



Session 5.09 Biologicals and vaccines: Progress and new approaches

Development of Alternatives for Quality Control of Biomedicines: The Example of Vaccines for Human Use

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Summary

The European Directorate for the Quality of Medicines (EDQM) co-ordinates the elaboration of the European Pharmacopoeia. Within the field of biomedicines, the EDQM has been able to implement high standards due to the contribution of experts and to the European Biological Standardisation Programme (BSP). The aim of the BSP is to establish reference methods and preparations with a focus on alternatives to animal testing: potency assay validation studies for vaccines illustrate the potential contributions of the BSP to animal welfare. The successful projects run for diphtheria and tetanus vaccines will be extended. Based on the assumption that sera from the same animals (guinea pigs) could be used for *in vitro* determination of antibodies to different components of combined vaccines, the potential for reduction and refinement of the routine vaccines batch potency assay by this approach is substantial and will be further explored by the BSP.

Keywords: European Pharmacopoeia, quality control, biomedicines, vaccines, alternatives

Introduction

Marketing of medicines is regulated in the European Union (EU) by the relevant directives¹. Medicines quality standards in 35 countries and organisations² – including the EU –, which are parties to the European Pharmacopoeia (*Ph. Eur.*) Convention³, are defined in monographs and general methods elaborated by medicines control experts groups under the co-ordination of the EDQM. New or revised monographs and general methods are submitted to public enquiry through publication in the European pharmacopoeial forum “Pharmeuropa” and then endorsed by the *Ph. Eur.* Commission (representatives of parties to the Convention) prior to being published and implemented. Adherence to the “European Convention for Protection of Vertebrate Animals used for Experimental and Other Purposes”⁴ in the field of medicines Quality Control (QC) is strongly promoted by the EDQM⁵.

Among the products covered by the *Ph. Eur.* are many biologicals which require extensive QC testing to demonstrate their quality, safety and efficacy. High quality standards for biomedicines have been implemented in Europe thanks to contributions of specialised *Ph. Eur.* experts groups and to the development of the Biological Standardisation Programme

¹ European Commission directives : 2004/27/EC amending 2001/83/EC, 2004/24/EC amending 2001/83/EC and 2004/28/EC amending 2001/82/EC

² see www.pheur.org

³ Council of Europe European Treaty Series No. 50, 22/07/1964 and No. 134. 01/11/1992

⁴ Council of Europe European Treaty Series No. 123, 18/03/1986

⁵ Artiges, A. (1999). Alternatives to animals in the development and control of biological products for human and veterinary use. The role of the European Pharmacopoeia. *Dev. Biol. Stand.* 101, 29-35.



(BSP), a research programme co-sponsored by the EU (European Commission Directorate General-Enterprise, Pharmacy Division) and the Council of Europe.

The aim of the BSP is to establish reference methods and materials for biomedicinal products with a special emphasis on validation of alternatives to animal testing. Division IV of the EDQM is responsible for co-ordination of the BSP.

This manuscript focuses on potential contributions of the BSP to animal welfare issues in the field of vaccines for human use regarding serological potency assay validation (table 1). Two projects, coded BSP019 and BSP035, were run using a guinea pig serological potency assay model for tetanus (T) vaccines for human use. A follow-up project (BSP034) dealt with diphtheria (D) vaccines and provided confirmation of the assumption that sera from the same animals could be used for *in vitro* determination of antibodies to both T and D components of combined vaccines. Future plans include the validation of ELISA methods for an acellular pertussis vaccine serological potency assay in guinea pigs.

Features of the T vaccine potency assay validation studies will be described here-in. Indeed these projects were pilot studies from which it was possible to identify and resolve a number of issues. These projects also demonstrated an exemplary collaboration with the EU through the sponsorship provided by the European Centre for Validation of Alternative Methods (ECVAM-IHCP-JRC) to pilot laboratories, a good model for effective regulatory acceptance and implementation of alternatives through *Ph. Eur.* general methods revisions and a test as regards the potential for harmonisation of international medicines QC standards in line with the European animal welfare policy.

