

3Rs Symposium

SESSION IV

The 3Rs Concept: The Future Developments

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The 3Rs Concept: The Future Developments The EDQM Biological Standardisation Programme Jean-Marc Spieser Head Division IV EDQM-Council of Europe

The Biological Standardisation Programme

- Within the context of development of alternatives to animal testing
 - Problems encountered and possible solutions
- In the framework of current QC for production batch testing for release by manufacturers and control authorities
- For biologicals for human and veterinary use

The Biological Standardisation Programme(BSP) : general presentation

- Its origin
- Description of the BSP
- Working methods
- Key issues based on example
- Resources
- Procedures
- Achievements and progress
- Perspectives

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BSP origin

- Programme started in 1992
 - Cooperation contract with EU Commission
 - In relation to the implementation of Directives on Biologicals :
 - vaccines - 89/342 and
 - blood/plasma derivatives - 89/381
 - especially Batch Release

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Description of BSP (1)

- A specific programme aimed at contributing to the standardisation of biologicals through
 - a harmonised approach and
 - unique tools
- To guarantee high level quality products
- For Europe and its citizens and in practice far beyond

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Description of BSP (2)

- **Three major activities**
 - **Establishment of common European Reference Standards -in sufficient amounts for OMCLs and Manufacturers- as working standards**
 - **Establishment of validated and standardised assay methods**
 - **Coordinated validation of alternative methods for QC in application of the 3Rs concept**

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BSP-alternative to animal testing

- **Legal environment exist (cf. presentation of Mme Lwoff)**
- **Incentive of scientific world to use state of the art in vitro methods to characterize biologicals**
- **Pressure of public opinion**
- **But must be**
 - **Scientifically sound**
 - **Practicable**
 - **Implementable**
- **Because it involves quality of products and patient safety + efficacy**

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BSP-working methods (1)

- **The stakeholders**
 - **PhEur Commission**
 - **Network of OMCLs**
 - **EU Commission**
 - **EMEA-CPMP/CVMP and working parties**
 - **(manufacturers)**

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BSP-working methods (2)

- **A TEAM WORK BETWEEN COMPETENT PARTNERS**

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BSP-working methods (3)

- **European Directorate for the Quality of Medicines - EDQM**
 - **Division IV-administration, co-ordination and execution of the programme (*interacts with division III-labs and division I PhEur experts groups where appropriate*)**
- **Steering Committee**
 - **11 members and 2 observers**

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BSP-working methods (4)

- **Project leader**
 - **Scientific Expert in the appropriate field generally originating from an OMCL**
 - **Advise EDQM for study (feasibility,protocol,report etc)**
- **DONATORS - manufacturers - of suitable products (candidate standards/testing samples)**

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BSP-working methods(5)

- **Submission of project proposal to Steering Committee**
- **Based on Collaborative Studies**
- **Invitation goes to**
 - OMCLs involved (ie OCABR)
 - Manufacturers present on European market
 - Third parties to foster global approaches and harmonisation
 - WHO, USA/FDA-CBER, USDA, Japan, Cadreac etc

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BSP-working methods (6)

- **Final report with clear conclusion on outcome of the Study**
 - Approved by participants (meeting if necessary) and Steering Committee
 - Adopted by PhEur Commission
 - Goes to stakeholders for consideration/implementation
 - new methods to relevant group of experts
 - Standards added to the catalogue of reference materials available from EDQM

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BSP-working methods (7)

- **Results and conclusions of the collaborative study are published in *Pharmeuropa-Bio* and if appropriate in a peer reviewed international scientific journal - publicly available to document in MA files the use of the new method/standard**
- **Note that *Pharmeuropa-Bio* is referenced in Medline since end 2000**

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Key issues based on examples why to develop alternatives to animal testing(1)

- **Very expensive testing**
 - Large number of animals required
 - Need for very skilled personnel
 - Number of performant labs diminishes
 - Difficulty to maintain competence
- **General global/systemic tests (weight gain/loss) or**
- **Very sophisticated**
 - Growth of tibial bone
 - Hypophyse ectomised animals etc

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Key issues based on examples why to develop alternatives to animal testing(2)

- **Technical development**
 - **Production**
 - Purification
 - GMP
 - Biotechnological processes
 - **Analytical**
 - Molecular Biology
 - Hplc
 - Immunological/immunochemistry
- **Well characterised molecules**

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Problematics encountered in the development of in-vitro methods(1)

