Overview of the EDQM Biological Standardisation Programme

European Pharmacopoeia Training Session on Biologicals
4-5 February 2020
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Presentation Overview

- EDQM Biological Standardisation Programme
- Scope
- Why Focus on Biologicals?
- ...and Standardisation
- Standardisation What Do We need?
- Goals of the EDQM BSP
- How Does the EDQM BSP Help?
- What EDQM BSP Does Not Do
- Organisation
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- Special Considerations
- Focus on Step 3: The Collaborative Study
- Focus on Step 3: The Collaborative Study Phases

EDQM Biological Standardisation Programme (BSP)

- Exists since 1991
- Contract with EU since 1994 - coincides with the creation of the OMCL network
- Sponsors
  - Council of Europe /EDQM
  - EU Commission

Dissemination of Results
Pharmeuropa Bio & Scientific Notes
Accessibility of BRPs and Leaflets
Examples of BSP Projects Past and Present
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Designed For Purpose
A Few Words About Method Development For 3Rs
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Thanks
Scope

- **Biotech products** (Group 6, MAB, P4BIO)
  (hormones, cytokines, anticoagulants (heparins), mAbs...)
- **Blood products, contaminants** (Group 6B)
  (immunoglobulins, coagulation factors...)
- **Vaccines, sera for human use** (Group 15)
- **Vaccines, sera for veterinary use** (Group 15V)
- **Miscellaneous** (specific working groups)
  (allergens, endotoxins, mycoplasma...)

Why Focus on Biologicals? (1)

**Nature of the products**

Biological products are
- complex molecules, mixtures
- produced by a biological process
  - human, animal, recombinant...
  - purified, fractionated, concentrated,...
- inherent with biological variation
  reflecting molecular heterogeneity
- Characterised by their biological activity and structure/function
Why Focus on Biologicals? (2)

Nature of the control/characterisation tests

Tests

In vivo
- direct : challenge assays, body temperature,...
- indirect : serology assays,...

In vitro
- cell culture assays
- immunoassays (ELISA, ToBI,...)
- nucleic acid amplification
- physico-chemical characterisation
- specific activity

→ inherent (biological) variation/heterogeneity
  - of the samples
  - of the reagents, references, readout systems, assay conditions,...

→ Historic reliance on animal models for routine testing

Variation sources
- animal strain & individuals,...
- immunisation design,...
- cell lines & culture conditions,...
- reagents, testing conditions,...
- sample preparation
- polydispersion, glycosylation,...
- conversion factors (IU-mg)

...and Standardisation?

Interest of standardisation

- Reliable, relatable results for manufacturers batch to batch
- Supports independent control by authorities and comparison of results
- Supports exchange and mutual recognition of control results
- Provides confidence in values for clinical use

Context
- Licencing decisions e.g. centrally authorised products
- Investigations e.g. adverse events, suspect adulterated or counterfeit product, ...
- Exchange of products in global markets
  - Access e.g. stock shortages (due to production issues, emergencies: natural disasters or outbreaks,...)
  - continuity of treatments (for travellers, for use of related products...)

→ need for comparison & trust in results obtained by different laboratories
→ global exchange of data and medicines in the interest of public health
Standardisation: What Do We Need? (1)

Common - reference standards
- critical reagents

Chemical Reference Substances (CRS) → Physico-chemical tests

Biological Reference Preparations (BRP) → Biological assays (majoritarily)

Biological Reference Reagents (BRR)

Reference standards for use in biological assays
→ are usually assigned an arbitrary unitage:
  ‘amount that induces a defined biological effect’
→ represent the average response
  from a large number of assays from various laboratories

Standardisation: What Do We Need? (2)

For biological assays common unitage is generally defined via a primary standard in International Units

International Standards (IS) are primary standards for biological activity

• Established through international collaborative studies – usually not method specific
• Assigned a value in International Units (IU), arbitrary units defined by the World Health Organization (WHO)
• Primary role to define the unit
• Stable formulation provides a long-term reference point for calibration of secondary standards for ‘regular’ use
Standardisation: What Do We Need? (3)

Ph. Eur. Reference standards for Biologicals are:
- working standards for use in conjunction with the Ph. Eur. texts
- officially adopted by the Commission of the Ph. Eur.
- calibrated against the corresponding WHO IS (when existing)
- assigned with a content
  • in IU (when available)
  • in Ph. Eur. Units or other relevant units when no IS are established
  • mg/vial, chromatogram (e.g. CRS, BRP for system suitability)