The tetanus studies were divided into 2 projects, BSP019 and BSP035, which were initiated in 1996 and completed in 2000. These projects were conducted under the scientific direction of Dr. C. Hendriksen (Netherlands Vaccine Institute, NL) and Dr. R. Winsnes (Norwegian Medicines Agency, N) and co-ordinated by EDQM.

A BSP019

A.1 Context

In 1996, the tetanus potency assay prescribed by the *Ph. Eur.* 4th edition was a quantitative direct challenge test in guinea pigs or mice (immunisation followed by toxin challenge), generating severe distress and pain in animals. The replacement of this test was therefore considered a priority for the BSP, which was sup-

ported by ECVAM-IHCP-JRC in this endeavour. Potential alternatives included serology, where the toxin challenge is replaced by an antibody detection test, or pure *in vitro* tests, e.g. antigen content measurement of the vaccine.

A.2 Candidate alternative method selection

In order to determine the method of choice for validation as an alternative to challenge, the following criteria were applied.

A Candidate method should:

- represent an animal welfare and technical improvement.
- be validated for use in QC in at least one laboratory, transferable to other laboratories.
- be based on Standard Operating Procedures (SOPs) and reagents available to EDQM and to potential users in the future.
- make use of established reference preparations, if relevant.
- have the potential to be accepted further to validation by regulatory authorities in view of implementation as an official standard in Europe and elsewhere to promote international harmonisation.

Analysis according to the criteria listed in A.1 revealed that only the serological potency assay complied with these prerequisites.

A.3 Animal model and *in vitro* method selection

In order to determine a suitable animal model and an *in vitro* method for antibody measurement, the following criteria were applied.

An animal model should:

- mimic the situation in humans.
- demonstrate the relationship between challenge and antibody response with documented variability sources (e.g. strain, breeding conditions, sex, etc.).
- generate sufficient amounts of sera to assay several components of combined vaccines on the same animals.

An *in vitro* method should:

- be validated (in-house).
- be easily transferable to other QC laboratories.
- be evaluated for vaccine QC, notably for its ability to discriminate between good and borderline vaccines.

The Toxin Binding Inhibition Test (ToBI) and Enzyme Linked Immunoassay (ELISA) for determination of tetanus antibodies in guinea pigs complied with the prerequisites set by organisers and project leaders.

Tab. 1: BSP projects for human vaccines serological potency assay validation

Product	Method	Monograph(s) – General Method	Project(s)
Acellular pertussis vaccine	Assay (serological method(s) based on ELISA)	2.7.16. Assay of pertussis vaccine (acellular)	BSP083
Diphtheria vaccine (adsorbed)	Assay (serological method(s) based on Vero cell test and/or ELISA)	2.7.6. Assay of diphtheria vaccine (adsorbed)	BSP006 BSP034
Tetanus vaccine (adsorbed)	Assay (serological method(s) based on ELISA and ToBI)	2.7.8. Assay of tetanus vaccine (adsorbed)	BSP019 BSP035

A.4 Organisation of the project – objectives

Further to the selection of potential alternatives to the challenge assay, the study was divided into different phases, aimed at addressing successive goals.

A.4.1 Prevalidation phase

The aim of the prevalidation phase was to optimise the methods and test design to suit the purpose of BSP019. Optimal immunisation doses and period, bleeding methods, ELISA and ToBI procedures were defined in one laboratory.

A.4.2 Phase I

All methods were transferred to a second laboratory, which confirmed the results obtained formerly, using the same testing procedures and design.

A.4.3 Phase II

In Phase II, 5 laboratories were requested to test a set of vaccines including 1 borderline vaccine in challenge and by using the candidate alternatives. Correlation between potencies (*in vivo* vs. serology) and between antibody titres and protection in the individual animal were shown.

