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

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BSP130 Phase III
Statistical considerations

Workshop on a novel *in-vitro* model as alternative to *in-vivo* toxoid vaccines testing:
Clostridium septicum vaccine as proof of concept

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Endpoint dilution assay

- E.g. TNE+ of toxins, 96-well plate

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
A	Blk	Blk	Blk	Blk	Blk	Blk	Blk	Blk	Blk	Blk	Blk	Blk
B	Ctrl	Ctrl	1/1	1/2	1/4	1/8	etc.					
C	Ctrl	Ctrl	1/1	1/2	1/4	1/8	etc.					
D	Ctrl	Ctrl	1/1	1/2	1/4	1/8	etc.					
E	Ctrl	Ctrl	1/1	1/2	1/4	1/8	etc.					
F	Ctrl	Ctrl	1/1	1/1.1	1/1.21	1/1.331	etc.					
G	Ctrl	Ctrl	1/1	1/1.1	1/1.21	1/1.331	etc.					
H	Ctrl	Ctrl	100 µL NBS									

Absorbance below (+) or above (-) cut-off

Median Ctrl = 0.56	+	+	+	-	-	-	-	-	-	-
	+	+	+	-	-	-	-	-	-	-
	+	+	+	+	-	-	-	-	-	-
	+	+	+	+	-	-	-	-	-	-
Cut-off = 0.23	+	+	+	+	+	+	-	-	-	-
	+	+	+	+	+	+	-	-	-	-

Endpoint	Pre-dil	Final
1/4	1/10	1/40
		Toxin S
1/8	1/10	1/80
		CSTx2
1/1.6105	1/800	1/1288

One assay requires 6 plates to test the 6 toxins (2 toxins per plate x 2 parallel plates).

If one plate is invalid (CV>20% or CSTx2 is off-target), repeat the assay for the 2 plated toxins.

If the plate is valid but toxin end-points are not (not consistent enough), the toxin is re-assayed.

Several invalid toxins can be grouped in the next assay.

Testing procedure

- Initial assay. Selection of starting dilution of final assays
- Final assays. Lower dilution step for better precision of EPs

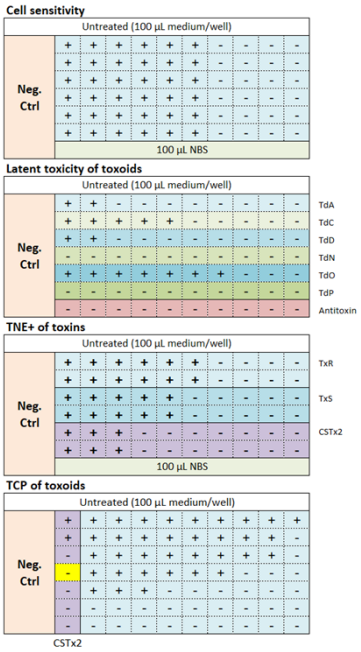
	Initial assay		3 final assays	
Test	**Dilution**	**Dil. step**	**Dilution**	**Dil. step**
Cell sensitivity	1/8000	2-fold	NA	NA
Latent tox.	1/5	2-fold	NA	NA
TNE+ CSTx2	1/525	1.3-fold	Optimal*	1.1-fold
TNE+ toxins	1/10	2-fold	Optimal*	1.3-fold
TCP toxoids	1/10 to 1/160	20-increment	Optimal**	10-increment

* Dilution just before EP dilution, e.g. EP = 1/80, optimal starting dil. = 1/40
** EP of initial assay – 40-increment, e.g. EP = 1/160, optimal starting dil. = 1/120

Validity criteria

- Common to all assays: $CV_{Neg} \leq 20\%$

Test	Criteria
Cell sensitivity	i) Positive results in consecutive wells: **PR** ii) Max. of **1** well between **6** final Eps:**W1(6)**
Latent toxicity	**PR** (each toxoid tested in single row)
TNE+ CSTx2	Per plate: **PR + W2(6)** Max. 2 wells between mean EPs of 2 plates
TNE+ Toxins	Per plate: **PR + W2(2)** Max. 2 wells between mean Tx EPs of 2 plates
TCP Toxoids	Per plate: **PR +** max. 2 wells between CSTx2 observed and expected value (yellow well) Max. 2 wells between mean Td EPs of 2 plates



