*Published in : Biologicals (2010), vol. 38, n°3, pp. 326–331*
*DOI: 10.1016/j.biologicals.2010.01.013*
*Status : Postprint (Author's version)*

# Viral safety and extraneous agents testingfor veterinary vaccines

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**KEYWORDS:** Veterinary vaccines, Viral safety, Viral contaminants, Testing Recommendations

*Published in : Biologicals (2010), vol. 38, n°3, pp. 326–331*
*DOI: 10.1016/j.biologicals.2010.01.013*
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Immunisation of animals with high quality vaccines is the primary means of control for many animal diseases. Considerable progress in the production of vaccines for animal use has been made over the past few decades with the increasing use of continuous cell lines as a substrate and the adoption of fermenta-tion technology for antigen production. However, the use of material of animal or human origin in the production of medicinal products includes the risk of contamination due to extraneous agents. Regulations and guidelines have been developed to assure the viral safety of human and animal medicinal products. These guidelines, however, are based on our knowledge regarding extraneous agents, the available testing technologies and experi-ence, all of which are constantly evolving. In the past few decades, several factors including changes in the regulatory approach, advances in molecular biology and increased globalization of veterinary vaccine production have culminated in the need for a review of the progress and requirements in this area.

To provide a forum for discussion and moving forward, the International Association for Biologicals (JABS) in partnership with the International Federation for Animal Health Europe (IFAH-Europe) organized an international workshop on "Viral Safety and Extraneous Agents Testing for Veterinary Vaccines," 25-27 October 2009, in Annecy, France. Scientists, vaccine manufacturers, regulators and suppliers gathered together to review and evaluate the current procedures and purity standards ensuring the safety of veterinary vaccines, and consider potential modifications in regu-latory policies, in light of the experience accumulated over the past few decades, new changes in production standards and the devel-opment of more sensitive testing methods.

This summary reviews the main issues discussed at the workshop and presents the recommendations produced by the participants. The first session of the workshop, chaired by Jacques Lechenet (Merial, France), set the scene, with a broad overview of past vaccine contamination incidents and a review of the current challenges in viral safety. In the second session, chaired by Phil-ippe Vannier (The French Food Safety Agency (AFSSA), France) and Paul-Pierre Pastoret (The World Organization for Animal Health (OIE)), the rationale and limitations of the current requirements and the sources of potential contamination were described. A third session, chaired by David Mackay (The European Medi-cines Agency (EMEA)), addressed decontamination and inactiva-tion treatments. The fourth session, chaired by Wim Hesselink (Intervet/Schering Plough Animal Health, The Netherlands), included presentations on specific viral contaminants and new tests. Final sessions, chaired by Carmen jungback (Paul-Ehrlich-Institut, Germany), Lukas Bruckner (Institute of Virology and Immunoprophylaxis, Switzerland) and Jacques Lechenet, included a round table on risk-benefit assessment, followed by conclusions and recommendations.

## Key issues and constraints regarding viral safety of veterinary vaccines

Viral safety is a key issue for veterinary vaccines. In the past, contamination of human and veterinary vaccines has resulted in a few spectacular incidents, leading industry to change certain

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practices and regulatory authorities to develop more stringent and detailed requirements [see article by Paul-Pierre Pastoret, p. 332-334].

The three main concerns of viral safety of immunological veterinary medicinal products are (1) the presence of extraneous agents, (2) the residual pathogenicity of live viruses used as active ingredients in live viral vaccines and (3) the inadequate inactivation of viruses used as active ingredients in inactivated viral vaccines. While this meeting focused on extraneous agents, topics and discussions also concerned the latter two points. Contamination by extraneous agents can result from the use of contaminated source materials or contamination during production. Viral purification is an expensive procedure: the quality and safety of veterinary vaccines is consequently based on the basic principle of building quality into vaccines.

Assuring the viral safety of a veterinary medicinal product thus involves the selection and testing of the raw materials, cell lines and seed materials used; assessing the extent to which manufacturing processes clear infectious viruses; and testing at appropriate steps throughout the process and at the level of the final product [see article by David Mackay p. 335-337].