- **They are new ! = learning phase**
- **Also very expensive (instruments and reagents)**
- **Availability of suitable reagents - quantity, quality, consistency and continuity in time typical example Hep B/A in vitro assays based on commercial kits which will be withdrawn from the market very soon**

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Problematics encountered in developing in-vitro methods(2)

- **Limitation of correlation studies as both methods the new in-vitro and the classical in-vivo do not measure the same properties of the product**
- **In-vitro not always functional tests**
- **Turns rapidly into a full validation but then need to include « bad samples »(degraded,altered using different techniques, failing batches .etc**

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Problematics encountered in developing in-vitro methods(3)

- **Full validation can mean as much as five years or more of dedicated lab work**
- **Involving both manufacturers and OMCLs**
- **Expensive !**
- **Need for new reagents and Standards IS and BRP no longer usable**
- **New expression of results and potency different from IUs**
- **Implications for the patient -dosing**

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BSP - working principles

- **Always feasibility study prior to**
- **Large collaborative study**
 - **Scientifically sound-generating enough data for statistical evaluation**
 - **Political involvement of majority of interested partners as it will help to transpose the new method in mandatory requirements- facilitates that decision making process through people who were part of the development itself and its future use**

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BSP - working principles(continued)

- **Our work does not always bring to a ready to use situation**
- **Still need for in-house/ individual product specific validation**
- **But applicability and robustness of the method will have been demonstrated**

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Standards

- PRIMARY STANDARD
 - IS OR IRP/IRR
∇ ↓
- REGIONAL STANDARD
 - PH EUR - USP - JP
∇ ↓
- NATIONAL STANDARD
 - D - UK - F - I ...
∇ ↓
- IN-HOUSE STANDARD
- MANUFACTURER - OMCL
 - ∇ ↓
- LOCAL OR REGIONAL TRADE/SCIENTIFIC ASSOCIATION

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Resources (1)

- **EDQM Div.IV**
 - **5 scientists including a Biometrician/statistician**
 - **Div.III Biolab**
- **Project Leaders of excellent quality and dedication**
- **Study participants voluntary (QAS in place)**

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Resources (2)

- **Funding**
 - EU Com.
 - Self financing (sales of standards/BRPs)
- **Support to project leaders**
- **Development and monitoring programme at EDQM**
- **Co-sponsorship on exceptional cases ECVAM, national programmes ZEBET ...etc**

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Procedures

- **Ensures efficiency and customer satisfaction**
- **Structure and instruction well defined for all key steps internal or external**
- **Monitoring in place where appropriate**
- **Storage and shipment Good Practices in place**

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Achievements and progress (1)

- **BSP since 10 years**
 - 63 projects initiated
 - 41 projects already finalised
 - 14 projects involving alternative to animal testing finalised by end of 2001
- **5 projects currently in development**

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Perspectives (continued)

- **IVMPs: intensification of development of tools for facilitating implementation of alternatives to animal testing**
 - **Methods**
 - **Specific reagents**
 - **New standards**

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Your EDQM team

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**New Approaches to Vaccine
Quality Control:
Veterinary Industries Experience**

KEITH REDHEAD
Intervet UK Ltd, Milton Keynes, UK



Two Approaches

-
- New assays/methodologies
 - Novel interpretation of existing data



In-VITRO

International Veterinary Industry Test Replacement Organisation

•Established in 1995

•CHAIRMAN: Roger Lucken



AIMS

- REDUCTION
 - Improve understanding of mechanisms at work in *in-vivo* assays to minimise numbers of animals
- REFINEMENT
 - Development, validation and standardisation of *in-vitro* serological assays to replace challenge tests
- REPLACEMENT
 - Subsequent replacement of serological assays with validated potency assays based on the quantitation of protective antigens



APPROACH

- Commitment and involvement in the development of alternative *in-vitro* test methods
- Standardisation of antitoxins
- Establishment of a supply of *in-vitro* standards
- Collaborative studies
- Liaison with European Pharmacopoeia and USDA
- Liaison with workers in research institutes and regulatory authorities
- Obtain approval of regulatory authorities world-wide



***Cl.chauvoei* Guinea Pig Potency Test Requirements**

An example of
REDUCTION



***Cl.chauvoei* Potency Test Requirements 1997**

TreatmentNumbersNo.survivorsResultRe-testControls50Valid testN/ACor



***Cl.chauvoei* Potency Testing for Stability**

***Cl.chauvoei* potency test result**Batch0 mths6 mths12 mths18 m



***Cl.chauvoei* Potency Testing for Combined Manufacturers**

Initial Testing No. of batches No. passing % passing 3



***Cl.chauvoei* Potency Testing for Combined Manufacturers**

Repeat Testing No. of batches No. passing % passing



***Cl.chauvoei* Potency Test Requirements 1998**

Test Type Numbers No. survivors Outcome Initial 10 10 Pass Initial 109 Pass Initial



