Clostridium septicum vaccine project (BSP130)

Results Overview

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Manage the future together

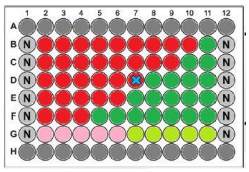
Summary of methods and data analysis

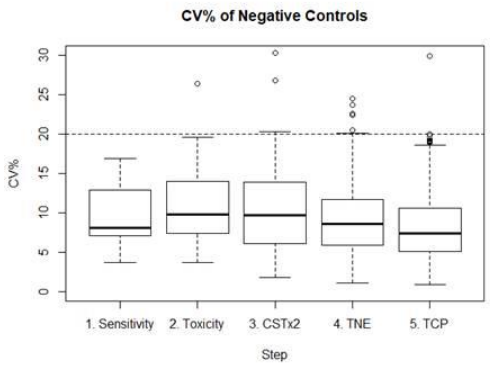
	Methods performed			Result analyses made		
	Phase II		Phase III	Phase II	Phase II & III	
	In vivo (mouse)	In vitro (Vero)	In vitro (Vero)	Correlation mouse/Vero	Intra-lab	Inter-lab
Sensitivity to C. septicum toxin	MLD	MLD	MLD	Factor*		Factor
Latent toxicity	MLD	MLD	**MLD**			Factor
Toxicity of toxins	MLD	MLD	**TNE+**	Corr	GCV**	GCV
Antigenicity of toxoids	TCP	TCP	TCP	Corr	CV	CV

Factor* Fold-differences
GCV** Geometric Coefficient of Variation
CV*** Coefficient of Variation

The general validity criterion in all in vitro assays

Plate layout example
„N": Negative control





Negative ctr. CV: Important because the variance of the cell number in the wells directly influences the results of the test items. E.g. 50% cell number → 50% OD = false end-point

Total invalidity ratio in Phase III: **16%**. Invalidity due to > 20% CV of negative wells (= the set acceptance limit): **7%**

Acceptance limit could be set to 25% without significantly influencing the test items' results if neg. ctr. well No ≥ 12.

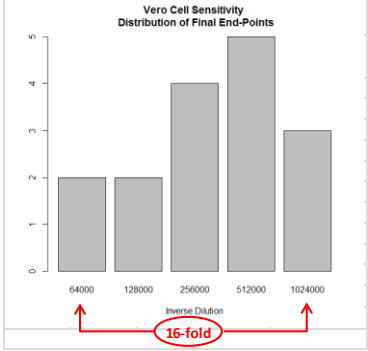
Sensitivity

- Large sensitivity differences found among the participants' mouse strains and also among their Vero cell lines. This may lead to high inter-lab variance in both the in vivo and the in vitro results.
- The sensitivity difference between the mouse versus Vero cell systems are in the range of 3 logs. This has implications in protocol design and result interpretation.

Phase II

Lab	In vivo		In vitro		Ratio vivo/vitro
	LD50 nL/mouse	Factor fold-diff.	LD50 nL/well	Factor fold-diff.	
1	237		-		-
2	288		0.198		1450
3	96		0.126		760
4	356	12-fold	0.405		880
5	1188		0.405		2930
6	617		0.757		820
7	-		0.196	24-fold	-
8	-		0.236		-
9	-		1.694		-
10	-		0.134		-
11	-		3.049		-

Phase III



Phase III

Latent toxicity of test toxoids

In vivo pre-test

All test toxoids included in Phase III have been **pre-tested in mice** for residual toxicity with **negative results**

In vitro MLD Frequency of Latent Toxicity End-Points (Inverse Dilution)

Material	Low	5	10	20	40	80	160	320	640	Median
TdA			5	3	7	1	2			40
TdC				3	8	8	1			40
TdD	3	9	4	2						5
TdN	16	2								Low
TdO						5	3	7	3	320
TdP	1		4	3	3	5	3			40
VI	20									Low

Toxicity of toxins with MLD (Phase II, in vivo)

