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
  1\(^{st}\) step: BSP 022 – batch release tests – 2001
  2\(^{nd}\) 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

<table>
<thead>
<tr>
<th></th>
<th>IPC</th>
<th>FPC</th>
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<tbody>
<tr>
<td><strong>Traditional tests</strong></td>
<td>toxicity (MLD or LD50 in mouse)</td>
<td>residual toxicity (MLD in mouse)</td>
</tr>
<tr>
<td></td>
<td>antigenicity (TCP in mouse)</td>
<td>potency (TNT in rabbit + mouse)</td>
</tr>
<tr>
<td><strong>3Rs conform tests</strong></td>
<td>toxicity (BSP 130) cell line (TNE)</td>
<td>residual toxicity (TCP in cell line (MLD))</td>
</tr>
<tr>
<td></td>
<td>antigenicity (BSP 130) TCP on cell line</td>
<td>potency (BSP 022) rabbit serology ELISA</td>
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**Legend**

- **IPC**: In-Process Control
- **FPC**: Final Product Control
- **BSP**: Biological Standardisation Programme (EDQM)
- **MLD**: Minimal Lethal Dose
- **LD50**: 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

- **Batch potency:**
  - Step 1: immunise rabbits
  - Step 2: titrate the immune sera

- **Test readout:**
  - mice (traditional)
  - cell line (3Rs compliant)

- **BSP 130**
  - TNT in mice (traditional)
  - ELISA (3Rs compliant)
  - BSP 022
# BSP 130 study evolution

## Three phases in 5 years

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
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| Phase I | - Preliminary protocols developed  
|         | - Study samples collected and prequalified  
| 2013    | - All done by MSD AH UK                                                   |
| Phase II| - Collaborative validation of *in vitro* MLD and TCP assays  
| 2014    | - Eleven laboratories participated from 8 countries  
|         | - Concordance with the *in vivo* tests established                          |
| Phase III| - Optimisation of the *in vitro* protocols by Ceva  
| 2016-2018| - 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

<table>
<thead>
<tr>
<th>Industrial</th>
<th>Non-industrial</th>
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<tbody>
<tr>
<td>Ceva</td>
<td>Hungary</td>
</tr>
<tr>
<td>MSD AH</td>
<td>UK</td>
</tr>
<tr>
<td>CZV</td>
<td>Spain</td>
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<tr>
<td>Syva</td>
<td>Spain</td>
</tr>
<tr>
<td>Merck AH</td>
<td>USA</td>
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<tr>
<td>MCI</td>
<td>Morocco</td>
</tr>
<tr>
<td>Merial (now BI)</td>
<td>France</td>
</tr>
<tr>
<td>BI</td>
<td>Mexico</td>
</tr>
<tr>
<td>Zoetis</td>
<td>Belgium</td>
</tr>
<tr>
<td>Dollvet</td>
<td>Turkey</td>
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<tr>
<td>Hipra</td>
<td>Spain</td>
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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 differences were corrected plus assay protocol was optimised
BSP 130 – Specific conclusions

- 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 conclusions

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 reactivation, 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) 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