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Meeting report

The consistency approach for quality control of vaccines – A strategy to improve quality control and implement 3Rs

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ABSTRACT

Current batch release testing of established vaccines emphasizes quality control of the final product and is often characterized by extensive use of animals. This report summarises the discussions of a joint ECVAM/EPAA workshop on the applicability of the consistency approach for routine release of human and veterinary vaccines and its potential to reduce animal use. The consistency approach is based upon thorough characterization of the vaccine during development and the principle that the quality of subsequent batches is the consequence of the strict application of a quality system and of a consistent production of batches. The concept of consistency of production is state-of-the-art for new-generation vaccines, where batch release is mainly based on non-animal methods. There is now the opportunity to introduce the approach into established vaccine production, where it has the potential to replace *in vivo* tests with non-animal tests designed to demonstrate batch quality while maintaining the highest quality standards.

The report indicates how this approach may be further developed for application to established human and veterinary vaccines and emphasizes the continuing need for co-ordination and harmonization. It also gives recommendations for work to be undertaken in order to encourage acceptance and implementation of the consistency approach.

1. Introduction

The European Centre for Validation of Alternative Methods (ECVAM, Institute for Health and Consumer Protection, European Commission Joint Research Centre, Ispra, Italy) and the European Partnership for Alternative Approaches to Animal Testing (EPAA) organised a joint workshop on the consistency approach and its potential to reduce the number of animal tests used in the quality control of human and veterinary vaccines. The workshop took place on 11–12 January 2010 in Brussels, Belgium.

In contrast to well-characterized biological products and small-molecule pharmaceuticals, vaccines can be difficult to characterize due to the complexity and variable heterogeneity of the molecular structure of their active components, and to the presence of excipients in their final formulations that may interfere with non-animal testing. Due to this complexity, regulators currently require that quality control (QC) is performed on each batch (lot/serial) of a vaccine (by manufacturers and on a regular basis by regulatory bodies such as Official Medicines Control Laboratories [OMCLs] in Europe) before it can be placed on the market. Safety and potency testing (as part of QC) often involve animal tests. Particularly, for established inactivated vaccines such as rabies, pertussis, diphtheria, tetanus and other clostridials, large numbers of laboratory animals are used.

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A new approach is needed to assure vaccine quality, safety and efficacy without the use of laboratory animals. At least some of the animal-based methods currently in use for vaccine quality control are inherently variable, might yield unsatisfactory results or are time-consuming and expensive. Additionally, animal use has moral implications and raises public concern. The 3Rs concept has long existed to promote humane research and testing [1]. 3Rs alternatives refer to a refinement of animal procedures, reduction in numbers of animals or replacement of laboratory animal use by non-animal methods. For vaccine quality control, 3Rs methods may have a direct effect by focusing on specific animal tests, or an indirect effect by reducing the requirement for testing in laboratory animals. Frequently used replacement alternatives in vaccine quality control are *in vitro* (tissue culture) methods and analytical (e.g. immunochemical and physicochemical) methods.

Significant progress has already been made in implementing 3Rs alternatives in vaccine quality control by employing direct methods such as the use of serology instead of challenge (e.g. diphtheria and tetanus toxoid vaccines) or antigen quantification tests (e.g. Newcastle disease vaccine and inactivated polio vaccine). Nevertheless, progress is slow and many methods requiring animals remain.

In current vaccine development, production and control there is better characterization of the product during development, improved optimization and standardization of the production process, intensive in-process control and product monitoring with superior analytical tools and the use of quality systems to guarantee consistency in both production and testing methods. Such an approach results in consistent vaccine batches with similar characteristics to those batches already shown to be safe and effective in the target population. Further monitoring of the crucial characteristics relevant to safety and efficacy in the context of quality systems such as Good Manufacturing Practice (GMP) has the potential to reduce the need for testing in animals.

