to the protection of the animals.
 - b. The level of effort, formality and documentation of quality risk management process is commensurate with the level of risk.
2. The manufacturer should have an adequate number of personnel with the necessary qualifications and practical experience to perform the necessary operations and achieve the intended quality of product.
 3. The manufacturer must have an organizational chart. People in responsible positions should have specific duties recorded in written job descriptions and adequate authority to carry out their responsibilities.
 4. The organization structure of the company shall be such that manufacturing, quality control and quality assurance shall be headed by different person, neither of whom shall report directly or indirectly to the other.
 5. Each person responsible for supervising the manufacture of a product shall have the education, training and experience or any combination thereof, to perform assigned functions in such a manner as provide assurance that the vaccine for animal use has the intended quality.

IV. PERSONNEL

All personnel employed in the premises should be capacitated and receive regular training in areas relevant to the successful manufacture of veterinary drugs or biological products including the basics of biosecurity. Initial training should be followed by suitable refresher courses in GMP.

1. Key Personnel:

a. Manufacturing Manager

The manufacturing manager should be qualified in vaccine manufacturing He/She should have full authority and responsibility on the manufacturing and storage area.

The manufacturing manager generally has responsibilities:

- To ensure that vaccine are produced and stored according to the appropriate documentation in order to obtain the required quality;
- To approve the instructions relating to productions, including the in-process control, and to ensure their strict implementation.

- To ensure that the production records are evaluated and signed by a designated person;
- To check the maintenance of department, premises and equipment;
- To ensure that the appropriate process validations and calibrations of control equipment are performed and recorded and the reports made available.
- To ensure that the required initial and continuing training of his/her department personnel is carried out and adapted according to need.

b. Quality Assurance Manager

The Quality Assurance Manager should have full authority and responsibility to manage in all quality system/ assurance duties, including:

- To ensure implementation (and, when needed, establishment) of the quality system;
- To participate in or initiate the development of the company's quality manual;
- To perform the oversight of the Quality Control;
- To initiate and participate in external audit (vendor audit);
- To initiate and participate in validation programmes to evaluate/ review batch records;
- To approve or reject, as she/he sees fit, finished products for sale.

c. Quality Control Manager

The Quality Control Manager should be given full authority and responsibility in all quality control duties. He/She should be qualified in vaccine quality control.

Quality Control Manager has the following responsibilities:

- To release or reject starting materials, packaging materials, and intermediate, bulk and finished products in relation to their specifications;
- To prepare detailed instructions for each test and analysis;
- To evaluate the adequacy of the conditions under which raw materials, intermediate products and finished biological preparations are stored;
- To ensure that all necessary testing is carried out;
- To ensure that the required initial and continuing training of quality control personnel is carried out and adapted according to need;
- To prepare and evaluate sampling instructions, specifications, test methods and other quality control procedure;
- To ensure that the appropriate validations are done;
- To check the maintenance of his/her department premises and equipment.

The Manufacturing/ Production, Quality Assurance and Quality Control Managers generally have some shared or jointly exercised responsibilities relating to quality:

- The monitoring and control of the manufacturing environment;
- Training;
- Process validation;
- Plant hygiene;
- The approval and monitoring of contract manufacturers;

- The designation and monitoring storage conditions for materials and products;
- The monitoring of compliance with the requirement of GMP of animal vaccine;
- The inspection, investigation, and taking samples in order to monitor factors which may affect product quality;
- The retention of records;
- The authorization of written procedures and other documents, including amendments.

2. **Others Important Personnel**

a. Supervisor

Each supervisor should possess adequate technical training and practical experience related to his/her assignment. A supervisor assists the Manufacturing, Quality Control or Quality Assurance Managers in executing direct supervisory duties and responsible to respective section. The supervisor should receive training and suitable refresher courses in GMP periodically.

b. Biosafety Officer

The biosafety officer generally has the following responsibilities:

- To ensure the implementation of the biosafety and biosecurity policies and guidelines of the company.
- To coordinate and have responsibilities for all facilities and activities in BSL 2 and BSL 3 Laboratory.
- Prepare the BSL 2 and BSL 3 Manuals
- Reviewing others Documents such as Biorisk Management SOP, Risk Assessment Protocol, Working Instruction for equipments and Testing Method. All these documents must be reviewing annually.
- To ensure accidents/incidents spillages etc. in the laboratory (or other containment facility) are appropriately investigated and followed up.
- To advise on the safe storage, transport and disposal of genetically modified organisms/ harmful or potentially harmful material and ensuring that the records kept are current and accurate.
- Managing the regularly meeting for all BSL-3 staff so all staff can be familiar with the most updated information in relation to the laboratory engineering control.
- Performing periodic site inspection on regular basis in all BSL 3 facilities to verify biorisk management requirements are met and ensuring the laboratory operates in a good standard in terms of engineering and administrative control works properly.

c. Other Technical Personnel

- All personnel should have an updated job description- Other technical personnel involved in the production process such as engineers, technicians, workers, , should understand his tasks and duties
- The staff engaged in the manufacturing process should be separate from the staff responsible for animal care.

All technical staff should be adequately trained on:

- i. Vaccine production.
- ii. Good manufacturing practice principles

- iii. Good laboratory practise
- iv. Basics of biorisk

NOTE:

- All personnel engaged in production, maintenance, testing and animal care should be vaccinated with appropriate vaccine, eg. rabies.
- Training record should be maintained and periodic assessments of the effectiveness or training programmes should be made.
- Visitors or untrained personnel should preferably not to be taken into the production and quality control areas. If this unavoidable, they should be given information in advance, particularly about personal hygiene and prescribed protective clothing. They must be closely supervised.
- Personnel should report any condition such as diarrhea, cough, colds, infected skin or hair, wounds, fever or unknown origin that may cause the shedding of abnormal number or types of organism into the working environment.
- 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 productions.

V. PREMISES

The design and construction of buildings for veterinary vaccine manufacturing/ Production Facilities, quality controls, including animal facilities must incorporate features which prevent hazards that might adversely affect the quality of the product. These design features should provide suitable environmental conditions, promote good sanitary practices, permit adequate cleaning and sanitation, prevent access to dust, insects and other animals, and allow employees to perform their assigned duties

A. Production Facilities

1. The quality and design of production facilities should meet appropriate standards for vaccine production.
2. Facilities used for the production of vaccines should be designed to protect the purity of the product throughout the production process and to safeguard the health of the personnel.
3. Facilities must be including dressing rooms and other facilities for personnel.
4. They facility should be easy to clean, adequate separation of preparation rooms, adequate ventilation, have ample clean hot and cold water, efficient drainage and plumbing equate to provide for all applicable production functions for storage of master seeds, ingredients, and other production materials; preparation of growth media and cell cultures; preparation of glassware and production equipment; inoculation, incubation, and harvest of cultures; storage of in-process materials;

inactivation, centrifugation, addition of adjuvant, and formulation of product; filling, desiccation, sealing of containers, labelling and storage of final product; quality control testing of in-process materials and final product; and research and development.

5. All rooms and air-handling systems must be constructed so as to prevent cross-contamination from other products and to prevent contamination by people or equipment.
6. Virulent or dangerous microorganisms must be prepared and stored in rooms separate from the remainder of the establishment. In particular, challenge organisms must be completely separated from vaccine strains.
7. All equipment that comes into contact with product must be sterilised using validated procedures.
8. Production facilities should be designed to prevent contamination of the external environment. Any material used during production has to be made safe before leaving the facility. If highly contagious microorganisms are propagated, the exhaust air must be treated to prevent escape of infectious agents.
9. Personnel must follow safety procedures such as showering, and avoid contact with susceptible animals after leaving the production facilities.
10. The plant should be so arranged as to eliminate disorder, crowding, and the potential of cross contamination and mix ups among different veterinary drugs, components, packaging and labeling materials.
11. Separate areas are generally required for different activities. The defined areas of operations which require such separations are as follows :
 - a. Room for seed lots and cell banks used for the production of biological product should be stored separated from other material and access should be restricted to authorized personnel
 - b. spore-forming organism shall be handled in facilities dedicated to this group of products until the inactivation process is accomplished.
 - c. Ancillary area
 - d. Storage area
 - e. Weighing area
 - f. Production area
 - g. Quality control area
12. Room required in the manufacturing of sterile products.

Clean areas for the manufacturing of sterile products are classified according to the required characteristics of the environment. Each manufacturing operation requires an appropriate environmental cleanliness level in the operational state in order to minimize the risks of particulate and/or microbial contamination of the product and/or materials being handled.

Refer to **Appendix 1** for the classification of clean area.

Refer to **Appendix 2** for the appropriate classification clean room for different activities in vaccine manufacture.
13. In the exiting premises, effective measures should be taken to avoid such contaminations.
14. Premises should be designed and laid out in such a way that the risk of mix up or contamination of one product or material by another is

minimized. This especially applies to premises for the handling of highly virulent materials.