The calibration of BRPs requires data from a large number of laboratories
→ need for coordinated international collaborative studies

Standardisation: What Do We Need? (4)

Common validated methods
- transferable
- acceptable intra-laboratory variation
- acceptable inter-laboratory variation
- suitable for various products

And what would we like?
To REPLACE, REDUCE, REFINE the use of animals (3Rs) for quality control testing
- Scientific, ethical and practical motivation
  • Develop better, more reproducible/precise tests with state of the art validation
  • Humane use of animals
  • Potential cost reduction and sustainability
Goals of the EDQM BSP

Support Quality Control of Biologicals

- Establishment of Ph. Eur. working standards (BRP/CRS) & reagents (BRR)
- Standardisation of methods for Ph. Eur.
- Application of 3R concept (refine, reduce, replace) to Ph. Eur. methods
- International harmonisation: collaboration with WHO, OIE and national authorities

How Does the EDQM BSP Help?

- Provides a ‘neutral’ independent space for exchange (anonymised participants/samples, central calculation)
  - Independent laboratories from national authorities e.g. OMCLs
  - Laboratories from different manufacturers
  - Qualified academic/other labs
  - National authorities and experts

- Uses large scale collaborative studies to:
  - Demonstrate the general applicability and recommended use of methods already validated in a ‘local/small scale’ context
  - Establish commonly assigned values for Ph. Eur. reference standards

- Provides a key link between practical work of laboratories and the Ph. Eur.
  - Successful methods and standards are included in the Ph. Eur. and have a legal standing

- Focus on the European market with an eye on global participation and acceptance (common reference material and method recognition where possible)
What EDQM BSP Does Not Do

- Method validation outside the QC field
- Method development from scratch
- Complete validation for individual products
- Method validation for single products or single manufacturers

Organisation

**Steering Committee**
Includes:
- Chairs of Ph. Eur. biological groups (6, 6B, 15, 15V)
- Interested parties’ representatives
  - European Medicines Agency (EMA); BWP, IWP
  - EU Commission
- Co-opted experts (human and vet)
- EDQM Director
- Observer from World Health Organisation (WHO)

**Project Leaders**
- Nominated technical experts for a given study, bound by confidentiality agreements

**EDQM DBO:**
- Technical secretariat; coordination & management of projects
Method of Work – Overview (1)

1. Proposal for new BRP/method (from Expert Group, OMCL, manufacturer...)
   - Relevant to the Ph. Eur.
   - Mature method with validation package (no research/development required)
   - Applicable to most products
   - No proprietary method/reagents

2. Decision by Steering Committee
   - Start of the project (if possible run jointly with WHO, US-FDA, USP to establish harmonised/common reference standards and methods)
   - Nomination of external expert as Project Leader

3. Launch of BSP project including collaborative study

Method of Work – Overview (2)

3. Reporting and updates on project to BSP SC for advice and decisions on critical steps
   - BSP SC meets face to face twice/year
   - Written/exchange as needed

4. Approval of final report by BSP SC, relevant Ph. Eur. Group of Experts


6. Publication in Pharmeuropa-Bio & SN
Special Considerations

- Studies can take a long time (in particular method validation) – requires stamina, perseverance and adaptability
- Assay standards: in most cases IS is needed for calibration
- Whenever possible collaboration with WHO/NIBSC for simultaneous establishment of IS & BRP
- As far as possible study participants include a mix of OMCL and Industry labs from Ph. Eur. member states/observers
- Respect of the 3Rs in study design

Focus on Step 3: The Collaborative Study

- Aims at
  - Confirming the suitability of and calibrating / assigning a unitage to a candidate RS
  - determining the transferability of a method and general applicability
- Includes
  - A pool of international participants: OMCLs, manufacturers, authorities
  - A variable number of laboratories depending on methods
    (<10 in vivo, up to 50 in vitro)
  - a common protocol, reagents (as needed) & reporting sheets
  - a central analysis of the datasets
  - a review of the study report at successive levels:
    participants, relevant group of experts, BSP Steering Committee, Ph. Eur. Commission
  - a publication
Focus on Step 3: The Collaborative Study Phases (1)

**Phase 1 - Preparation** with Project Leader(s)
- Procure candidate starting material
- Produce batch(es) of candidate standard
- Pre-test in small number of labs
- Stability studies
- Elaborate study protocol
- Interaction with statistician for study design
- Invite participant laboratories
  - OMCLs, manufacturer (EU and other)
- Prepare for shipping (permits etc.)

