Feedback Workshop Session 1 – Activities of study stakeholders in the field of 3Rs

Rapporteur:

Catherine Milne, EDQM, Council of Europe

Stakeholder Presentations

- **European Directorate for the Quality of Medicines & HealthCare, Council of Europe** Catherine Lang
- **The European Partnership for Alternative Approaches to Animal testing (EPAA),** Irene Manou
- **The European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) of the European Commission Joint Research Centre** Marlies Halder

Summary (1)

- Each stakeholder provided background on their organisation and an overview of 3R related activities
- All have longstanding experience and varied activities in promoting 3Rs albeit through different means and with different scopes
- All demonstrated that European governments, organisations and industry are focused on and invested in progressing the 3Rs
- Activities are complimentary and co-operative and all are dedicated to reducing, refining, replacing the use of animals in experimental testing in particular in regulatory testing

e.g. - Ph. Eur. removal of TABST and ATT – EURL ECVAM lead on VICH TABST text, and EPAA harmonisation of 3Rs in biologicals

- BSP programs such as BSP130

Summary (2)

- 3Rs for quality control testing including in vaccines in the veterinary and human fields is a common target
- All use evidence-based scientific approaches and collaborative work including interaction with stakeholders
- All have a final goal of dissemination of findings and implementation and use of the 3R approaches
- Each organisation has contributed to the advancement of BSP130 within their own scope of activity
- Additional information on each available in the meeting package and on their websites

Feedback Workshop Session 2 Clostridium septicum vaccine project (BSP130) Study aims and results

Rapporteur:
Marie-Emmanuelle BEHR-GROSS, EDQM, Council of Europe

BSP programme

- The BSP applied research programme, procedures and international collaborative projects were described (co-sponsoring EC-CoE). Where possible, cooperation of other stakeholders (eg EPAA, JRC, OIE, WHO …) is sought
- Importance of the programme for evaluating candidate alternatives to animal testing for use in quality control of biomedicines was highlighted. (It was precised that the methods than can be evaluated in a BSP project should already be fully developed and validated in one laboratory and have a demonstrated transferability.)
- Subject of WS: BSP130 Validation of cell line assays for toxicity and antigenicity testing of *Clostridium septicum* vaccine antigens

BSP130 Part 1

- The principles of the study were presented: replace mice by a cell line as toxicity indicator in the current in-process toxicity (MLD) and antigenicity testing (TCP)
- Cell line assays are suitable replacements for the mouse in MLD and TCP tests for *Cl. septicum* antigens
- Use of cell line tests could be the basis for an objective measurement of toxicity and more accurate quantification of antigenicity
- The *in vitro* assays can give significant savings in animal usage, shorten the duration of QC testing, allow more accurate and reproducible blending of final vaccines and provide a basis for harmonisation

BSP130 Part 2 Method optimisation

- The needs for and the principles of method optimisation for cell line measurement of toxicity and antigenicity were explained
  - Intra- and inter-laboratory variations high in part 1 (despite concordance when results were pooled)
  - Optimised MLD, TNE+ (eq *in vivo* L+50) and TCP procedures developed at CEVA Phyllaxia
- The stepwise structure of the collaborative study was justified
  Training/Sensitivity of cell line/MLD for latent toxicity determination/TNE+ determination of ref. toxin/Testing of panels by TNE+ and TCP
- The choice of assay validity criteria and end-point determination method(s) were justified, examples of plate setup(s) and calculations as well as key performance parameters were shown.

