THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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COUNCIL OF EUROPE
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BSP130 follow up Workshop
9 & 10 March 2021

Novel *in vitro* model as alternative to *in vivo* toxoid vaccines testing: Clostridium septicum vaccine as proof of concept

Sponsors: EDQM, EPAA and JRC

Contributions prepared by the EDQM/EPAA/JRC for the meeting

- **Publications (Session 2)**
  - Phase 1 participants workshop Proceedings (2015)
  - BSP130 part 1 results (PB&SN) (2020)
  - Publication of BSP130 part 2 results (PB&SN pending)
- **Field enquiries (Session 3)**
  - Study participants (June-October 2020)
  - Manufacturers (June-October 2020)
- **Ph. Eur. revised draft monographs (Session 4) (July-September 2020)**

Presentation on field enquiries

Participants Study performance and learnings
PA/PH/BIO (20) 23 DEF

Speaker: ME Behr-Gross (EDQM)

Manufacturers Questions to manufacturers regarding implementation of *in vitro* methods
PA/PH/BIO 20 (24) DEF

Speaker: Catrina Stirling (EPAA)

Acknowledgements Natalia Sinitskaya and Sally Woodward (DBO)

Participants survey

Participants survey (8 respondents) - 1

How clear was the scope of the BSP130 Project (Scale 1-5)
5: 7 labs; 4: 1 lab

Which phase(s) did you participate in?

Attendance & usefulness of participants' Workshop „Validation of alternative/3Rs methods for the in-process quality control of Clostridium Septicum Vaccines" (Egmond aan Zee, NL) 15-16/09/2015

Lab	Phase 1	Phase 2	Phase 3	Phase 1 Workshop	Workshop usefulness
A	+	+	+	+	+
B	-	-	+	-	
C	+	-	-	-	
D	+	+	+	+	+
E	+	+	+	+	
F	+	-	+	-	
G	-	-	+	+	+
H	+	+	+	+	+
8	6	4	7	5	4

Participants survey (8 respondents) - 2

Do you have any comments/suggestions regarding the overall structure of the project?

None of relevance

Do you have any comments/suggestions regarding the performance of the following steps in Phase II and III?

Vero cell sensitivity (3 comments)

- Instructions were very clear; lab templates were found helpful. Would suggest to lay open the calculations performed in Excel cells.
- During the experimental phase, it was clear that Vero cell culture is affected by specific concentration of *C. septicum* toxin causing cells degeneration, which is decreased along with the higher dilutions of the toxin. The same results were obtained through 3 repetitions, which leads to think that Vero cell culture could be used as titration method of *C. septicum* toxin.
- It was surprising to see the higher sensitivity of the cells compared to that observed in mice.

Participants survey (8 respondents) - 3

Do you have any comments/suggestions regarding the performance of the following steps in Phase II and III?

Residual toxicity of the test toxoids and the reference antiserum (4 comments)

- Instructions were very clear; lab templates were found helpful. Would suggest to lay open the calculations performed in Excel cells.
- Some sera samples were toxic for Vero cell culture in the low dilution.
- Although most of the toxoids showed a residual toxicity, a limit was not implemented for the conformity/ non-conformity on the residual toxicity assays. Each laboratory will have to implement a limit based on the correlation with the mice assays.
- Regarding the latent toxicity of toxoids, tested in vitro in Vero cell lines. In all toxoids except TdN, the technique shows some latent toxicity. Does it mean that the toxin is not completely inactivated, or there are an effect of chemical inactivator? Which would be the cut-off on the *in vitro* assay to confirm that toxoid is completely inactivated and no latent toxicity are present?

Participants survey (8 respondents) - 4

Do you have any comments/suggestions regarding the performance of the subsequent steps in Phase II and III?

In vitro MLD/TNE+ determination of the reference toxin (2 comments)

- Instructions were very clear; lab templates were found helpful. Would suggest to lay open the calculations performed in Excel cells.
- Different results were obtained

In vitro TCP determination of the test toxoids (3 comments)

- Instructions were very clear; lab templates were found helpful. Would suggest to lay open the calculations performed in Excel cells.
- No technical problem
- Has this *in vitro* method (*in vitro* TCP) been evaluate to study the stability of the antigens/toxoids?