## Definition of viral safety: a challenge to manufacturers and regulators

Defining viral safety poses a real challenge to manufacturers and regulators. It is increasingly understood that the ideal of viral safety -absolute freedom from extraneous agents or residual pathoge-nicity -is neither possible nor realistic. This is in part due to the process of testing starting materials or final product for extraneous agents which is, by definition, based on probability since only a portion is tested. There are also technical limits to testing and inactivation techniques, and neither can confer a full guarantee of freedom from extraneous agents. The nature of the extraneous agents and our level of knowledge about them are also important when it comes to assuring viral safety. Generally speaking, there are three types of extraneous agents: 'known known' agents that are both known and suspected in a sample and should be tested for; the 'known unknowns' or agents that are known and can be tested for, but are not necessarily suspected in a sample; and the 'unknown unknowns' meaning agents that are recognized but cannot currently be tested for, or agents that are as yet unknown. The last group is the most difficult, in terms of detection and determining an appropriate response, although unknown agents may be detected by molecular techniques. Considering the above limitations, David Mackay proposed a realistic definition of viral safety as the maximum possible assurance of a sufficiently low risk of harm arising from any virus present in a veterinary medicinal product.

jean-Claude Rouby (AFSSA-ANMV, France) stressed the diffi-culty of detecting 'low level' contaminants and even of defining what can be considered as a 'low level.' The level of risk linked to the potential presence of extraneous agents was discussed, and more specifically, how the situation is handled in practice [article p. 354-357].

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## Current regulations

Guidelines and regulations have been established in Europe and in the USA to ensure viral safety of veterinary vaccines. In the European Union (EU), extraneous agents testing is addressed by different regulations of the European Pharmacopoeia (Ph. Eur.) and guidelines issued by the Committee for Medicinal Products for Veterinary Use (CVMP) under the European Medicines Evaluation Agency (EMEA). Lukas Bruckner explained how the Ph. Eur. approach to the prevention of contamination through extraneous agents testing embraces the entire production process, from raw materials to the final product. This includes reliable sourcing and testing of raw materials; standardized, controlled production processes using good manufacturing practices ( GMP); and tests confirming the quality of the final product. The general monograph Vaccines for Veterinary Use and texts such as Chapter 5.2.5 Substances ofAnimal Origin for the Production of Immunological Veterinary Medicinal Products address requirements for the purity of raw materials; the details regarding which tests should be used for specific products are specified in monographs [see article p. 338,339].

Requirements in the USA were described by Donna Gatewood (US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, USA); they are based on the Virus-Serum-Toxin Act (VSTA) of 1913 as amended in 1985 and Code of Federal Regulations Title 9 (9CFR), Part 113. The Center for Veteri-nary Biologics (CVB) is responsible for promulgating regulations and enforcing the provisions of the VSTA. The 'master seed' and 'master cell' concepts are used (as in the EU), requiring extensive testing of these materials by the manufacturer; the tests are then confirmed by the CVB. All cells and viruses must be tested for bacteria, mycoplasma, and cytopathic and haemagglutinating agents. They must also be tested for bovine virus diarrhoea virus, reovirus and rabies virus by fluorescent antibody. Each seed is also tested for other specific contaminating agents based on the origin of the seed, its passage history and its intended use.

## Attempts at harmonization

With the increased globalization of veterinary vaccine produc-tion, registration and marketing, there is also a consequent need for globally harmonized standards. The International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) was launched in 1996 with the aim of harmonising technical requirements for veterinary product registration in three regions: the EU, the USA and japan (Australia, New Zealand and Canada are observer countries in the group). The first guideline of the Expert Working Group on the harmonization of the guidelines for extraneous agents testing was meant to be on tests for mammalian live viral vaccines. Wim Hesselink explained that following the last meeting in 2003, it was decided to adjourn until japan has introduced the seed lot system, as used in Europe and the USA. japan accomplished this in 2008, however, the next VICH meeting is not scheduled until 2010. Understandably, many consider that the negotiation process is too slow. Improving the processes at the international level so that negotiations for harmonization can move forward is a challenge facing this area.

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Currently, the differences in requirements between Europe and the USA are such that no one test may satisfy both sets of regula-tions, as pointed out by Sarah Sheridan (BioReliance, Scotland -see article p. 340-345). In order to comply with both, it may be necessary to perform additional tests and/or justify methods chosen from one set of regulations over another. Interestingly, no direct comparison of the accuracy of the two approaches, with respect to which one is better at assuring freedom from extraneous agents, has ever been done.

