

**QUALITY ON THE MOVE  
DYNAMICS OF THE EUROPEAN PHARMACOPOEIA**

**Workshop Session  
Biological Standardisation**

**Moderators: R. Dobbelaer & J. M. Spieser**

**08:30-10:00**

## The European Pharmacopoeia/EDQM Biological Standardisation Programme (BSP)

K.H. Buchheit (EDQM, Strasbourg)  
Roland Dobbelaer (SIPH, Brussels)

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### BSP - General Goals

For the quality control of biologicals

- Establishment of Ph. Eur. working references (BRPs)
- Development / improvement / standardisation of test methods
- Support application of 3R concept (Refine, Reduce, Replace *in-vivo* test methods)
- Contribution to international harmonisation (ICH, VICH) by collaboration with WHO, FDA, Japan

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### BSP - Procedure (1)

1. Proposal for new method or BRP
2. Decision by Steering Committee
3. Procurement materials
3. Collaborative study (7-50 participants, OMCLs, manufact.), external project leader + EDQM co-ordinator
4. a) BRP: Adoption EP Commission  
b) Method: Gr. of Experts --> Monograph
5. Publication of results (Pharmeuropa-Bio, scientific workshops, scientific journals)
6. Monitoring of BRP quality

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## BSP - Procedure (2)

### Average Duration of Projects

- BRP replacement batches: ca 1 year
- New BRPs: ca 2 years
- New methods: > 3 years

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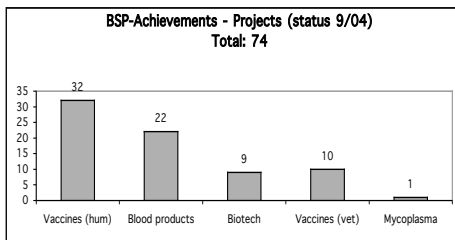
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## BSP-Achievements (1)

### Projects

- 74 projects                      50 projects completed
- 24 projects ongoing        7 projects in preparation



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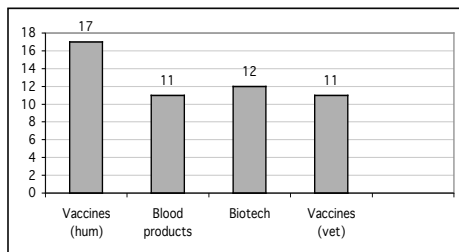
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## BSP-Achievements (2)

### BRPs

Total: 51 BRPs



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### **BSP-Achievements (3)**

#### *Methods*

- **Tetanus vaccine: serol. potency assay**
  - Included in Ph. Eur. General Chap.
  - Critical reagents available from EDQM
- **Hepatitis A vaccine: in vitro assay**
- **IPV: immunogenicity assay**
- **IFN-alfa: HPLC method**

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### **BSP-Highlights in 2004 (1)**

#### *Diphtheria vaccine: Alternative Assay (BSP034)*

- **Current EP assay: direct challenge (intradermal, lethal)**
  - high number of suffering animals
- **Goal: Serological assay, same sera for tetanus and diphtheria vaccines**
  - halve animals number, less suffering
- **Results presented at Intern. Symposium, Budapest, Oct. 2004;**

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### **BSP-Highlights in 2004 (2)**

#### *Newcastle Disease Vaccine: In Vitro Assay (BSP055)*

- **Current EP assay: direct challenge or serology**
- **Goal: Antigen content assay (ELISA); provide necessary BRP & reagents (e.g. coating- & detection antibody)**
  - no use of animals !
- **Correlation with challenge and serological potency**
  - 9 different vaccines from 5 manufacturers
- **"Model" project**
- **EP monograph under revision to incorporate new assay**

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### **BSP-Highlights in 2004 (3)**

#### ***Vaccinia Immunoglobulin (VIG) BRP (BSP066)***

- **Goal: Establish VIG BRP for development of smallpox vaccine and VIG potency assay (treatment of vaccine complications)**
  - only British standard available (hepatitis B positive)
  - Fast track project (fight against bio-terrorism)
  - start 6/03, currently data evaluation & reporting