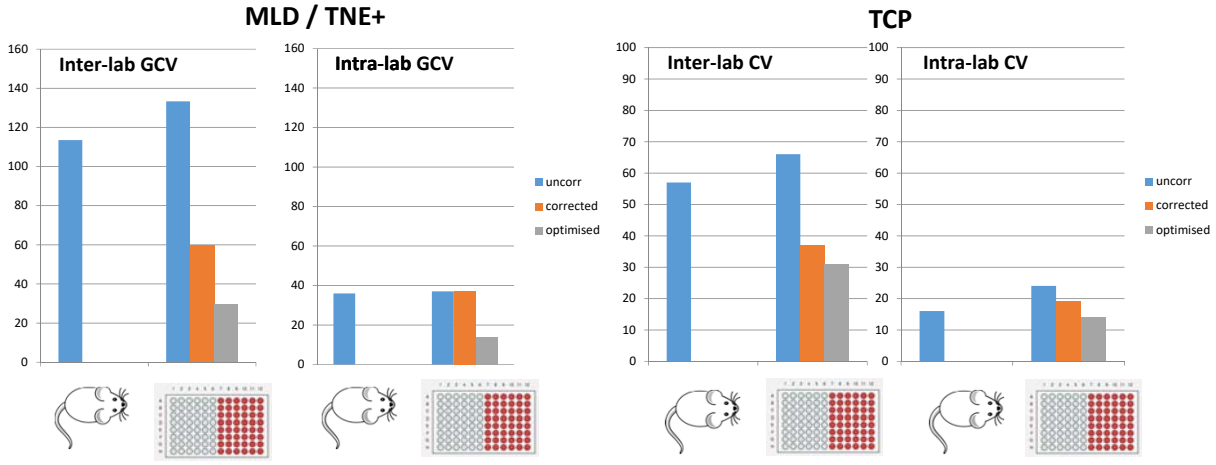
BSP130 Part 2 Statistics

- The principles taken into account for calculating and reporting the results of individual assays for the optimised MLD, TNE+ and TCP were presented
  - Endpoint dilution assay
  - Testing procedure for initial and final assay(s)
  - Validity criteria
  - Spreadsheets (centrally provided): generated calculated results
- The pooling of participants results was done as follows
  - TCP: Arithmetic mean (AM) Coefficient of variation (CV%)
  - MLD: Geometric Mean (GM) Geometric coef. of variation (GCV %)

BSP130 Part 2 Results overview

- A summary of the methods and data obtained in the whole study by *in vivo* and/or *in vitro* methods was shown for
  - Sensitivity
  - Latent toxicity
  - MLD of toxins: absolute, relative (corrected), optimised
  - TNE+ of toxins: absolute, relative, optimised
  - TCP of toxoids : absolute, relative, optimised
- The principles used in the study for comparing in vivo and *in vitro* methods outcomes were explained
  - Ranking (individual lab results)
  - Concordance (pooled data from all labs)

Main outcome: Improved test performance achieved in Phase III



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 or with a ref. antitoxin
optimised = sensitivity differencies were corrected plus assay protocol was optimised

Feedback Workshop Session –
Session 3: Outcome of a Field Survey on Clostridium vaccines control

Rapporteur:
Marlies Halder, European Commission, Joint Research Centre, Italy

Highlights - Presentations - 1

- Feedback from BSP130 participants: experience and learnings
  - Marie Emmanuelle Behr-Gross, EDQM
    - 8 labs replied (4 involved in 3 phases of BSP)
    - Feedback is very positive regarding structure, organization, support, supply, communication, study protocols
    - Superior sensitivity of Vero cells underlined
    - All interested in participating in future BSP studies on 3Rs methods inter-laboratory validation

Highlights - Presentations - 2

- Results of an enquiry on alternative methods implementation by manufacturers - Catrina Stirling, EPAA
  - High interest in implementing in vitro methods for C. septicum
    - 2 already have
  - Definite plans to use approach for other cytotoxic clostridial toxins
  - Participation in BSP130 gave access to methodology, reagents, etc
    - Satisfied with support
  - Great interest in participating in further BSP studies
  - Support from/awareness of management OK in most cases

Poll - Summary

Live on line poll with no official character used for audience participation and general audience trend indentification

BSP 130 study awareness
- 52% no
- 48% yes

Will publication of BSP 130 study results support implementation (manufacturers reply)
- 58% yes
- 1% no
- 40% unable to answer at this stage