15. Premises should provide sufficient for suitable operations to be carried out, to allow efficient work flow, and permit effective communication and supervision.
16. Locker rooms should be separated from the experimental animal house, but directly next to the manufacturing area.
17. Floor in the processing areas should be made of impervious materials, laid to an even surface. They should be free from cracks and open joints and should allow prompt and efficient removal of any spillages and disinfection.
18. Buildings should be effectively lit and ventilated, with air control facilities (temperature, humidity, and filtration)
19. Air intakes and exhausts, and associated pipe work and trunking, should be situated so as to avoid product contamination hazards.
20. Waste material should not be allowed to be accumulated. It should be collected in suitable receptacles for removal to collection points outside the buildings and disposed in special containers.
21. Manufacturing areas should not be used as passage ways for personnel or transport of materials, or for storage (except for materials in process).
22. Positive-pressure areas should be used to process sterile products, but negative pressure is acceptable in specific areas where pathogens are processed. In general, any organisms considered to be pathogenic should be handled with specifically designed areas under negative pressures, in accordance with containment requirements for the product concerned.
23. Specific decontamination systems should be considered for effluent when infectious and potentially infectious materials are used for production.
24. All buildings and rooms shall be clean and sanitary at all times. If rooms intended for the manufacture of biological are used for other purposes, they shall be cleaned thoroughly and, if necessary, sanitized before the manufacture of biological substance is resumed.

B. Quality Control Facilities

1. A manufacture's quality-control laboratory shall be separated from the production area and ideally should be in separate building.
2. The control laboratory should be designed and equipped and of such a size as to be self-contained entity, with adequate provision for the storage document and samples, preparation of record and performance of the necessary test.
3. Sufficient space should be given to avoid mix-ups and cross-contamination
4. The design of the laboratory should take into account the suitability of construction materials, prevention of fumes and ventilation. There should be separate air supply to laboratories and production area.

C. Animal Facilities

1. Animals shall be accommodated in separate buildings with self-contained ventilation systems.

2. Facilities for animal care shall include isolation units for quarantine of incoming animals and provision for vermin-free food storage.
3. Animal inoculation rooms shall be separated from postmortem rooms.
4. Animal facilities shall be facilities for disinfection of cages, and with access to an incinerator for disposing of waste and of dead animals.
5. Animal testing facilities should be sufficiently secure to ensure that unauthorized entry is prevented and that animals cannot break in or escape. This applies particularly to facilities where live challenge tests are being carried out.
6. Animal houses accommodating animals used for, or intended to be used for, production purposes should be provided with appropriate containment and/or clean area measures and should be separated from other animal accommodation.
7. Animal houses accommodating animals that are used for quality control test which involve pathogenic biological agents should be adequately contained to prevent escape of the pathogenic agents to the environment.
8. The sanitary status of the animals used for production should be defined, monitored, and recorded. Animals should be handled in ways defined in specific monographs (e.g. specific pathogen free [SPF] flocks), where these are available and relevant.
9. Animals, biological agents and tests carried out should be identified in such a way as to prevent any risk of confusion and to control all foreseeable hazards.
10. Procedures should be in place to ensure that animals cannot be incorrectly identified or otherwise mixed up by staff. Attention should be also be given to decontamination and environmental requirements, where applicable.
11. Staff employed in animal facilities must be provided with special clothing, changing facilities and showers

D. Ancillary Area

1. Rest and refreshment rooms should be separated from production and quality control laboratory areas.
2. Facilities for changing clothes and for washing and toilet purposes should easily accessible and appropriate for the number of users. Toilets should not directly communicate with production or storage area. Changing rooms should be directly connected to but separated from production areas.
3. Maintenance workshop should as far as possible be separated from production areas. Whenever parts and tools are stored in the production area, they should be kept in rooms or lockers reserved for that use.

VI. MACHINERY (IDENTIFIED, MAINTAINED, QUALIFIED)

1. Machinery used in manufacturing, handling or storage shall be of appropriate design and adequate size to facilitate the operations for which it is intended and for its cleaning and maintenance.
2. Equipment shall be constructed in such a way that surface coming into contact with any raw, intermediate or bulk product should not alter their identity or purify beyond the established limit.
3. Automatic, mechanical, or electrical equipment that will perform a function satisfactorily may be used in the manufacturing. If such equipment is so used, it shall be routinely calibrated, disinfected or checked according to a written QC protocol and records calibrations and inspections shall be maintained.
4. Utensils shall be cleaned and maintained regularly to prevent malfunctions or contaminations which could alter the identity, quality or purity of a product. Written procedures shall be established and followed for cleaning and maintenance of utensils used in the manufacturing processing or storage of the product.
5. All containers of biological substances, regardless of the stage of manufacture, shall be identified by securely attached labels.
6. Whenever possible, equipment used for processing sterile products should be chosen so that it can be effectively sterilized by steam or dry heat or others method.
7. 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 a sepsis have not been maintained during the work.
8. All equipment such as sterilizers, air handling and filtration systems, air vent and gas filters, water treatment system, generation, storage and distribution systems should be subject to validation and planned maintenance; their return to use following maintenance should be approved and recorded.

VII. PRODUCTION

1. The primary elements for effective processing of veterinary vaccine products are:
 - a. The existence of written general operation procedures.
 - b. The existence of explicit manufacturing introductions for each product manufactured.
 - c. The strict adherence to the above procedures.
 - d. Precise and timely documentation of critical data to substantiate adherence to standard procedures or if necessary, with recording of any deviation and its justification
2. All procedures must be properly validated when any new master processing procedure is adopted. Steps should be taken to demonstrate that the adapted procedure is suitable for routine production and that the defined process using the materials and equipment specified will consistently field a product of the required quality.
3. Significant changes in processing method, equipment or material should be accompanied by further validation steps to ensure that the changed conditions continued to yield a product of consistent quality.

4. Functionally documentation for manufacturing is generally subdivided into:
 - a. Master processing procedure for each product
 - b. Processing order (manufacturing order)
 - c. Batch record.
 - d. Back-up documentation.
5. Starting Materials
Specifications for starting materials should include details of their source, origin and method of manufacture and of the control applied to ensure their suitability for use.
6. Seed Lot and cell bank systems
 - a. To prevent the unwanted drift of properties, the vaccines obtained by microbial culture, cell culture or propagation in embryos and animal should be based on a system of master and working seed lots and/or cell banks.
 - b. The number of passages between seed lot or cell bank and the finished product should be consistent with the marketing authorization dossier.
 - c. Seed lots and cell banks should be adequately characterized and tested for contaminants. Seed lots and cell banks should be established, stored and used in such a way to minimize the risks of contamination or alteration
 - d. Establishment of the seed lot should be performed in a suitably controlled environment to protect the seed lot. During the establishment, 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.
 - e. Evidence of the stability and recovery of the seeds should be documented.
 - f. Storage containers of seed lot should be hermetically sealed, clearly labeled and kept at an appropriate temperature.
 - g. Only authorized personnel should be allowed to handle the material and this handling should be done under the supervision of a responsible person.
 - h. All containers of master or working seed lots should be treated identically during storage.
7. Release of a finished product is conditional on satisfactory results being obtained in the tests on starting materials.
8. When an inactivation process is performed during manufacture, measures should be taken to avoid the risk of cross-contaminations between treated and untreated product.

VIII. PROCESSING

1. All personnel must be qualified and trained for the functions they are performing.
2. All materials used in processing must be verified before use.
3. The environment of an area must be monitored and controlled to the degree required for the operation to be performed.
4. The condition of the equipment to be used must meet specified requirements.
5. Equipment must be certified in writing as clean before use.
6. All operations performed are in accordance with the written general and specified procedures.
7. The growth promoting properties of culture media should be demonstrated.
8. 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.
9. All intermediate and bulk products must be properly labeled and quarantined until released by quality control.
10. All required process control documentation must be accurately recorded.
11. In all stages of processing particular attention should be paid to the problem of cross-contamination, since even if it is of a nature and at a level unlikely to affect health directly, it may be indicative of satisfactory manufacturing practices.
12. Processing records of regular production lots must provide a complete account of the manufacturing history of each lot of a biological preparation, showing that it has been manufactured, tested, dispensed into containers and distributed in accordance with the licensed procedures.

IX. QUALITY CONTROL

1. Each batch of raw materials, packaging and labeling material intermediate, bulk and finished products should be quarantined until the batch has been sampled, tested or examined as appropriate before released for use. Any batch of those materials which complies with the appropriate written specification may be approved and released in writing for use.
2. Any batch of such materials which does not comply with the specification shall be rejected. Periodic revisions of specifications are necessary based on the latest edition of standard (reference), any other official compendia, and through comparison studies on relevant literature.
3. The consistent production of pure, safe, potent, and efficacious vaccines requires quality assurance procedures to ensure the uniformity and consistency of the production process.
4. Vaccine purity, safety, potency, and efficacy must be ensured by consistency in the production process. Consistent product quality (batch-to-batch uniformity) must be built in at each stage.
5. Final product testing is used as a check to verify that the controls on the production procedures have remained intact and that the released product meets the specification previously agreed with the licensing authority.

6. An important part of documentation deals with Quality Control and the following details should be readily available to the Quality Control department:

a. Specification

Each specification should be maintained and/or approved by the quality control unit. Periodic revisions of the specifications are necessary to comply with the latest edition of the reference/ pharmacopoeia/ official compendia.

b. Sampling

- Samples should be representative of the batches on material from which they are taken and should be taken in accordance with a written sampling plan.
- Sampling should be carried out so as to avoid contamination in order adverse effects on quality.
- Sampling instructions include:
 - The method of sampling
 - The equipment to be used
 - The amount of sample to be taken
 - Instruction for any required subdivision of the sample
 - The type of sample container to be used
 - for aseptic sampling
 - for routine sampling
 - Any special precautions to be observed
 - Each sample container should have a label indicating:
 - Name of material sampled
 - The batch number reference
 - The number of the container from which sample has been taken
 - The signature of the person who has taken the sample
 - The date of sampling
 - The storage conditions
 - Instruction for cleaning and storage of sampling equipment

c. Testing

Samples to be collected or examined and tested are as follows:

- Raw materials samples
- Packaging and labeling materials samples
- Intermediate and bulk production sample
- Finished product samples

d. Analytical reports and/or certificates

e. Data from environmental monitoring, where required

f. Validation records of test methods, where applicable

g. Procedure for and records of the calibration of instruments and maintenance of equipment

7. Retained Sample

An appropriate identified reserve sample representative of each lot in each shipment shall be retained for a period specified by the ASEAN Member State.

