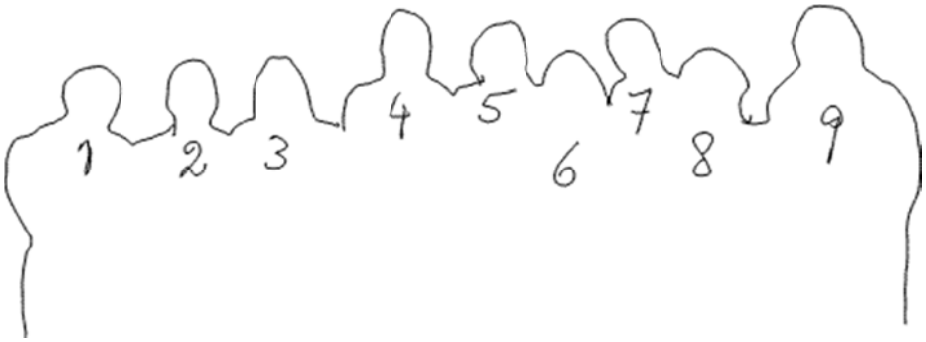
Minutes botulism CoVetLab – club5 meeting

Date: 9 February 2011; 11:00 – 17:00h

Place: VLA-Weybridge

Host: Maurice Sauer



1 Maurice Sauer, 2 Mikael Hedeland, 3 Viveca Baverud, 4 Øystein Angen, 5 Brank Kokotovic, 6 Janine Gielbert, 7 Jo Erkens, 8 Jemma Thorne, 9 Jan Langeveld

Present: MS, JG, Jt, and Yue Tang (not on picture) (VLA-Weybridge, Addlestone, United Kingdom); ØA, BK (DTU, Kopenhagen, Denmark); VB, MH (SVA, Uppsala, Sweden); JL, JE (CVI-WageningenUR, Lelystad, The Netherlands).

1. Introductions to each other, information on resources:

VLA

Maurice Sauer: chemist/biochemist - need to find alternatives for botulism mouse bioassay, long history of outsourcing botulism toxin tests to CVI-WUR; Maurice and Jan have had a few years collaboration in TSE field multiplex diagnosis of TSE strains like BSE, scrapie etc. (Maurice could you indicate which mass spectrometric tools you have for the project triple quadrupole (QqQ, Agilent), MALDI-ToF/ToF (Bruker) & Q-ToF MS instruments). Bovine blood samples of outbreaks in UK are commonly not or very weakly positive in mouse bioassay – are cattle more sensitive than mouse bioassay?!

Yue Tang: biochemist/molecular biologist. Attending morning session, is currently working on proteomics developments (together with Maurice)

Jemma Thorne: research scientist in Maurice's lab, biochemistry. Working up the EndopepMS assay – using synthetic peptide substrates for the toxin and analysing the toxin specific products by LC-QqQ mass spectrometry (together with Janine Gielberts)

Janien Gielberts: chemist, mass spectrometry specialist, currently focussing on characterisation of proteins using quantitative (QqQ) and qualitative (Q-ToF) mass spectrometry.

DTU

Øystein Angen: microbiologist; approval/permission for botulism work has to be renewed within ¾ year; perform lots of botulism toxin bio-assays), the number of positive feed samples was very limited last year; sample types – feed, birds (mostly C-type toxin); cattle (C-type), tried to acquire funding for mass spectrometric and fluorimetric assays previously, without success. (Maybe this consortium might be of help to retry? Dead cattle: cause a dead cat in hey on attic, water leakage probably distributed the toxin in stable, chicken samples from Norway, DTU has all strains and (rabbit) antisera for diagnosis in bioassay. Peter Heegaard performs the mass spectrometry (*The biological samples that Mikael presented had been sent to and investigated at CDC with MALDI-TOF only. At SVA LC-ESI-MS/MS runs were carried out on standard toxin spiked to buffer*). ØA mentions the inactivation option for botulism samples by heating.

Branko Kokotovic: microbiologist with broad experience in veterinary field, has experience with other Clostridia like difficile and others; did until now not directly grow the Clostridium botulinum serotypes.

SVA

Viveca Båverud: microbiologist and used molecular biology techniques; RT-PCR for toxine genes in Clostridium cultures from coecum samples, is subtyping in pulsed-field electrophoresis towards dendrograms (2 publications appeared, request for copies). In 2008 lot of outbreaks in chicken-broiler farms; cattle samples - problems in silage. Whole genome sequencing related to mink and vaccine.

Mikael Hedeland: chemist/mass spectrometrist. Some time ago: toxin samples sent to CDC USA for substrate incubations; investigated these in LC-ESI-MS/MS and Maldi-Tof at SVA (*LC-ESI-MS/MS*) Level 0.23 LD_{50} in 10 µl; 1 ng/mL = 1pg/µl. Sensitivity high enough in Maldi-Toff. Publication to appear. Supplier of toxin material: The firm selling the C and D toxin is www.metabiologics.com. D- and C-serotype.

