Together, beyond animal health

EDQM BSP 130

Validation of cell-based assays for in-process toxicity and antigenicity testing of Clostridium septicum vaccine

AFSA/HfA/IABS webinar 2022

3Rs implementation in veterinary vaccine batch-release testing:
Current state-of-the-art and future opportunities

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Topics discussed

- **Background of the BSP 130 project**

  3Rs implementation in clostridial veterinary vaccines' batch release and IPC tests

  1st step: BSP 022 – **batch release tests** – 2001

  2nd step: BSP 130 – **in process control tests** – 2018

- **Correlation of IPC and FPC tests with protection in polyvalent adjuvanted vaccines**

  In vitro antigen quantification vs. biological tests

- **BSP 130 evolution, sponsors and participants**

- **Results and conclusions**

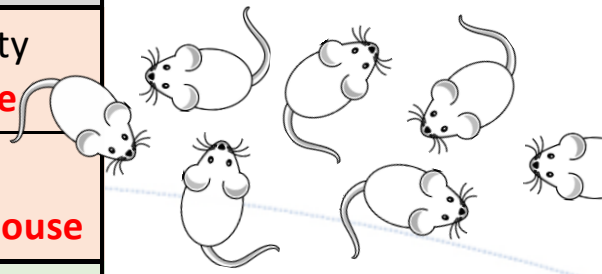
- **Ph. Eur. Adoption – 2022**

Background of the BSP 130 project

3Rs implementation in clostridial veterinary vaccines' batch release and IPC tests

Polyvalent, adjuvanted toxoid vaccines

	IPC	FPC
Traditional tests	toxicity MLD or LD_{50} in mouse	residual toxicity MLD in mouse
	antigenicity TCP in mouse	potency TNT in rabbit + mouse
3Rs conform tests	toxicity (BSP 130) cell line (TNE)	~~residual toxicity in cell line (MLD)~~
	antigenicity (BSP 130) TCP on cell line	potency (BSP 022) rabbit serology ELISA



Legend

IPC: In-Process Control
FPC: Final Product Control
BSP: Biological Standardisation Programme (EDQM)
MLD: Minimal Lethal Dose
LD_{50}: Lethal Dose 50%
TCP: Total Combining Power
TNT: Toxin Neutralisation Test

Correlation of IPC and FPC tests with protection in polyvalent adjuvanted vaccines – implications in 3Rs

1. **Monovalent, non-adjuvanted vaccines**

   IPC: in vitro antigen quantification (e.g. ELISA)
   FPC: in vitro antigen quantification (e.g. ELISA)

   } **Good correlation with protection**

2. **Polyvalent, adjuvanted clostridial toxoid vaccines**

   IPC: in vitro antigen quantification (e.g. ELISA)
   FPC: in vitro antigen quantification (e.g. ELISA

   } **Poor correlation with protection due to**
   - antigen competition
   - **measurable** antigen quantity varies in FP

Solution: **Overall biological / immunological effect** should be measured instead

IPC: LD_{50}, MLD for toxicity
TCP for antigenicity
→ **test readout** → **mice** (traditional)
→ **cell line** (3Rs compliant) → **BSP 130**

Batch potency: Step 1: immunise rabbits
Step 2: titrate the immune sera
→ **TNT in mice** (traditional)
→ **ELISA** (3Rs compliant) → **BSP 022**

BSP 130 study evolution

Three phases in 5 years

Phase I
2013

- Preliminary protocols developed
- Study samples collected and prequalified
- All done by MSD AH UK

Phase II
2014

- Collaborative validation of *in vitro* MLD and TCP assays
- Eleven laboratories participated from 8 countries
- Concordance with the *in vivo* tests established

Phase III
2016-2018

- Optimisation of the *in vitro* protocols by Ceva
- A new set of test samples collected and prequalified by Ceva
- 15 laboratories participated from 9 countries

BSP 130 study sponsors

EPAA, a voluntary collaboration between the EC, European trade associations, and companies from seven industry sectors