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### **Future BSP Studies**

- **Hepatitis A vaccine BRPs (product- and non-product specific)**
- **von Willebrand factor CoBA BRP**
- **Tetanus Ig: alternative assay**
- **Equine influenza antiserum BRP (replacement)**
- **Erythropoietin BRP replacement batches**

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### **Impact of BSP**

- **Public health (safety/potency of biologicals)**
- **Animal welfare**
- **Economical use of resources**

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**Happy 10th anniversary**

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Council of Europe

**Biological standardisation:  
what for and for whom?**

Budapest, October 4-6, 2004  
A.M. Georges, EVM/GSK Bio

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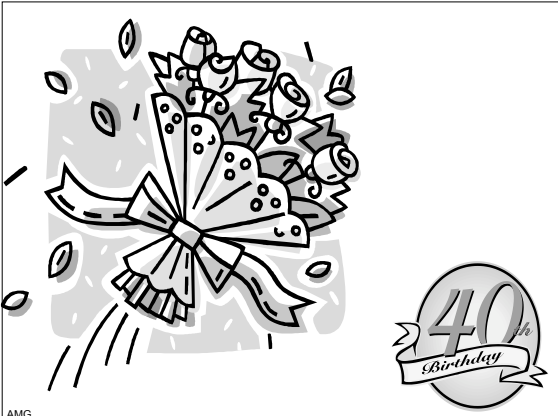
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**Biological standardisation  
MENU**

- Aims
- Benefits
- Possible improvements and needs
- Anticipated needs for and advantages of worldwide globalisation

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## Aim of Biological Standardisation

- Harmonising the control of quality of Biological Medicinal Products in Eur. Pharm. Countries
  - EU standard reference preparations
  - Alternative methods
    - Developed taking into account technical progress, validated, simplifying existing ones, reducing animal use ....
    - Integrated in Eur. Pharm.
  - Updated Monographs
- Cooperation to international harmonisation

WHAT FOR?

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## Biological Standardisation, by whom?

- Council of Europe/ EU agreement
- EDQM coordinated
- Collaborative studies
- Discussion, exchanges...
  - All partners involved: OMCL's.....industry...
  - FACILITATING MUTUAL RECOGNITION
  - BUILDING CONFIDENCE
  - TRANSFER OF TECHNOLOGY

BY WHOM?

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## Benefit of Biological Standardisation

- ***Speaking (together) about the same things***
  - Starting from various methods, materials, calculations, interpretations....
  - Exchanges and collaborative studies succeed in
    - ....Demonstrating "comparability" or equivalence of tests and products (suitability, effectiveness of methods)
    - ...Simplifying tests (D and T potency, neurovirulence, MAPREC...) and reduction of animal testing
    - Establishing reference preparations (+ exceptions)

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## Benefit of Biological Standardisation

- ***Speaking together (about the same things)***

- Networking and collaborative studies
  - Improve quality of work and develop high level of knowledge and requirements
  - Create contacts between all partners and facilitate confidence building (EDQM, EU, OMCL's, Industry)
- Success of EDQM network of OMCL's and of recognition of batch release

LEARNING FROM EACH OTHER

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## Benefit of Biological Standardisation

European Level

- ***Contribution to the achievement of the Free Circulation within the EU***

FOR WHOM?

- Allows harmonisation of dossiers for marketing authorisation application
  - Identical specifications and methods of analysis, references...
  - Required in all Regulatory procedures, Centralised, Decentralised, National
- Facilitates mutual recognition of batch release between OMCL's
- Avoids repetitive testing (time, workload, cost)
- **Reduces time for accession of medicines to patients**

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## Biological Standardisation: further needs and improvements?

