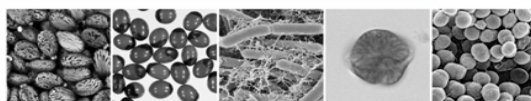




General Public

Annual newsletter 4

EuroBioTox



MAY 2023

EUROBIOTOX UPDATES

EuroBioTox stakeholder meeting

EuroBioTox - a research project funded by DG HOME under Horizon 2020 - has created a comprehensive mechanism to increase quality assurance in security-related biotoxin analysis in Europe. It has provided a solid platform for the technical advancement of expert laboratories and practitioners from the security, health, and food sectors. It brought together 63 laboratories, industrial partners and end-users from 23 countries, spreading know-how of the work with biological toxins from experts to practitioners in the EU.

At its core was the development and bulk-manufacture of certified reference materials, the setting up of a repository of validated tools and reagents, the conduct of proficiency tests, and capacity building through dedicated training. The project started in June 2017 and will end in May 2023. In order to ensure that the capacity built up by the programme will be sustainable, a **Stakeholder Meeting** was organised on 7th Sept 2022 in Brussels with 26 invited experts from DG HOME, DG SANTE, DG HERA, DG INTPA and DG ECHO as well as representatives from UNODA, OPCW and the related EU project EMERGE. Proposals were put forward on how the achievements of the project could be consolidated beyond 2023 and different options were laid down and prioritized in a roundtable discussion.



Participants of the Stakeholder meeting. Sept 22

Main achievements in a few figures

It is a great joy for the EuroBioTox partners to publish **the fourth and last annual newsletter**.

Thanks to the involvement of the EuroBioTox partners significant achievements were made during the six years of this project.

With respect to the production of reference materials, we made significant progress in 2021 and 2022: all of the materials are far in progress and show an appropriate quality in terms of purity and activity.

The SEB CRM project has been finalized, and the material is now available for sales by the Joint Research Center of the European Commission.

The other four CRMs (ricin, abrin, BoNT/A and BoNT/B) will be released in 2023. Detection tools are available since early 2020 for authorised labs in the EuroBioTox network.

19 different training courses on different toxins and methods were implemented, a number of them as remote trainings. All in all, 41 laboratories have been trained successfully.

All of the 11 large proficiency tests planned at the beginning of the project were organised and conducted.

In the framework of WP4, dedicated to safeguarding the quality (analytical method vali-

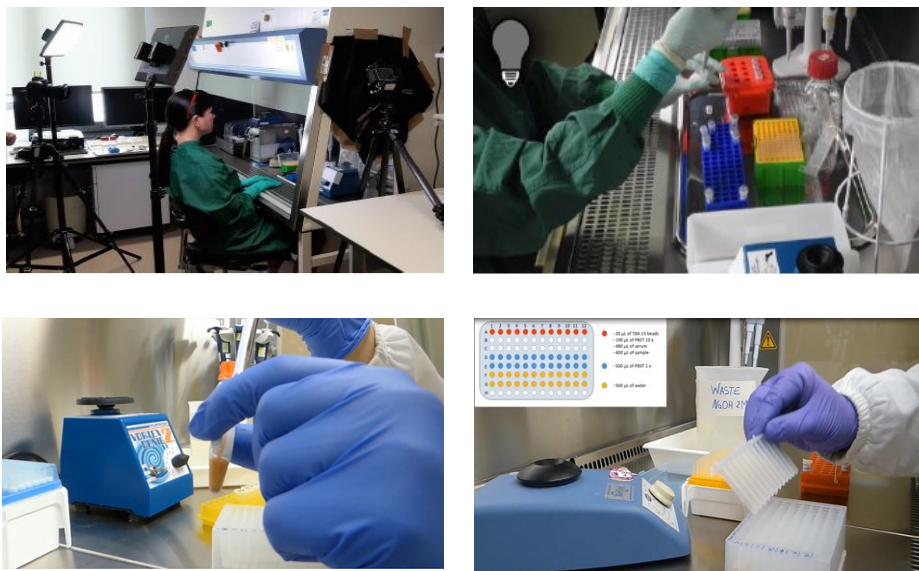
ations, procedures throughout the project.), several documents were finished (production of a standardised PT protocol detailing each step of a PT organization, validation protocols and method SOPs).

During the last year of EuroBioTox, the experimental focus was laid on the evaluation of five potential animal replacement methods for BoNT detection to eventually replace the mouse bioassay.

- ⇒ 5 CRMs (23810 vials)
- ⇒ 19 training courses
- ⇒ 11 Proficiency Tests
- ⇒ 190 material shipments
- ⇒ >35000 website visitors
- ⇒ > 50 oral presentations

Training course movies

The implementation of the advanced training courses foreseen for the 3rd reporting period was heavily influenced by the Corona-crisis. EuroBioTox beneficiaries decided to organize web-based training courses instead of face-to-face courses. Therefore, to demonstrate to the attendees how to handle protocols, movies summarizing all the steps of the workflow were shot before the course and shown to the participants. These movies were provided to the participants that need to implement the trained protocols to their laboratory.



