Simple, Rapid, and Sensitive Analysis System for the Detection of Major Ciguatoxin Congeners

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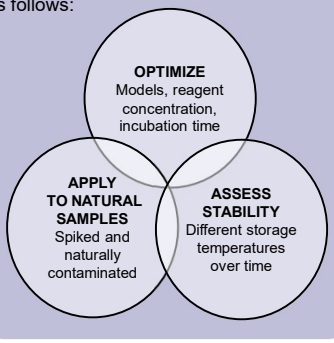
Introduction

1. Ciguatera poisoning (CP) is caused by consuming seafood that has accumulated ciguatoxins (CTXs) produced by genera *Gambierdiscus* and *Fukuyoa*.[1]
2. CTX congeners target the voltage-gated sodium channels (VGSCs) and cause gastrointestinal, cardiac, and neurological symptoms.[1]
3. It is a public health threat due to severe symptoms, limited diagnostic and treatment options, and expansion of ciguatera to previously unreported areas. Therefore, better detection methods are needed.[1]
4. An immunoassay-based protocol by Leonardo and colleagues uses magnetic beads (MBs) and sandwich ELISA to detect major CTX congeners (CTX1B and CTX3C series).[2]

Aim and Objectives

To develop a simple, rapid, and sensitive method for the specific detection of major CTX congeners (CTX1B and CTX3C series) based on a previously established protocol. The objectives of the study were as follows:



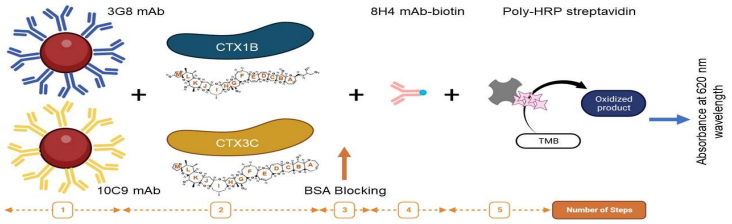


Fig 1. Schematic representation of the original protocol

Methodology

1. Activation of the MBs using and conjugation to the 3G8 and 10C9 capture monoclonal antibodies (mAbs).[2]

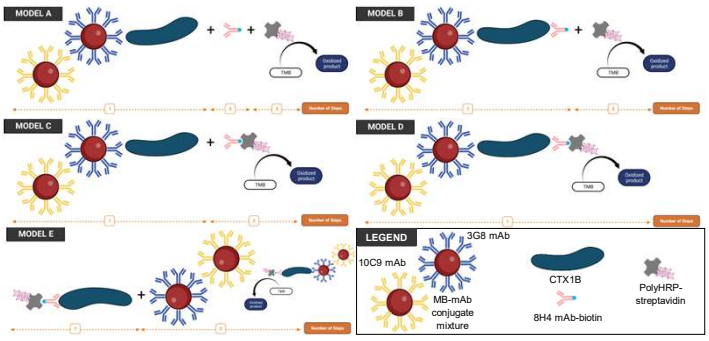


Fig 2. Schematic representation of models A–E. All steps followed by colorimetric measurement at 620 nm.

2. Optimization:
   a) Models A–E tested with polyHRP-streptavidin concentrations 1:250 and 1:1000.
   b) Reagent concentrations of polyHRP-streptavidin (1:1000, 1:250, 1:100), and 8H4mAb-biotin (1:2000, 1:1000, 1:500) for model D.
   c) Range of incubation times followed by calibration curves at 30 and 60 minutes for model D.
3. Application of the optimized model D-30 and D-60 to a negative fish matric spiked with 0.01 µg CTX1B eq./kg tissue. This was followed by the application of model D-30 to naturally contaminated samples. Compared with the original protocol
4. Stability of MB-mAb immunoconjugates was assessed at 4°C and -20°C for 120 days, following the original protocol.[2]

Results

1. Optimization

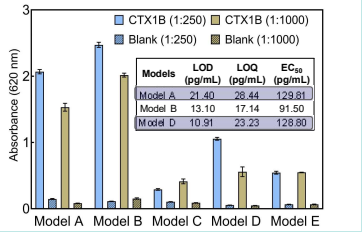


Fig 3. Model optimization: Absorbance of models A–E at polyHRP-streptavidin concentrations 1:250 and 1:1000 (n=3). Model D performs comparable to the original model (Model A).