8. In Process Control

To ensure batch uniformity and integrity of veterinary vaccine product, written procedure shall be established and followed, describing in process controls,

and tests for examination to be conducted on appropriate samples of in-process materials of each batch.

9. Packaging and labeling control

All packaging operations should proceed in accordance with the instructions given using the materials specified in the packaging procedures.

The label on the container shall show:

- The name of vaccine product
- A list of the active ingredients and the amount of each present, with the statement of the net contents, eg. number of dosage units, weight or volume
- The batch/ lot number
- The expiry date
- Recommended storage condition or handling precautions
- Direction for use
- Warning or precaution that may necessary
- The name and address of the manufacturer

10. Stability Study

- a. The stability of finished product should evaluate, and when necessary, Quality Control also evaluates the stability of starting materials and intermediate products.
- b. Expiry date and shelf-life specification should be established on the basis of stability tests related to storage conditions.
- c. The on-going stability programme should be described in a written protocol and results formalized as a report. The programme should be include elements such as:
 - a complete description of the drug involved in the study;
 - the complete set of testing parameters and methods, describing all tests for potency, purity, and physical characteristics and documented evidence that these tests indicate stability;
 - provision for the inclusion of a sufficient number of batches;
 - the testing schedule for each drug;
 - provision for special storage conditions;
 - provision for adequate sample retention;
 - a summary of all data generated, including the evaluation and the conclusions of the study
- d. Stability should be determined prior to marketing and following any significant changes in processes, equipment, packaging material, etc.

X. HANDLING OF PRODUCT COMPLAINTS AND PRODUCT RECALL

1. Any complaints and other information concerning potentially defective products must be carefully reviewed according to written procedures.
2. In order to provide for all contingencies system should be designed to recall, if necessary, promptly and effectively products known or suspected to be defective from the market.
3. Vaccine vigilance (feedback from industry, alertness)
 - a. There should be documented procedures for the handling of all complaints, recalls and returned product.

- b. There should be in place a documented procedure for receiving, recording, reviewing and, where appropriate, acting upon all quality-related complaints received about veterinary chemical products manufactured or handled on the premises.
 - c. The procedure should include the need to consider a recall in the event of a complaint concerning a possible product defect.
 - d. A follow up action should be taken after investigation and evaluating of the product complaint and report. The action may include:
 - corrective action where applicable;
 - recall of the batch or all the finished products; and
 - other appropriate action.
4. Suspected product defects
- a. If a product defect is discovered or suspected in a batch, other batches should be checked in order to determine whether they also might be affected.
 - b. Attention should be paid to other batches that may contain reworks of the defective batch.
5. Recalls
- a. It should be notified if a manufacturer is considering recall action following possibly faulty manufacture, product deterioration, or any other serious quality problem with a product.
 - b. The competent authorities of all countries to which defective products may have been distributed should also be notified.
 - c. There should be a written procedure for the initiation and management of any recall activity.
 - d. Procedures should be regularly checked and updated when necessary.
 - e. Institution of recall:
 - A product recall should be instituted immediately after discovery of adverse reaction of the product;
 - Vaccine with high healthy risk should be prevented from further usage by having them under embargo as well as recalling the products immediately. The recall point should reach the consumer level;
 - The manufacturer documentation system for product recall should ensure that recall and embargo have been adequate quickly, effectively, and completely carried out.
 - Procedure and guideline to recall a product should be established to enable the recall and embargo be quickly and effectively
 - Recalled products should be identified and stored separately in a secure area while awaiting a decision on their fate.
 - Recalled products cannot be re-sold.
 - f. The effectiveness of the arrangements for recalls should be evaluated from time to time.
6. Records
- a. The handling product complaints and report including result of the evaluation of investigation and the follow actions taken should be recorded and reported to the relevant management or department.
 - b. Complaints record should be reviewed regularly for any indication of specific or recurring problem requiring attention and possibly the recall of marketed products

- c. Any complaint concerning a product defect should be recorded with all the original details and thoroughly investigated.
- d. The record and report of product recall including the result of product recall and embargo action should be properly documented.
- e. The distribution records should be readily available to the personnel(s) responsible for recalls, and should contain sufficient information on wholesalers and directly supplied customers (with addresses, phone and/or fax numbers inside and outside working hours, batches and amounts delivered), including for exported vaccine or for samples.
- f. The progress of the recall process should be recorded and a final issued, reconciliation between the delivered and recovered quantities of the products.

XI. DOCUMENTATION

1. A documentation system must be prepared for manufacturing activities.
2. The documentation system consists of written procedures and instructions, descriptions, specifications and records which can be batch-related or not.
3. The documentation system should be able to record the complete history of each batch of manufactured finished vaccine product. It should be able to record executed activities in production, quality control, maintenance, storage, distribution and other specific matters linked to GMP. It should be adequate to permit investigation and tracing of defective product.
4. Documentation should contain all necessary, but not superfluous data, to be kept up to date.
5. Any amendments should be formally authorized.
6. Batch related documents and records as well as reference samples of finished products and starting materials should be retained at the establishment for a time period as specified by the ASEAN member Country.
7. Essential documents in veterinary manufacturing:
 - a. GMP or quality assurance Manual
 - b. Specifications:
 - Raw material specification
 - Packaging material specification
 - Intermediate and Bulk product specifications
 - Finished product specification
 - c. Production document:
 - Master production document
 - Master processing procedures
 - Master packaging procedures
 - Batch processing records
 - Batch packaging records.
 - d. Procedures and record:
 - SOP and records for the receipt of each delivery starting material, primary and printed packaging material.
 - SOP for internal labeling quarantine and storage starting materials, packaging materials and other materials.

- SOP for sampling, which include the authorized person, the methods and equipment to be used, the amounts to be taken and any precautions to be observed to avoid contamination of the material or any deterioration in its quality.
 - SOP for testing materials and products at different stages of production (e.g. Starting materials, intermediate, bulk and finished product) and equipment to be used. The test performed, analytical reports and/or certificates should be recorded.
 - SOP and records for release and rejection for materials and products, and in particular for sale of the finished product.
 - Distribution of each batch of a product records, in order to facilitate the recall of the batch if necessary.
 - SOP and the associated records of action to be taken or conclusions reached, where appropriate, for:
 - Validation, e.g. process, procedures, analytical procedures, computerized systems;
 - Equipment assembly, qualification and calibration;
 - Maintenance, cleaning and sanitization (eg. equipment, rooms, etc);
 - Personnel matters including training, clothing, hygiene;
 - Environmental monitoring;
 - pest control;
 - Complaints; and
 - Recall
 - SOP should be available for major items of manufacturing and test equipment.
 - Log books should be kept for major or critical equipment recording, as appropriate, any validations, calibrations, maintenance, cleaning or repair operations, including the dates and identify of people who carried out these operations.
 - SOP of stability study and record of stability tests for finished products, if necessary, raw materials.
- e. Other supporting documents:
- Inventory card
 - Standard calibration procedures and record for specific instruments.
 - Self inspection record
 - Record of efficacy test

XII. AUDIT (SELF INSPECTION, QUALITY AUDIT/ EXTERNAL AUDIT, SUPPLIER'S AUDIT AND APPROVAL)

A. Self Inspection

The purpose of self inspection is to review regularly the start and adequacy of the industrial manufacturer's compliance to GMP set forth. Self inspection programs are designed to seek out any defects in the quality assurance system and establishing corrective action. These methods of self inspection shall apply to all manufacturing facilities.

1. Evaluation

The self inspection shall be carried out using a self inspection check list which is classified as follows:

- a. Conform/Yes: for condition which conform to GMP standard.
- b. Does not conform/No: for condition which does not conform.
- c. Comments are required if the evaluation does not conform to GMP standard.
- d. Product evaluation is to be carried out if necessary.

2. Team of Self Inspection

The team consists of at least 3 persons who are experts in their fields and have already studied GMP rules and have been appointed by the management. They may or may not be from the company. The team members should be from different areas of experience and it is advisable they are from:

- a. Quality Assurance / Quality Control (QA/QC)
- b. Production and engineering
- c. Production, Planning and inventory control
- d. General affairs, etc.
- e. Such experts should be independent in their inspection.

3. Item of Inspection

In order to provide certain minimum and uniform standard of self inspection, the following check lists are furnished for all those conducting inspection in the veterinary drug manufacturing facilities:

- a. Personnel
- b. Premises including personnel facilities
- c. Raw/ starting material and packaging storage
- d. Weighing, dispensing room
- e. Production: housekeeping, equipment, safety, processing, control production area, water system, filling/labeling/ packaging area.
- f. Finished goods warehouse: housekeeping, quarantine environmental control.
- g. Quality control facilities: housekeeping, space for all activities environment, instruments.
- h. Equipment
- i. In process control
- j. Documentation
- k. Sanitation and hygiene
- l. Validation and re-validations programmes
- m. Calibration of instrument
- n. Recall procedures
- o. Complaint management
- p. Label control
- q. Result of previous self-inspections and any corrective steps taken

4. Frequency of self-inspection

- a. Self inspection can be conducted partially (one product line, facility, standard operating procedure, etc) in accordance with the manufacturing needs.
- b. The frequency may depend on company requirements but should preferably at least once a year. The frequency should be stated in the procedure

5. Self-inspection report should include:
 - a. Self-inspection results;
 - b. Evaluation and conclusions;
 - c. Recommended corrective actions.