The EU adopted GMP in order to assure more consistent production, validation of production processes and testing. The situation in the USA, however, is different, since there is no official GMP requirement for the production and control for veterinary vaccines. Donna Gatewood explained, however, that the combina-tion of the US requirements for production, record keeping and testing culminates in a GMP-like environment.

## Conditions for the respect of GMP

Respect of GMP implies that vaccine producers and suppliers of raw materials adhere to certain standards, i.e., ISO certification, GMP for production sites and registration of culture media with a Certifi-cate of Suitability from the European Directorate for the Quality of Medicines and Healthcare. One of the major impacts ofglobalization on the viral safety ofveterinary vaccines is the global sourcing of raw materials. Whereas the producer once sourced locally, raw materials are now traded on the global market. Therefore, a very clear process must be in place to guarantee that raw materials are traced. Producers are responsible for auditing their suppliers, and assuring that raw materials adhere to the required levels of safety and traceability. Incoming materials should be controlled in a single Enterprise Resource Planning system that allows for multiple assignments (lot number, expiration date, etc.). The specifications that need to be checked vary according to the ingredient, based on the level of risk and the way the raw material is produced. The risk of viral contamination of raw materials can be reduced, for example, by avoiding purchases in countries where an infectious animal disease is currently declared [see article by Laurence Faretra-Peysson, BD Diagnostic Systems Europe; p. 352,353].

Establishments that are approved by national competent authorities for producing veterinary biologicals are subject to in-depth inspections of the entire premises. Participants in the IABS workshop wondered, however, how much one can rely on GMP inspection. They agreed that GMP inspectors require specific training and recommended closer cooperation between licensing authorities, site and GMP inspectors and official medicinal control laboratories (OMCLs).

## Inactivation

Testing for inactivation is a mandatory step in the production of killed viral and bacterial antigen for vaccines, in order to show that they are indeed inactivated. However, as pointed out by Frank Milward (Merial, USA), inactivation kinetics studies showed that, in most cases, the extent of inactivation goes beyond the level of the most sensitive test systems. While inactivation testing provides a certain level of assurance, the most critical aspect of inactivation from a risk management perspective is actually the control of execution of the inactivation procedure (quality

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assurance) and its validation via kinetic studies. From a regulatory point ofview, Ralph Woodland (Veterinary Medicines Directorate, UK) explained that regulators are looking for assurance that the finished product will be safe. Extrapolation from inactivation kinetics studies can therefore be allowed only to a very restricted extent. Starting materials should be free from contamination and, if necessary, potential contaminants removed or killed with appropriate validated measures.

## From animal to molecular tests

Traditionally, the safety ofveterinary vaccines has relied on tests conducted in animals. However, an effort is being made to replace animal testing with in vitro methods. The Ph. Eur. applies the 'three R's rule': reduce, refine and replace the use of animals in the manufacture and testing of veterinary medicinal products. Tradi-tionally, the batch testing of avian live viral vaccines for extraneous agents was based on antibody testing after the application of an overdose to two-week-old chickens. The new procedure recommended by the Ph. Eur. is based on culturing the vaccine after neutralization with mono-specific antisera in a panel of in vitro host systems, including SPF embryos and various cell cultures. However, serious limitations have been noted in the practical applicability of these tests, which have not been validated for the detection of low levels of virus contamination in the presence ofan overwhelming amount of vaccine virus. As reported by Hans Philipp (Lohmann Tierzucht, Germany), technical problems encountered with the new methods introduced to reduce animal testing result in only a small proportion of batches being tested with the new method, with the majority of batches still tested in animals [see article p. 350,351 ]. Participants in the IABS meeting concluded that there is a need for practicable tests that are less complicated and less expensive.