1st approach: „Absolute" MLD – Differences in the sensitivity of the mouse strains was NOT corrected

2nd approach: „Relative" MLD – Sensitivity differences corrected by CSTx → **Inter-lab CV improved**

"Absolute" MLD, expressed as the inverse of the final dilution caused death in both mice							
	CSTx	TxA	TxB	TxC	TxD	TxE	TxF
Overall GM	834	73	74	5	12	128	110
Inter-lab GCV	-	113	117	91	81	69	75
Median intra-lab GCV	-	25	47	33	33	25	28

"Relative" MLD, expressed relative to the CSTx							
	(CSTx)	TxA	TxB	TxC	TxD	TxE	TxF
Overall GM	-	0.088	0.089	0.006	0.014	0.153	0.132
Inter-lab GCV	-	49	91	65	82	72	84
Median intra-lab GCV	-	25	47	33	33	25	28

Toxicity of toxins with MLD (Phase II, in vitro)

1st approach: „Absolute" MLD – Differences in the sensitivity of the Vero cell lines were NOT corrected

2nd approach: „Relative" MLD – Sensitivity differences corrected by CSTx → **Inter-lab CV improved**

"Absolute" MLD, expressed as the inverse of the final dilution caused death to Vero cells							
	CSTx	TxA	TxB	TxC	TxD	TxE	TxF
Overall GM	216813	15901	13292	680	2694	25201	25617
Inter-lab GCV	-	145	176	183	173	143	174
Median intra-lab GCV	-	29	25	50	28	24	26

"Relative" MLD, expressed relative to the CSTx							
	(CSTx)	TxA	TxB	TxC	TxD	TxE	TxF
Overall GM	-	0.073	0.061	0.003	0.012	0.116	0.118
Inter-lab GCV	-	55	77	60	59	43	50
Median intra-lab GCV	-	29	25	50	28	24	26

Toxicity of toxins with TNE+ (Phase III)

- **MLD** is highly dependent on Vero sensitivity to the toxin → high inter-lab CV in Phase II
- **TNE+** replaced MLD in Phase III, corrected for Vero sensitivity → improved inter-lab AND intra-lab CV

Phase II

"Relative" MLD, expressed relative to the CSTx							
	(CSTx)	TxA	TxB	TxC	TxD	TxE	TxF
Overall GM	-	0.073	0.061	0.003	0.012	0.116	0.118
Inter-lab GCV	-	55	77	60	59	43	50
Median intra-lab GCV	-	29	25	50	28	24	26

Phase III

TNE+ values in IU/mL							
	CSTx2	TxR	TxS	TxV	TxW	TxY	TxZ
Overall GM	290	15	27	27	165	225	85
Inter-lab GCV	20	51	37	49	20	28	21
Median intra-lab GCV	5	20	16	15	13	14	11

Antigenicity of toxoids (Phase II, TCP)

TCP in mice						
	TdG	TdH	TdJ	TdK	TdL	TdM
Overall GM	142	48	21	178	69	71
Inter-lab GCV	33	42	51	31	73	110
Median intra-lab GCV	15	20	0	10	21	31

TCP on Vero cells, uncorrected for sensitivity						
	TdG	TdH	TdJ	TdK	TdL	TdM
Overall GM	104	46	36	125	72	77
Inter-lab GCV	84	61	51	58	67	77
Median intra-lab GCV	35	20	13	25	26	24

TCP on Vero cells, corrected						
	TdG	TdH	TdJ	TdK	TdL	TdM
Overall GM	142	52	30	157	76	63
Inter-lab GCV	41	40	42	25	30	46
Median intra-lab GCV	24	20	10	15	17	28

TCP already includes a reference antitoxin → mouse or Vero line sensitivity differences compensated → low impact on the inter-lab CV

Still, further method corrections could improve the variance.