Participants survey (8 respondents) - 5

How clear and detailed did you find the study protocols? Scale 1-5

Phase I: 5: 2 labs; 4: 2 labs; 3: 1 lab

Phase II: 5: 1 lab; 4: 2 labs

Phase III: 5: 4 labs; 4: 2 labs; 3: 1 lab

Were you satisfied with the supply of the test samples/reference material?

- **Good conditions/delivery (Scale 1-5):** 5: 6 labs; 4: 2 labs
- Suitable quantities: Yes: 7 labs No: 1 lab (reference antitoxin in too limited quantity)
- Customs clearance: Yes: 8 labs

Did you request extra technical support from the project leaders/statistician and/or scientific/administrative assistance during the project?

Yes: 6 labs; No:2 labs

Were you satisfied with the support provided?

Participants survey (8 respondents) - 6

Were you satisfied with the support provided? Scale 1-5

5: 5 labs; 4: 1 lab

Was the communication with the management team (project leaders/ coordinators/ statisticians) during the study sufficient/suitable?

Yes: 8 labs

Have you any suggestions to improve communication? (1 comment)

No: 7 labs; Yes: 1 lab: If possible to have regular meetings or workshops

Would you have required more in-person meetings and/or lab trainings and/or any other support? (1 comment)

No: 7 labs; Yes: 1 lab : For application of the test in practice, more technical validation should be done

Were you satisfied with the support provided?

Participants survey (8 respondents) - 7

Could you please precise why you would have required the above-mentioned support?(2 comments)

- **Various results were obtained, which could be related to the test performance, personal, cell culture or other factors, so in these concerns control samples must be supplied as a reference along with technical guideline.**
- **Might have needed more support if a junior technician would have done the work (was not the case here)**

Please enter a brief description of your experience RE the BSP130 study (3 replies)

- The study took a lot of hard work but it is a technique with very rewarding results because it is easy to perform and to read. Also, the project leaders and the statistician were very supporting during every step of the project.
- SL performed all the experiments: test well designed (control, calculation sheet)
- Despite only participating in Phase III of the BSP130, it has been a very enriching experience. Being able to participate in the validations and being able to test the possible future methods available for the manufacture of antigens is undoubtedly very interesting, as well as the possibility of providing feedback at the end.

Were you satisfied with the support provided?

Participants survey (8 respondents) - 8

Would you be interested in participating in another BSP project in the future if you would consider it to be relevant in terms of 3Rs/alternatives to animal testing? Yes: 8 labs

If yes, could you please specify which topics you would consider as priority?
Clostridia: 5 labs; other in vitro tests for veterinary vaccines*:4 labs; rabies vaccine: 2 labs; ELISA quantification techniques: 1 lab (*many components cited, multiple answers per lab)

Could you please specify your future plans in terms of 3Rs/alternatives to animal testing?

- Mass spectrum analysis tuberculins/leptospira vaccines (ongoing)
- We are trying to find *in vitro* alternatives for the antigens we use. Any testing to advance on this point, would be included in our plans
- Unfortunately our laboratory is not a research lab so we do not have any new method in our portfolio
- Our lab is part of Vac2Vac consortium
- We plan to implement the alternative methods currently available

Manufacturers survey

Manufacturers survey (9 respondents) - 1

Considering all the pros and cons would you recommend to your company to replace the current mouse minimum lethal dose (MLD) and total combining power (TCP) tests with the *in vitro* equivalents for the in-process control of cytotoxic antigen during *C. septicum* vaccine production? Yes: 8 labs; No: 1 lab

If no, could you please specify the reasons?

Use in vitro test to determine MLD and assess toxicity clearly YES. In case of TCP, I'm not sure. Obviously it represents an improve in terms of 3R, but I think there are other techniques like sandwich ELISA to quantify that are easier to perform and that you can translate to the batch potency test in vitro to reduce animals in batch testing.