The consistency approach is based upon thorough characterization of the vaccine during development and the principle that the subsequent batches are similar to clinically evaluated batches and is the consequence of the strict application of a quality system (typically based on GMP). Through the application of this principle, which has already been implemented for most highly defined novel human vaccines (e.g. Human Papilloma Virus (HPV) virus-like particles (VLP) and polysaccharide conjugate vaccines such as *Haemophilus* b, *Pneumococcus* and *Meningococcus* vaccines), agreed that characteristics can be tested by analytical methods during the manufacturing process of a batch and shown to be similar to those of batches demonstrated to be safe and effective in clinical trials. The concept of consistency may now also be applied to the production of less defined established vaccines (e.g. tetanus) in order to replace laboratory animal tests with *in vitro* tests and analytical methods indicative of the quality and quantity of the product whilst maintaining the highest quality, efficacy and safety standards to ensure that each batch of final product is safe and effective in the target population.

The workshop discussed the applicability and implementation of the consistency approach to replace laboratory animal tests, primarily for established human and veterinary vaccines. Present at the current workshop were experts from manufacturers, licensing authorities, academia and animal welfare organisations. The outcome of these discussions and the recommendations given by the workshop participants are summarized in this report.

2. Definitions of the consistency approach

At a previous workshop [2], the following performance description of consistency as a new approach to batch release testing of the established vaccines was agreed: *The consistency approach implies the use of a set of parameters to constitute a product profile (e.g. antigen content, antigen integrity, etc.) that can replace current release tests. The product profile is established to the satisfaction of the regulators at the time of licensing, and is monitored throughout production under a strict quality system. The product profile ensures that each batch or lot released is similar to a manufacturer-specific vaccine of proven clinical efficacy and safety, with respect to all characteristics agreed upon at licensing between manufacturer and regulator.*

This approach may lead to a reduction in animal use, since a narrow set of animal tests performed on each final batch, with potentially limited power to predict vaccine behaviour in the target populations, may be replaced by a battery of meaningful tests with enhanced capacity to measure equivalence with batches of proven safety and efficacy.

In advance of the workshop, a questionnaire was distributed to potential participants to provide a 'snapshot' of awareness and understanding of the consistency approach amongst the various stakeholders. There were 19 respondents (8 industry, 11 regulatory including EDQM). Whilst not representative of global opinion, the questionnaire indicated that there was a high level of awareness of the consistency approach as applied to the quality control of vaccines, but the understanding of what is meant by the consistency approach was variable. However, it was clear to all workshop participants that the production process is key to the provision of the critical quality elements of a vaccine product. In addition, it was recognised that quality control has to be based on the use of an appropriate battery of meaningful QC (consistency) tests with relevant limits for production parameters, applied under the strict performance of the production process.

The appropriate battery of consistency tests should be scientifically relevant, measuring the crucial characteristics associated with the consistency of the production process and with safety and efficacy of the product. Selected characteristics may vary from batch-to-batch, and the appropriate tests should measure any variation from a batch with proven safety and efficacy, with an understanding of what magnitude of variation is relevant. To this end, limits for production and quality parameters should be established as well as alert criteria. Tests are required both during the production process (in-process testing), and on the final product. The tests would be specific to a particular antigen (product specific) and a particular production process (manufacturer specific), and need to be identified, validated and approved for each individual product. Furthermore, consistency in production and tests to characterize the product need to be re-established if the production process is significantly modified.

Strict control of the production process leads to a robust, reproducible and consistent process and product. The tools used in the pursuit of consistency include manufacturing to GMP requirements (process validation, production procedures, operator's certification, change control, documentation, and strict keeping of storage conditions) and the application of a quality system (qualified and validated methods/equipment, testing of raw materials/excipients, and stability). Thus, the building of product consistency is achieved through the ways of working (strict application of GMP), regular product quality reviews (trending), and the existence of a strong quality system (QA).