Antigenicity of toxoids (Phase III, TCP)

In vitro TCP further optimised → **Inter-lab AND intra-lab CV improved, BUT!...**

… „weak" toxoids with low TCP values are still measured with higher CV (see the red frame below)

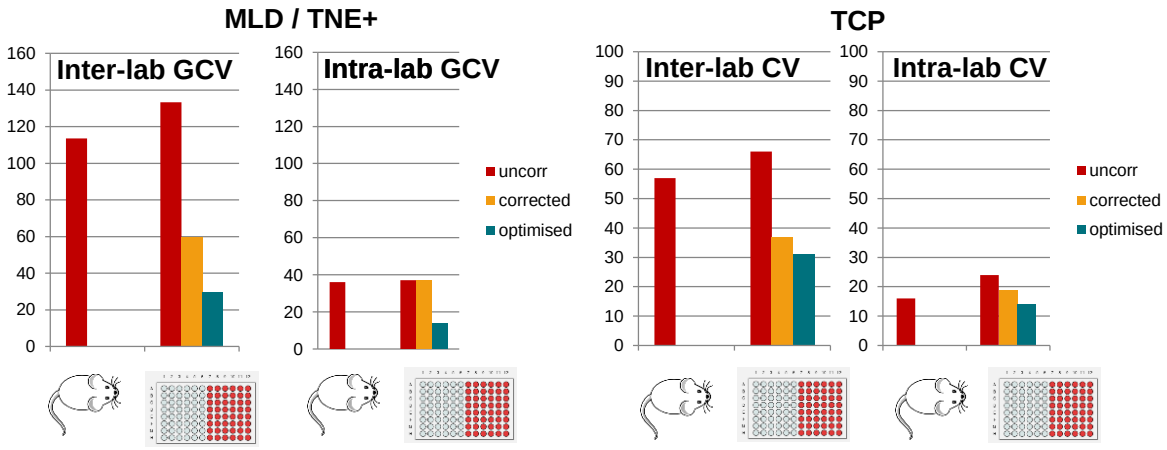
Phase II

TCP on Vero cells, corrected						
	TdG	TdH	TdJ	TdK	TdL	TdM
Overall GM	142	52	30	157	76	63
Inter-lab GCV	41	40	42	25	30	46
Median intra-lab GCV	24	20	10	15	17	28

Phase III

TCP on Vero cells, optimised						
	TdA	TdC	TdD	TdN	TdO	TdP
Overall GM	76	7	32	17	112	105
Inter-lab CV	13	51	39	52	15	15
Median intra-lab CV	7	21	22	22	7	7

Improved test variance achieved in Phase III



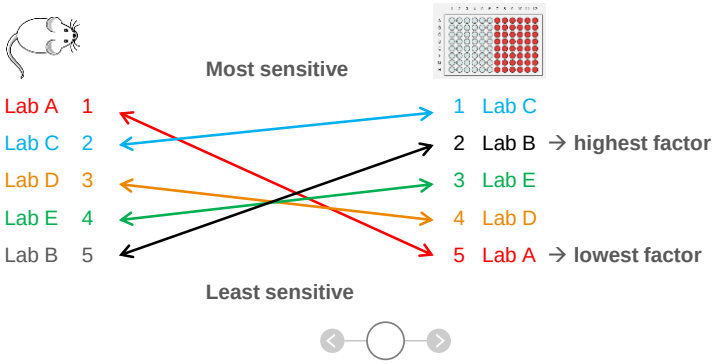
uncorr = „uncorrected" - different sensitivities to the toxin among participants influenced the results
corrected = different sensitivities were corrected by expressing toxicity relative to a reference toxin or with a ref. antitoxin
optimised = sensitivity differencies were corrected plus assay protocol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

Correlation between the in vivo and in vitro results in Phase II

Methodological difficulties:

The random distribution of mouse strains and Vero cell lines with highly different sensitivity to *C. septicum* toxin among the participants made the establishment of correlation at multiple labs level a real challenge.