A.5 Issues and knowledge acquired from Phases I and II

A number of issues were raised and solved during phases I and II. Amongst them, it is useful to take note of the following observations.

A.5.1 Number and experience of testing laboratories

For obvious animal welfare reasons, it is recommended to limit the numbers of animals used for *in vivo* testing in alternatives validation projects. It is however recommended to enrol a sufficient number of very experienced laboratories to avoid delays and repetition of animal experiments due to test failure (e.g. BSP019, phase IIa, 3 labs: failure of *in vivo* test in 1 laboratory and of *in vitro* test in another laboratory, therefore extension to 2 more laboratories needed, phase IIb).

A.5.2 Borderline products and selection of test vaccines.

The testing of borderline products is usually not possible, as such products are frequently unavailable. It is generally accepted that artificially weakened products (e.g. by dilution) are used instead. In BSP019 however, a borderline vaccine could be studied, in parallel with 6 other vaccines complying with *Ph. Eur.* potency specifications.

A.5.3 Test-scheme

To generate appropriate dose-response relationship in the serological model, it is necessary to explore the behaviour of the method using a multi-dilution immunisation scheme, perhaps with more doses than would be used in routine QC. For *in vivo* testing, where difference in challenge toxin, strains and origin of animals and conditions of breeding may interfere, test-schemes must be elaborated on an individual basis for each laboratory.

A.5.4 Sourcing of essential reagents

Sourcing of non-commercial reagents needs to be addressed ahead of the start of a collaborative study. Sufficient amounts must be produced and made available to the EDQM.

A.5.5 Statistical analysis

The impact of the use of different software and statistical models on estimated parameters must be evaluated, despite the labour-intensive approach this implies.

In BSP019 the following models were used:

- probit analysis for challenge data.
- 4 and 5 parameters models, dichotomisation and probit analysis for tetanus antitoxin concentrations.
- logistic regression for protective concentration.
- probit analysis after dichotomisation.
- Sign test and correlation coefficients (Pearson) were used for correlation.

A.5.6 Continuation of study

Critical evaluation of the first phases enabled making the decision on whether to extend to the large international validation study.

B BSP035

B.1 Context

In view of the duration and large amount of data generated in phases I and II of BSP019, it was decided to start a new project to run an enlarged international collaborative study for the validation of the *in vitro* part of the serological potency assay. This part of the study is referred to as Phase III.

B.2 Aim

The aim of Phase III was to assess intra- and inter-laboratory variations in estimation of T-antibodies by ELISA and ToBI.

B.3 Issues and knowledge acquired from Phase III

B.3.1 Participants

Enrolment of participants experienced with the test methods is deemed necessary. This implies that both public Official Medicines Control Laboratories (OMCLs) and private (manufacturers) sector QC laboratories participate on a geographical basis that may be larger than Europe. In BSP035, 25 laboratories from 13 European countries, USA, Australia and India took part.

B.3.2 Test samples

Production of a large panel of antisera implies knowledge of marketed products and support from manufacturers for sourcing of test vaccines and antisera. A choice has to be made among available vaccines. In BSP035, 14 vaccines and references, corresponding to different components and potencies, enabled production of a panel of 28 sera.

B.3.3 Essential reagents

Identification of suppliers of essential reagents for the collaborative study and for future validation studies at QC laboratories is needed. For BSP035, RIVM (NL) and NIBSC (UK) supplied EDQM with essential reagents for ToBI and ELISA. A stock of essential reagents for ToBI was made available to the EDQM by RIVM further to completion of the study and a tetanus toxoid batch suitable for ELISA and ToBI was donated by SSI (DK).

B.3.4 Standardisation of *in vitro* tests

It is useful to determine the extent of standardisation needed in the study and also for subsequent use in routine QC: in general this can be addressed by prescribing use of in-house procedures in parallel to centrally provided SOPs. In BSP035, only a few laboratories performed in-house tests. After completion of BSP035, SOPs and essential reagents were therefore provided to all potential users upon request.