Screenshots from training course movies

Set up of 2 e-learning courses

During the 3rd reporting period, several e-learning courses were set up by QUB.

The aim of this task is to procure online e-learning materials which facilitate the dissemination of information regarding the analytical and immunological methods used by the EuroBioTox consortium for studying the toxins under their remit (i.e. SE, STX, BoNTs, ricin and abrin).

The target audience is primarily the lay community with little understanding of both the toxins and the methods used for their detection.

The learning is completed through the use of video lectures, quizzes, and extra 'further watching/reading' sections. These extra sections contain videos, interactive platforms and reading materials hand-selected from the internet to compliment the course content.

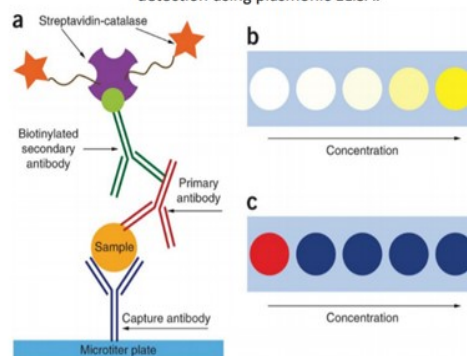
All of this is hosted on the CANVAS online learning website.

Do not hesitate to ask for a URL to have access to these courses to:

katrina.campbell@qub.ac.uk

Model sandwich immunoassay for toxin analysis

(a) the enzyme label is linked to the immunocomplex by biotin-streptavidin interactions. (b) Visual detection with conventional colorimetric ELISA. (c) Visual detection using plasmonic ELISA.



Exemplary screenshots from the CANVAS EuroBioTox e-learning

Advanced immunological course on BoNT detection

An advanced training course for the detection of BoNT by singleplex/multiplex ELISA methods was organised by RKI and CEA in February 2021. There were 15 participants from ten scientific institutions from six countries. This course consisted of four afternoon sessions held on 23–26 February 2021 together with spare time exercises. Lectures started with introductions into variability of BoNT sero- and subtypes and BoNT detection methods including ELISA validation procedures and analysis of performance criteria. On day two, trainees were provided with ELISA



Trainees and instructors of EuroBioTox training course in February 2021

raw data and templates to perform an ELISA validation calculation independently based on a comprehensive protocol provided. The focus was laid on how to thoroughly validate sandwich ELISA methods on the example of the BoNT/A in-house ELISAs from CEA and RKI in order to prepare for an accreditation of the method. Evaluation of the results was done on the next day. The third day continued with an introduction into the multiplex bead-based suspension array technology (Luminex assay) and the corresponding data analysis. On the fourth day, two training movies were provided on how to practically conduct the multiplex method according to a detailed protocol. The feedback was very positive and several participants were planning to implement the validation procedure for the method.

Advanced training course on BoNT detection by functional Endopep-ELISA



Trainees and instructors of EuroBioTox training course in March 2021

An advanced training course for the detection of BoNT by a functional multiplexed endopeptidase assay was organised by RKI using distance teaching on 16–18 Mar 2021. Nine participants from six countries participated in the course. This course consisted of three virtual afternoon sessions together with a practical exercise on data analysis.

The first day started with introductions into the variability of BoNTs and functional assays in general including information on the nuts and bolts of the functional assay trained as well as details on the multiplexed endopeptidase assay based on neopeptide-specific antibodies. To demonstrate the technology three teaching movies were shown and discussed: i) coupling of proteins (antibodies) to Dynabeads; ii) coupling of proteins (antibodies) MagPlex beads; iii) performance of the functional multiplexed endopeptidase assay.

On the second day, an introduction into data analysis was given and basics of assay validation were explained; a practical exercise on data analysis ended the day.

On the last day there was a general discussion of the trained method including the use of alternative capture reagents.

LC-MS training course on SEB detection

An advanced training course for SEB identification by LC-MS peptide fingerprinting from complex matrices was organised by CEA using distance teaching on 12–14 April 2021 (course 13).

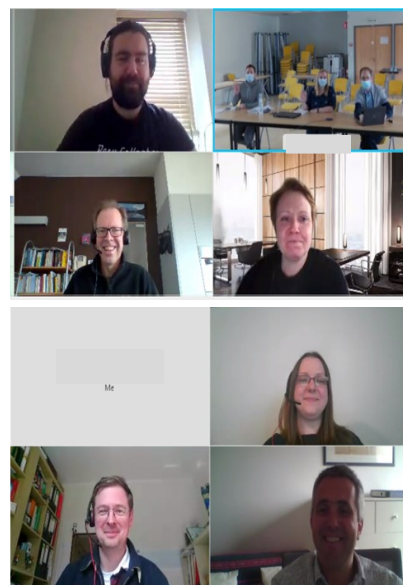
There were six participants from three core partner institutions. This course consisted of three online sessions including practical exercises on data analysis.