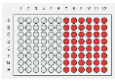
Sensitivity ranking of the mouse strains and the Vero cell lines, and the lab-specific „correlation factors"



Correlation between the in vivo and in vitro results in Phase II

Methodological difficulties solved, concordance demonstrated:
Lab-specific unique correlation factors eliminated by ranking the test toxins (and toxoids) in the mouse test and in the cell line assay by the participating laroratories.

MLD test



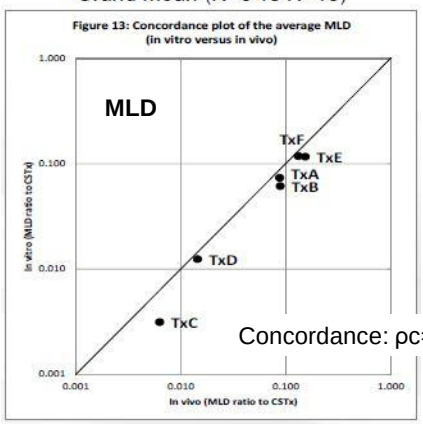
Most toxic

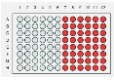
by all labs	Tx A	1	⟷	1	Tx A	by all labs
by all labs	Tx C	2	⟷	2	Tx C	by all labs
by all labs	Tx B	3	⟷	3	Tx B	by all labs
by all labs	Tx E	4	⟷	4	Tx E	by all labs
by all labs	Tx D	5	⟷	5	Tx D	by all labs

Least toxic

Correlation between the in vivo and in vitro results in Phase II

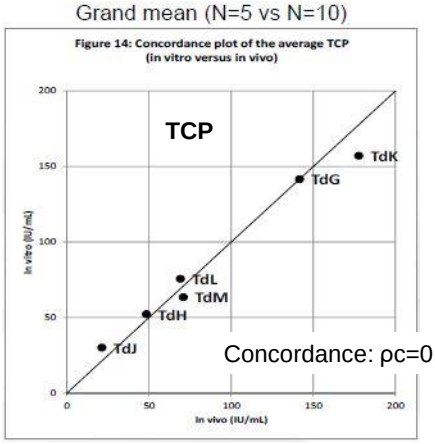
Grand mean (N=5 vs N=10)

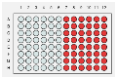




Concordance: ρc=0.961

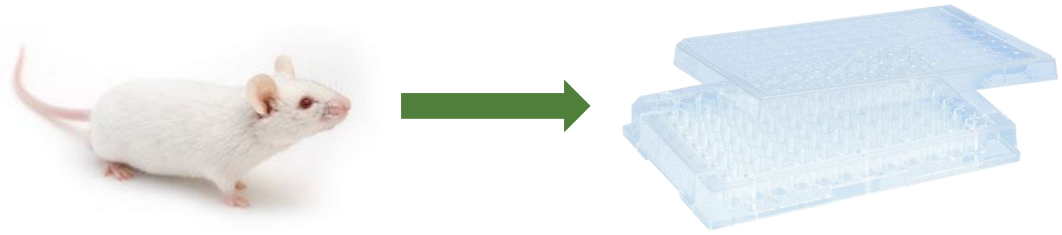
Grand mean (N=5 vs N=10)





Concordance: ρc=0.980

Thank you for your attention



Implications for vaccine manufacturers and regulators

- How to establish correlation between the in vivo and in vitro methods in one lab?
  - An individual correlation factor should be established between the results of the mouse and cell line methods when both methods are used in parallel for testing the same samples. This factor is supposed to be constant, and applicable to the individual lab only. Each lab, therefore, should establish its own correlation factor which then can be used for transformation between the in vivo and in vitro results.
- How to establish an acceptance limit in the in vitro residual toxicity test for toxoids?
  - The acceptance limit for the residual toxicity of toxoids in the mouse test is clear: No mouse should die attributable to the toxoid injection.
    The in vitro assay with up to 1000-fold higher sensitivity will definitely detect some residual toxicity in toxoid samples that already passed the mouse residual toxicity test. Each lab should set its own in vitro acceptance limit according to the „correlation factor" already established as described above.