Europe: actions

- Need for evaluation of clinical parameters (suitability, Flu serological testing)
- Need for comparisons between products (DPaP: unknown as internal references)
- Single standards? (Hep B?)
- Efforts toward "exclusion" of animal testing

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## Biological Standardisation: further needs and improvements?

European level

- Distributions of references to OMCL's shall be done by EDQM, not by companies (OMCL's more independent; companies to send references to EDQM, not to OMCL's)
- References established by EDQM (workload, cost, validation) shall be used by manufacturers (ex: antisera Pertussis antigens)
  - Developed after evaluation of needs
  - By collaborative studies
  - Why not used?
- Companies sending references to EDQM have to pay for getting those for their own use?

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## Biological Standardisation: further needs and improvements?

Europe and the world

- International organisations should collaborate with EDQM rather than with individual control laboratories
- Non European countries should be more and more encouraged to participate in collaborative studies

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## Biological Standardisation: GLOBALISATION

For whom? Europe and the world

- Harmonisation within EU acceding countries achieved
- Collaboration to ICH ongoing
  - Harmonisation of Eur. Pharm., USP (CFR?), JP?
- Need for globalisation, i.e. other third countries
  - ASIA
  - LATIN AMERICA
  - (AFRICA).....Wish to participate

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## Vaccines: Batch release actually tested in third countries

- **Extra administrative and lab. work. (Time, cost, human resources)**
  - 3 times QC testing: manufacturing company, EU OMCL, importing country
  - numerous samples, various reagents (burden)
  - discussions re/ divergent results (rejection)
  - local equipment, animals, experience
  - new high-tech products
  - assistance to third countries official laboratories
  - Increased number of animals

EVM members manufacture 85% of vaccines used in the world

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## Biological Standardisation: GLOBALISATION

For whom?  
Europe and the world

- Benefit of globalisation
  - Facilitating dissemination of information
  - Speaking the same language
  - Allowing recognition of EU tests and references and Eur. Pharm
  - Facilitating actions of and harmonisation with WHO
  
  - Facilitating approval of EU authorised products in third countries (same dossier)
  - Facilitating recognition of batch release carried out in the EU by third countries

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## GLOBALISATION

Europe and the world

- High quality level for the world
- ...Through EDQM and WHO
- Contribution to Confidence and...PEACE



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*Aventis Pasteur* 

**Challenges for vaccines of the future:  
standardization of new testing**

□

European Biological Standardisation Programme

**A. Sabouraud**

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*Aventis Pasteur* 

**Standardization of vaccine testing : why ?**

- To ensure that methods used in one lab for release, process validation, stability deliver accurate and reproducible results.
- To ensure that the same method used in different labs (ex: Manufacturer vs OMCL) give same results

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*Aventis Pasteur* 

**Standardization of vaccine testing : how?**

- **Standardization of the method (operating conditions)**
  - EP monographs and/or in-house assays
  - Assay validation (accuracy, LOD, LOQ, interm. precision,...): # of replicates
  - Robustness studies (multifactorial plan) during the test development
  - Calculation programs – data statistical analysis
  - Transfer validation studies
- **Use of international or in-house standards (calibrated and monitored)**
  - collaborative studies (EDQM)

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### EP monographs and standardization

- Most of current vaccines are manufactured using « generic processes » (D, T, IPV, MMR, ...)
  - monograph is a good tool
- General monographs: Assays are described very differently in level of content
- New vaccines will be more manufacturer-specific (viral vector, coupling chemistry for PS, ...)
  - limitations of monograph system (one monograph per product?)

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### New techniques and OMCL testing

- Is there a need to duplicate release testing at manufacturer and OMCL for sophisticated techniques (NMR, mass spec)?
- Could development of expertise centers in the OMCL network be a good approach for specific techniques?

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### Examples of new analytical approaches

- Molecular sizing of polysaccharides or PS conjugates
- Quantitation of polysaccharides
- Nuclear magnetic resonance
- Mass spectrometry
- Host cell protein quantitation
- DNA quantitation and sizing in viral vaccines

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Aventis Pasteur sanofi **aventis**  
L'essence d'être la santé.