B.3.5 Analysis of study data

In most collaborative studies, calculations are to be performed by participants and repeated in a central statistical analysis.



However, in case of validation of a new method, this may not be required. In BSP035, only a central analysis was performed. Based on the conclusion of this analysis, a suitable statistical model was prescribed for use of the test in routine QC.

B.4 Conclusions of Phase III

Several conclusions could be reached in BSP035:

- In terms of test reliability: ELISA vs. ToBI generate different results that may indicate that there are differences in the antibodies measured.
- In terms of test suitability: ELISA and ToBI are both considered suitable for monitoring tetanus antitoxin levels in guinea pig sera.
- In terms of suitability for vaccines batch release: ELISA and ToBI-based serological methods are suitable, provided that the test is carefully designed and variability of different steps is monitored.

C Implementation of serological potency assay as a regulatory test for tetanus vaccine for human use

The adoption of the newly validated potency assay as a *Ph. Eur.* standard was the result of several steps subsequent to the collaborative studies.

C.1 Communication

A symposium took place on 22-23 June 2000 to address the need for changes in T vaccine QC standards⁶. Publication of the reports of the collaborative studies in the Pharmacopoeial forum⁷ allowed the results of BSP019 and BSP035 to be shared widely with the scientific community and the regulatory authorities worldwide. The project leaders and EDQM experts and representatives also participated in scientific and WHO meetings on vaccines standardisation.

C.2 Technical support to potential users

SOPs and essential reagents for ToBI and ELISA are provided upon request to manufacturers and OMCLs.

C.3 Inclusion in *Ph. Eur.* Group 15 work programme

After completion of BSP035, the group of 15 experts of the *Ph. Eur.* started to revise the general method 2.7.8. Assay of tetanus vaccine (adsorbed), resulting in the inclusion in 2001 of a new method referred to as “method C: measurement of antibodies in guinea pigs”. The revised text was published for public enquiry in *Pharmeuropa* 14.3 in July 2002, and adopted by the *Ph. Eur.* Commission in March 2003. The new method was published in the *Ph. Eur.* (4.7) in September 2003.

D Promotion of reduction and refinement of animal testing for tetanus vaccines, follow-up studies

Humane end-points and single dilution design of challenge constituted a step forward towards the 3Rs. Moving from challenge to serological potency assay represents another significant step, but to encourage manufacturers and OMCLs to move to this test, economical and technical incentives must be found. The BSP was therefore asked to look into the possibility of testing several components on the same sera. Most vaccines currently marketed are combined, and this is currently considered the most promising approach. Follow-up studies have been undertaken since 2001 and are listed below.

D.1 BSP034

BSP034, a project on D vaccine serological potency assay started in 2001 and was completed in 2004. A Vero cell assay and an ELISA-based serological potency assay were validated. By demonstrating that T and D antibody contents can be measured in sera from the same guinea pigs, the study has shown that determining potency for D and T components using a refined test requiring fewer animals was possible^{8,9}.

Currently, method 2.7.6. Assay of diphtheria vaccine (adsorbed), is undergoing revision. The public enquiry started with publication in *Pharmeuropa* 17.3 in July 2005.

D.2 BSP083

BSP083 started in 2005 with the aim of validating ELISAs for detection of antibodies to acellular pertussis components (pertussis toxin, fimbrial haemagglutinin, pertactin, Fim. 2/3 antigens).

D.3 Perspectives

Scientific data still needs to be generated in order to determine whether the guinea pig “same animal” model can also be used to monitor batch potency for other components of combined vaccines, e.g. whole cell pertussis, poliomyelitis and hepatitis B vaccines.

E General conclusion

Performance of collaborative validation studies BSP019, BSP034 and BSP035, involving manufacturers and authorities on a worldwide basis, was initiated by the EDQM in the field of human vaccines, with obvious positive consequences for implementation of 3R-compliant QC policies. Experience from the BSP benefits new projects and has enabled the introduction of alternatives in the *Ph. Eur.* BSP projects have also contributed to international harmonisation, by allowing non-European authorities and the WHO to be proactively involved at all stages of the projects.