The detection of extraneous agents depends on the amount of agent present in the raw material as well as the methods used for sampling and detection. The development of molecular techniques over the past 20 years, particularly PCR, is starting to have a major impact. The Ph. Eur. allows for the use of nucleic acid techniques (NAT), if the method is shown to be comparable to the conventional method with regard to specificity and sensitivity, while the CVB is currently exploring the use of PCR as a potential 'new technology' for use in extraneous agents testing. Hans-Peter Ottiger (Institute of Virology and Immunoprophylaxis, Switzerland) described the modern molecular techniques developed for purity testing of avian viral vaccines and their benefits such as increased speed, reduced cost, reduced need for animal testing and the potential for high sensitivity and specificity. He explained how nucleic acid testing was introduced in the Institute of Virology & Immunoprophylaxis (IVI) in Switzerland, and how the PCR technology was evaluated in this Official Medicines Control Laboratory, resulting in a simple and reliable procedure for batch control [see article p. 381-388]. Andreas Motitschke (Paul-Ehrlich-Institut, Germany) showed that the sensitivity of these PCR tests is comparable to or even slightly better than the Ph. Eur. serological test and that they thus fulfil the requirements of the Ph. Eur. and can be used as alternative tests for the detection of extraneous viruses in final batches of inactivated avian vaccines [see article p. 389-392]. However, it was noted in discussions that many of the required tests of the Ph. Eur. have not been validated, making comparison with new tests difficult. Another difficulty is that the interpretation of PCR tests is not always straightforward since PCR detects

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nucleic acid, which may be from inactivated virus, as well as viable virus. Jean-Marc Spieser (European Directorate for the Quality of Medicines and Health Care -EDQM) indicated that more data on this subject is needed before a proposal for deleting certain tests from the Ph. Eur. can be considered. Participants in the IABS meeting proposed that the new methods be assessed, not in comparison with the 'old methods,' but rather regarding the current needs. The availability of reference materials and reagents was also identified as an issue for comparison of sensitivity in different laboratories; in certain cases, reagents have been devel-oped, but are not available for routine application.

## Interpretation of molecular tests

Another major point of discussion throughout the meeting was the need for a general implementation scheme for the validation and interpretation of molecular methods for extraneous agents testing, with close coordination between science, legislation and quality management. One problem is that PCR detects genome and not infectious agent; therefore it is possible that viruses detected by PCR in products have been inactivated during processing steps such as heat inactivation, irradiation and chemical inactivation. Positive results require careful interpretation and confirmation with B. Dodet et al / Biologicals 38 (2010) 326-331

traditional tests. The relevance of the contamination needs to be weighed as well; the detection of certain contaminants, such as Newcastle or foot-and mouth disease virus, are cause for alarm even if they are inactivated. Participants agreed that there is a need to better define the steps of the manufacturing process in which nucleic acid techniques can be applied in the framework of a deci-sion tree considering the output of positive results. The emphasis with these technologies should be on starting material testing.

David Onions (BioReliance, USA) described new methods based on massively parallel sequencing, developed for the identification of viruses and other adventitious agents without prior knowledge ofthe nature ofthe agent. These methods, using random priming to detect viruses in the supernatant from cell substrates or in virus seed stocks, detect viruses missed by other methods such as degenerate or family-specific PCR. They provide enormous amounts of data, requiring sophisticated bioinformatic algorithms for the analysis and verification of virus targets (see article p. 377-380].

## The discovery of new viral contaminants

One of the results of the development ofmolecular techniques is the discovery of new and/or known contaminants in vaccines and substrates for which authorization is sought or that have been marketed for a considerable period of time. Paul-Pierre Pastoret pointed out that we will probably continue to discover new contaminants; only 5000 viruses have been identified so far, out of an estimated 150 000 -and viruses are constantly evolving. One newchallenge is how to handle newcontaminants when knowledge of their pathogenicity is limited, as illustrated by the case ofTorque teno virus presented by Gregory Nitzel (Ffizer, USA) and retrovirus contaminations

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presented by Takayuki Miyazawa (Kyoto University, Japan) and Marie Dewannieux (Institut Gustave Roussy, France).

Torque teno viruses (TTVs), isolated for the first time in 1997, are small single-stranded circular DNA viruses that have been found in humans and numerous domestic and wild animals, including non-human primates. Although some reports indicate a potential link between TTV infection and some autoimmune and liver diseases in humans, and post-weaning multi-systemic wasting syndrome in swine, TTV is currently considered as non-pathogenic. Recently, PCR testing for TTV DNA confirmed its presence in biologicals containing starting materials (e.g., serum) of porcine origin, including swine vaccines, which is not surprising considering the high prevalence of TTVs in swine. Since the majority of pigs are already TTV positive and there is no proof linking TTV to disease, the presence of TTV is not currently considered as a reason for concern.