Manufacturers survey (9 respondents) - 2

Have you already implemented in-house any of the *in vitro* methods for *C. septicum* vaccines control? Yes: 2 labs; No: 6 labs

If yes, could you please specify which one(s)?

- The *in vitro* Toxin Neutralisation Equivalence plus (TNE+), as a replacement for the *in vivo* MLD test for quantification of the toxicity of toxin Yes: 1 lab; No: 1 lab, Not applicable: 7 labs
- The *in vitro* MLD, as a replacement for the *in vivo* MLD test for detection of residual toxicity associated with toxoid No: 2 labs, Not applicable: 7 labs
- The *in vitro* TCP, as a replacement for the *in vivo* TCP test for quantification of the antigenicity of toxoid Yes: 1 lab; No: 1 lab, Not applicable: 7 labs

Manufacturers survey (9 respondents) - 3

Do you plan to implement any of the methods in the future? Yes: 6 labs; No: 2 labs

If yes, could you please specify which one(s)?

- Residual toxicity and antigen content
- An *in vitro* assay to replace mice serum neutralisation will be implemented by the end of 2020. Other *in vitro* assays to replace current *in vivo* TCP and L+ are planned for the future.
- *In vitro* toxicity to replace MLD, and to check residual toxicity of toxoids.
- Potency
- If it is confirmed that this method is applicable to our C. *septicum* toxoid, and when necessary resources will have been dedicated to ensure the follow up of the project, we are interested by implementing the *in vitro* MLD, as a replacement for the *in vivo* test for detection of residual toxicity associated with toxoid.
- All with the priority being towards the non-toxicity assay.

Manufacturers survey (9 respondents) - 4

Does your company plan to extend this approach to other cytotoxic antigen production in your portfolio? Yes: 6 labs; No: 2 labs

If yes, could you please specify the antigens:

- Most clostridial toxins are included in this plan
- *C. perfringens* and *C. novyi*
- *C. chauvoei*, C. *perfringens D, C. tetani, C.novyi B, C. botulinum*, *Corynebacterium pseudotuberculosis* and 2 species of Leptospirosis
- The approach is applicable to all those antigens that are prepared based on a toxic protein that you have to inactivate. Use of in vitro quantification of toxicity would be considered to reduce use of animals
- *Clostridium sp.* antigens

If no, what are the main hurdles preventing the extension?

- Experience
- The principles developed on *C. septicum* with BSP130 may be applicable to the other clostridial toxoids but it has not been proven to date. We strongly encourage to check the feasibility on the other clostridial toxoids through other EDQM collaborative studies to allow all parties to share their knowledge and resources while ensuring the harmonisation and easier worldwide implementation.

Manufacturers survey (9 respondents) - 5

Did your company participate in the BSP130 study? Yes: 5 labs; No: 3 labs

How would you rate the general knowledge of the management about the BSP130 project at your company? Scale 1-5

5: 2 labs; 4: 2 labs; 1: 1 lab

Comments: Allowed us to have a good knowledge of the technique, to have access to the necessary reagents and it was an excellent opportunity to participate in an inter-laboratory validation

How would you rate the support and acknowledgement you received for your BSP130 participation from the management at your company? Scale 1-5

5: 3 labs; 4: 1 lab; 3: 1 lab

Manufacturers survey (9 respondents) - 6

Are you interested in obtaining more information about the BSP130 study? Yes: 3 labs

Would your company be interested in taking part in collaborative studies for the validation of new *in vitro* methods for veterinary vaccines quality control?

Yes: 3 labs

Will your company participate in the public enquiry of the European Pharmacopoeia on clostridial vaccines monographs?

Yes: 6 labs ; No: 2 labs

Will your company be represented at the Workshop 'Novel in vitro model as alternative to in vivo toxoid vaccines testing: *Clostridium septicum* vaccine as a proof of concept'?

Yes: 7 labs ; No: 1 lab

Thank you for your attention

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