The workshop participants therefore considered that the previous definition should be clarified to better emphasize the need for and importance of a consistent production process. The following definition was suggested: *The consistency approach is a concept which includes the strict application of GMP rules and guidelines, process validation and in process and final product tests and is aimed at verifying if a manufacturing process produces final batches which are consistent with one that fulfils all the criteria of*

Quality, Safety and Efficacy as defined in the marketing authorization, ultimately resulting in replacement of routinely used in vivo tests.

3. The role and views of stakeholders

Vaccine manufacturers and licensing authorities are stakeholders that play a central role in the acceptance of the consistency approach. Manufacturers are responsible for vaccine development and control strategies. Product characterization at this stage as well as selection of tests for this purpose will direct the set up of quality control for batch release. Licensing authorities provide the regulatory framework for the methodologies and are responsible for approving new applications and variations based on requirement specifications and information generated by manufacturers during development and validation.

These stakeholder groups are keen to promote 3Rs alternatives, for reasons of ethics, but also to make quality control less time-consuming, more economical and scientifically more relevant as reflected by the many 3Rs methodologies developed. This interest in alternatives is also shared by the OMCLs which are in charge of releasing every batch of a vaccine before distribution onto the market as required by Article 114 of Directive 2001/83/EC relating to medicinal products for human use, as amended by Directive 2004/27/EC [3], and Article 82 of Directive 2001/82/EC relating to medicinal products for veterinary use, as amended by Directive 2004/28/EC [4]. Other approaches include the review of current regulations and guidance documents to identify changes that facilitate the adoption of non-animal methods, the expedition of applications for variations which may result in the replacement of animal tests and eliminating the need for *in vivo* retesting of batches by licensing authorities. Overviews of 3Rs progress and possibilities have been published [5–8] or have been the subject of conferences (e.g. the EDQM Dubrovnik conference [9]).

Specifications for human and veterinary vaccine batch approval might differ between various parts of the world. Dominant requirements are those of the Ph. Eur. Monographs, published by the European Directorate for the Quality of Medicines and Healthcare (EDQM), the World Health Organisation Expert Committee on Biological Standardisation (WHO-ECBS; human vaccines), the World Organisation for Animal Health (OIE; veterinary vaccines), and the Code of Federal Regulations for the FDA and USDA. Post-approval, the agreed methodologies may be used to conduct product testing by independent standardization bodies (e.g. OMCLs for batch testing and trending within Europe). The standard approach to vaccine approval enables individual manufacturers to develop 3Rs alternatives for their own products, provided those methods are fully validated. Such 3Rs alternatives may then be authorized by the competent authority. The participating standardization bodies like the EDQM also encourage and offer support in the development of these methods and a common harmonized approach. However, the workshop participants acknowledge that not all authorities will necessarily accept proposed 3Rs alternative methods in every region or country where vaccines will be marketed. Reluctance to change may be due in some countries to the lack of national expertise and facilities to conduct sophisticated *in vitro* and analytical testing. This can result in manufacturers performing a number of *in vivo* tests on the same antigens for release through different regulatory agencies (e.g. mouse histamine sensitisation tests for acellular pertussis vaccine).

Replacement of *in vivo* tests for vaccine batch release is generally agreed to be the ultimate objective of all stakeholders. However, the development of non-animal test methods may be hampered by an incomplete understanding of the detailed biological process involved in immunization and the complex interactions that occur between the vaccine components and the living organism. The participants recognize that it has to be accepted first that *in vitro* tests and analytical methods do not necessarily provide the same information as the *in vivo* tests but are rather a marker of consistency. Of course correlation with current *in vivo* tests is preferable, but also the analysis of historical data in terms of quality, safety and efficacy for a specific vaccine from a specific manufacturer could help validate the applicability and context of a new non-animal approach. Nevertheless it is important to recognize that prediction of, for example, protective immunity based on *in vitro* and analytical tests alone can be particularly challenging in certain cases (e.g. rabies vaccine). Manufacturers would welcome guidance regarding the minimum criteria required to have an alternative method approved by the competent authority.