Focus on Step 3: The Collaborative Study Phases (2)

**Phase 2 – Collaborative study** with all participants
- Distribute common samples
  - calibrants: current WHO IS and/or Ph. Eur. Ref. Standard
  - candidate replacement batch(es)
  - additional samples, critical reagents
- Distribute common study protocol
  - materials and methods
  - special instructions
  - reporting sheets
  - deadlines and contact details
- Return of results and central analysis
  - Assignment of a unitage to the candidate
  - Evaluation of assay variation
  - Consider continuity with IS and previous BRP
- Draft study report - anonymised data sets
Focus on Step 3: The Collaborative Study Phases

**Phase 3 – Reporting Phase with manufacturers (primarily)**

- **Optional phase based on specific need and study outcome**
  - e.g. new reference standard, new method
- **Collect and analyse data from routine production lots**
  - Provides additional input on suitability
  - Helps to determine criteria for inclusion in Ph. Eur., specification setting

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**Dissemination of Results**

BSP studies results are:

Reported to:
- Study Participants - pre-check of draft report before further distribution
- BSP Steering Committee
- Involved Expert Groups
- Ph. Eur. Commission

Published in:
- Pharmeuropa-Bio & Scientific Notes
- Peer reviewed scientific journals (some studies)

In some cases (e.g. new methods, issue of global interest) a symposium is organised by EDQM
Complete study report with all methodological details published in electronic format, FREELY ACCESSIBLE VIA EDQM WEBSITE

Accessibility of BRPs and Leaflets

BRPs/BRRs/CRS are available via the EDQM catalogue and can be searched for on-line
https://crs.edqm.eu/

You can also download the European Pharmacopoeia Daily Reference Standards catalogue:
### Examples of BSP Projects Past and Present (1)

**160 BSP projects**

123 projects on BRP/CRS/BRR establishment  
about 100 batches of more than 60 different BRP/CRS  
BRPs/CRS monitored throughout their lifetime

- **Human vaccines**  
  Antigens, toxoids, toxins  
  Antisera,...

- **Veterinary vaccines**  
  Antigens  
  Challenge strains  
  Antisera,...

- **Blood products**  
  Immunoglobulins  
  Clotting factors  
  Contaminants,..  
  Normal and specific Ig (tetanus, hepatitis,...)  
  Coagulation factors VII, VIII, IX, albumins,..  
  HAV & HEV RNA, prekallikrein...

- **Biotech products**  
  Erythropoietin, somatropin, heparins, etanercept, infliximab...

- **Miscellaneous**  
  Mycoplasma strains, allergens, endotoxins...

### Examples of BSP Projects Past and Present (2)

**Validation of improved & alternative 3R methods**

43 projects on method validation  
>25 projects on 3R methods - to refine, reduce, replace animal use

- **Human vaccines**  
  Serology methods, alternative 3R  
  Diphtheria, tetanus, and  
  Hepatitis A, rabies  
  Inactivation, immunogenicity & antigen content,..  
  Inactivated Poliomyelitis Virus immunogenicity & antigen content,..  
  Immunogenicity & antigen content,..  
  Alternative 3R in vitro assays  
  Streptococcus pneumoniae, swine erysipelas, Newcastle disease,..  
  Improved agglutination method, alternative 3R potency assays  
  Isoagglutinins direct method, Tetanus immunoglobulins,..  
  Alternative 3R & improved physico-chemical tests  
  Somatropin, heparins, erythropoietin, INF alfa-2,..
# Uses of BSP References in the Ph. Eur. (1)

### Physico-chemical tests

- Content in mg/vial
- Chromatogram(s)/spectrum

- **Identification Tests**
  - Molecular size distribution
  - related proteins
  - Impurities
  - assay for protein

- **Zone electrophoresis** (2.2.31)
- **Capillary electrophoresis** (2.2.47)
- **Peptide mapping** (2.2.55)
- **PAGE** (2.2.31)
- **SEC** (2.2.30)

- **Erythropoietin for physicochemical tests CRS**
- **Erythropoietin for SEC system suitability CRS**
- **Heparin Low-molecular mass for calibration CRS**
- **Somatropin CRS**
- **Somatropin/desamidosomatropin resolution mix CRS**
- Human immunoglobulin for molecular size BRP
- Human immunoglobulin for electrophoresis BRP
- Human albumin for electrophoresis BRP

→ as reference solution for comparison of profile, mobility, ...