B. Quality Audit/ External Audit

1. It may be useful to supplement self inspections with quality audit. A quality audit consists of an examination and assessment of all or part of a quality management system with specific purpose of improving it.
2. A quality audit is usually conducted by external or independent specialist or a team designated by the management for this purpose.
3. Such audits may also be extended to suppliers and contactors (see contract manufacture).

C. Supplier's Audit and Approval

1. The person responsible for Quality Management (Quality Assurance) should have responsible together with other relevant departments for approving suppliers who can reliably supply starting and packaging materials that meet established specifications.
2. A list of approved suppliers of starting and packaging materials should be established and reviewed.
3. Before suppliers are approved and included in the approved suppliers list or specifications, they should be evaluated. The evaluation should take into account a supplier's history and the nature of the materials to be supplied.
4. All established suppliers should be evaluated regularly.

XIII. CONTRACT MANUFACTURE

1. Contract manufacture must be correctly defined, agreed and controlled in order to avoid misunderstandings which could result in product or work of unsatisfactory quality.
2. There must be a written and signed contract between Contract Giver and The Contract Acceptor which clearly establish the duties of each party.
3. The Contract Giver
 - a. Responsible for assessing the competence of the Contract Acceptor. Audits of contract acceptor/ manufacturer by the contract giver should be carried out to ensure that work is carried out to requirement in accordance with GMP. The depth and frequency of inspections should be determined on a risk management basis.
 - b. Provide the Contract Acceptor with all the information necessary to carry out the contracted operations correctly in accordance with the marketing authorization and any other legal requirements.
 - c. Ensure that the Contract Acceptor is fully aware of any problem associated with the product or the work or tests which might pose a hazard to his premises, equipment, personnel, other materials or other products.

- d. Ensure that all processed products and materials delivered by the Contract Acceptor comply with their specifications or that the products have been released by the authorized person.
4. The Contract Acceptor
- a. Must have adequate premises and equipment, knowledge and experience, and competent personnel to carry out satisfactory the work ordered by the Contract Giver. Contract manufacture may be undertaken only by manufacture holding GMP certificate.
 - b. Ensure that all products or materials delivered to him are suitable for their intended purpose.
 - c. The contract acceptor should not pass to a third party of the work entrusted to him under the contract without the Contract Giver's prior evaluation and approval.
 - d. Arrangements made between the contract acceptor and any third party should ensure that the manufacturing and analytical information is made available in the same way as between the original Contract Giver and Contract Acceptor.
 - e. The contract acceptor should refrain from any activities which may adversely affect the quality of the products manufactured for the Contract Giver.
5. The Contract
- a. A contract should be drawn up between the Contract Giver and the Contract Acceptor which specifies their respective responsibilities relating to the manufacture and control of the product. It should also defined the GMP responsibilities of each party for each aspects of the work. All GMP agreement should be kept up-to-date.
 - b. Technical aspects of the contract should be drawn up by competent persons suitably knowledgeable in vaccine technology, analysis and GMP.
 - c. All arrangements for manufacture and analysis must be in accordance with the marketing authorization and agreed by both party.
 - d. The contract should permit the Contract Giver to visit the facilities of the Contract Acceptor
 - e. Arrangement for contracted steps of manufacture must not compromise the quality of the product.
 - f. The contract should specify the way in which the authorized person (e.g Head of Quality Management/ Quality Assurance) releasing the batch for sale ensures that each batch has been manufactured and checked for compliance with the requirements of marketing authorization.
 - g. The contract should describe clearly who responsible for purchasing materials, testing and releasing materials, undertaking production and quality controls, including in-process controls, and who has responsibility for sampling and analysis.
 - h. Manufacturing, analytical and distribution records, and reference samples should be kept by, or be available to, the Contract Giver. Any records relevant to assessing the quality of a product in the event of complaints or suspected defect must be accessible and specified in the defect/recall procedures of the Contract Giver.

- i. Where a contractor authorized to manufacture under the license of another manufacturer, the license holder must exert direct control and oversight of the quality management of the contracted steps.
- j. The contract should describe the handling of starting materials, packaging materials, intermediate and bulk products and finished products if they are rejected. It should also describe the procedure to be followed if the contract analysis shows that the tested product must be rejected.

XIV. QUALIFICATION AND VALIDATION

1. Manufacturers should identify what validation work is needed to prove control of the critical aspects of their particular operations.
2. Significant changes to the facilities, the equipment and the processes, which may affect the quality of the vaccine, should be validated.
3. A risk assessment approach should be used to determine the scope and extend of validation.
4. Planning For Validation
 - a. All validations activities should be planned
 - b. The key elements of a validation programme should be clearly defined and documented in a Validation Master Plan (VMP) or equivalent document.
 - c. The VMP should contain data on at least the following:
 - validation policy
 - organizational structure of validation activities
 - summary of facilities, systems, equipment and processes to be validated
 - change control
 - reference to exiting documents
5. Documentation
 - a. A written SOP should be established that specifies how qualification and validation will be conducted. The SOP should be reviewed and approved by Quality Assurance Manager
 - b. A report should be prepared, summarizing the result obtained, commenting on any deviations observed, and drawing the necessary conclusions, including recommending changes to correct deficiencies.
6. Qualification
 - a. Design Qualification
 - The first element of the validation of new facilities, systems or equipment could be design qualification (DQ).
 - DQ should be compliance with GMP, and should be demonstrated and documented.
 - b. Installation Qualification
 - Installation Qualification (IQ) should be performed on new or modified facilities, systems and equipment
 - c. Operational Qualification
 - Operational Qualification (OQ) is performed after IQ has been completed, reviewed and approved.

- OQ at least should included: tests that have been developed from knowledge of processes, systems, and equipment; test to include as “worst case” condition.
 - The completion of a successful OQ should allow the finalization of calibration, operating and cleaning procedures, operator training and preventive maintenance requirement. It should permit a formal “release” of the facilities, systems and equipment.
- d. Performance Qualification
- Performance qualification (PQ) is performed after both IQ and OQ have been completed, reviewed and approved.
 - PQ at least should included: tests using production materials, qualified substitutes or simulated product, that have been developed from knowledge of the process and the facilities, systems or equipment;
 - In some cases, PQ may be appropriate to perform it in conjunction with OQ.
- e. Qualification of Established Facilities, System and Equipment
- Evidence should be available to support and verify the operating parameters and limits for the critical variables of the operating equipment.
 - The calibration, cleaning, preventive maintenance, operating procedures and operator training procedures and records should be documented.
7. Process Validation
- a. Process validation should normally be completed prior to the distribution and sale of the vaccine (prospective validation). In exceptional circumstances, where this is not possible, it may be necessary to validate processes during routine production (concurrent validation). Processes in use for some time should also be validated (retrospective validation)
 - b. Facilities, systems, and equipment to be used should have been qualified and analytical testing methods should be validated.
 - c. Staff taking part in the validation work should have been appropriately trained.
 - d. Facilities, systems, equipment and process should be periodically evaluated to verify that they are still operating in a valid manner.
8. Cleaning Validation
- a. The objective of validation of cleaning procedure is in order to confirm the effectiveness of cleaning procedure.
 - b. Validated analytical method having sensitivity to detect residues or contaminants should be used.
 - c. Typically three consecutive applications of the cleaning procedure should be performed and show to be successful in order to prove that the cleaning procedure is validated.
9. Validation of Analytical Procedures
- a. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.
 - b. The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which should be considered are listed below:
 - accuracy;
 - precision;
 - intermediate precision;

- specificity;
- detection limit;
- quantitation limit;
- linearity; and
- range.

10. Change Control

- a. Written procedures should be in place to describe the actions to be taken if a change is proposed to a starting material, products component, process equipment, process environment (or site), method of production or testing or any other that may affect vaccine quality or reproducibility of the process.
- b. All changes that may affect product quality or reproducibility of the process should be formally requested, documented and accepted.
- c. The likely impact of the change of the facilities, systems and equipment on the product should be evaluated, including risk analysis.

11. Re-Validation

- a. Facilities, systems, equipment and processes, should be periodically evaluated to confirm they remain valid.
- b. Where no significant changes have been made to the validated status, a review with evidence that facilities, systems, equipment, processes and analytical method meet the prescribed requirements fulfils the need for re-validation.

XV. REFERENCE

1. WHO Environmental Monitoring Of Clean Rooms In Vaccine Manufacturing Facilities, 2012
2. WHO Good Manufacturing Practices: Main Principles For Pharmaceutical Products, 1997
3. A WHO Guide To Good Manufacturing Practice (GMP) Requirements, 1997
4. WHO Good Manufacturing Practices For Biological Products, 1992.
5. OIE : Terrestrial Manual: Manual Of Diagnostic Tests And Vaccines For Terrestrial Animal, 2012
6. Guideline On Good Manufacturing Practice, National Agency of Drug And Food Control (Republic Indonesia, 2012)
7. Australian Code of Good Manufacturing Practice for Veterinary Chemical Products, 2007
8. British Pharmacopoeia (Veterinary) 2012

APPENDIX

APPENDIX 1

CLEAN ROOM CLASSIFICATION

Classification should be clearly differentiated in accordance with the maximum permitted airborne particle concentration for each grade given in the following table:

| Grade \ Particle size | At Rest* | | In operation** | |
|-----------------------|------------------------------------------------------|--------|----------------|-------------|
| | Maximum Permitted number of particles/m ² | | | |
| | ≥ 0.5 µm | ≥ 5 µm | ≥ 0.5 µm | ≥ 5 µm |
| A | 3.520 | 20 | 3.520 | 20 |
| B | 3.520 | 29 | 352.000 | 2.900 |
| C | 352.000 | 2.900 | 3.520.000 | 29.000 |
| D | 3.520.000 | 29.000 | Not defined | Not defined |

* *At rest* state is the condition where the installation is installed and operating, complete with production equipment but no operating personnel present.