4. The applicability and implementation of the consistency approach: general considerations

The production processes for biological products such as inactivated vaccines show an intrinsic variability associated with the culturing, purification and inactivation of pathogens (or pathogen-products) making each vaccine batch unique. The presence of additional antigens, preservatives and adjuvants, make the final composition of vaccines even more complex. Therefore, each vaccine batch is extensively tested for quality control purposes with emphasis on final product testing. In the production and quality control of recently developed vaccines there is improved characterization of the product during development, state-of-the-art production processes and intensive in-process monitoring through the use of analytical tools and/or *in vitro* tests at almost every manufacturing step and of strict quality systems such as GMP and QA. The application of these modern tools to vaccine production results in the release of consistent high-quality batches. Consequently, the consistency approach considers each batch to be one of a series having the focus for testing shifted from final batch to the production process. The consistency approach promotes the use of these improved analytical tools and *in vitro* tests to create a product profile and to assess the quality of vaccine batches by demonstrating the similarity of their profiles to a reference batch of proven quality (i.e. efficacy and safety).

The establishment of a product profile requires the measurement of relevant antigen characteristics during production such as quantity, identity, antigenicity, purity, configuration, size and functionality. This can be achieved using a battery of tests with the ability to discriminate between batches of standard and sub-standard quality. Various types of analytical tools for profiling a vaccine batch are available (for review [7]): physicochemical (e.g. size-exclusion chromatography, spectroscopy), immunochemical (e.g. ELISA), *in vitro* assays (lymphocyte proliferation, cytokine production, MIMIC™). The application of an appropriate battery of analytical tests and *in vitro* methods to profile vaccine batches provides a non-animal alternative to *in vivo* tests for batch release.

For some recently developed and well-characterized human vaccines batch release testing is already performed using only non-animal methods that were validated during the product development phase. Vaccines such as polysaccharide conjugate vaccines (*Haemophilus* b, Pneumococcal and Meningococcal) or HPV-VLP vaccines are tested using physicochemical and/or immunochemical methods and are released on the basis of batch-to-batch consistency. These examples demonstrate the applicability of the consistency approach for batch release of vaccines.

5. The applicability and implementation of the consistency approach: human vaccines

In contrast to recently developed vaccines, established inactivated vaccines such as diphtheria, tetanus, whole-cell pertussis and rabies are less well-defined products for which *in vivo* testing for potency and safety are still performed. The consistency approach requires that products need to be well-characterized using relevant analytical tools and agreed crucial product-specific parameters have to be monitored. Pioneering work in establishing consistency testing for diphtheria vaccine was published by a research group of the Netherlands Vaccine Institute in two seminal reports [10,11]. Due to the detoxification procedure of the toxin, using chemicals such as formaldehyde, diphtheria toxoid vaccines are difficult to characterize and their safety and potency are routinely assessed by *in vivo* methods. In these publications, the authors show that the quality of diphtheria toxoid vaccine, in terms of safety and potency, can be determined using a battery of *in vitro* and analytical methods, thereby demonstrating for toxoid vaccines that moving to the consistency approach is a realistic option, allowing reduction and eventually replacement of animal use for quality control.

The idea that the quality of established human vaccines could be determined by non-animal methods was further substantiated by results presented during this workshop. For example, an ELISA developed by the group of D. Sesardic (NIBSC) to characterize diphtheria toxoid in complex combined vaccine products showed good results with regard to antigen quantification and degree of adjuvant adsorption as a measure of consistency [12]. The French Agency for Health Products (AFSSAPS) has developed an immunoassay that can measure the Hc fragment of tetanus toxoid and could be used to determine tetanus toxoid vaccine quality and to monitor the vaccine consistency [13]. Properties of other vaccine antigens have also been successfully exploited such as pertussis toxin's binding to sialylated proteins and its ADP ribosyltransferase activity [14]. Sanofi-Pasteur presented results from two in-house validated non-animal tests to detect active pertussis toxin in acellular pertussis vaccines: a carbohydrate binding ELISA using fetuin and the quantification of ADP ribosyltransferase activity in vaccines. The different non-animal tests described here could be part of a battery of tests to generate product profiles and be used for quality testing and monitoring of batch-to-batch consistency.