### New Analytical approaches Molecular sizing of polysaccharides

UV or RI

Elution Kd  
Relative value  
 $Kd = (V_e - V_0) / (V_t - V_0)$

MALLS  
(LS, RI, UV)

- Mw – extrapolation to 0 angle  
- Polydispersity (Pd)  
- Radius of gyration (Rg)

- Mw – correction with viscosity data (RALLS)  
- Mw – without correction (LALLS)  
- Polydispersity (Pd)  
- Radius of gyration (Rg)

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### Standardization of PS molecular sizing methods

**HP- or LP-SEC with RI or UV detection**

➤ limitations:

- variability of Kd due to high inter-column variability
  - ⇒ use of PS such as dextrans for column qualification
- Kd not an actual measure of molecular size but reflects hydrodynamic volume
  - ⇒ use of MALLS or RALLS detection

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### Standardization of PS molecular sizing methods (cont'd)

**HP-SEC with RALLS on PRP-T**

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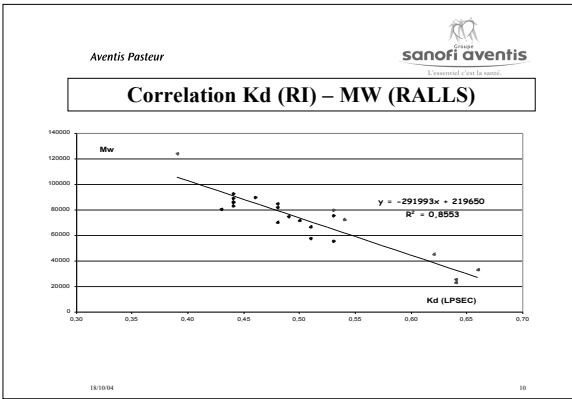
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Aventis Pasteur 

**Standardization of PS molecular sizing methods**

HP-SEC with RALLS detection

➤ advantages:

- MW measurement independent of separation characteristics of the column
- access to new parameters such as polydispersity and gyration radius
- standard : synthetic PS (monodisperse) such as pullulans

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Aventis Pasteur 

**Quantitation of polysaccharides by HPAEC-PAD**

HPAEC-PAD = method of choice for PS

➤ Sample pretreatment : NaOH or HF hydrolysis (conditions to be optimized)

➤ Chromatography

➤ Standards:

- Polysaccharide (production lot calibrated using colorimetric method for ex.)
- monosaccharide (if existing)

Issue to be resolved in test standardization : stability of saccharides to hydrolysis

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### Nuclear magnetic resonance (<sup>1</sup>H-NMR):

**Objective: quantitation of polysaccharides and solvents in manufacturing intermediates**

▪ **Quantitation of residual solvents by CPG-HS (ethanol, acetone, ether):**

> GC with Head-Space injection vs <sup>1</sup>H-NMR

▪ **Quantitation of polysaccharide**

> HPAEC-PAD vs NMR

> **Standard used for quantitation: DMSO 0.01% in D2O**

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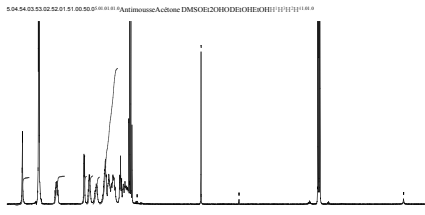
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### <sup>1</sup>H-NMR spectra (PS and impurities) of PRP




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### NMR: comparative results (25 batches)

	GC or colorimetric method Mean (SD)	NMR Mean (SD)
PRP (%)	77.8 (4.3)	73.6 (4.3)*
Ethanol (%)	11.9 (2.6)	11.8 (1.6)*
Ether (%)	0.2 (0.1)	0.3 (0.1)*
Acetone (%)	0.2 (0.)	0.05 (0)*