F Acknowledgement to BSP019, BSP034 and BSP035 contributors

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⁶ *Pharmeuropa* (Oct 2000). International Symposium, Strasbourg, 22-23 June 2000. Tetanus vaccines for human use. Proceedings of the Symposium

⁷ *Pharmeuropa Bio* (Oct. 2001). Special Issue Biol. Bio 2001-2.

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F), Dr. N. Hug (Chiron Vaccines, I) and Dr. St. Knapp (Chiron Behring GmbH, D) for donation of reagents and test samples.

G Concluding remarks

Experience gained in the BSP programme contributes to revising and updating the regulatory frame of biomedicines in Europe in line with the European Convention for the Protection of Vertebrate Animals for Experimental and other Scientific Purposes⁴. However, additional efforts are needed, notably at the level of policy makers, to promote research and development on alternative methods and international harmonisation for medicines control in Europe.

Nevertheless, a number of 3R issues linked to QC control of biomedicines are out of the BSP scope. Indeed for many products:

- harmonisation of regulations in different regions has not yet been achieved.
- implementation of *in vitro* methods is dependant on economical parameters (e.g. in the developing countries animal tests are cheaper than *in vitro* tests).
- lack of resources to move to new tests is frequently reported, notably by public sector laboratories.

- the start of new studies is strongly hindered by a lack of knowledge and research into potential alternatives.

To promote the 3Rs in medicines control, the cooperation of policy makers, public health and regulatory authorities, standardisation bodies, medicines control experts, scientists as well as animal welfare organisations is sought. In particular, there is an absolute need for research and development and potential sponsors have to be identified amongst OMCLs, manufacturers, national and international research platforms, European Commission (FP6 Thematic priority 1), ECVAM-IHCP-JRC and the various animal protection organisations.

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Contributions of the European OMCL Network and Biological Standardisation Programme to Animal Welfare

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Summary

In 1991 a contractual co-operation in harmonising medicines control started between the European Union and the European Directorate for the Quality of Medicines (EDQM)-Council of Europe (CoE): The EDQM has been charged to co-ordinate a network of national Official Medicines Control Laboratories (OMCL) and a research programme referred to as Biological Standardisation Programme (BSP). In line with the CoE convention on the protection of animals, the BSP establishes European Pharmacopoeia (*Ph. Eur.*) standards for biomedicines quality control with a special emphasis on 3Rs alternative methods. Sixteen projects on vaccines and one on blood products have been initiated in this field. The programme, run in the spirit of international harmonisation, involves the OMCL network, public and private sector medicines control laboratories in Europe, the Americas, Asia and Australia and non-European standardisation bodies. Completed projects on Newcastle disease and clostridial veterinary vaccines and on diphtheria and tetanus human vaccines led to new *Ph. Eur.* general methods and standards, thus showing that the BSP promotes regulatory acceptance of alternatives. Further studies deal with botulinum toxin, vaccines for human use (inactivated poliomyelitis virus, hepatitis A, hepatitis B and pertussis) and tetanus immunoglobulin. For the future, the programme hopes to benefit from synergies between fundamental, medical and pharmaceutical sciences experts for promoting animal welfare aspects in control whilst guaranteeing quality, safety and efficacy to biomedicines potential users.