→ for system suitability

# Uses of BSP References in the Ph. Eur. (2)

### Biological assays / tests

- Content in IU, titre, .../ampoule or vial
- In specific unit e.g. cfu/mL, arbitrary Ph. Eur. unit (when no IU)

- **Potency**
- **System Suitability**
- **Contaminants**

→ **Examples for the determination of a biological activity**

#### Standard for potency assay

- e.g. Tet, Dip, IPV, infliximab, rabies vet, FVIII, FIX....
- (2.7.8, 2.7.6, 2.214, 2.29, 2.24, 2.846...)

- BRP calibrated in IU/vial (U/vial, ...)
- Method described in Ph. Eur.
- Run sample together in the same test as the calibrator

#### Reference Sera (in IU if possible)

- e.g. Bordetella pertussis mouse antisera (ELU/vial),...
- (2.7.16)

Statistical analysis compares sample to reference to express relative potency in standardised units

Validity criteria in monograph

Allows expression of results in calibrated comparable units
### Uses of BSP References in the Ph. Eur. (2)

→ **Examples for system suitability/limits/evaluating level of contaminants**

**Measles, mumps, rubella, varicella**
- (0162, 0213, 0538, 0648, 1057, 2442)
- BRP established with viral units (PFU)/vial
- **Viral titration assay**
- **Method performance monitored for consistency with independent reference**
- **Validity criteria in monograph**

**Mycoplasma – 5 reference strains**
- (2.6.7)
- Low passage reference strains for culture method
- BRP established with CFU/mL
- **- Suitability of culture media**
- **- Inhibitory substances**
- **- Positive control**
- **Qualifies the test reagents and conditions to ensure method performance and representative Results**
- **Validity criteria in monograph**

**NAT assays** (HAV, HCV, HEV RNA, B19 DNA) (1646)
- BRP calibrated in IU against IS
- **- Positive control**
- **Acceptable level and validity criteria in monograph**

**anti-D, antiA/antiB, PKA in albumin, Endotoxin...** (2.6.26, 2.6.20, 2.6.15, 2.6.14...)
- **- Positive/negative control**
- **- System calibrators**
- **Acceptable level and validity criteria in monograph**

### Uses of BSP References in the Ph. Eur. (4)

**Biological Reference Reagents (BRRs, BRPs*)**
- **- Critical reagents needed for the performance of the method**
- **- Often linked to in vitro methods e.g. specific mAbs for immunochemical methods but also others**

**Examples:**
- **- Coating antigens – hepatitis A in vitro assay, Erysipelas coating antigen**
- **- Newcastle Disease vaccine – mAbs for coating and detection**
- **- Challenge strains e.g. swine erysipelas bacteria**

* Historic nomenclature
Leaflet 1

- **Cat. code**
- **Official name**
- **Approx. content** (only for customs)
- **Assigned potency**
- **Instructions for reconstitution, ...**

Leaflet 2

- **Storage conditions** (unopened vials)
- **Publication of the BSP study report** (open access)
- **Adopted suitability for intended use**
**Designed For Purpose**

The BRPs/CRS are established for an intended purpose

The collaborative studies are designed to verify the suitability for that purpose only and sufficient information is provided in the study report and leaflets to use the BRP/CRS in that context

• Can I use the BRP to calibrate something else/in a different method than that in the Ph. Eur.?  
• Can you tell me if there is X, Y or Z in the formulation because I want to use the BRP for...’insert favourite research project here’...?

Users are advised not to use the BRP/CRS/BRRs outside the scope of intended use

Consult the leaflet carefully and be sure it is the right tool for the job

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**A Few Words About Method Development For 3Rs**

Important goal and a commitment of the Ph. Eur. and EDQM/COE

Several important considerations....