Assays for potency evaluate, directly or indirectly, whether a batch of vaccine contains sufficient antigen with suitable characteristics to induce, with reasonable probability, a protective immune response in the target population. Potency testing of established products tends to be an *in vivo* evaluation of protective immune response (via laboratory animal challenge), with a questionable correlation to the efficacy response in the target population. As potency depends upon the purity, structure, amount and age of the relevant antigen, as well as the presence and type of adjuvant, it is crucial to be able to quantify and characterize immunodominant and/or neutralising epitopes in production samples (antigen only), inactivated materials (antigen only) and final product (antigen formulated with adjuvant). In addition, the mechanism for immunogenicity (ability to induce an immune response) should also be understood.

To facilitate antigen quantification and characterization by non-animal methods, the antigen should be relatively pure. Therefore, well-defined vaccines comprising recombinant proteins (e.g. Hepatitis B) or sub-units (e.g. polysaccharide conjugate) are the most suitable candidates for the consistency approach. However, for established vaccines such as diphtheria vaccines, the total antigen content and the percentage of adsorbed antigen in the product have been shown to be superior parameters for monitoring trends and consistency between batches [12]. Such tests represent a significant advantage over *in vivo* potency assays due to their lower inherent variability. The critical parameters for particular antigen-adjuvant combinations may be very variable. The type and amount of antigen and adjuvant, the degree of adsorption to adjuvant, as well as the effect of adsorption on antigen conformation could have a significant impact on the ability to assess quality by non-animal methods.

6. The applicability and implementation of the consistency approach: veterinary vaccines

In several aspects veterinary and human vaccines are fundamentally different. Many veterinary vaccines, whilst administered to young and healthy animals to prevent disease and improve welfare, are used primarily for economic benefit through improved health of livestock (e.g. livestock clostridial vaccines). The veterinary vaccine market value is less than the human one resulting in lower R&D investment despite a greater number of pathogens. In contrast to human vaccines, veterinary vaccines are often made using crude extracts from eggs or cells and are hardly purified before being formulated resulting in less well-defined products than the human vaccines. On the other hand, veterinary vaccine development has less stringent regulatory requirements and a better understanding of how a veterinary vaccine behaves in the target population can be obtained due to the ability to test veterinary products in the target species.

As with human vaccines, quality control is mandatory for veterinary vaccines, including *in vivo* potency tests such as challenge and serology assays for final product testing of inactivated vaccines. However, there is a very strong emphasis in the veterinary industry on the adoption of *in vitro* potency assays to replace animal testing and also on the development of assays allowing in-process testing of antigens in accordance with the consistency approach. This was exemplified by presentations given during the workshop. Details of the development of *in vitro* assay for in-process testing to quantify clostridial toxins and toxoids were presented by K Redhead (Intervet/Schering-Plough Animal Health, UK). These quantifications, which are a regulatory requirement, are usually done using mice to assess toxicity end-points. In the presented *in vitro* assay, in-process quantification of clostridial toxins and toxoids was achieved by measuring cell death in specifically sensitive cell lines. This new *in vitro* approach has been validated in-house, correlated with the relevant *in vivo* assays and will be submitted to the Regulatory Authorities. Results presented by L Phillips and J Rodriguez (Novartis Animal Health Canada) showed that their non-animal test (ELISA) for IPNV (Infectious Pancreatic Necrosis Virus) antigen could measure IPNV final product potency and discriminate between potent and sub-potent batches. These fast- and low-cost antigen quantification tests showed excellent reliability and could be used in a consistency approach to profile these products. Furthermore, in the US, analytical antigen quantification by relative potency assay using a reference preparation which has been validated *in vivo* is routinely used as the final product potency test for many veterinary vaccines.