Keywords: biological standardisation programme, alternative method development, three Rs, validation acceptance, biomedical products, routine release, quality control

The BSP programme and its achievements

A contractual co-operation between the European Union and the European Directorate for the Quality of Medicines (EDQM)-Council of Europe (CoE) started in November 1991 with the aim of improving harmonisation within the European framework for the control of medicines. For that purpose, the EDQM has been charged with setting up and co-ordinating the activities of a network of national Official Medicines Control Laboratories (OMCL) (Paulsen-Sörmann et al., 2000; Milne et al., 2002) and to develop a scientific research programme referred to as the Biological Standardisation Programme (BSP). In agreement with the Council of Europe convention on the protection of animals¹ and with the general policy adopted by the European Pharmacopoeia (*Ph. Eur.*) (Artiges, 1999), the OMCL network has committed itself to promoting the 3Rs (Russell and Burch, 1959), in particular in the field of biomedicines control. The BSP, supported by scientific advisors from this network, establishes *Ph. Eur.* standard preparations and methods with a special emphasis on validation of “alternative” methods for reduction, refinement and replacement of animal testing. Public and private sector medicines control laboratories from Europe, the Americas, Asia and Australia participate actively in the BSP pro-

jects thus enabling

- a) to successfully validate alternative methods for biomedicines quality control purposes
- b) to enhance regulatory acceptance of the validated alternative methods
- c) to establish reference materials (reagents or Biological Reference Preparations) for alternative methods specified in the *Ph. Eur.*
- d) to promote an internationally harmonised scientific and ethical approach to biomedicines control.

17 projects dealing with “3Rs-compliant” control methods or standards for human vaccines (13), veterinary vaccines (3) and blood products (1), have been initiated within this framework. Integration of new general methods and adoption of new standards by the European Pharmacopoeia have taken place after successful completion of projects; amongst those the following are the most recent achievements: new methods for the control of Newcastle disease (Claasen et al., 2004) and clostridial veterinary vaccines (Lensing et al., 2000; Lucken et al., 2002) and for diphtheria (Sesardic et al., 2003; Winsnes et al., 2003) and tetanus^{2,3,4} vaccines for human use. Ongoing projects deal with various topics: botulinum toxin, human vaccines (inactivated poliomyelitis virus, hepatitis A, hepatitis B and pertussis) and tetanus immunoglobulin. Whenever possible, BSP contributions and plans for 3Rs are communicated to the life sciences community through publications and at meetings. Indeed, synergies between fundamental, medical and pharmaceutical sciences are

¹ European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. Council of Europe, ETS 123, 18.III.1986.



deemed indispensable for promoting animal welfare aspects in biomedicines control whilst guaranteeing quality, safety and efficacy for potential users.

Experiences from the last 20 years of developing alternatives to animal testing by EDQM

It should be noted that the methods used for the control of medicines are subjected to very strict procedures defined by the Codes for human⁵ and veterinary⁶ medicines, including detailed validation, independent assessment, authorisation and supervision of their application. Any change in the authorised methods must follow these procedures, and demonstration of equivalency in terms of quality, safety and efficacy is a prerequisite. Consequently, it is of prime importance that all the actors concerned, i.e. manufacturers, control authorities and regulators, are involved at an early stage in a close collaboration to achieve an efficient and cost saving development of alternative methods. This development has to be based on experimental work with the support and active contribution of all concerned.

The international dimension as well as the inclusion in the studies of so-called “failing” batches (subpotent, impurity loaded, etc.) should always be considered when defining the project plan. Any other approach of the issue will require unnecessary duplication of validation and acceptance.

A clear separation should be made between routine Quality Control (QC) for batch release and investigational work aimed at demonstrating biological activity and safety aspects.

EDQM, through its function of European Standards setting body and its privileged and officialised liaison with the national and international agencies and institutions regulating medicinal products (EMEA, FDA, WHO, PEI, NIBSC, AFSSAPS, etc.), has a unique role to play in the setting up and implementation of alternative methods to animal testing.

The development of alternatives to animal testing is closely linked with the technical development in the domain of production of biologicals, which nowadays includes highly sophisticated purification and biotechnological processes. The application of detailed and controlled working procedures based on good manufacturing practices (GMP) throughout the entire production chain, from the starting material to the final product and its individual packaging, contributes also to a higher quality of biologicals.

Furthermore, technical developments in the field of analytical procedures should enable animal free or animal friendly QC

tests based on molecular biology, immunological/immunochemistry and separative methods to be implemented.

