Risk analysis in the manufacture of veterinary biologicals

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Summary: ‘Primum non nocere’ must be the first quality of veterinary immunological medicinal products. Throughout the manufacturing process, stage by stage, various ingredients and/or operations could be responsible for safety problems observed when the product is administered. During the preliminary stages, various ingredients – e.g. master seed strains (virus, bacteria or parasite), cell substrates (cell-lines or primary cells) and substances of animal origin – could be contaminated by extraneous agents. These represent the most important risks.

During the process – i.e. preliminary stages, microorganism growth, downstream processing (harvest, purification, concentration, inactivation, etc.), formulation, filling, freeze-drying and finally packaging – environmental conditions (working areas, equipment, etc.) might also be defective and responsible for product contamination.

The author examines all aspects of risks and their assessments through consideration of 'good manufacturing practice' based on quality assurance and quality control systems.


INTRODUCTION

After marketing authorisation has been obtained for immunological veterinary medicinal products (IVMPs), these must be produced in accordance with the principles of ‘good manufacturing practice’ (GMP) as stated in European Economic Community (EEC: now European Union [EU]) Directive 91/412/EEC (2). This means that all the production stages have to be performed under very strict conditions. The biological domain of pharmaceutical product manufacture differs from the manufacture of other medicinal products in the following specific ways:

- The active ingredient is almost always produced by the manufacturer.
- It is necessary to handle living organisms, some of which may be pathogenic for humans and/or animals.
- The range of products is wide, due to the large number of animal species and related pathogenic agents.

The specific risks to be assessed throughout the manufacturing process are discussed below.

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PRODUCTION STAGES

The three main stages in the vaccine production process (Fig. 1) are described below.

**Preparation of active ingredients**

Active ingredient preparation is one of the most crucial stages of the production process and involves handling various starting materials, including the following:

- living organisms
- cell substrates
- media supplemented with substances of animal origin
- water.

All these elements are at risk.

The main culture and other stages – such as harvest, separation, concentration, extraction, purification, inactivation (killed products), and all downstream processes – must be conducted in suitably-adapted working premises with appropriate, controlled and calibrated equipment (6, 7). Personnel must be properly trained (qualified) and provided with the appropriate (protective) clothing. All the elements involved here – personnel, working area, equipment and starting materials – are at risk.

**Formulation**

This stage appears to be less critical in terms of risk. Formulation involves the addition of active ingredient(s) to adjuvants and/or stabilisers and preservatives. The specific process consists in either emulsion (oil-adjuvanted products) or blending. The requirements for personnel, premises and equipment are identical to those for the preparation of active ingredients.

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**FIG. 1**

Manufacturing veterinary biologicals
Packaging

This stage includes the following types of operations:

- open stages (e.g. filling and freeze-drying), which require the same precautionary measures against potential contamination as those described above for active ingredient preparation and formulation;
- closed stages (e.g. labelling and storage), where the major risk is incorrect identification of a product.

Other considerations

For freeze-dried products, reconstitution is required at the time of administration. Some diluents consist of substances of animal origin which therefore represent the same risks outlined for the previous stages.

The correct use of the product (dose, route of administration, etc.) and the associated risks are the responsibility of the veterinarian, provided that the instruction leaflet is sufficiently clear.

RISKS AND ASSESSMENTS

Starting material

Live materials

Seed lots

The mass production of IVMPs obtained from microbial cultures is based on a seed lot system (3, 4, 8). Master seed lots (virus [MSV] or bacteria [MSB]) and working seed lots (virus [WSV] or bacteria [WSB]) are controlled and validated before any use for pilot and industrial production. The major risk is contamination of the strains.

Seed lots should be adequately characterised and tested for contaminants. The test for contaminants must be as complete as possible. For each master seed lot or working seed lot, a specific file should be kept, describing all the tests performed and recording the results.

The list of potential contaminants is documented primarily in terms of the animal species of origin (as far as this is known), and possibly in terms of the animal species which will eventually be vaccinated (if not the same as the species of origin of the product) (9).

Another risk is the misuse of a master seed lot or working seed lot. For this reason, each seed lot should be stored, identified and used in such a way as to minimise the risks of contamination or alteration. Storage containers should be hermetically sealed and clearly labelled.

Cell substrates

a) Cell-lines

At present, most viral amplifications are obtained on cell-lines. These productions are based on a cell bank system. Master cell banks and working cell banks are controlled and validated before any use for pilot and industrial productions.
The major risk for seed lots is also contamination by an unexpected agent. The contamination could arise from within the original cells or may possibly be introduced by additional media or handling.