Despite the excellent results obtained with clostridial antigens and IPNV vaccine, the antigens present in many veterinary vaccines are often difficult to characterize. This can be due to the use, in the cultivation phase, of crude extracts from cells or eggs, some components of which often interfere with the performance of analytical tools, to the inactivation method or the presence of various adjuvants. Antigens that are not produced in eggs, but in cell cultures, such as recombinant subunit vaccines and toxoids, are the best candidates for the consistency approach at present; through the use, for example, of monoclonal antibody based tests for antigen quantification and profiling. Many veterinary vaccines

incorporate adjuvants and, unfortunately, the presence of these often remains a barrier to the full characterization of final product. As described for human vaccines, the degree of adsorption of the antigen to the adjuvant as well as the type and amount of antigen and adjuvant and the effect on antigen conformation could have a significant effect on the ability to assess product quality by non-animal methods. A wide range of adjuvants is used in veterinary vaccines including water and oil emulsions, vitamin-E derivatives, aluminum compounds and saponins. Whilst the characterization and quantification of antigens and adjuvants is possible, there are few tools available to characterize interactions between antigen and adjuvant. Such interactions can have a major influence on potency, safety and stability. In addition, the antigen-adjuvant interaction and the antigen inactivation method can also interfere with antigenic mass determination. Metz et al. have shown that formaldehyde, an inactivating agent used in the production of many toxoid vaccines, induces important modifications to various amino acids [15]. These types of modification occur also in formaldehyde-inactivated toxoids (e.g. diphtheria toxoid) and change toxoid epitopes making these unavailable for detection with specific antibodies.

A better understanding of how a vaccine behaves in the target population can assist validation and correlation of the relevant non-animal tests to existing *in vivo* tests (e.g. testing by ELISA can detect sub-potent batches of Infectious Pancreatic Necrosis vaccine as demonstrated in model studies using the target fish species). Furthermore, a risk-benefit analysis for assessing the potential consequences of regulatory changes favouring the use of analytical tools and/or *in vitro* tests is more applicable to veterinary vaccines. These factors combined with improved pharmacovigilance in the field would aid transition to non-animal potency testing.

In the future, it is anticipated that there will be more emphasis on consistency of production, better analytical and *in vitro* test methods and the use of test panels (fingerprint approach or product profile), combined with a risk/benefit based approach for regulatory changes. For existing products, using new tests in parallel with *in vivo* tests may expedite a transition. However, it is often not clear what would be an adequate enough demonstration of consistency to allow the removal of the relevant *in vivo* testing. There is an expectation that for new products greater emphasis will be placed on the development of antigen quantitation assays rather than *in vivo* potency tests during the pre-registration development phase.

7. Hurdles

Limitations to the acceptance and implementation of the consistency approach for human and veterinary vaccines are mostly regulatory and technical. Regulatory limitations mainly relate to test validation and guideline harmonization.

The validation of product-specific physicochemical, immunochemical or cell-based tests is crucial to demonstrate the relevance and reliability of these methods and is a required step for their implementation as batch release tests. The purpose of these tests may be to determine the potency of a vaccine batch. Obtaining good agreement between the non-animal method and the current *in vivo* test can be problematic since many animal tests show high variability (e.g. the immunisation-challenge test for rabies vaccines [16]). Therefore, the workshop participants expressed their consensus that it is not a valid approach to categorically require demonstration of correlation between *in vivo* and non-animal test results. Subject to the approval by the competent authorities alternative test methods may be used without validation against the method shown in the monograph.