RD114, a long-known endogenous feline retrovirus that was recently detected as a contaminant of some live feline vaccines, can infect several feline cell lines as well as cells from other mammalian species, including dogs, as presented by Takayuki Miyazawa (Kyoto University, Japan). Since RD114 grows efficiently in several canine cells, there is concern with respect to its infectivity. Further studies are needed to evaluate its pathogenesis in dogs.

Marie Dewannieux (Institut Gustave Roussy, France) explained that most mammalian species contain a few infectious endogenous retroviruses (ERVs), and it is anticipated that some of these ERVs have a broad host range. Although they are usually silent, ERVs can easily be reactivated with the expression of the vaccine virus, the chemical treatment of cell culture, or changes in cell culture conditions. Whole genome sequencing of an increasing number of species may help identify species with no ERVs, or that only contain ERVs considered to be 'safe' for the purposes of certain vaccines, which could be used for providing 'safe' cell lines. It was noted, however, that a number ofvaccine viruses only grow on cells from one species [see article p. 366-370].

Gabor Kulcsar (Directorate of Veterinary Medicinal Products, Hungary) presented the experience of the Official Control Authority Batch Release (OCABR) and their research activities. They tested randomly selected veterinary vaccines used in Hungary between 1992 and 2009. Only one Aujesky's disease vaccine was found positive for pestivirus by RT-PCR, out of 72 batches of vaccines against various diseases for several animal species produced by different companies. In vivo tests did not confirm this contamination. PCR detected TTV nucleic acid in 5 poultry and 10 mammalian vaccines of the 35 batches tested. The presence of TTV in these vaccines was confirmed by direct sequencing [see article p. 346-349].

Regarding the risks associated with vaccines produced in insect cells, David Onions mentioned that about half of the known mammalian viruses are arthropod-transmitted, and a significant proportion replicate in the arthropod host. Viruses normally considered lytic in mammalian cells can also establish latent or silent infections in insect cells. He mentioned that the newly developed deep sequencing technique could be used to exclude any latent or integrated contaminants in insect cell lines and baculo-virus. Christa Drexler (Intervet/Schering Plough Animal Health, the Netherlands) noted that no specific recommendations are in place for the assessment of insect viruses or insect cell lines, in spite of their increasing use for vaccine production. Some of the

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guidelines for testing animal cell lines and virus seeds cannot be directly applied to insect cells and viruses. For the two swine vaccines produced in insect cells by Intervet/Schering Plough Animal Health, the general EU guidelines for testing vaccines produced on verte-brate cells are followed as much as reasonably possible. The testing performed, the general measures to reduce the risk of accidental contamination -particularly adequate selection, testing and treatment of starting materials of biological origin -and stringent inactivation protocols are considered to sufficiently limit the potential risks associated with vaccines produced in the baculovi-rus insect cell system.

## Revisiting the list of extraneous agents

In the EU, the 'Table of Extraneous Agents to be Tested" (1] provides the list of agents to be investigated. The list, however, dates from 1999, has never been revised and is probably not complete. CarmenJungback suggested that the provisions of the Ph. Eur. should be adjusted in light of the increased quality of the starting materials and vaccines due to the use of the risk-assessment approach and progress made by manufacturers. More specifically, she suggested that for SPF flocks and master seed viruses, the list of extraneous agents to be tested for is revised regularly according to epizootic data; that subtypes/substrains be specified, when justified; and that requirements be introduced for sensitivity thresholds [see article p. 362-365]. The value of certain required tests that have not been validated has been shown in practice. These tests should be validated. Some tests may have to be modified if they are not applicable for specific cases, and the system should be flexible enough to make this possible. It was noted that comparative data on validation and control testing using different methods or procedures is needed if Ph. Eur. tests are to be modified or deleted.

A revision of the Table ofExtraneous Agents to be Tested' is on the agenda of the Immunological Working Party of the CVMP. Participants considered that this would be a good opportunity for a common list between Europe, the USA and Japan, and recom-mended that this request be submitted to the VICH. Participants wondered whether the OIE was or could be involved in such an approach.