Cell banks should be adequately characterised and tested for contaminants. The United States of America Federal Code of Regulations (8) and the World Health Organisation (WHO) requirements (10) state that controls must be performed on the following:
- cell cultures (validated cell-lines and primary kidney cells)
- laboratory animals (new-born mice, adult mice, guinea-pigs, rabbits, etc.)
- embryonated eggs.

Additional tests should be performed, including the following:
- bacterial, fungal and mycoplasma tests
- tests for tumorigenicity
- tests for oncornaviruses (evidence of reverse transcriptase activity)
- electron microscopy.

Cells and animals are tested for specific contaminants. For example, the list of potential contaminants of the VERO cell-line – currently used for the amplification of many viruses (human and veterinary) – is rather impressive.

b) Primary cells

No longer in common use, primary cells are required for the multiplication of some viral agents. As with cell-lines, there is obviously a risk of contamination by a viral or bacterial agent. The cells should be obtained from specific pathogen-free (SPF) animals which have been carefully and regularly tested for contaminants. The production cells are also carefully tested, although the results of these tests may be obtained after their use in the manufacturing process. Release of the final product, however, is conditional on satisfactory results of the tests.

c) SPF eggs

Avian live vaccines are prepared on SPF eggs or SPF chicken embryo fibroblasts. The risk of contamination and the means of assessment of this risk are the same as those described above for other live materials. SPF laying birds are carefully monitored, and purity controls must be reinforced for this type of vaccines, as stated in the relevant European Pharmacopoeia (EP) monographs. The monograph on avian live infectious bronchitis vaccine (1) may be cited as an example.

Non-living materials

Media

Amplification of living organisms (viruses, bacteria) requires specific media prepared with various chemical substances and antibiotics. Some of these products are covered by a specific EP monograph, and therefore raise no problems.

a) EP registered products

Media covered by specific EP monographs include sodium hydrogen carbonate, sodium chloride, disodium phosphate dihydrate, gentamicin sulphate, kanamycin acid sulphate, aluminium hydroxide and sucrose.

b) Non-EP registered products

Monopotassium phosphate, vancomycin hydrochloride, sodium glutamate, polyoxyethylene fatty acid, ether of fatty alcohols and of polyols, etc. are controlled using in-house techniques, and (when possible) in accordance with EP monographs.
c) Commercial media
These media (e.g. MEM, RPMI) are prepared, controlled and distributed by commercial suppliers, and are sterilised by filtration.
None of these defined products presents high risks in the production of IVMPs.

\textit{Substances of animal origin}

Amplification of viruses and bacteria requires media and additional substances of animal origin, i.e. sera, peptones and/or protein hydrolysates. Most of these media and other substances are of bovine origin.

This kind of material represents the major risk in the production of IVMPs. Two types of potential contaminants must be considered, namely conventional agents and non-conventional agents.

\textbf{a) Conventional agents}

Two approaches are currently used for the treatment of substances of animal origin, namely inactivation of potential contaminants by inactivants (mainly $\beta$-propriolactone) and, more recently, gamma-ray irradiation. For the latter approach, one of two processes may be used:

\textit{i) Batch mode}

The product is arranged at fixed locations around the radiation source and cannot be loaded or unloaded while the radiation source is exposed.

\textit{ii) Continuous mode}

An automatic system conveys the products into the radiation cell, past the exposed radiation source (along a defined path and at an appropriate speed) and out of the cell.

It is important to establish the amount of radiation necessary to inactivate the suspected contaminating viruses, to ensure effective treatment and minimise deleterious side-effects.

In both cases, treatments must be validated. It has been defined that the treatment should eliminate the potential agent or reduce its titre by at least $10^6 \text{ ID}_{50}$ (50% infective dose) (3).

\textbf{b) Non-conventional agents (prions)}

There is a risk that substances of bovine, ovine and caprine origin may be contaminated with an agent responsible for spongiform encephalopathy, and great care must be taken in choosing the source of such substances for use in the manufacture of IVMPs.

Adequate measures to assess such a risk include selection of the geographical origin of the product (i.e. the product must not come from an infected country) and selection of the appropriate type of tissue, when possible, in accordance with the official classification of tissues. Bovine material should not be used in products for species (e.g. cattle, sheep, goats) which are known to be naturally susceptible to bovine spongiform encephalopathy (BSE), scrapie, or similar diseases.