Lectures covered targeted proteomics, MS quantification, method validation, peptide selection for SEB and MS data analysis using different MS software tools.

A teaching movie summarising all the steps of the proteomic workflow had been prepared at CEA and was shown to the participants and discussed.

The last day of the training was dedicated to independent data analysis exercise of raw LC-MS data by the participants.

The participants were overall satisfied with the training course and had no further suggestions for changes or improvements of the course.



Screenshots from participants of
the training course 13

Training course on BoNT detection by functional MS

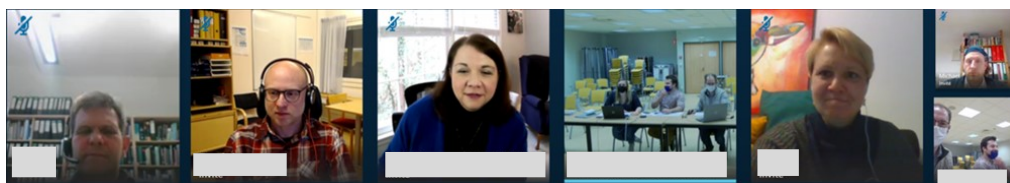
An online advanced training course for the detection of BoNT via the Endopep MS assay was organised by CEA from 08 to 09 December 2021.

There were 10 scientific institutions represented at the training course from seven countries. The aim of the training course was to train participants to analyse BoNT containing samples based on ENDOPEP MS assay and to implement this approach in their own laboratory.

During day 1 of the training course, lectures were given on BoNT state of art, ENDOPEP-MS method and history and on method evaluation. Instructors from CEA were supported by CDC and RKI.

A teaching movie summarising all the steps of the workflow had been prepared at CEA and was shown to the participants and heavily discussed.

The starting level of the participants on ENDOPEP MS assay was low for almost all participants but regarding to the feedback it seems that the benefit from the course was important for all trainees.



Screenshots from participants of the training course

Training course on ricin detection by functional MS

CEA organised an online advanced liquid chromatography coupled to mass spectrometry training course on ricin detection by functional MS. Overall 7 participants from 4 institutions and 4 countries from beneficiaries attended the web based EuroBioTox course 9 on 3rd-5th November 2021.

The focus was laid on how to set up the adenine release LC-MS workflow from sample preparation to data analysis using Xcalibur.

Lectures covered MS quantification, method validation, peptide selection for ricin and MS data analysis using different MS software tools. A teaching movie summarising all the steps of the proteomic workflow had been prepared at CEA and was shown to the participants and discussed.

The last day of the training was dedicated to independent data analysis exercise of raw data by the participants.



Screenshots from participants of the training

Training course on ricin quantification by ELISA

An advanced training course for the detection of ricin by the singleplex/multiplex ELISA methods was organised by CEA and RKI using distance teaching. There were 9 participants from seven scientific institutions. This course consisted of four afternoon sessions held on 9-12 March 2021 including practical exercises. Lectures started with introductions into ricin detection methods including activity assays, ELISA and Lateral Flow Immunoassay detection. ELISA validation procedures were also presented in detail including discussion of assay quality criteria.

Trainees were provided with raw data of a ricin ELISA validation in buffer and complex matrices and templates to perform an ELISA validation calculation and were asked to analyse these raw data. The participants' results were evaluated, presented and discussed using the information previously given during the different introductions.

An introduction into cell-based cytotoxicity assays was given and method protocols were provided. A practical description of the cell-based method was done using training movies. Then raw data from cell-based assay for ricin containing samples were analysed and evaluated.

The feedback was very positive and several participants planned to apply the trained validation procedures in their laboratories.



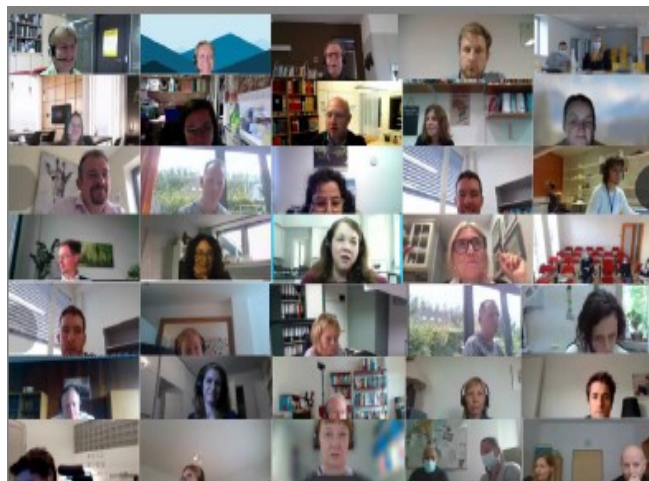
Trainees and instructors of EuroBioTox training course 7

Meetings

Due to the proceeding of the Corona crisis, no face-to-face meetings among the partners could be organised in the 3rd reporting period. Instead, a number of **virtual meetings** were held as Webex video conferences and are summarised below.