Furthermore, global harmonization of test guidelines would greatly simplify the implementation of *in vitro* assays and analytical methods for the consistency approach to batch release. In a globalized market, human and veterinary vaccines are produced, registered and marketed worldwide. Vaccine manufacturers must deal with different sets of testing guidelines that vary in different parts of the world: e.g. the European Pharmacopoeia in Europe, the Code of Federal Regulations in the USA and WHO guidelines. These discrepancies between testing guidelines lead to different testing protocols in the license applications for a product depending on its market localization. The absence of international regulatory harmonization will result in multiple product variations when alternative testing methods are being submitted to the regulatory authorities for approval. These multiple product variations can be expensive and the participants believe that the cost may be prohibitive especially for the numerous veterinary vaccines, and that these costs will hamper full implementation of the consistency approach. To support the implementation of the consistency approach, global harmonization of testing requirements (guidelines) is highly desirable. The participants of the workshop believe that international harmonization both within and beyond the EU might best be managed in a step-wise manner, seeking co-ordination within the EU and with the US to produce some well-founded proposals. As described, regulatory coordination is required for full implementation of the consistency approach in human and veterinary vaccines quality control.

Technical limitations include the lack of defined minimum acceptance criteria for the introduction of alternative tests (e.g. degradation studies, relevance of antibodies used, and need for correlation to an existing *in vivo* test). *In vivo* tests are often perceived as the 'gold standard', and any new alternative, whilst encouraged, is expected to provide 'the same level of information as that obtained in experiments using animals' [17] or to provide assurances 'equal to or greater than' those provided by the existing method [18]. It is not clear if this indicates a perfect translation in non-animal test of all biological mechanisms from *in vivo* tests or an indication of consistent manufacturing.

Another hurdle that was identified during the workshop is the determination of stability of vaccines using *in vitro* and analytical methods. This information is needed to measure the shelf-life of a product. For this, it is essential to understand the changes in the antigen taking place during shelf-life and desorption/adsorption kinetics of antigen-adjuvant interaction. Addressing these stability and validation issues will require additional research and development of new techniques.

Finally, a specific barrier to the implementation of the consistency approach for veterinary vaccines was acknowledged. The human vaccine market is much more profitable than the veterinary vaccine market. This is despite there being a much greater number and variety of veterinary vaccines, many of which have a very small market, but nevertheless provide an important welfare benefit for the vaccinated animals. There is a perception that the potentially time-consuming and complex process to develop, validate and correlate consistency assays may jeopardise the continued existence of some commercially minor but nevertheless important veterinary vaccines. The net result is less funding available for research and development of 3Rs alternatives to support veterinary vaccines and the existence of fewer analytical tools. Therefore, some *in vivo* tests remain potentially cheaper, easier and more convenient for manufacturers of veterinary vaccines.

8. Co-ordinating and supporting implementation

In order to overcome the hurdles to the implementation of the consistency approach, the participants of the workshop proposed

to set up an international technical platform for established human and veterinary vaccines that include manufacturers, licensing authorities, academics and standardization bodies (WHO and EDQM). The role of this platform would be to formulate proposals on the issues identified in the above section. The goals of this technical platform for established vaccines are the following:

1. Prioritization of objectives based on current scientific knowledge.
2. Collaborative development and validation of new 3Rs alternative testing methods. The technical platform will be used as a forum for manufacturers to share current data, to discuss the requirements for developing and validating a battery of *in vitro* and analytical safety and potency tests, and to determine how to develop and validate relevant tests for complex vaccines (adjuvanted, multiple antigens, and raw extracts). Additionally, small-scale manufacturers will benefit from a technical platform as all the data/tests and approaches will be available to them.
3. Definition of acceptance criteria for new proposals for alternative testing. As indicated above, the lack of defined acceptance criteria for the introduction of alternative tests is a technical limitation to the implementation of the consistency approach.
4. Proposal of an implementation mechanism for alternatives, including harmonization. Which of the tests in use need to be harmonized, as in some circumstances there are multiple *in vivo* methods, and identifying the best one would refine animal use.
5. Establishment of a working group focussing on veterinary vaccines which would address specific issues for this type of product, with emphasis on reviewing existing *in vivo* tests in order to replace these with validated alternative non-animal tests wherever possible.
6. Validation of new analytical and *in vitro* methods (although not necessarily by comparison with existing *in vivo* methods) and their submission to EDQM for collaborative study.
7. Use of the technical platform as an opportunity to efficiently increase interactions between the regulators and manufacturers, not only to discuss alternatives for products in development, but also to clarify expectations for the development of alternative methods to replace existing *in vivo* tests.