CVI

Jan Langeveld: biologist/biochemist with protein biochemstry specialism. Worked in several places on basement membranes, auto-immune syndrome, lipid enzyme biochemistry, and since 1990 in veterinary

field on vaccines and diagnostics. Specialist in vet. prion diseases. Collaborated with Maurice since 2007 on multiplex assays for prions/TSEs (therefrom the idea for the club 5 project description title). CVI has all has all strains and antisera for diagnosis in bioassay for serotype confirmation. Further a set of about 30 C/D type toxin specific monoclonals have been generated by Alieda van Essen and Fred van Zijderveld. Antisera to cleavage sites of toxins in substrates of C and D are under development (first results expected halfway March).

Jo Erkens: research assistant, biochemist, working with Jan since ±2003. Has long experience in hormonal RIA, conventional ELISA and prion research. Has since last year developed background knowledge and worked out a synaptosomal system for toxin capture (to be presented today).

2. The CoVetLab proposal:

Jan Langeveld (the project: *In vitro* multiplex botulism bioassays using neurotoxin capture systems; plans; CVI resources).

Rough project plan:

milestone / deliverable	subject	month	
1	method for synapstosome preparations	1	Nov/2010
2	method for synapstosomal toxin binding BoNT/C and D	4	Mrc/2011
3	mass spectrometric data on substrate cleavage (captured, non-captured)	3&8	Jan & July/2011
4	antisera specific for cleavage products	7	Sep/2011
5	spiked field samples tested	12	Sep/2011
6	1st report	12	Sep/2011
7	new plans for year 2 CoVetLab	12	Sep/2011
8	plans for acquiring external funding	10-12	July-Sep/2011

Jemma Thorne (together with Janine Gielberts: mass spectrometric recognition accomplished in the different toxin serotypes using synthetic peptides (as Boyer). Preliminary analysis performed on recombinant C-toxin analysis since native BoNT C not available to VLA at present.

Mikael Hedeland (the N-terminal fragment of C-type substrate by mass spectrometry).

Jo Erkens (mAb and polyclonal samples in ELISA, protocol development for mink synaptosomal material and BoNT/D binding assays).

3. Plans & suggestions for further work:

Major focus (in 1st year): Develop capture system synaptosomal preparations. Develop the tools for mass spectrometry of the endopeptidases. For club 5 project: BoNT/C and BoNT/D.

Actions (see also table below):

- CVI will perform more research on optimal conditions for toxin binding, and supply synaptosomes to all three other partners. Protocols for incubations will be accompanying. Shipment of synaptosomal preparations due at end April (for the time being).
- CVI will also ship to partner subtrate and cleavage material from synaptosome-toxin incubations.
- Toxin inactivation: inactivation of resulting in vitro test material by heating, which would make shipping to each other simple. Check the reliability of this heat inactivation. Further check the stability of the peptide substrate and cleavage products therefrom. Other suggestion is to test the high titer toxin samples in a 1/100 dilution if feasible.
- Next step after initial mass spectrometric confirmation: test presence/effect of matrix samples on effectiveness of spiked toxin: liver (VLA), serum (DTU, SVA?, VLA), coecum (SVA?), gut content (VLA?, SVA?), feed material (CVI, DTU, VLA). Animal species of origin.
- Peptidic substrate choices: mainly based on Boyer's work. When choosing a peptidic substrate, consult the mass spectrometrist for proper choices.

		practical roles					
				toxin testing			
leader	**partner**	**synaptosome capture***	**immuno-capture****	**mass spectrometry**	**ELISA**	**fluori-metry**	**field samples for spiking with toxin*****
Langeveld	CVI, Lelystad, NL	x (supplier)		no	x	x	x
Sauer	VLA-Weybridge, UK	x	to be started	x (Gielbert)			x (at CVI?)
Angen	DTU, Copenhagen, DK	x		x (Heegard)			x
Baverud	SVA, Upssala, SW	x		x (Hedeland)			x
		** synaptosome preparation shipped from CVI*	*** immuno capture system shipped from VLA*				*** coecum, serum, liver, gut content, feed

Dear colleagues: could you indicate which matrix sample(s) you would like to use for being spiked and tested. Keep the choice limited. I would think of one sample from each type of tissue in the ***footnote if realistic. Mikael proposes: "Regarding our wishes for sample matrices, we prefer serum to begin with and then feed and liver".

4. **Health and safety issues**: are C and D safe enough for humans? Maurice considers these also as potentially also harmful for humans. VLA is expecting to receive soon license for botulism toxin testing. Viveca refers to a case of lethal effect in human being by BoNT/C.

5. **Future**: collaborate plans and confidentiality issues. No discussions yet. Jan indicated that today' s meeting was an open meeting with no confidentiality issue since techniques were still immature and not yet really proven, but that in future he might want to have a confidentiality. Patents were not so realistic respecting the large amount of patents already in the field. It was expected that all partners are interested in publishing in scientific journals.

6. **Other**: Such as: club 5 meeting in France, June 9th, ANSES at Sophia-Antipolis (near Nice). Jan will represent the group's progress.

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Jan langeveld, 17February2011