• Complex task  
• Global ‘blunt’ measure (life/death) → specific parameters/parts of system  
• Paradigm shift may be required (globally applied specification vs consistency approach)  
• Critical mass on consensus to embark can be long in coming  
• Not always possible to directly compare to existing test  
• Usually requires access to specific reagents that may not be freely available  
• Should as far as possible be relevant for all products on the market  
• Not always a straight line from idea to fruition
Recent Example of a Successful Study (1)

**BSP130 – Clostridium septicum - in vitro replacement of in vivo methods**
- co-sponsored with EPAA (European Partnership for Alternative Approaches to Animal Testing)
- **BSP Project Leaders:** Keith Redhead (formerly at MSD-AH), Lukas Brukner (formerly at IVI), Botand Siklodi (Ceva)

Clostridial vaccines – major group of veterinary vaccines sold world wide for farm animals - Compromise less than 5% of veterinary vaccines but responsible for over 40% of animal use for QC testing

**Antigen** *Clostridium septicum*, a common component of veterinary vaccines – Proof of Concept for other strains

Grow organism → Remove cells → Active toxin → Inactive toxoid → Formulated vaccine

**QC tests** Minimal lethal dose (**MLD**) for toxicity (T) testing

Total combining power (**TCP**) for antigenicity (A) testing

Traditional MLD, TCP differ in set up and objective but both use mice as final read-out for toxicity

**Goal:**
- replace mice with cell culture (vero cell assay) for read out

Recent Example of a Successful Study (2)

Pre-BSP: Method development – MSDAH validated method accepted by regulators
- 2013 - dedicated EPAA workshop brings together interested parties and sets ‘critical mass’ in motion

**BSP-SC endorses project start in June 2013**

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**Phase 1**
- Preliminary protocols developed
- Study samples collected and prequalified
- in vivo and in vitro

- Lab work carried out in 1 lab (PL:K.R.)
- 11 labs from 7 countries participate
- 6 batches of toxin and 6 of toxoid tested
- Common antitoxin and toxin provided
- Rationalised design to minimise animal use

**Phase 2**
- Collaborative validation of in vitro MLD and TCP
- Concordance with in vivo tests established

**Phase 3**
- Optimisation of in vitro protocols
- Qualification of new samples (PL: B.S.)
- TCP and MLD refined/adapted (**MLD → TNE+**)
- New samples collected and pre-qualified
- New collaborative study – in vitro only

- 14 labs from 11 countries participate
- 6 batches of toxin and 6 of toxoid tested
- Common antitoxin and toxin provided
Recent Example of a Successful Study (3)

Conclusions

• Assays can be successfully transferred
• Good comparison in vivo - in vitro
• Vero cells 1000x more sensitive than mouse
• Both TNE+ and TCP in vitro are better than in vivo at discriminating toxins/toxoids of different strengths
• Both have better intra-lab precision and reproducibility than in vivo

Recommend in vivo \(\rightarrow\) in vitro in Ph. Eur.

Group 15V are preparing revised text(s) to be published in Pharmeuropa for public comment, following preliminary consultation with industry

Symposium planned for December 2020 at EDQM

Focus on implementation for C. Septicum and other strains
- Interested manufacturers, OMCLs, regulators; Europe and international, encouraged to attend

How to Participate in a BSP Project ?

✓ evaluate a proposed new method/standard
✓ contribute to the calibration of a new batch of reference standard

• As participant
  • laboratory from manufacturer / OMCL
    - if you perform the test
    - if you use the Ph. Eur. RS

• As project leader
  • expert in the field
    - with access to a laboratory
    - with wide knowledge of products / methods
    - if you developed a new method

• As donator of starting material
  • manufacturer

\(\rightarrow\) Check the EDQM website for ongoing / future studies (BSP work programme)
\(\rightarrow\) Contact the EDQM/DBO/BSP (via Helpdesk on the EDQM website or direct contact)
Conclusion

BSP contributes to the Ph. Eur.

- Validation of methods (particularly 3Rs)
- BRP/CRS/BRR that support the application of Ph. Eur. methods

And in a wider sense

- To the standardisation of biological medicines
- To public health protection

Many Thanks to All Supporters

BSP Steering Committee Members
Project Leaders
Project Participants
Donators of Material
International Collaborators

EDQM DBO Team
Scientific Project Administrators: Marie-Emmanuelle Behr-Gross, Angèle Costanzo, Sébastien Jouette, Natalia Sinitskaya, Eriko Terao
Assistant: Sally Woodward
Statisticians: David LeTallec, Elena Regourd
Head of Section: Catherine Milne
Head of Division: Michael Wierer
Head of Department: Laurent Mallet

..and EDQM collaborators  
DLab, DRSL, DAF, ITPD, PRDD....
Thank you for your attention

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