** *In operation* state is the condition where the installation is functioning in the defined operating mode with the specified number of personnel working.

Grade A:

The local zone for high risk operations, e.g. filling zone, open ampuls or vials. Normally conditions are provided by a laminar air flow work station. Laminar air systems should provide a homogenous air speed in a range of 0.36 – 0.54 m/s at the working position in open clean room applications.

The maintenance of laminarity should be demonstrated and validated. A unidirectional air flow (UDAF) and lower velocities may be used in closed isolators and glove boxes.

Grade B:

For aseptic preparation and filling, this is the background environment for Grade A zone.

Grade C and D:

Clean areas for carrying out less critical stages in processing of sterile product.

WHO recommended limits for microorganisms during operation

| Grade | Air sample (CFU/m ³) | 90 mm diameter settle plates (CFU/4 hours) | 55 mm diameter contact plates (CFU/plate) | Glove print (5 fingers) (CFU/glove) |
|-------|----------------------------------|--------------------------------------------|-------------------------------------------|-------------------------------------|
| A | <1 | <1 | <1 | <1 |
| B | 10 | 5 | 5 | 5 |
| C | 100 | 50 | 25 | - |
| D | 200 | 100 | 50 | - |

APPENDIX 2.

MANUFACTURING ACTIVITIES ACCORDANCE TO CLEAN ROOM CLASSIFICATION

*Recommended Clean Room Grades For General Activities in The Manufacture of Prequalified Vaccines:

| General Activities | | |
|----------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| Activity | Open Systems | Closed Systems |
| Raw materials receipt and storage | UNC (unclassified) | N/A (not applicable) |
| Raw materials sampling | - Non-growth promoting materials: sampling hoods with dust control/fume control in UNC ⁽¹⁾ - Growth-promoting materials: sampling hoods with HEPA air supply and dust control in D - Sterile materials: in specialized area ⁽²⁾ | N/A |
| Preparation of glassware and accessory equipment for sterilization by heat | D | N/A |
| Preparation of glassware and accessory equipment after heat sterilization | D (fully enclosed wrapping, such as autoclave bags) or C (with barrier protection such as flask openings covered with aluminium foil) | UNC (pharma-sealed containers) |
| Preparation of media to be sterilized by heat | Component weighing, mixing: D | N/A |
| Preparation of media to be sterilized by filtration | Component weighing, mixing: C | Media final filtration: UDAF in D (a closed system is normally required) |
| Storage of media after sterilization | C for sealed but “open” containers | D for closed containers |
| Preparation of excipients to be sterilized by heat | Component weighing, mixing: D | N/A |
| Preparation of excipients to be sterilized by filtration | - Component weighing, mixing: C - Excipient final filtration: A in B | Excipient final filtration: D |
| Production of master and working seeds | UDAF or Class II BSC in C ⁽³⁾ | Isolator or Class III BSC in D |
| Seed storage | N/A | UNC |
| Thawing and small-scale expansion of seeds | Open manipulating of seeds/ inoculation of flasks, plates, slants: UDAF in D. Alternative use of a Class II BSC acceptable. | - Manipulation in isolator in Class III BSC: D - Incubation: closed containers in D |
| Inoculation of production media | UDAF in D | D |
| Large-scale replication | Open systems are discouraged ⁽⁴⁾ | D |
| Harvesting | C | D |
| Pre-inactivation dissociation/ purification | C | D |
| Inactivation | C | D |
| Purification post-inactivation | C | D |
| Storage of post-inactivation bulks | Not recommended | D |
| Formulation of filling bulks prior to sterile filtration | C | D |
| Final sterile filtration | A in B | D |
| Formulation of filling bulks prior to sterile filtration | A in B | D |
| Storage of sterile filling bulks | N/A | D ⁽⁵⁾ or UNC depending on closure |

| General Activities | | |
|----------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Activity | Open Systems | Closed Systems |
| Filling | - Filling bulk tank with open connections to be located in A in B - Filling operation in A in B | - Closed filling bulk tank: D - Filling in isolator or Class III BSC: A in D |
| Transfer of fully stoppered liquid vaccines prior to capping | - Capping areas within aseptic core (A/B) separated from filling zone: A in B - Capping areas outside aseptic core, separated from aseptic filling zone: UDAF for transfer, and UDAF in D for capping/ crimping | - N/A |
| Transfer of fully partially stoppered vials from filling to lyophilization | - On a continuous belt: Grade A in Grade B - In a mobile unit: Grade A air with cart in a Grade B surround - Transfer of open ampoules from lyophilizer to sealing: Grade A in Grade B | - In closed validated transfer containers: UNC |
| Loading area of lyophilizer | - Grade A in Grade B | - N/A |
| Transfer of fully stoppered vials from lyophilization to capping area | - Transfer systems without additional air supply: B - Transfer in a mobile unit providing Grade A air: D ⁽⁶⁾ | - In closed validated transfer containers: UNC |
| Capping lyophilized vials | - Grade A ⁽⁷⁾ | - N/A |
| Visual inspection | - UNC | - UNC |
| Labeling | - UNC | - UNC |
| Packaging | - UNC | - UNC |
| Quality control laboratories | - Sterility test: A in B | - Sterility test: isolator in D |

* Recommended clean room grades for general activities in the manufacture of prequalified vaccines are provided as guidance and do not intend to be restrictive

Vaccine-specific Production Activities:

| Subunit and Conjugate Vaccine | | |
|-----------------------------------------------------------|-------------------------------------------------------------------|--------------------------------------------------------------|
| Activity | Open Systems | Closed Systems |
| Cell disruption or dissociation | C | D |
| Component purification | C | D |
| Component sterile filtration | - Intermediates sterilization: C - Final sterilization: A in C | D |
| Activation and conjugation reactions | C | D |
| Conjugate purification | C | D |
| Conjugate sterilization | N/A | Intermediate sterilization: C Final sterilization: A in B |
| Inactivated Viral Vaccines with Sterile Filtration | | |
| Activity | Open Systems | Closed Systems |
| Viral seed/ cell seed storage | N/A | UNC |
| Tissue collection and disruption (primary cell) | C | N/A |
| Cell expansion | UDAF in C | D |
| Thawing and small-scale expansion of seeds | UDAF in C | N/A |
| Preparation of inoculum | UDAF in D | D |
| Inoculation of production cells | UDAF in D | D |
| Viral replication | C | D |
| Media changes/ additions | UDAF in D | D |

| Activity | Open Systems | Closed Systems |
|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|
| Harvesting | C | D |
| Concentration/ buffer changes | C | D |
| Pre-inactivation purification | C | D |
| Inactivation | C | D |
| Post-inactivation purification | C | D |
| Formulation before final sterile filtration | UDAF in C | D |
| Sterile filtrations | A in B | C |
| Formulation after final filtration | A in B | C |
| Filling | - Oral or nasal administration: A in B ⁽⁸⁾ - Parenteral administration: A in B | Filling in isolators requires a grade D background |
| Vaccines Prepared Without Sterile Filtration | | |
| Activity | Open Systems | Closed Systems |
| Preparation of materials to be heat sterilized | D | N/A |
| Preparation of materials to be filter sterilized | C | N/A |
| Preparation of growth cells | UDAF in C | D |
| Preparation of inoculum | UDAF in C | D |
| Replication | C with opens manipulations in UDAF/ C | D |
| Harvesting, purification | C with opens manipulations in UDAF/ C | D |
| Treatment by non-sterilizing temperatures | C with opens manipulations in UDAF/ C | D |
| Filling, lyophilization (see general activities), capping | - Bulks containing live bacterial for oral administration: A in B ⁽⁹⁾ - Bulks containing live viruses for oral or nasal administration: A in B ⁽⁸⁾ - Bulks containing live mycobacteria or viruses, or heat-killed bacteria for SC, ID or IM administration: A in B ⁽¹⁰⁾ | Filling in isolators requires a grade D background |
| Egg-Based Vaccines | | |
| Activity | Open Systems | Closed Systems |
| Egg incubation and candling | UNC | N/A |
| Egg inoculation and sealing | UDAF in C | N/A |
| Inoculated egg incubation | Unsealed eggs: C ⁽¹¹⁾ | Sealed eggs: D |
| Egg harvesting | UDAF in C (in cases where the product is sterile filtered, UDAF in D may be acceptable) | N/A |
| Pre-inactivation viral purification | C, or UDAF in D | D |
| Pre-inactivation bulk storage | C | D |
| Post-inactivation viral purification | C | D |
| Expression of Sequences in Genetically Modified Bacteria, Yeast, or Insect Cell | | |
| Activity | Open Systems | Closed Systems |
| Storage of production cell | UNC | UNC |
| Expansion of production cell | - D for systems with selective media - C for systems without selective media | D |

| Harvesting | - D for systems with selective media - C for systems without selective media | D |
|----------------------------------------|-------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| Purification | C | D |
| Formulation | - Pre-sterilization: C - Post-sterilization: A in B | D |
| Chemically Synthesized Antigens | | |
| Activity | Open Systems | Closed Systems |
| Chemical synthesis, purification | GMP for active pharmaceutical ingredients | GMP for active pharmaceutical ingredients |
| Conjugation reactions | D | D |
| Formulation | - D if prior to heat sterilization - C if prior to sterile filtration - A in B if after sterilization | D |