This is now current practice at the R & D laboratory level, especially within the EU. However, implementation of such methods as routine practices through appropriate scaling up (e.g. 1 million individual doses of packaged vaccines) requires enormous investment and full validation at each stage of the industrial scaling up.

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² Collaborative Study for the Validation of Serological Methods for Potency Testing of Tetanus Toxoid Vaccines for Human Use. Part 1. *Pharmeuropa Bio.* 2000 Aug;2000(1):83-124.

³ Collaborative Study for the Validation of Serological Methods for Potency Testing of Tetanus Toxoid Vaccines for Human Use. Summary of all three Phases. *Pharmeuropa Bio.* 2001 Oct;2001(2):73-9.

⁴ Collaborative Study for the Validation of Serological Methods for Potency Testing of Tetanus Toxoid Vaccines for Human Use. Part 2. *Pharmeuropa Bio.* 2001 Oct;2001(2):45-72.

⁵ Code for human medicines: Directive 2001/83(EC) as amended by Directive 2004/27(EC) and 2003/63(EC)

⁶ Code for veterinary medicines: Directive 2001/82(EC) as amended by Directive 2004/28(EC)



USDA 3Rs Initiatives in Veterinary Biologics

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Summary

This paper provides a brief overview of the U.S. system of regulatory oversight of the production of veterinary biologics (vaccines, bacterins, diagnostic test kits intended for use in animals). Alternative test methods have been accepted by the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Services (APHIS), Veterinary Services, Center for Veterinary Biologics (CVB). Both historical and current initiatives in this area are discussed.

Keywords: veterinary biologics, alternative test methods

Background

Federal regulation of veterinary biologics in the United States began in 1913 with passage of the Virus-Serum-Toxin Act. This law was enacted largely because of public concern over the importation of contaminated veterinary vaccines from Europe and in reaction to complaints about worthless and contaminated hog cholera products being sold throughout the country. This law requires the Department of Agriculture to ensure that veterinary biologics (vaccines, bacterins, antiserums and similar products) sold in interstate commerce are pure, safe, potent, and efficacious (CVB "Background").

For nearly 50 years, the biologics programme was carried out by veterinary field inspectors located in the commercial biologics manufacturing establishments and by programme staff in Washington, DC. In 1961, the biologics programme was allocated ten percent of the space at the National Animal Disease Laboratory (now Center) that had just been established in Ames, Iowa. Since October 1996, the veterinary biologics programme has been carried out by the Center for Veterinary Biologics (CVB), a unit of Veterinary Services, APHIS, USDA. There are three units of the CVB: Laboratory; Licensing and Policy Development; and Inspection and Compliance (CVB "Background").

"3Rs Initiative"

The Center for Veterinary Biologics recognises the responsibility to minimise animal pain and distress in research and testing procedures. In 2003, special emphasis was placed on the consideration of alternatives when the following goal was included in the strategic plan: to "significantly refine, replace and reduce animal testing of veterinary biologics" (CVB "Strategic Goals"). These "3Rs" must be accomplished to the degree possible while maintaining credible animal disease and immunity models to correlate *in vitro* and *in vivo* assays of the safety, potency and purity of veterinary biologics.

Replacement

In the 1960s and early 70s, all vaccine serial releases required the vaccination and challenge of target species or surrogate laboratory animals. This testing required a large number of animals to experience pain or distress in order to prove the vaccine released for use by the public was effective. That is, the control animals must succumb to the disease while the vaccinated animals must survive.

The "master seed" principle introduced in the 1970s was a major step towards reducing animal use and is the first example of *in vitro* potency testing. The vaccinate/control challenge potency test for modified live virus (MLV) vaccines was replaced by quantification of the live organisms (titration). Given that approximately half of the regulated biologics products are MLV vaccines, this resulted in an almost 50% decrease in animal use.