Finally, new 3Rs alternative testing methods for both human and veterinary vaccines can already be introduced through the Ph. Eur. However, the participants reason that greater emphasis on the consistency approach in the general monographs would probably encourage the acceptance of alternative tests by currently reluctant stakeholders.

9. Conclusions

The workshop participants believe that the consistency approach is the way forward for in-process testing and final product batch release although various obstacles must be overcome before this approach can be fully implemented. The considerable progress already achieved in the development of analytical tools and the characterization of recently developed human vaccines demonstrates the applicability of the consistency approach for quality control purposes. However, it must be borne in mind that further research is required to develop suitable analytical and *in vitro* methods for antigen quantification and characterization of established vaccines and that additional research is also needed to gain a greater understanding of adjuvant function and interaction with antigens and the impact on the stability, safety and potency of vaccines. The consistency approach is not a generic method readily applicable to all products. Its suitability must be determined by the vaccine manufacturers on a product by product basis, and while the scale of this task should not be underestimated, neither should the potential benefits in terms of both increased consistency of vaccines and reduced animal testing.

9.1. General recommendations

1. When considering the consistency approach for 3Rs alternatives to current batch release requirements:
   (a) Established human vaccines should be differentiated from new-generation vaccines that consist of well-characterized products/antigens. For the latter, the QC routine release testing in the Marketing Authorization File should be primarily based upon *in vitro* assays and analytical methods. For established vaccines, a transition procedure towards *in vitro* and analytical QC routine release testing should be set up.
   (b) Whenever possible, human and veterinary vaccines should be treated the same way. However, it should be recognised that for particular veterinary vaccines there may be a justified case for taking an alternative approach to the controls applied to human products.
2. The introduction, validation and application of alternative tests needs to be stimulated, co-ordinated and prioritised within the current regulatory framework (for instance facilitating variations to authorised products and updating Ph. Eur. Monographs).
3. Within the European Commission Framework Programmes (DG RTD), funding should be provided for the development of non-animal-based methods (e.g. *in vitro*, analytical, immunochemical) to be used in the quality control of vaccines and the implementation of the consistency approach. Until now, funding has been focused on 3Rs alternative methods for safety testing of cosmetics, chemicals, pharmaceuticals and other consumer products but not on the quality control of vaccines.

9.2. Specific recommendations

1. A technical platform set up by EPAA, should be created to deal with general strategies and policies to introduce the consistency approach, to define minimal acceptance criteria for the consistency tests (*in vitro* and analytical) and to set up specific technical task forces (see below) to address specific vaccines, tests, and their validation. Funding programmes should be established (see above).
2. Technical task forces should be created to review methods for potency tests currently in veterinary and human Ph. Eur. Monographs and to propose non-animal tests. These task forces should prioritise the replacement of unvalidated *in vivo* tests with validated alternative tests wherever possible. Funding programmes should be established (see above).
3. The general notice/general monograph of the Ph. Eur should more explicitly state that the consistency approach can be used to reduce/replace animal use for batch release testing.
4. For veterinary vaccines, stimuli to introduce non-animal tests should be created: e.g. licensing authorities should be encouraged to waive fees for scientific advice or applications for variations which are 3Rs related. Any such variations should be 'fast-tracked' by use of co-ordinated regulatory review.
5. A technical veterinary vaccine forum should be created to discuss items that are specific for veterinary vaccines and the technical aspects of *in vitro* and analytical testing. This forum

must include regulators, enabling greater interaction between the regulators and the manufacturers.

6. Ethical review and risk-benefit analysis should be applied to any new marketing authorization or variation application. Consideration should be given to setting up a mechanism for undertaking an ethical review and risk-benefit analysis to evaluate the need and regulatory benefits associated with proposed products and variations to existing products.

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Appendix

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