- (1) UDAF in C or D or UNC (unclassified) refers to the situation where unidirectional airflow system may not be classified as Grade A (due to the lack of Grade B surrounding) but can provide significant additional protection to operations.
- (2) Raw materials may not be brought into production areas while under quarantine. Sterile materials that require sampling should be sampled in a testing area if sterility of the raw material is required for sterility of final product.
- (3) The use of surrounding areas of Grade C or higher is recommended for open manipulation of seeds.
- (4) The use of open systems for replication of production microorganisms is discouraged due to the lack of control it offers. However, with the use of highly selective media or other growth conditions that limit the growth of contaminating microorganisms, some manufacturer may choose to continue this practice.
- (5) Final filling bulks are often closed but not fully sealed, such as during the use of screw cap bottles. Additional protection for this such closure may be necessary during transport through uncontrolled areas.
- (6) The carts utilized for this transfer may not be the same as those used to transfer from filling to lyophilization within the Grade B surround.
- (7) Vial capping can be undertaken as an aseptic process using sterilized caps or as a clean process outside the aseptic core. Vials should be protected by Grade A conditions up to the point of leaving the aseptic processing area and thereafter stoppered vials should be protected with a Grade A air supply until the cap has been crimped.
- (8) Due to the pharmacopoeial sterility test requirement, at least Grade B dynamic (in operation) limits should be met in both static and dynamic conditions.
- (9) Due to the presence of formulations promoting the stability of viable microorganisms, at least Grade B dynamic (in operation) limits should be met in both static and dynamic conditions.
- (10) Depending on the design and historical state of control of the surrounding area, Grade B (dynamic) limits may be accepted for area qualification during both static and dynamic conditions
- (11) National regulatory agencies may accept specialized procedures for seasonal or pandemic influenza vaccines

APPENDIX III

VETERINARY VACCINE*

**Vaccines for Veterinary Use, Ph Eur monograph 0062, 2010 in British Pharmacopoeia (Veterinary) 2012*

Vaccines for veterinary use are preparations containing antigenic substances and are administered for the purpose of inducing a specific and active immunity against disease provoked by bacteria, toxins, viruses, fungi or parasites. The vaccines, live or inactivated, confer active immunity that may be transferred passively via maternal antibodies against the immunogens they contain and sometimes also against antigenically-related organisms. Vaccines may contain bacteria, toxins, viruses or fungi, living or inactivated, parasites, or antigenic fractions or substances produced by these organisms and rendered harmless whilst retaining all or part of their antigenic properties; vaccines may also contain combinations of these constituents. The antigens may be produced by recombinant DNA technology. Suitable adjuvants may be included to enhance the immunising properties of the vaccines.

I. BACTERIAL VACCINES AND BACTERIAL TOXOIDS

1. Bacterial vaccines and bacterial toxoids are prepared from cultures grown on suitable solid or liquid media, or by other suitable means; the requirements of this section do not apply to bacterial vaccines prepared in cell cultures or in live animals. The strain of bacterium used may have been modified by genetic engineering. The identity, antigenic potency and purity of each bacterial culture used are carefully controlled.
2. Bacterial vaccines contain inactivated or live bacteria or their antigenic components; they are liquid preparations of various degrees of opacity or they may be freeze-dried.
3. Bacterial toxoids are prepared from toxins by diminishing their toxicity to a very low level or by completely eliminating it by physical or chemical means whilst retaining adequate immunising potency. The toxins are obtained from selected strains of specified micro-organisms grown in suitable media or are obtained by other suitable means, for example, chemical synthesis.
4. *The toxoids may be:*
 - liquid;
 - precipitated with alum or another suitable agent;
 - purified and/or adsorbed on aluminium phosphate, aluminium hydroxide, calcium phosphate or another adsorbent prescribed in the monograph.
5. Bacterial toxoids are clear or slightly opalescent liquids. Adsorbed toxoids are suspensions or emulsions. Certain toxoids may be freeze-dried.
6. Unless otherwise indicated, statements and requirements given below for bacterial vaccines apply equally to bacterial vaccines, bacterial toxoids and products containing a combination of bacterial cells and toxoid.

II. VIRAL VACCINES

1. Viral vaccines are prepared by growth in suitable cell cultures (5.2.4), in tissues, in micro-organisms, in fertilised eggs or, where no other possibility is available, in live

animals, or by other suitable means. The strain of virus used may have been modified by genetic engineering. They are liquid or freeze-dried preparations of one or more viruses or viral subunits or peptides.

2. Live viral vaccines are prepared from viruses of attenuated virulence or of natural low virulence for the target species.
3. Inactivated viral vaccines are treated by a validated procedure for inactivation of the virus and may be purified and concentrated.

III. VECTOR VACCINES

Vector vaccines are liquid or freeze-dried preparations of one or more types of live micro-organisms (bacteria or viruses) that are non-pathogenic or have low pathogenicity for the target species and in which have been inserted one or more genes encoding antigens that stimulate an immune response protective against other micro-organisms.

IV. PRODUCTION

A. PREPARATION OF THE VACCINE

1. The methods of preparation, which vary according to the type of vaccine, are such as to maintain the identity and immunogenicity of the antigen and to ensure freedom from contamination with extraneous agents.
2. Substances of animal origin and other substances used in the production of vaccines for veterinary use must comply with the requirements.
3. All materials prepared in a manner that avoids contamination of the vaccine.

A.1. Substrates for production

1. Cell cultures used in the production of vaccines for veterinary use comply with the requirements.
2. Where a document refers to chicken flocks free from specified pathogens (SPF), these flocks comply with the requirements.
3. For production of inactivated vaccines, where vaccine organisms are grown in poultry embryos, such embryos are derived either from SPF flocks or from healthy non-SPF flocks free from the presence of certain agents and their antibodies, as specified in the document. It may be necessary to demonstrate that the inactivation process is effective against specified potential contaminants. For the production of a master seed lot and for all passages of a micro-organism up to and including the working seed lot eggs from SPF flocks are used.
4. Where it is unavoidable to use animals or animal tissues in the production of veterinary vaccines, such animals shall be free from specified pathogens, as appropriate to the source species and the target animal for the vaccine.

A.2. Media used for seed culture preparation and for production

1. At least the qualitative composition must be recorded of media used for seed culture preparation and for production. The grade of each named ingredient is specified. Where media or ingredients are claimed as proprietary, this is indicated and an appropriate description recorded. Ingredients that are derived from animals are specified as to the source species and country of origin, and must comply with the requirements. Preparation processes for media used, including sterilization procedures, are documented.

2. The addition of antibiotics during the manufacturing process is normally restricted to cell culture fluids and other media, egg inocula and material harvested from skin or other tissues.

A.3. Seed lots

1. Bacterial seed lots

General requirements

The genus and species (and varieties where appropriate) of the bacteria used in the vaccine are stated. Bacteria used in manufacture are handled in a seed-lot system wherever possible. Each master seed lot is tested as described below. A record of the origin, date of isolation, passage history (including purification and characterisation procedures) and storage conditions is maintained for each master seed lot. Each master seed lot is assigned a specific code for identification purposes.

Propagation

The minimum and maximum number of subcultures of each master seed lot prior to the production stage are specified. The methods used for the preparation of seed cultures, preparation of suspensions for seeding, techniques for inoculation of seeds, titre and concentration of inocula and the media used, are documented. It shall be demonstrated that the characteristics of the seed material (for example, dissociation or antigenicity) are not changed by these subcultures. The conditions under which each seed lot is stored are documented.

Identity and purity

Each master seed lot is shown to contain only the species and strain of bacterium stated. A brief description of the method of identifying each strain by biochemical, serological and morphological characteristics and distinguishing it as far as possible from related strains is recorded, as is also the method of determining the purity of the strain. If the master seed lot is shown to contain living organisms of any kind other than the species and strain stated, then it is unsuitable for vaccine production.

2. Virus seed lots

General requirements

Viruses used in manufacture are handled in a seed-lot system. Each master seed lot is tested as described below. A record of the origin, date of isolation, passage history (including purification and characterisation procedures) and storage conditions is maintained for each seed lot. Each master seed lot is assigned a specific code for identification purposes. Production of vaccine is not normally undertaken using virus more than 5 passages from the master seed lot. In the tests on the master seed lot described below, the organisms used are not normally more than 5 passages from the master seed lot at the start of the tests, unless otherwise indicated.

Where the master seed lot is contained within a permanently infected master cell seed, the following tests are carried out on an appropriate volume of virus from disrupted master cell seed. Where relevant tests have been carried out on disrupted cells to validate the suitability of the master cell seed, these tests need not be repeated.

Propagation

The master seed lot and all subsequent passages are propagated on cells, on embryonated eggs or in animals that have been shown to be suitable for vaccine production, and, where applicable, using substances of animal origin that meet the requirements.

Identification

A suitable method to identify the vaccine strain and to distinguish it as far as possible from related strains must be used.