\textit{Water}

Preparation of media requires 'ultrafiltered' or, preferably, distilled water. If water purity is monitored according to the appropriate EP monographs, the only specific risk
is potential bacterial contamination, which will be detected by several sterility tests (on
the water batch itself or, later, on the active ingredient and the finished product).
Treated water should be monitored regularly for chemical and biological contamination.

Final containers and stoppers

Glass containers
A specific EP monograph distinguishes between type I and type II glass containers,
on the basis of hydrolytic resistance.

Plastic containers
The quality of polypropylene is covered by a specific EP monograph.

Stoppers
Butyl stoppers are controlled using in-house techniques.

None of the above elements presents any risk, except to the product (e.g. heavy
metal ions could be released, potentially damaging the live vaccinal agent).

Personnel

Documentation
Personnel should know and regularly consult all technical documentation (see
below).

Training
For all production and control stages, the manufacturer should select personnel with
the necessary qualifications and practical experience. Specific training should be
provided to all personnel involved in production areas or in control units, and to other
personnel whose activities could affect the quality of the product. Detailed hygiene
programmes should be established and adapted to the different needs within the plant.
All personnel should undergo a medical examination upon recruitment, and at regular
intervals thereafter.

Clothing
Personnel should wear clothing of a type and quality appropriate for the process
being performed and the working area, to protect the product from contamination
(Fig. 2). Clothing should be appropriate for the air grade of the area where the
personnel will be working. It may be useful for clothing worn in open operations areas
to be of a different colour from that worn in closed systems areas.

Working areas

General considerations
Production of sterile IVMPs should be conducted in ‘clean’ areas, where both
personnel and goods enter through airlocks. ‘Clean’ areas should be maintained to an
appropriate standard of cleanliness and supplied with air which has been thoroughly
filtered to an appropriate degree. The specific question of controlled areas represents
the second most important problem in terms of risk.

Each manufacturing operation requires an appropriate level of air cleanliness to
minimise the risks of particulate or microbial contamination of the product or materials
being handled (6, 7, 10).
Live biological agents should be handled in contained areas. The level of containment should depend on the pathogenicity of the microorganism and whether it has been classified as exotic for the country concerned.

Inactivated biological agents should be handled in 'clean' areas.

Non-infected cells isolated from multicellular organisms and filtration-sterilised media should also be handled in 'clean' areas.

Open circuit operations involving products or components not subsequently sterilised should be carried out under a grade A laminar air-flow work station in a grade B area (both graded in accordance with European GMP, on the basis of the size of particles and the number per cubic metre).

Contained premises should be easily disinfected and must have the following characteristics:

a) no direct venting to the exterior
b) ventilation with air at negative pressure and air extraction through 'high-efficiency particulate air' (HEPA) filters (two sets of HEPA filters in series for areas where exotic organisms or pathogenic agents are handled)

c) system for collection and treatment of liquid effluents

d) procedure for sterilisation or incineration of solid wastes

e) changing rooms designed and used as airlocks, equipped with washing and showering facilities

f) airlock system for the transit of equipment

g) barrier double-door autoclave to secure removal of waste material and introduction of sterile items

h) specific areas for very resistant agents

i) thorough disinfection between production campaigns using different vaccinal viruses.

Requirements for the installation and maintenance of production areas

In 'clean' areas, all exposed surfaces should be smooth and impervious to avoid accumulation of dust, particles or microorganisms. The surfaces should be easily cleaned and disinfected. The installation of pipes and ducts should not create recesses, while installation of sinks and drains should be avoided. Changing rooms should be designed as airlocks, and used to provide separation of the different stages of changing; this will help to minimise microbial and particulate contamination of protective clothing. A filtered air supply should maintain a positive pressure relative to surrounding areas. It should be demonstrated that air-flow patterns do not present a contamination risk (e.g. no spread of particles from persons, operations [e.g. inoculation, harvest, concentration, purification], machines, etc.). A warning system should be provided to indicate failure in the air supply. Indicators of pressure differences should be fitted between areas where these differences are important. Pressure differences should be recorded regularly.

Sanitation of 'clean' areas is particularly important, and should be performed frequently. Appropriate, validated disinfectants and detergents should be used. Fumigation may be useful to reduce microbiological contamination. Clean areas should be monitored by means of microbial counts at planned intervals, and the results should be considered when determining batch release (6, 7, 10).

Equipment and sterilisation

Production equipment (fermenters, inactivators, containers, vessels, concentration and filtration devices and, indeed, any apparatus involved in sterile production) should be carefully decontaminated and sterilised before use. The equipment should be handled carefully after the final cleaning process, to prevent recontamination (Fig. 3).