BSP 130 study quality
- 68% satisfied
- 9% neutral
- 23% unable to answer at this stage

Will publication of BSP 130 study results support acceptance (regulators reply)
- 49% yes
- 2% no
- 49% unable to answer at this stage

Significance of BSP13 outcome & workshop for 3Rs implementation
- 87% significant/very significant
- 0% not significant
- 13% unable to answer at this stage

Feedback Workshop Session 4 – Regulatory process

Rapporteur:

Catherine Lang, EDQM, Council of Europe

Presentations

- **European Pharmacopoeia (Ph. Eur.) monographs overview and revision proposals**
  - **Achievements and issues for Ph. Eur. Group of Experts on Veterinary Vaccines and Sera (15V),**
    Lukas Bruckner (Expert and former chair of Group 15V)
  - **Feedback from industry and way forward,**
    David John (AnimalhealthEurope)

- **Regulators' perspective**
  - **Europe,** Esther Werner (PEI)
  - **USA,** Angela Walker (CVB APHIS USDA)

Summary (1)

- **Conclusions drawn for Clostridium septicum in EDQM BSP 130**

➔revised Ph. Eur. monograph 0364 published in **Pharmeuropa 32.3** (public enquiry until Sept. 2020)

- Proposals
  - **Residual toxicity** (*in process*)
  - **Antigen content** (*in process*), any suitable in vitro method (TCP preferred because functional) - New test to monitor consistency of production to replace mice by suitable cell cultures (e.g. Vero cells) in the Minimum Lethal Dose (MLD) and Total Combining Power (TCP) assays as indicator of toxicity
- **Residual toxicity of the final product** (performed in mice) deleted

- **Batch potency test**: several *in vitro* tests are available and should therefore be used (no animal test anymore)
- **Identification** using animals deleted to encourage manufacturers to use in vitro methods

Group 15V revised Ph. Eur. monograph 0363 & 0362 Clostridium perfringens and Clostridium novyi (type B) vaccines in the same way

because BSP130 considered as "proof of concept".

Summary (2)

- Manufacturers comments on the 3 revised Ph. Eur. monographs published in **Pharmeuropa 32.3** (public enquiry until Sept. 2020)
  - Revision welcome and appreciate the efforts of Group 15V to introduce the *in vitro* alternative into the monograph
  - Residual Toxicity Test: "We proposed a slight rephrasing to indicate that whilst tissue culture tests are the preferred way forward we do not exclude other suitable methods, in line with other monographs."
  - Next steps: Implementation of the revised monographs, acceptance of the alternative test on a global basis.
  - Regulators perspective (Europe and USA): welcome the introduction of *in vitro* methods, guidance documents available

Challenges

- Resources needed to switch to *in vitro* methods (development, validation)
- Communication with the Competent Authority at an early stage
- For multicomponent cytotoxic vaccines, the replacement of mice by cells should ultimately be validated for **all** cytotoxic components (and not only for 1 or 2 components)
- *In vivo* and *in vitro* methods have to be run in parallel for some time
- Variations to be submitted
- Acceptance of *in vitro* alternatives globally

Next steps?

- Only a few comments from manufacturers during the public enquiry, the major comment being to indicate that tissue culture is the way forward, **but without excluding other (*in vivo*) methods**

=>Does this mean that manufacturers need to keep the possibility to use animals as indicator of toxicity? or is it to open to any *in vitro* method?

=> Does this mean that BSP130 results can be transposed to Clostridium perfringens and Clostridium novyi (type B) vaccines (with a « minimum » of validation work)?