Bacteria and fungi

The master seed lot complies with the test for sterility

Mycoplasmas

The master seed lot complies with the test for mycoplasmas (culture method and indicator cell culture method).

Absence of extraneous viruses

Monographs may contain requirements for freedom from extraneous agents, otherwise the requirements stated below apply.

3. Preparations of monoclonal or polyclonal antibodies containing high levels of neutralising antibody to the virus of the seed lot are made on a batch basis, using antigen that is not derived from any passage level of the virus isolate giving rise to the master seed virus. Each batch of serum is maintained at 56°C for 30 min to inactivate complement. Each batch is shown to be free of antibodies to potential contaminants of the seed virus and is shown to be free of any non-specific inhibiting effects on the ability of viruses to infect and propagate within cells (or eggs, where applicable). If such a serum cannot be obtained, other methods are used to remove or neutralise the seed virus specifically.
4. If the seed lot virus would interfere with the conduct and sensitivity of a test for extraneous viruses, a sample of the master seed lot is treated with a minimum amount of the monoclonal or polyclonal antibody so that the vaccine virus is neutralised as far as possible or removed. The final virus-serum mixture shall, if possible, contain at least the virus content of 10 doses of vaccine per 0.1 mL for avian vaccines and per millilitre for other vaccines.

For mammalian vaccines, the seed lot or the mixture of seed lot and antiserum is tested for freedom from extraneous agents as follows:

- The mixture is inoculated onto cultures of at least 70 cm² of the required cell types.
- The cultures may be inoculated at any suitable stage of growth up to 70% confluency.
- At least 1 monolayer of each type must be retained as a control. The cultures must be monitored daily for a week.
- At the end of this period the cultures are freeze thawed 3 times, centrifuged to remove cell debris and re-inoculated onto the same cell type as above. This is repeated twice. The final passage must produce sufficient cells in appropriate vessels to carry out the tests below.
- Cytopathic and haemadsorbing agents are tested for using the methods described in the relevant sections on testing cell cultures and techniques such as immuno-fluorescence are used for detection of specific

contaminants for the tests in cell cultures. The master seed lot is inoculated onto:

- primary cells of the species of origin of the virus;
 - cells sensitive to viruses pathogenic for the species for which the vaccine is intended;
 - cells sensitive to pestiviruses.
- If the master seed lot is shown to contain living organisms of any kind, other than the virus of the species and strain stated, or foreign viral antigens, then it is unsuitable for vaccine production.

A.4 Inactivation

Inactivated vaccines are subjected to a validated inactivation procedure. The testing of the inactivation kinetics described below is carried out once for a given production process. The rest of this section applies to each production run. When conducting tests for inactivation, it is essential to take account of the possibility that under the conditions of manufacture, organisms may be physically protected from inactivation.

a. *Inactivation kinetics*

The inactivating agent and the inactivation procedure shall be shown, under conditions of manufacture, to inactivate the vaccine micro-organism. Adequate data on inactivation kinetics shall be obtained. Normally, the time required for inactivation shall be not more than 67% of the duration of the inactivation process.

b. *Aziridine*

If an aziridine compound is used as the inactivating agent then it shall be shown that no inactivating agent remains at the end of the inactivation procedure. This may be accomplished by neutralising the inactivating agent with thiosulfate and demonstrating residual thiosulfate in the inactivated harvest at the completion of the inactivation procedure.

c. *Formaldehyde*

If formaldehyde is used as the inactivating agent, then a test for free formaldehyde is carried out as prescribed under Tests.

d. *Other inactivating agents*

When other inactivation methods are used, appropriate tests are carried out to demonstrate that the inactivating agent has been removed or reduced to an acceptable residual level.

e. *Residual live virus/bacteria and/or detoxification testing*

A test for complete inactivation and/or detoxification is performed immediately after the inactivation and/or detoxification procedure and, if applicable, the neutralisation or removal of the inactivating or detoxifying agent.

e.1 Bacterial vaccines

The test selected shall be appropriate to the vaccine bacteria being used and shall consist of at least 2 passages in production medium or, if solid medium has been used for production, in a suitable liquid medium or in the medium prescribed in the monograph. The product complies with the test if no evidence of any live micro-organism is observed.

e.2 Bacterial toxoids.

The test selected shall be appropriate to the toxin or toxins present and shall be the most sensitive available.

e.3 Viral vaccines

- The test selected shall be appropriate to the vaccine virus being used and must consist of at least 2 passages in cells, embryonated eggs or, where no other suitably sensitive method is available, in animals.
- The quantity of cell samples, eggs or animals shall be sufficient to ensure appropriate sensitivity of the test.
- For tests in cell cultures, not less than 150 cm² of cell culture monolayer is inoculated with 1.0 mL of inactivated harvest.
- The product complies with the test if no evidence of the presence of any live virus or other micro-organism is observed.

The final bulk vaccine is prepared by combining one or more batches of antigen that comply with all the relevant requirements with any auxiliary substances, such as adjuvants, stabilisers, antimicrobial preservatives and diluents.

B. CHOICE OF VACCINE COMPOSITION AND CHOICE OF VACCINE STRAIN

For the choice of vaccine composition and choice of vaccine strain, important aspects to be evaluated include safety, efficacy and stability. These requirements may be made more explicit or supplemented by the requirements of specific documents.

For live vaccines, a maximum virus titre or bacterial count acceptable from the point of view of safety is established during development studies. This is then used as the maximum acceptable titre for each batch of vaccine at release.

B.1 Potency and immunogenicity

The tests given under the headings Potency and Immunogenicity serve 2 purposes:

- the potency tests establishes, by a well-controlled test in experimental conditions, the minimum acceptable vaccinating capacity for all vaccines within the scope of the definition, which must be guaranteed throughout the period of validity;
- well-controlled experimental studies are normally a part of the overall demonstration of efficacy of a vaccine the test referred to immunogenicity suitable as a part of this testing.

B.2 Route of administration

During development of a vaccine, safety and immunogenicity are demonstrated for each route of administration to be recommended. The following is a non-exhaustive list of such routes of administration:

- intramuscular;
- subcutaneous;
- intravenous;
- ocular;
- oral;
- nasal;
- foot-stab;

- wing web;
- intradermal;
- intraperitoneal;
- *in ovo*.

B.3 Methods of administration

During development of a vaccine, safety and immunogenicity are demonstrated for each method of administration to be recommended. The following is a non-exhaustive list of such methods of administration:

- injection;
- drinking water;
- spray;
- eye-drop;
- scarification;
- implantation;
- immersion.

B.4 Categories of animal

Sometimes the test is to be carried out for each category of animal of the target species for which the product is recommended or is to be recommended. The following is a non-exhaustive list of categories that are to be taken into account.

Mammals:

- pregnant animals/non-pregnant animals;
- animals raised primarily for breeding/animals raised primarily for food production;
- animals of the minimum age or size recommended for vaccination.

Avian species:

- birds raised primarily for egg production/birds raised primarily for production of meat;
- birds before point of lay/birds after onset of lay.

Fish:

- broodstock fish/fish raised primarily for food production.

B.5 Antimicrobial preservatives

- a. Antimicrobial preservatives are used to prevent spoilage or adverse effects caused by microbial contamination occurring during use of a vaccine which is expected to be no longer than 10 h after first broaching. Antimicrobial preservatives are not included in freeze-dried products but, if justified, taking into account the maximum recommended period of use after reconstitution, they may be included in the diluent for multi-dose freeze-dried products.
- b. For single-dose liquid preparations, inclusion of antimicrobial preservatives is not acceptable unless justified and authorised, but may be acceptable, for example where the same vaccine is filled in single-dose and multidose containers and is used in non-food-producing species.
- c. For multidose liquid preparations, the need for effective antimicrobial preservation is evaluated taking into account likely contamination during use and the maximum recommended period of use after broaching of the container.

- d. During development studies the effectiveness of the antimicrobial preservative throughout the period of validity shall be demonstrated to the satisfaction of the competent authority.
- e. The efficacy of the antimicrobial preservative is evaluated and in addition samples are tested at suitable intervals over the proposed in- use shelf-life. If neither the A criteria nor the B criteria can be met, then in justified cases the following criteria are applied to vaccines for veterinary use: bacteria, no increase from 24 h to 7 days, 3 log reduction at 14 days, no increase at 28 days; fungi, no increase at 14 days and 28 days.
- f. Addition of antibiotics as antimicrobial preservative is generally not acceptable.

B.6 Stability

- a. Evidence of stability is obtained to justify the proposed period of validity. This evidence takes the form of the results of virus titrations, bacterial counts or potency tests carried out at regular intervals until 3 months beyond the end of the shelf life on not fewer than 3 representative consecutive batches of vaccine kept under recommended storage conditions together with results from studies of moisture content (for freeze-dried products), physical tests on the adjuvant, chemical tests on substances such as the adjuvant constituents and preservatives, and pH, as appropriate.
- b. Where applicable, studies on the stability of the reconstituted vaccine are carried out, using the product reconstituted in accordance with the proposed recommendations.

C. MANUFACTURER'S TESTS

Certain tests may be carried out on the final bulk vaccine rather than on the batch or batches prepared from it; such tests include those for antimicrobial preservatives, free formaldehyde and the potency determination for inactivated vaccines.