## From risk assessment to benefit-risk assessment: a case-by-case approach

The benefit-risk assessment was at the heart of the round-table discussion. Since immunization of animals is the primary means of control for many animal diseases, the acceptable degree of risk must be weighed against the benefits to be gained by having the product available to prevent disease losses. Risk-assessment requires the integration of many parameters, as jean-Claude Rouby pointed out, including the nature of the extraneous agent, the target species and the limits ofdetection of the tests; the nature and geographical origin of the raw materials, the manufacturing processes and steps during production; and the application ofGMP. The risk is higher when linked to zoonotic agents, agents targeted by eradication programs, exotic diseases, or agents inducing big economic losses such as avian infectious bronchitis virus, blue-tongue virus or porcine reproductive and respiratory syndrome virus [see article p. 354-357).

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In Denmark, where a disease eradication program has long been a central component of animal production, the evaluation of extraneous agents in medicinal products is driven by a risk-based approach, as described by Peer Lyng Frandsen (Danish Medicines Agency, Denmark). Contaminants derived from the same species as the target species of the vaccine are considered as constituting the greatest risk (unless the contaminating agent is ubiquitous and harmless in the target species); the risk is considered to be lower when the contaminant is specific to a species that is not targeted by the vaccine. It was stressed, however, that caution is required since cross-species contaminations are possible. Bovine spongiform encephalitis, for example, was shown to cross the species barrier from bovines to humans.

On the other hand, the debate is still open about how to handle extraneous agents ofunknown pathogenicity (e.g. RD-114 or bovine polyoma virus) and/or for which there are no scientific tools for detection and culture (e.g., porcine ITV). jean-Marc Spieser insisted that it cannot be assumed that these contaminants are harmless without further studies. Some effects may be delayed, and their identification is essential for risk assessment. Participants agreed that in-depth analysis should be done to clarify the potential effect of new viruses, such as RD-114 and TTV, on the quality and safety of vaccines. It was suggested that a mechanism for funding research on new agents should be developed. An official protocol with a decision tree needs to be established, for use by authorities and manufacturers, for detecting extraneous agents in finished prod-ucts and for determining what to do if they are detected; it was suggested that JABS convene a collaborative focus group to address this issue.

Different approaches to dealing with contaminated starting materials were also presented. In the case of bovine serum, regu-lations have been developed to deal with the problem of potential contaminants, especially bovine viral diarrhoea virus (BVDV). According to EMEA/CVMP/743/00 [2] and Ph. Eur. Monograph 2262 [3], virus contaminated bovine serum is accepted if the level of contamination is bellow the inactivating capacity of gamma radi-ation. IfBVDV is detected, the impact of BVDV antibodies in the sera, potential interference with the inactivation procedure, should be assessed, and the BVDV test results must be negative after inacti-vation by gamma irradiation. Taking this one step further, Gergely Hamar (CEVA-Phylaxia Veterinary Biologicals Co. Ltd, Hungary) showed how this principle can be applied to chicken serum for use in the production of a poultry vaccine [see article p. 358-361 ).

Discussions also addressed potential strategies for dealing with the discovery of extraneous agents in marketed products. One may logically ask, for example, why not switch to a safer cell line if a contaminant is discovered in a cell line used for production of a vaccine that is already on the market? However, in many instances, such a change is considered by regulatory authorities as creating a new product, which has to go through the whole product development and homologation process. This is a concern, especially for vaccines with small markets, which could lead to their withdrawal from the market altogether. In this case, the benefit-risk assessment is necessary to compare the risk linked to vaccination with the risk linked to the contamination of unvacci-nated animals.

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Participants agreed that there is a need for an overarching approach on how to deal with novel agents and positive test results, with communication between primary regulatory agencies on a global level, industry, and competent authorities. A permanent survey of the situation and the state of art is necessary, and involvement of the competent authorities as early as possible in the process is essential.