The efficacy of all washing, decontamination and sterilisation procedures should be validated, and validation repeated at regular intervals thereafter, and when any significant change is made in the process or equipment. The heat cycle used in sterilisation should be recorded. Generally, if the product cannot be sterilised by heat in the final container, solutions or liquids should be filtered through a sterile filter of nominal pore size (0.22 μ).
Downstream processing – formulation – packaging

In view of the wide variety of products, the large number of stages involved in the manufacture of IVMPs, and the nature of the biological processes, careful attention must be paid to all operating procedures and their validation. This involves constant in-process monitoring of all completed stages of production.

Many different stages or processes are involved, e.g. inoculation, culture, harvest, separation, concentration, extraction, purification, inactivation, blending, formulation, filling, freeze-drying and labelling.

Most of these processes require transfer of materials (e.g. sterile media, cultures, active ingredients, bulks or final products), which should be performed in a pre-sterilised closed system. Where this is not possible, transfer operations must be protected by laminar air-flow work stations. Care must be taken to ensure that vessels are correctly connected when cultures are added.

Inactivation

Inactivation is the third critical stage in manufacturing killed IVMPs. Several of the steps involved must be under perfect control. The inactivation process includes the transfer of the product from the first vessel to the second after addition of the inactivant. In principle, products are inactivated following addition of inactivant accompanied by sufficient stirring. The inactivation kinetics must be established during the development of the inactivation process, and inactivation must be checked by personnel responsible for quality control (careful testing of samples, and evaluation of their representativity
for validating storage, filtration, inactivant, neutralisation; these are absolutely necessary elements).

**Filling and freeze-drying**

Filling and freeze-drying are two open operations where there may be a risk of viral or bacterial contamination. Containers for bulk product should be sealed prior to filling, appropriately labelled and stored under specified conditions of temperature.

The product should be placed in bulk containers as soon as possible after production. There should be a system to ensure that containers do not leak and are properly sealed after filling.

Freeze-dryers should be loaded in an appropriate 'clean'/contained area. Double-door freeze-dryers should be sterilised after each cycle unless opened in a 'clean' area. When this equipment is being operated as part of a production campaign using a specific vaccinal virus, it should be sterilised at least after each campaign.

**Labelling**

Labelling is the last operation before shipping. An error of labelling can be a real problem in the field, as the vaccine might be used in a species other than that for which it is intended. Care must be taken to avoid such errors and particularly to check packaging chains before and after packaging.

**QUALITY CONTROL AND QUALITY ASSURANCE**

As noted above, quality control and quality assurance are always present and necessary.

**Quality control**

Quality control forms part of GMP, and is concerned with sampling, specifications and testing. Documentation ensures that the necessary relevant and validated tests have been performed (5).

**In-process control**

In-process control is obligatory, and is useful for starting materials and for important stages of downstream processing and formulation. In addition to the classic controls of sterility and dosages, some in-process controls are particularly important, e.g. purity and inactivation testing (see above).

**Final controls**

All controls on the finished product (e.g. physicochemical tests for sterility, identity, purity, safety, titration or potency) should be listed in the registration file (5).

**Quality assurance**

Quality assurance consists of planned and systematic actions necessary to provide adequate confidence in a product, and to prove that it will satisfy quality requirements. Quality in design and production ('good laboratory practice' [GLP] plus GMP), using formal quality assurance procedures, ultimately provides the best guarantee for the quality of IVMPs.
Four main domains are currently covered by quality assurance, as outlined below.

**Documentation**

All general and specific operations, instructions, processes, tests, etc. must be precisely described and documented in a specific form known as 'standard operating procedures' (SOP).

All documents, specifications, manufacturing formulae, records and SOPs should be designed, prepared, reviewed and distributed with care. They should be approved, signed and dated by appropriate and authorised persons, and all records should be kept up to date.

Records of processing and packaging provide a history of each batch of product, including distribution and any relevant circumstances or deviations pertinent to the quality of the finished product.

**Training**

All personnel employed in areas where IVMPs are manufactured should be given training in hygiene and microbiology. Responsible personnel should be formally trained in one or all of the following fields: bacteriology, biology, biochemistry, immunology, medicine, virology and veterinary medicine.

**Internal audits**

Internal auditing is certainly one of the most useful and powerful tools to improve quality in the production of IVMPs. The main purposes are as follows:

- to compare what is observed with what is expected and/or specified
- to conduct a continuous critical analysis of the unit organisation and the handled documents
- to enable quality control by applying GMP.

Defects observed during internal audits are classified as critical, major or minor. The follow-up of these audits is important and should be carefully organised.