C.1 Residual live virus/bacteria and/or detoxification testing

- a. For inactivated vaccines, where the auxiliary substances would interfere with a test for inactivation and/or detoxification, a test for inactivation or detoxification is carried out during preparation of the final bulk, after the different batches of antigen have been combined but before addition of auxiliary substances; the test for inactivation or detoxification may then be omitted on the final bulk and the batch.
- b. Where there is a risk of reversion to toxicity, the test for detoxification performed at the latest stage of the production process at which the sensitivity of the test is not compromised (e.g. after the different batches of antigen have been combined but before the addition of auxiliary substances) is important to demonstrate a lack of reversion to toxicity.

C.3 Batch potency test

- a. For most vaccines, the tests cited under Potency or Immunogenicity are not suitable for the routine testing of batches.
- b. For live vaccines, the minimum acceptable virus titre or bacterial count that gives satisfactory results in the potency test and other efficacy studies is established during development. For routine testing it must be demonstrated

for each batch that the titre or count at release is such that at the end of the period of validity, in the light of stability studies, the vaccine, stored in the recommended conditions, will contain not less than the minimum acceptable virus titre or bacterial count determined during development studies.

- c. For inactivated vaccines, if the test described under Potency is not used for routine testing, a batch potency test is established during development. The aim of the batch potency test is to ensure that each batch of vaccine would, if tested, comply with the test described under Potency and Immunogenicity. The acceptance criteria for the batch potency test are therefore established by correlation with the test described under Potency. Where a batch potency test is described in a monograph, this is given as an example of a test that is considered suitable, after establishment of correlation with the potency test; other test models can also be used.

Batch

- a. Unless otherwise prescribed in the monograph, the final bulk vaccine is distributed aseptically into sterile, tamper-proof containers which are then closed so as to exclude contamination.
- b. Only a batch that complies with each of the requirements given below under 3 Batch tests or in the relevant individual monograph may be released for use. With the agreement of the competent authority, certain of the batch tests may be omitted where in-process tests give an equal or better guarantee that the batch would comply or where alternative tests validated with respect to the Pharmacopoeia method have been carried out.
- c. The identification test can often be conveniently combined with the batch potency test to avoid unnecessary use of animals. For a given vaccine, a validated *in vitro* test can be used to avoid the unnecessary use of animals.
- d. It is recognised that for an established vaccine the routine application of the safety test will be waived by the competent authority in the interests of animal welfare when a sufficient number of consecutive production batches have been produced and found to comply with the test, thus demonstrating consistency of the manufacturing process. Significant changes to the manufacturing process may require resumption of routine testing to re-establish consistency. The number of consecutive batches to be tested depends on a number of factors such as the type of vaccine, the frequency of production of batches and experience with the vaccine during development safety testing and during application of the batch safety test. Without prejudice to the decision of the competent authority in the light of information available for a given vaccine, testing of 10 consecutive batches is likely to be sufficient for most products. For products with an inherent safety risk, it may be necessary to continue to conduct the safety test on each batch.

Animal tests

Tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. The criteria for judging tests in monographs must be applied in light of this. For example, if it is indicated that an animal is considered to be positive, infected etc. when typical clinical signs occur then as soon as it is clear that result will not be affected the animal in question shall be either euthanised or given suitable treatment to prevent unnecessary suffering. Alternative test methods may be used to demonstrate compliance and the use of such

tests is particularly encouraged when this leads to replacement or reduction of animal use or reduction of suffering.

Physical tests

A vaccine with an oily adjuvant is tested for viscosity by a suitable method and shown to be within the limits set for the product. The stability of the emulsion shall be demonstrated.

Chemical tests

Tests for the concentrations of appropriate substances such as aluminium and preservatives are carried out to show that these are within the limits set for the product.

pH

The pH of liquid products and diluents is measured and shown to be within the limits set for the product.

Water

Where applicable, the freeze-drying process is checked by a determination of water and shown to be within the limits set for the product.

V. BATCH TESTS

All hen eggs, chickens and chicken cell cultures for use in quality control tests shall be derived from an SPF flock.

1. Identification

For inactivated vaccines, the identification prescribed in monographs is usually an antibody induction test since this is applicable to all vaccines.

2. Formaldehyde

Where formaldehyde has been used in the preparation, the concentration of free formaldehyde is not greater than 0.5 g/L, unless a higher amount has been shown to be safe.

3. Phenol

When the vaccine contains phenol, the concentration is not greater than 5 g/L.

4. Sterility

- Vaccines comply with the test for sterility. Where the volume of liquid in a container is greater than 100 mL, the method of membrane filtration is used wherever possible. Where the method of membrane filtration cannot be used, the method of direct inoculation may be used. Where the volume of liquid in each container is at least 20 mL, the minimum volume to be used for each culture medium is 10% of the contents or 5 mL, whichever is less. The appropriate number of items to be tested is 1% of the batch with a minimum of 4 and a maximum of 10.
- For live bacterial and for live fungal vaccines, the absence of micro-organisms other than the vaccine strain is demonstrated by suitable methods such as microscopic examination and inoculation of suitable media.
- For avian live viral vaccines, for non-parenteral use only, the requirement for sterility is usually replaced by requirements for absence of pathogenic micro-organisms and for a maximum of 1 non-pathogenic micro-organism per dose.

5. Extraneous agents

These measures include:

- production within a seed-lot system and a cell-seed system, wherever possible;

- extensive testing of seed lots and cell seed for extraneous agents;
- requirements for SPF flocks used for providing substrates for vaccine production;
- testing of substances of animal origin, which must, wherever possible, undergo an inactivation procedure;
- for live vaccines, testing of the final product for infectious extraneous agents; such tests are less extensive than those carried out at earlier stages because of the guarantees given by in-process testing.

In case of doubt, the tests intended for the seed lot of a live vaccine may also be applied to the final product. If an extraneous agent is found in such a test, the vaccine does not comply with the monograph.

Avian live viral vaccines must comply with the tests for extraneous agents in batches of finished product.

6. Mycoplasmas

Live viral vaccines comply with the test for mycoplasmas (culture method).

7. Safety

- a. In general, 2 doses of an inactivated vaccine and/or 10 doses of a live vaccine are injected by a recommended route. It may be necessary to reduce the prescribed number of doses under certain circumstances or amend the method of reconstitution and injection, for example for a combined vaccine, where it is difficult to reconstitute 10 doses of the live component in 2 doses of the inactivated component. The animals are observed for the longest period stated in the document quality control. No abnormal local or systemic reaction occurs. Where several batches are prepared from the same final bulk, the safety test is carried out on the first batch and then omitted for further batches prepared from the same final bulk.
- b. During development studies, the type and degree of reactions expected with the vaccine are defined in light of safety testing. This definition is then used as part of the operating procedure for the batch safety test to evaluate acceptable and unacceptable reactions.
- c. The immune status of animals to be used for the safety test is specified in the individual monograph. For most vaccines, one of the 3 following categories is specified:
 - c.1. the animals must be free from antibodies against the virus/bacterium/toxin etc. contained in the vaccine;
 - c.2. the animals are preferably free from antibodies but animals with a low level of antibody may be used as long as the animals have not been vaccinated and the administration of the vaccine does not cause an anamnestic response;
 - c.3. the animals must not have been vaccinated against the disease that the vaccine is intended to prevent.

As a general rule, category 1 is specified for live vaccines. For other vaccines, category 2 is usually specified but where most animals available for use in tests would comply with category 1, this may be specified for inactivated vaccines also. Category 3 is specified for some inactivated vaccines where determination of antibodies prior to testing is unnecessary or impractical. For poultry vaccines, as a general rule the use of SPF birds is specified.

- d. For avian vaccines, the safety test is generally carried out using 10 SPF chickens, except that for vaccines not recommended for use in chickens it is carried out using 10 birds of one of the species for which the vaccine is recommended, the

birds being free from antibodies against the disease agent for which the vaccine is intended to provide protection.

8. Potency

The vaccine complies with the requirements of the test mentioned under Immunogenicity when administered by a recommended route and method.

Expiry date

Unless otherwise stated, the expiry date is calculated from the beginning of the virus titration or bacterial count (for live vaccines) or the beginning of the potency test (for other vaccines). For combined vaccines, the expiry date is that of the component which expires first. For vaccines stored by the manufacturer at a temperature lower than that stated on the label, the stability for the entire storage period is demonstrated by an appropriate study. The expiry date is then calculated from the date that the vaccine is stored in the conditions stated on the label.

The expiry date applies to vaccines stored in the prescribed conditions.

VI. STORAGE

Store protected from light at a temperature of $5 \pm 3^{\circ}\text{C}$, unless otherwise indicated. Liquid preparations are not to be allowed to freeze, unless otherwise indicated.

VII. LABELLING

The label states:

- that the preparation is for veterinary use;
- the volume of the preparation and the number of doses in the container;
- the route of administration;
- the type or types of bacteria (and where applicable the antigenic components) or viruses used and for live vaccines the minimum and the maximum number of live bacteria or the minimum and the maximum virus titre;
- where applicable, for inactivated vaccines, the minimum potency in International Units;
- where applicable, the name and amount of antimicrobial preservative or other substance added to the vaccine;
- the name of any substance that may cause an adverse reaction;
- for freeze-dried vaccines:
 - the name or composition and the volume of the reconstituting liquid to be added;
 - the period within which the vaccine is to be used after reconstitution;
- for vaccines with an oily adjuvant, that if the vaccine is accidentally injected into man, urgent medical attention is necessary;
- the animal species for which the vaccine is intended;
- the indications for the vaccine;
- the instructions for use;
- any contra-indications to the use of the product including any required warning on the dangers of administration of an overdose;
- the doses recommended for different species.