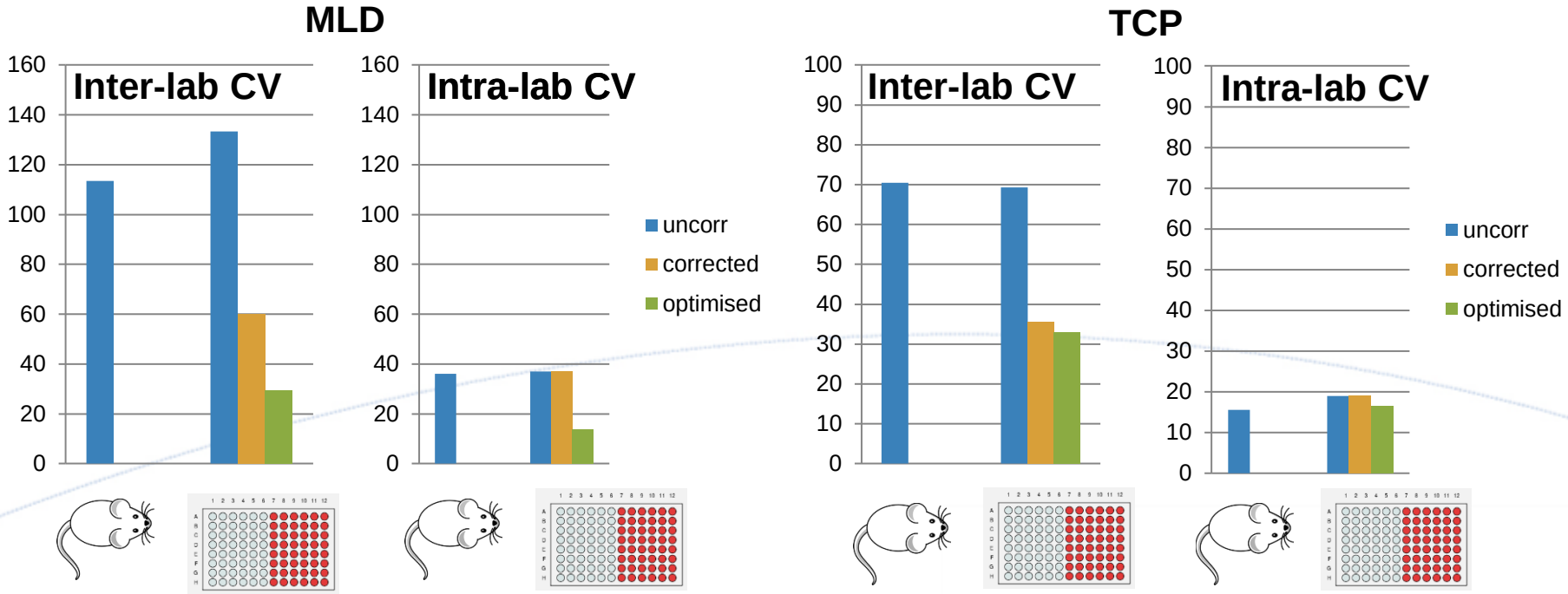
European Directorate for the Quality of Medicines and Healthcare, Council of Europe

BSP 130 study participants

Industrial		Non-industrial	
Ceva	**Hungary**	**EDQM**	**Europe**
MSD AH	**UK**	**PEI**	**Germany**
CZV	**Spain**	**CVB, USDA**	**USA**
Syva	**Spain**	**NEBIH**	**Hungary**
Merck AH	USA	IVI	Switzerland
MCI	Morocco	Bornova Vet Inst	Turkey
Merial (now BI)	France		
BI	Mexico		
Zoetis	Belgium		
Dollvet	Turkey		
Hipra	Spain		

Labs in **bold** participated in both Phase II and Phase III

BSP 130 study results: Inter- and intra-lab variance



uncorr = „uncorrected" - different sensitivities to the toxin among participants influenced the results
corrected = different sensitivities were corrected by expressing toxicity relative to a reference toxin
optimised = sensitivity differencies were corrected plus assay protocol was optimised

BSP 130 – Specific conlusions

- Cell line assays are suitable replacements for the mouse MLD and TCP tests for *C. septicum* antigens
- Cell line MLD could be the basis for an objective measurement of toxicity
- Cell line TCP assay has better discriminatory power in antigenicity measurement than the mouse test
- The *in vitro* assays are relatively easily transferable between laboratories
- The *in vitro* assays can give
  i) significant **savings** in animal usage,
  ii) shorten the **duration** of QC testing,
  iii) allow **more accurate** and reproducible blending of final vaccines and
  iv) provide a basis for **harmonization**

The BSP130 study can be used as a „template" for running other *in vitro* replacement assay validations

BSP 130 – General conlusions

International validations of newly developed *in vitro* alternative methods are **large projects** ⇨ **Sufficient resources needed**

From the organisers/sponsors

- Established international organisations for managing/coordinating long lasting projects and that are trusted by the industry
- Sufficient financial support from intergovernmental sponsors

From industrial participants

- Dedicated people and/or teams with sufficiently allocated time
- Trust to share results and test samples with potential competitors

From public sector participants (e.g. OMCLs)

- Governmental support for applied research activities

Study reports and Ph. Eur. adoption

Publications on the outcomes of BSP130 in **Pharmeuropa Bio & Scientific Notes**

Phases 1-2 in 2020

Phase 3 in 2021

Adoption of Clostridium monographs by **Ph. Eur. Commission** in June 2021,
Published in **Ph. Eur. 10.8** with **implementation date: 1st of July 2022**

Adopted monographs: 0362 for C. novyi

0363 for C. perfringens

0364 for C. septicum

2-3. *MANUFACTURER'S TESTS*

2-3-1. **Residual toxicity**. A test for detoxification is carried out immediately after the detoxification process and, when there is risk of reversion, a 2nd test is carried out at as late a stage as possible during the production process. The test for residual toxicity (section 2.6.3) may be omitted by the manufacturer.

2-3. *MANUFACTURER'S TESTS*

2-3-1. **Residual toxicity**. Residual toxicity is assessed immediately after detoxification by a suitable *in vitro* method (e.g. in Vero cells). The result complies with the value specified for the product.

2-3-2. **Antigen content**. The antigen content is determined by a suitable *in vitro* method such as total combining power (TCP) using cells (e.g. Vero cells) as indicators of toxicity, an enzyme-linked immunosorbent assay (ELISA) or any other validated method.

Thank you for your attention