**Validation**

For all processes and analytical tests, validation studies should reinforce GMP and should be conducted in accordance with defined procedures. Results and conclusions should be recorded.

When any new manufacturing formula or method of preparation is adopted, the defined process, using the materials and equipment specified, should be shown to yield a product of the required quality. The effect of significant amendments on the manufacturing process or analytical tests should be verified. Processes and procedures should be tested regularly to ensure that they remain capable of achieving the intended results.

**Batch release by the ‘qualified person’**

The role of the ‘qualified person’ is to provide an independent review of the entire manufacture of the product, and to determine the suitability of the product for release by examining the manufacturing procedures and test systems to the extent required by the quality assurance/quality control scheme. The ‘qualified person’ is better able to assess data relating to individual batches than the producer or controller, as the former operates independently.
When the ‘qualified person’ has performed this independent review and taken the responsibility for releasing a batch, the batch will be released on the basis of the following documents:

_a) from the manufacturer:_
- batch protocol
- release document;

_b) from the competent authority:_
- test result
- release certificate.

As each IVMP manufacturing plant is regularly inspected, the ‘qualified person’ releases a batch of product and submits documents to the competent authority. With regular inspection, there is some degree of confidence: the competent authority does not need to control the product and accepts the manufacturer’s batch release with the submitted documents. The competent authority checks that the batch record contains all relevant information on the production and quality control testing of the batch.

The batch is finally released by the competent authority. This is the final and official approval.

CONCLUSION

This study has concentrated on several points which represent risks in the production of IVMPs. Maximum security is ensured by in-process controls and final control, combined with GMP and quality assurance. The strong points of GMP are as follows:

- assessment of potential cross-contamination and possible confusion
- batch system production
- batch documentation
- processes and analytical methods
- validation (mainly of processes and analytical methods)
- independent examination of all data by the ‘qualified person’, leading to batch release.

ANALYSE DES RISQUES DANS LA FABRICATION DE PRODUITS BIOLOGIQUES À USAGE VÉTÉRINAIRE. – Y. Moreau.

Résumé: « Primum non nocere »; telle doit être la première qualité des médicaments vétérinaires immunologiques. A chacune des étapes de la fabrication, divers ingrédients et/ou opérations peuvent être à l’origine de problèmes sanitaires rencontrés lors de l’administration du produit. Au cours des phases préliminaires, divers composants — par exemple les lots de semence (virus, bactéries ou parasites), les substrats cellulaires (lignées cellulaires ou cellules de première explantation) ainsi que des substances d’origine animale — peuvent être contaminés par des agents extérieurs. C’est là que réside le risque le plus important.
Pendant la fabrication — étapes préliminaires, croissance des microorganismes, traitement en aval (récolte, purification, concentration, inactivation, etc.), formulation, remplissage, lyophilisation et conditionnement final — les conditions du milieu (zones de travail, équipements, etc.) peuvent également présenter des défauts et être à l'origine de la contamination des produits.

L'auteur examine les risques sous tous leurs aspects ainsi que leur évaluation à la lumière des « bonnes pratiques de fabrication » fondées sur la garantie de qualité et les systèmes de contrôle de qualité.


ANÁLISIS DE RIESGOS EN LA FABRICACIÓN DE PRODUCTOS BIOLÓGICOS DE USO VETERINARIO. — Y. Moreau.

Resumen: «Primum non nocere»: ésta debe ser la virtud principal de los productos inmunológicos veterinarios. En cada una de las etapas del proceso de fabricación hay diversos ingredientes y/o operaciones susceptibles de provocar los problemas de seguridad observados cuando se administra el producto. Durante las fases preliminares existen varios ingredientes — por ejemplo las cepas de referencia (ya se trate de virus, bacterias o parásitos), los sustratos celulares (líneas celulares o células primarias) y las substancias de origen animal — que pueden ser contaminados por productos extraños. En ellos reside el riesgo más importante.

Durante el proceso de fabricación — es decir, las etapas preliminares, la fase de crecimiento del microorganismo y su subsiguiente procesado (cosecha, purificación, concentración, inactivación, etc.), la formulación, el rellenado, la liofilización y finalmente el envasado — las condiciones ambientales (las áreas de trabajo, el material utilizado, etc.) pueden ser asimismo defectuosas y causar la contaminación del producto.

Señalando como elementos básicos de una «buena práctica de fabricación» la garantía de calidad y los sistemas de control de calidad, el autor examina todos los aspectos de los riesgos existentes y de su evaluación.


REFERENCES