Today CVB is evaluating enzyme-linked immunosorbent assays (ELISAs) to determine their usefulness as replacement tests for animal vaccinate/control challenge potency tests. An ELISA test of specific interest is one that measures the presence of a specific protective antigen found on *Leptospira* bacteria. CVB has contracted with a researcher at Michigan State University to determine the viability of the ELISA system as a replacement for the more expensive, time-consuming and painful/distressful hamster potency test.

In addition, CVB is considering new regulations to allow in-process potency testing for serial release. This is being done to take advantage of *in vitro* tests that might not otherwise be possible due to adjuvant and other ingredient interference. These tests hold the potential for improved reproducibility and reliability. There will be some additional tests required to address qualitative and quantitative issues, such as verifying identity and formulation on the final product, but these too would be *in vitro* tests.

Reduction

A significant undertaking in the area of reduction is occurring under the auspices of the Office International des Epizooties



(OIE) through the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). Officially launched in 1996, the third public conference was recently held in Washington, DC in May 2005. Members include the European Union, the U.S. and Japan; Canada, New Zealand and Australia participate as observers.

One of the objectives is to establish and implement harmonised regulatory requirements which meet high quality, safety and efficacy standards and minimise the use of test animals and costs of product development. Thirty-four guidelines have been finalised to-date. An example in the area of animal test requirements is the guideline developed for "Target Animal Safety" (VICH GL41) (FR 2004). It was published for public comment in the U.S. in December 2004. Comments received will be reviewed by the Expert Working Group, and then submitted to the VICH Steering Committee for finalisation and implementation. Once accepted, this will allow manufacturers to conduct one standard test that all the member countries have agreed is adequate for the given purpose.

Refinement

Two recent notices provide examples of acceptance of refinement techniques in biologics testing by CVB.

In April 2004, CVB issued notice of their policy concerning the use of humane endpoints in animal challenge tests. While the regulations still appear to indicate the animals must die from the challenge in order to be considered a valid test, CVB has modified their interpretation to state: "Moribund animals exhibiting clinical signs consistent with the expected disease pathogenesis that are unable to rise or move under their own power may be humanely euthanised and considered as deaths." Death is no longer a required endpoint (CVB Notice 04-09).

This notice also contains the acceptance of an early endpoint for the rabies vaccine challenge test: "Animals exhibiting paresis, paralysis, and/or convulsions may be humanely euthanised and considered as deaths."

More recently the CVB issued a notice regarding "Mouse Safety Testing". This is an announcement that CVB is considering revision of the test requirement outlined in Title 9 of the Code of Federal Regulations, Section 113.33(a)(1) for live virus vaccines. Currently the test requires inoculation of two groups of mice, one intracerebrally and the other intraperitoneally. CVB is proposing to revise the regulation to require that only one group of mice be inoculated, by either the intraperitoneal or subcutaneous route. The potential for pain or distress to the animal by these routes of injection is much lower. Until the regulation is revised, CVB will allow exemptions to the requirement on a case-by-case basis (CVB Notice 05-01).

Future Endeavours

In April 2004, CVB collaborated with the Institute for International Cooperation in Animal Biologics to sponsor a meeting on "Technology and Approaches to Reduce, Refine and

Replace Animal Testing". The objective of this meeting was to discuss policy, provide guidance, and highlight new technologies with the potential for use in developing *in vitro* assays for vaccine potency, consistency, and stability. New approaches to measuring the quantity and quality of antigens in vaccines were discussed with an emphasis on assessing their potential for use in veterinary biologics. The meeting also contained presentations on factors to consider when developing *in vitro* assays and on the statistical approaches used to evaluate their results (available at <http://www.cfsph.iastate.edu/IICAB/meetings/april2004.htm>).

Several interesting new technologies were presented, such as proteomics, Diachemix' differential fluorescence polarisation assays and the Biacore® surface plasmon resonance detection system. CVB is eager to evaluate their potential to augment or replace the animal testing standards of today, with the intent to continue incorporating such 3Rs alternatives into the regulatory framework in the future.

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