BIOLOGICAL TOXINS DETECTION: A RAPID DIAGNOSIS IN AN EMERGENCY RESPONSE

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INTRODUCTION

Toxins are chemical substances of biological origin and can be considered chemical or biological warfare agents. Four toxins are included in the list of bioterrorism agents: botulinum toxin (BoNT), saxitocin, ricin, and staphylococcal enterotoxin B. BoNT is included in Category A, while the other three are considered Category B.

Botulism is a potentially fatal disease caused solely by the action of serologically distinct neurotoxins (BoNT/A-G) that prevent acetylcholine release at neuromuscular junctions, resulting in paralysis. These neurotoxins are produced by a anaerobic spore- forming bacterium *Clostridium botulinum* (*Clostridium botulinum*). Theorically, a single gram could kill more than 1 million people by the inhalational route (1). However, botulinum toxin as a weapon is its low stability, especially when disseminated in open air.

The Emergency Response and Biopreparedness Unit is the Portuguese national reference laboratory for biological events or catastrophes and has know-how, Biosafety Level (BSL)-3 facilities, capacity to work 24 hours per day, 7 days per week and trained human resources to increase lab capacity in emergency situations.
Participation in European projects such as EquaTox allowed the upgrade of biosafety procedures, technical skills and the implementation of the best techniques to detect the biological toxins that can be used as biological weapons.

Knowledge of the signs and symptoms associated with these diseases caused either by inhalation or ingestion of these toxins, by the medical community, as well as the available laboratory diagnosis, is fundamental. The effective response to a bioterrorism attack will only be possible if the clinical suspicion is quickly confirmed by the laboratory and the source of infection is promptly eliminated following an epidemiological investigation.

MATERIAL & METHODS

For BoNT detection, have been implemented three different approaches (Figure 1) for several type of samples (serum, stool, gastric fluid, food, environmental samples, tissue, swabs and pus):

(1) Pathogen detection: culture of Clostridium.

(2) Toxin detection: detection of the different toxin serotypes using commercial ELISAs (enzyme-linked immunosorbent assays), quick lateral flow tests, and, if necessary, with the collaboration of the Food and Nutrition Department of our Institute, mouse bioassay can also be performed in order to confirm positive in vitro assay.

(3) Nucleic acids detection: amplification of BoNT genes for sequencing or real-time polymerase chain reaction using specific probes for the genes *bont/A*, *bont/B*, *bont/E*, and *bont/F* that cause human botulism.

In framework of the EU project EQuATox a first international proficiency test on the detection and quantification of BoNT was conducted (2). On this context, our laboratory tested 13 samples materials included BoNT serotypes A, B and E that had different and complex matrices and were tested by commercial ELISA and by mouse bioassay.

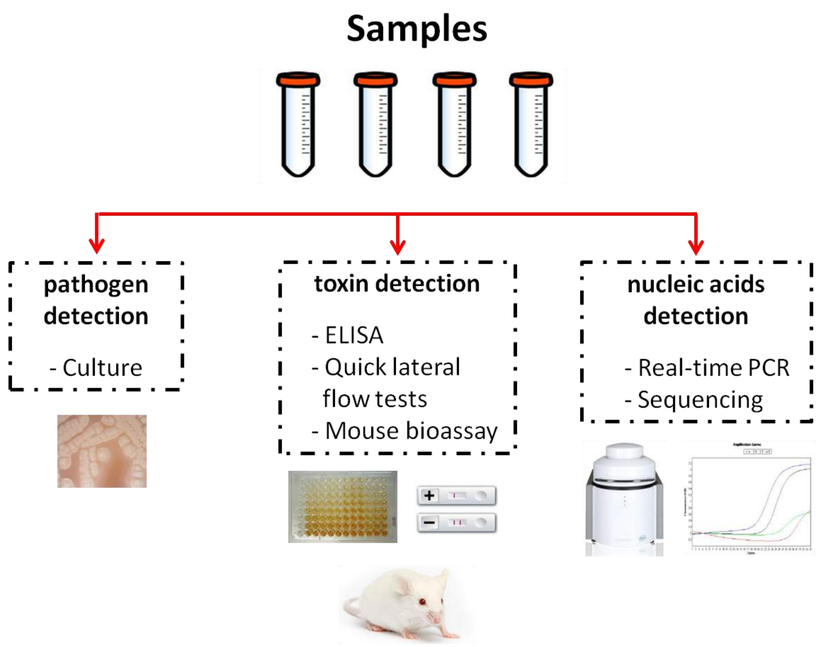

Figure 1 - Laboratorial diagnosis of *C. botulinum* and the neurotoxins

RESULTS

All samples were qualitatively correctly identified thereby delivering superior result compared to single in vitro methods.

Of the 13 samples studied, we achieved confirmation of correct results in 12 samples; in one, it was possible to detect toxin but not to serotype it (Table 1). The results were achieved by ELISA but mostly based on the mouse bioassay, which remains the gold standard assay for BoNT detection, despite many attempts to replace the use of animals. The precise detection and identification of biological toxins is difficult, since they occur naturally in different variants.

Table 1: Results of the 13 samples tested

Sample	BoNT	BoNT/A	BoNT/B	BoNT/E	BoNT/F
1	1	1	-1	-1	-1
2	1	1	-1	-1	-1
3	-1				
4	1	-1	-1	1	-1
5	1	1	-1	-1	-1
6	1	-1	1	-1	-1
7	1	1	-1	-1	-1
8	1	1	-10	10	-1
9	1	1	-1	-1	-1
10	1	1	-1	-1	-1
11	1	1	-1	-1	-1
12	1	1	-1	-1	-1
13	1	1	-1	-1	-1

Correct positive (1); Correct negative (-1)
False positive (10); False negative (-10)
Na/ not analysed

CONCLUSIONS

Participation in European projects, such as the EQuaTox Project, focused essentially on the detection and identification of biological toxins, allows the updating of laboratory procedures and participation in external quality controls, which leads to the implementation of the most appropriate techniques and the most appropriate response algorithm to the situation in question.

Other types of assays that are faster and equally accurate need to be developed because if a biological threat arises from this toxin, a speedy response could be crucial to minimize the impact on public health.

TOXINS 2017: Basic Science and Clinical Aspects of Botulinum and Other Neurotoxins, 18-21 January 2017 Madrid

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