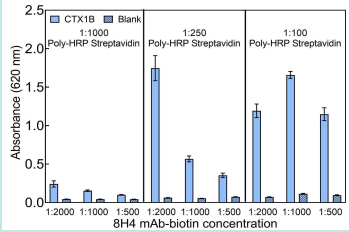


Fig 4. Concentration optimization: Absorbance of model D at different polyHRP-streptavidin and 8H4 mAb-biotin concentrations (n=3). Concentration pair 1:2000-1:250 for 8H4 mAb-biotin and polyHRP-streptavidin respectively gives the best signal.

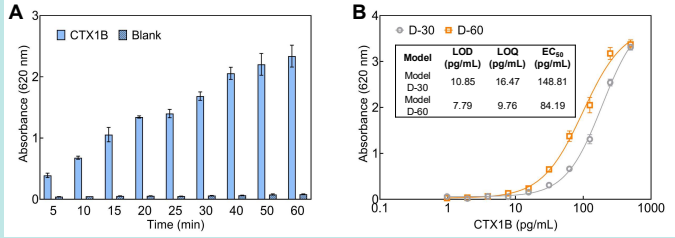


Fig 5. Incubation time optimization: (A) Absorbance of model D at different incubation times (n=3) and (B) Calibration curves of model D with incubation time of 30 and 60 minutes (n=3). Curves are background subtracted. Incubation time is a compromise between higher signal and reduced time. Model D-30 has appropriate signal with added benefit of reduced time.

2. Application to natural samples

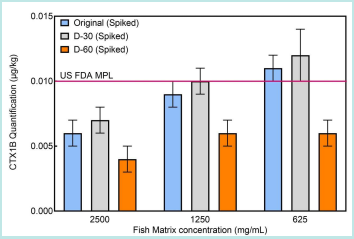


Fig 6. Spiking: Toxin quantification (µg/kg) by three models across spiked fish matrix dilutions (n = 3). MPL = Maximum Permitted Limit. Model D-30 performs comparable to the original protocol and within the US FDA limit of 0.01 µg CTX1B eq./kg. tissue.

Methods	Fishes			
	V. louti (04/DIV/03)	*V. louti* (15/41)	*L. bohar* (02/DIV/38)	*L. bohar* (03/DIV/21)
Original protocol (µg CTX1B equiv./kg tissue)	0.37 ± 0.03	0.24 ± 0.02	1.00 ± 0.10	0.10 ± 0.02
D-30 (µg CTX1B equiv./kg tissue)	0.36 ± 0.01	0.21 ± 0.02	1.22 ± 0.08	0.10 ± 0.01
CBA (µg CTX1B equiv./kg tissue)	0.21	2.08	1.13	0.67
MBA	Positive (++)	Positive (+++)	Positive (+++)	Positive (++)

Fig 7. Naturally-contaminated fishes: Toxin quantification (µg/kg) by the original protocol, D-30 model, CBA, and MBA. The CBA and MBA results were provided by IRTA, La Ràpita and CITEB, La Réunion, respectively. Model D-30 provides toxin quantifications comparable to the original protocol. Deviations in quantifications for *V. louti* (15/41) and *L. bohar* (03/DIV/21) when compared with CBA and MBA results.

3. Stability assessment

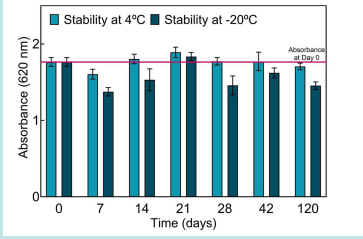


Fig 8. Stability: Absorbance of MB-mAb immunoconjugates over 120 days at storage temperatures 4°C and -20°C. Immunoconjugates were found to be stable for at least 120 days with acceptable inter-assay relative standard deviation.

Conclusion

1. Major CTX congeners detected in a one-step immunoassay leading to a 9-fold reduction in assay time, followed by colour development and quantification.
2. Strategy able to detect CTX1B at US FDA guidelines of 0.01 µg CTX1B eq./ kg tissue.
3. Quantification of CTX in contaminated fishes similar to the original protocol.
4. Stability of MB-mAb for at least four months allows to prepare immunoconjugates in advance and shortens the assay time.

Acknowledgement

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2. Leonardo, S., Gaiani, G., Tsumuraya, T., Hirama, M., Turquet, J., Sagristà, N., Rambla-Alegre, M., Flores, C., Caixach, J., Diogène, J., O'Sullivan, C. K., Alcaraz, C., & Campàs, M. (2020). Addressing the Analytical Challenges for the Detection of Ciguatoxins Using an Electrochemical Biosensor. *Analytical Chemistry*, *92*(7), 4858–4865.

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