\*: not statistically significant

Conclusion: DMSO suitable as a standard for quantitative NMR

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### Testing of Host Cell Proteins

#### Test standardisation issues (1): (HCP)

➤ Qualified anti-HCP reagent (antisera)

- Immunogen nature: native or denatured cell lysate  
(NB: generally accepted that sensitivity higher with denatured cell lysate for WB)
- animals for antisera production  
protein non immunogenic in some species
- reagent characterization

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### Testing of Host Cell Proteins

#### Test standardisation issues (2): (HCP)

➤ Source of anti-HCP reagent

- commercialized reagent versus in-house reagent (main criteria sensitivity)  
NB: large amounts are required for product development
- how to bridge (calibrate) between reagents
- no international standard (crucial need)  
EDQM project ?

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### Cell substrate DNA quantitation and sizing

#### Challenge for new viral vaccines using continuous cell substrates (vero, PerC6...)

- Limit the residual DNA content to 100 pg (FDA) or 10 ng (WHO – EMEA) per dose
  - DNA quantitation by Threshold or qPCR
- Reduce Vero DNA size (enzymatic treatment using DNase, Benzonase)
  - DNA size distribution by qPCR

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### Cell substrate DNA quantitation and sizing (cont'd)

Threshold method (immuno-enzymatic method to single strand DNA)

• **Advantages :**

- Sensitive (10 pg/ml)
- Quantification of all types of DNA with a very good specificity

• **Disadvantages :**

- Operating conditions difficult to standardize (sample-dependent)
- Variability, quantification range (5 to 150 pg/well), invalidity rate, low throughput
- Inhibition by fragments below 80 bp
- Sterile samples (without bacterial DNA)
- no information on the DNA size

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### Cell substrate DNA quantitation and sizing (cont'd)

#### qPCR

• Specific DNA quantification by real-time detection of PCR-amplified product by emission fluorescence monitoring

• Detection of a target sequence (Beta-actin for Vero DNA) - small fragment (59 bp for Vero DNA)

• Detection using a probe (Taqman...) or an intercalating agent (SybrGreen)

• Standard curve : 100 ng to 1 pg/reaction

• Possibility to use an internal control to evaluate the reaction yield (Duplex qPCR)

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### Cell substrate DNA quantitation and sizing (cont'd)

#### qPCR

• **Advantages :**

- Specific detection (Viral DNA/cellular DNA), sensitive (theoretical : 10 pg/ml) and fast (2 days)
- Single-stranded and double stranded DNA detected up to the defined size
- High throughput (up to 70 samples/qPCR)
- Wide range of detection : 100 ng/reaction to 1 pg/reaction

• **Disadvantages :**

- Do not quantify fragments below the size of the PCR product (59 bp for Vero DNA)

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**DNA content by qPCR (viral vaccine under development)**

Lot	qPCR	Threshold
1	150 pg/ml CV=41%	≤770 pg/ml
2	400 pg/ml CV=18%	≤770 pg/ml
3	280 pg/ml CV=28%	≤770 pg/ml
4	310 pg/ml CV=7%	≤410 pg/ml

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**DNA size distribution (copies/ml) (viral vaccine under development)**

Samples	59 bp fragment	108 bp fragment	407 bp fragment	620 bp fragment
1	499	0	0	0
2	410	0	0	0
3	1442	93	0	0
4	844	0	0	0

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**Conclusions**

- new high-tech technologies are now available for a better characterization of vaccines and open a new era in the vaccine development
- these instrumental techniques require specific technical competencies
- qualification of equipment is quite complex and most of the time difficult (CFR 11)
- Some of them could be used for routine release technique
- standardization of techniques remains a challenge
- definition, production, characterization, calibration, monitoring of standards are crucial and need input from EDQM

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### Acknowledgements

- S. Uhrich
- L. Mallet
- F. Brunel
- F. Guinet-Morlot
- M. Chevalier

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