Reporting of results

- Spreadsheets. Check of validity criteria and calculation of EPs

1.3-Fold Dil. Step	1.3-Fold Dilution Assay	TxR	TxS	TxV	TxW	TxY	TxZ
	Inverse dil. in 1st well	20	40	40	320	320	160
	Serial dilution step	1.3	1.3	1.3	1.3	1.3	1.3

Use the dilutions calculated in the sheet "Initial Assay".

Detector Toxin	TNE+ Conc. in **IU/mL** *	242
	Inverse dil. in 1st well **	826
	Serial dilution step	1.1

* Calculated using the reporting sheet *BSP130 TNE Value of Detector Toxin*

Absorbance Values (Test WL minus Reference WL)

0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.04	0.00
0.60	0.59	0.00	0.00	0.00	0.00	0.01	0.52	0.55	0.55	0.59	0.49
0.62	0.62	0.00	0.00	0.00	0.00	0.05	0.61	0.60	0.54	0.53	0.53
0.61	0.63	0.00	0.00	0.00	0.00	0.62	0.64	0.58	0.59	0.59	0.59
0.53	0.63	0.00	0.00	0.00	0.01	0.59	0.58	0.61	0.58	0.52	0.52
0.56	0.62	0.00	0.00	0.00	0.01	0.57	0.57	0.56	0.59	0.55	0.53
0.54	0.60	0.00	0.00	0.00	0.01	0.53	0.58	0.56	0.55	0.55	0.49
0.56	0.52	0.53	0.52	0.54	0.57	0.49	0.62	0.58	0.57	0.52	0.54

Validity criteria

Plate	Blank	Cut-off	CV%	<=20%	Detector Toxin CSTx2 EP 1	EP 2	Status	Mean	Status	Plate
1	0.004	0.300	7%	Valid	220	220	Valid	220	Valid	Valid
1'	0.006	0.273	7%	Valid	220	220	Valid	220		Valid
2	0.007	0.273	10%	Valid	220	220	Valid	220	Valid	Valid
2'	0.005	0.243	18%	Valid	220	220	Valid	220		Valid
3										Valid
3'										Valid

End-points

Toxin	Plate	Status	EP 1	EP 2	Status	Mean	Status	EP Final
TxR	1	Valid	57	57	Valid	57	Valid	57
	1'	Valid	57	57	Valid	57		
TxS	1	Valid	88	88	Valid	88	Valid	88
	1'	Valid	88	88	Valid	88		
TxV	2	Valid	68	68	Valid	68	Valid	68
	2'	Valid	68	68	Valid	68		
TxW	2	Valid	703	703	Valid	703	Valid	703
	2'	Valid	703	703	Valid	703		
TxY	3	Valid	914	914	Valid	914	Valid	914
	3'	Valid	914	914	Valid	914		
TxZ	3	Valid	352	352	Valid	352	Valid	352
	3'	Valid	352	352	Valid	352		

Pooling of participants results

TCP (toxoid)

- "x-increment" dilutions

➔**Additive scale**

  - Arithmetic means (AM)
  - Coefficients of variation (CV%)

TNE+ (toxins)

- "x-fold" dilutions

➔**Multiplicative scale**

  - Geometric means (GM)
  - Geometric CVs (GCV%)

How to calculate GM and GCV(%)

Run	TNE+		Log(TNE+)
1	1082		6.987
2	1233	Step 1 →	7.117
3	1155		7.052
4	1082	Ln()	6.987
5	1406		7.249

Multiplicative scale — Step 2 ↓ — Additive scale

Step 3

Geometric mean	1186	← Exp()	7.078	Arithmetic mean
Geometric CV	11%	← SQRT(EXP(SD²)-1)	0.110	Standard deviation
Lower 95% CL	1035	← Exp()	6.942	Lower 95% CL
Upper 95% CL	1359	← Exp()	7.214	Upper 95% CL

Thank you for your attention

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