- According to EU Directive 2010/63 alternative methods must be applied, when available
  =>European manufacturers cannot use *in vivo* methods anymore once *in vitro* methods are available

  =>need for a global harmonisation to avoid duplication of testing

Feedback Workshop Session 5 Potential application of the Approach to other Toxoid Vaccines

Rapporteur:

Marie-Emmanuelle BEHR-GROSS, EDQM, Council of Europe

Experience in extending the approach of cell-based TCP and MLD assays to *Clostridium perfringens* vaccines

- Principle: Development of *in vitro* assays addressing specific toxicity of ***Clostridium perfringens*, type C (non-inactivated) antigen**
- Frame: Vac2vac
- Results:
  - Selection of A10 and THP-1 as potential toxin senitive cell lines
  - Demonstration that toxicity of *C. perfringens* C non-inactivated antigen on **THP-1** cells is β-toxin specific (*C. perfringens* type C major antigen)
  - Optimisation: PMA, FBS and cell density/number in plates,
  - Issue: residual formaldehyde might interfere with test result
  - An optimised protocol for the THP-1-based MLD assay has been prepared and transferred to the industry partners (MSD AH and BI) for further assessment and validation

Experience in extending the approach of cell-based TCP and MLD assays to different *Clostridium* vaccines

- Principle: To develop, validate and implement toxicity assays replacing the control animal tests routinely performed within QC laboratories for *C. perfringens D*, *C. chauvoei*, *C. septicum* and *C. novyi* antigens
- Frame: Company developed these projects independently
- Results:
  - MDCK cells are widely established to be sensitive to epsilon toxin, from *C. Perf. D.*
  - Vero cells previously demonstrate sensitivity to *C. septicum* and *C. novyi* antigens.
  - MDCK selected as sensitive cell line for *C. chauvoei* (need for replacement of mouse and guinea pig tests), **specificity checked**
  - Full in house validation performed for toxin and toxoid tests (intermediate precision, robustness, repeatability, inter-operator variability, …)

Experience with different *Clostridium* vaccines

- Ongoing work
  - Concurrent *in vitro* testing of QC samples then correlation of cell assay results to *in vivo* pass/ fail result.
  - Determine the titre endpoints that represent the pass/fail threshold of each assay.
  - Establishment of MDCK as sensitive cell line for *C. chauvoei* (cells and supernatant testing)
- Technical challenges: Alleviating the impact of formaldehyde on cell monolayers.
  Neutralisation chemical used but still cell death and crystal violet interaction: resolved with pre-strain wash step for MDCK or dilution for VC

Poll – Summary 1

Live on line poll with no official character used for audience participation and general audience trend indentification

Awareness of the Vac2Vac Clostridia studies
- 20% yes aware
- 12% not aware of results
- 68% not aware

Did you take part in the public enquiry in Pharmeuopa 32.2 on the clostridia monographs
- 10% yes
- 90% no

Familiar with Pharmeuropa publications and notifications ?
- 12% yes
- 52% somewhat
- 23% not familiar

If response in *(question above)* was yes, how did you send your comments ?
- 13% via National Pharmacopoeia Authority (NPA)
- 50 % via industry associations
- 6% directly to EDQM via HELPDESK
- 31 % don't know

Aware of the Ph.Eur. public enquiry and the proposed changes to the clostridia monographs before this workshop ?
- 27% yes
- 19% aware but not of the proposed changes
- 54% no

Poll – Summary 2

Live on line poll with no official character used for audience participation and general audience trend indentification

"Immediate" implementation of the changes proposed in the revised C. septicum monograph ?
- 45% yes
- 6% no
- 49% don´t know

Organisation willing to participate in future collaborative studies in the clostridial vaccines field?
- 34% yes
- 11% no
- 55% don´t know

Further studies needed to implement changes in the draft Cl. novyi and Cl. perfringens monographs ?
- 10% yes, for cell-based methods
- 8 % yes for reference preparations
- 46 % yes form methods and reference preparations
- 5 % no
- 31 % don´t know

Organisation willing to participate in initiatives promoting international harmonisation in Cl.vaccines QC ?
- 48% yes
- 7 % no
- 45 % don't know

Willing to support EDQM in replacing multicomponent Clostridia Rabbit Antiserum BRP1 or other BRPs ?
- 32% yes
- 9% no
- 58% don´t know