## Recommendations

The participants of the conference on "Viral Safety and Extra-neous Agents Testing for Veterinary Vaccines," organized by the International Association for Biologicals (JABS) and the Interna-tional Federation for Animal Health (IFAH)-Europe, held in Annecy, France, 25-27 October 2009,

**Considering that:**

- viral safety is an important element for veterinary vaccines, the principal elements related to viral safety in the context of veterinary vaccines being:
    - extraneous agents in either raw materials used for produc-tion or in the finished product;
    - residual pathogenicity oflive viruses used as active ingredients;
    - incomplete inactivation of inactivated viruses used as active ingredients;
- risk control for extraneous agents includes control of sourcing, testing of starting materials of animal origin and/or subjecting them to validated inactivation procedures, validation of the capacity of the manufacturing process of the product to remove and/or inactivate viruses, and, if still deemed necessary, testing of the final product;
- the amount of information required for marketing authoriza-tion and requested by regulators has increased;
- globalization of sourcing of starting materials, veterinary vaccine production, registration and marketing has increased;
- production standards have changed, with the introduction of GMP requirements including traceability, built-in quality concepts, and heavy upstream control;
- more rapid and/or sensitive testing methods for detection have been developed;
- the potential of endogenous retroviruses being present in seed materials may have been underestimated;
- novel agents such as TTV and other non-cultivatable viruses are a challenge for vaccine testing;

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**Recognizing the need for:**

- global harmonization of requirements for demonstrating the safety of biological products;
- global harmonization of standards and reference materials;
- modification of the current mandatory tests, with more flexi-bility when they are not applicable to specific cases, and the introduction of less expensive and less complicated tests;
- reduction of extraneous agents tests, in particular those on finished products involving experimental animals, when sufficient upstream measures to guarantee viral safety are in place;

**Recommend:**

- mobilising resources for the harmonization of standards and reference materials, towards the introduction of requirements for sensitivity thresholds for extraneous agents tests for SPF flocks, seeds and other starting materials of animal origin, and involving all stakeholders and competent authorities as early as possible in the process;
- collecting data from control testing using different methods or procedures, to define sensitivity thresholds for various tests in order to introduce more flexibility;
- collecting more information on traceability and knowledge on health status of the geographic origin of starting materials, and consolidating data from epidemiological monitoring concern-ing the status of epizootic/enzootic diseases in the geographic origin of starting materials;
- increasing the use of benefit-risk assessments in regulatory policies, and establishing decision trees;
- exploring the role of modelling inactivation of active compo-nents of vaccines in meeting regulatory requirements. This may provide assurance with respect to studies conducted at different scales with different titres;
- establishing a permanent survey of novel agents such as ITV and retroviruses, and a framework between industry and competent authorities for determining on a case-by-case basis, based on a standard approach, or decision tree, whether new agents require immediate action (such as testing of seeds and other starting materials) or not and for anticipating how to deal with positive test results. The involvement of competent authorities as early as possible in the process is essential;
- exploring and developing inactivation methods for non-seed starting materials of animal origin that are effective against small viruses and do not affect the biological and/or immu-nological properties of the material;
- establishing good controlled test systems for insect cell lines and insect viruses, based on a risk assessment of the specific vaccine product involved;

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- regularly revising the list of pathogens to be tested for, both in the EU and USA, according to epizoological data, including specific avian purity testing as described in Ph. Eur.; specifying subtypes/substrains wherever justified; and, after a thorough risk assessment, defining the levels of contamination that can be accepted in starting materials;
- revising testing of WSVs and batches/finished product regu-larly, considering the following parameters: the level of GMP and CLP, the geographic location of the facility, the quality of the facilities/laboratories, the quality and health status of personnel, the purity of starting materials used for production and an evaluation of the reduction/elimination or inactivation of EA within the production process. Antigen testing should be revised and reduced, based on risk assessment.
- validating testing methods in collaborative studies with the involvement of official control laboratories;
- elaborating an implementation scheme for the validation and interpretation of molecular methods for extraneous agents testing, with close coordination between science, legislation and quality management;
- including the product-enhanced reverse transcriptase (PERT) assay for cell bank testing in the Ph. Eur., as has been done for cells used for the production of human vaccines, with the explicit indication that a positive test result should be further investigated and judged according to the intended use of the cell and the nature of the vaccine product;
- defining the steps of the manufacturing process where PCR and a certain type of PCR such as q RT-PCR, can be applied, in the framework of a decision making tree considering the output of positive results.

**To this end, the participants of the meeting strongly urge IABS to promote or convene the organization of workshops or other forms of working groups including all stakeholders to review specific issues and make specific recommendations to be submitted to regulatory agencies.**

**The Organizing Committee of the Workshop and the Veter-inary Biologicals Committee of IABS will actively endorse implementation of the specific steps coming forth from these recommendations.**

# References

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