The 2nd large face-to-face meeting

with all beneficiaries, outer network partners, members of the Advisory Board, Security Board and representatives of EC/DG HOME was originally scheduled to take place at Anses, Paris, and was already announced to take place on 25-29 May 2020. However, due to the ongoing Corona crisis, a virtual meeting was held as Webex video conference on 06-07 Oct 2020: A 2-day video conference with all core and outer partners was arranged to review progress obtained in all WPs. A particular focus was laid on the discussion of results on three PTs performed in the first PT round (STX PT1, SEB PT1, ricin/abrin PT1).



Participants of the 1st virtual EuroBioTox meeting among all core and outer partners organised on 6-7 Oct 2020

In-situ PT evaluation meeting (FOI):

An additional virtual meeting was organised on 21 January 2021 with core and outer partners where all PT participants in the *in-situ* PT were invited. The PT organizer (FOI) presented the PT design, stability and homogeneity data, and the overall result of the PT. Two PT participants were invited to present their results. The participants were able to ask questions and make comments on the PT.

Organisation of face-to-face meetings between all 13 core partners:

A number of small technical meetings among individual core partners were held in a virtual format and were necessary to discuss and solve technical issues.

- 28 May 2020: A 1-day virtual meeting among all core partners was held to discuss an update of all WPs and evaluate the potential impact of the Corona crisis on project implementation
- 07-08 June 2021: A 2-day virtual meeting with all core partners was organised. The meeting was arranged to update on the activities in all WPs, especially focusing on necessary next steps in WP1, WP3 and WP7



Core partner meeting on 07-08 June 2021

Final Meetings



Sciensano, Brussels. Oct 22

Two meetings were held to finalise the EuroBioTox project. The first one was held in October 22 in Brussels, and the second one in April 23 in Maisons-Alfort. During these last two meetings, the latest experimental progress on WP1 (Certified Reference Materials) and WP7 (Animal replacement methods) was discussed, as well as the results obtained during the latest Proficiency tests (WPP3). An important part of the discussions was the follow-up of the EuroBioTox project and how to maintain the network and the expertise acquired during the project.



ANSES, Paris. April 23



We would like to THANK all the laboratories involved in EuroBioTox, for their investment and for helping to make the EuroBioTox project a success ! With some sweets from the EuroBioTox brand...

Proficiency Tests

So far, all of the eleven large proficiency tests (PT) planned at the beginning of the project were organised and conducted.

By the end of the 2nd reporting period, four PTs covering qualitative and quantitative detection of STX, SEB as well as ricin and abrin were conducted, the latter exercise targeted both toxins in one combined exercise. During the 3rd reporting period, three additional PTs were planned, organised and conducted: the 2nd PT on qualitative and quantitative detection of STX, the first PT on qualitative and quantitative detection of BoNT and one PT focussing on *in-situ* detection of protein toxins ("*in-situ* PT"). During the 4th reporting period, the last PTs were planned, organised and conducted: the 2nd PT on qualitative and quantitative detection of BoNT, SEB, ricin and abrin with increasing level of difficulty.

All in all, the implementation of the European Proficiency Testing Scheme under EuroBioTox allowed us to draw meaningful conclusions on the current status quo of detection capabilities and to highlight significant progress and the potential for further improvement.

2 additional EuroBioTox publications !

During the third reporting period, the EuroBioTox partners were able to publish two more publications on EuroBioTox contents highlighting abrin detection by immunoassay and mass spectrometry. If you are interested, publications are available as open-access publications.

Livet S, Worbs S, Volland H, Simon S, Dorner MB, Fenaille F, Dorner BG, Becher F. Development and Evaluation of an Immuno-MALDI-TOF Mass Spectrometry Approach for Quantification of the Abrin Toxin in Complex Food Matrices. *Toxins* 2021 Jan 13;13(1):52. doi: 10.3390/toxins13010052

Worbs S, Kampa B, Skiba M, Hansbauer EM, Stern D, Volland H, Becher F, Simon S, Dorner MB, Dorner BG. Differentiation, Quantification and Adentification of Abrin and Abrus Precatorius Agglutinin. *Toxins* 2021 Apr 18;13(4):284. doi: 10.3390/toxins13040284.

And many more coming soon....