Establishment of a Multi-component Reference Serum for Veterinary Clostridial Vaccines

An example of REDUCTION and REFINEMENT



PHASE 1

Validation of manufacturers serum pools and correlation of different *in-vitro* methods

Preparation of Protocol

Donation and checking of sera

Freeze-drying, coding and distribution of serum pools

Testing of serum pools (in-vivo and in-vitro) by different manufacturers

Analysis and reporting of results

Preparation of serum pool blends

Freeze-drying, coding and distribution of blends

Testing of blends by an in-house and common in-vitro method

Analysis and reporting of results



PHASE II

Validation of final serum pool blend

Preparation of protocol

Checking, freeze-drying, coding and distribution of blended sera

Collaborative Study

Analysis and reporting of results

Adaptation and revision of monographs and guidelines



Assigned Antitoxin Activities for Clostridial Rabbit Antiserum BRP

- *Cl.perfringens* beta 10.5 IU per vial
- *Cl.perfringens* epsilon 11.0 IU per vial
- *Cl.septicum* 7.5 IU per vial
- *Cl.novyi* type B 11.0 IU per vial
- *Cl.tetani* 8.0 IU per vial



Development of Antigenic Mass Assays

An example of REPLACEMENT



Antigen Mass Determination (Bacterial)

Two purposes:

1. Antigen quantitation for vaccine blending
2. 'Potency' testing of final vaccine



Background

From QC for final product to QA of production process

Move away from using animals -

- welfare
- cost
- reliability/consistency
- speed



Approach

- Ph.Eur. monographs allow for validated alternatives
- Potency of product demonstrated by efficacy in target species
- Reduced strength vaccine efficacious
- Assay (ELISA) to measure protective antigen
- Assay to discrimination between full and reduced antigen contents
- Vaccine based on a fixed antigen content
- Assay to demonstrate consistency of product
- Batch to batch variation < test to test



Optimal Methodology

- Vaccine efficacy proven full and reduced
 - Efficacious vaccine as reference
 - Pre-treatment of vaccine (adjuvant)
 - Antigen capture (direct, polyclonal, Mab)
- Antigen detection (protective Mab or polyclonal)
- Antigen quantitation (not significantly <100% reference)



Regulatory Requirements

Based on VICH1 and 2 guidelines

Methodology - SOP, appropriate

Accuracy - International reference or 'in-house', monovalent or polyvalent

Precision - Repeatability, intermediate precision, reproducibility

Specificity - One antigen among many, spiking experiments



Regulatory Requirements

Detection Limit - Sensitivity, < reduced vaccine

Quantitation Limit - not applicable

Linearity - May not be demonstrable

Range - Full to reduced

Controls - Reduced reference preparation, vaccine base

Measurement/Statistics - OD's, multicalc, parallel line, t-test

Robustness - Incubation temp, incubation time, agitation rate, conjugate concentrations



Regulatory Requirements

Reference preparations and reagents - Availability, preparation methods, stability

Validation - Relevance, correlation

Limits - Maximum, as well as minimum, criteria for antigen content.

Adjuvant - Quantity and quality assessment



Potential Problems

- **Reduced vaccine non-efficacious**
 - Increase antigen content**
 - Improve detection limit**



Potential Problems

- **Reference preparation replacement**
 - Replacement efficacy study**
 - Replace within shelf-life**
- **Adjuvant interference**
 - Pre-treatments**
 - Alternative capture methods**
- **No antibodies to protective antigens**
 - Surrogate correlates of protection**



Potential Problems

- **Assay does not distinguish between full and reduced antigen vaccines**
 - Accuracy of antigen blending**
 - Improve assay**
- **Test batch results different to reference**
 - Similarity of reference and test vaccines**
 - Consistency of test vaccine antigen content**
- **Lack of specificity**
 - How bad and how relevant**



Potential Problems

- Lack of Linearity
If full > reduced may not be necessary
- Batch to batch variation > test to test variation
Consistency of antigen content



Conclusions

3R's can be implemented in new approaches to vaccine QC in several ways:

REDUCTION - Use of historical data and statistical analysis to ensure minimum number of animals used. From TNT to serology

REFINEMENT - From challenge tests to serological assays

REPLACEMENT - From QC to QA. From in-vivo potency to antigen quantification with proven efficacy and consistency of production.



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