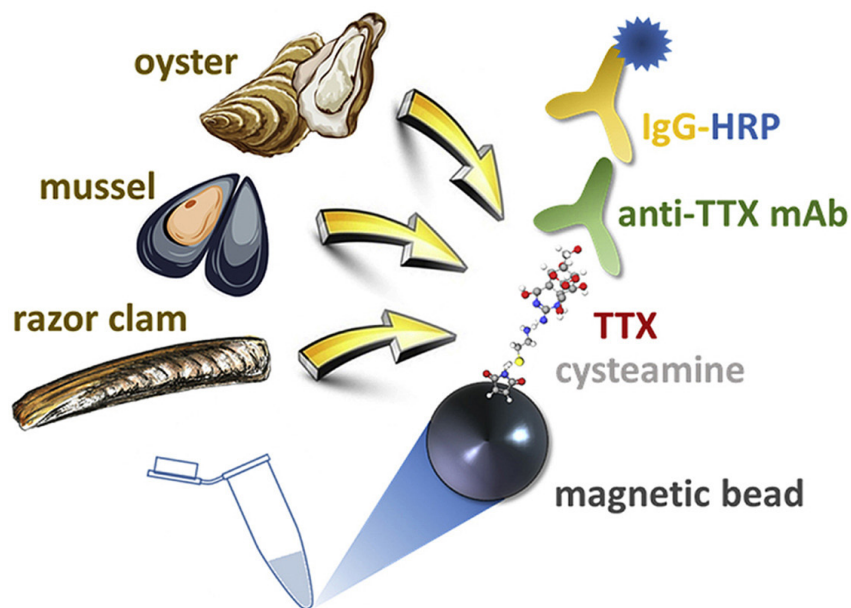


## Rapid screening: A fast screening method for the detection of Tetrodotoxin (TTX) in seafood



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### SUMMARY

Tetrodotoxin (TTX) is a potent neurotoxin responsible for food poisoning incidents, mainly related with the consumption of pufferfish (*Tetraodontidae*) in tropical or subtropical regions of Asia and the Pacific Islands. Since 2007, TTX has been found in shellfish from European countries such as the United Kingdom, Greece, the Netherlands, Spain and Italy, though with low concentrations to date. This output concerns a new extraction and immunoassay method to quickly screen for the presence of TTX in shellfish. The novel immunoassay utilises an antibody which specifically recognises TTX and several of its analogues. The method allows for the detection of TTX at concentrations as low as  $1 \mu\text{g}/\text{kg}$  in oysters and razor clams, and  $3.3 \mu\text{g}/\text{kg}$  in mussels – levels well below the European Food Safety Authority guidance threshold.



### KNOWLEDGE NEED

TTX presence in shellfish is not currently regulated in Europe. There is no maximum permissible level, but the European Food Safety Authority (EFSA) affirms that concentrations below  $44 \mu\text{g}$  of TTX equivalents/kg shellfish meat, based on a large portion size of 400 g, do not result in adverse effects in humans. There is a need for an effective method that would make it possible to screen shellfish for this potent neurotoxin and protect human health if and when outbreaks occur.



### RESULTS

The two-step method includes a simple and easy-to-implement extraction protocol, where TTX is extracted from a seafood sample, followed by a novel immunoassay process. The presence of TTX and its analogues can be detected in a rapid, simple, specific, sensitive and reliable way using immunoassays. Unlike conventional immunoassays, performed on plates, this method exploits magnetic beads as antigen immobilisation supports. The use of magnetic beads reduces the shellfish matrix effects, thus allowing the detection of TTX at concentrations as low as  $1 \mu\text{g}/\text{kg}$  in oysters and razor clams, and  $3.3 \mu\text{g}/\text{kg}$  in mussels. The analysis, which can be performed in 70 minutes, has been validated for mussels, razor clams and oysters, with proven inter- and intra- assay precision. This novel method guarantees shellfish safety, and protects human health. The protocol has been published in a scientific article, and all supporting data are available in open access. This method has been prototyped in the form of a kit.

### IMPACTS

This knowledge has significant potential to improve shellfish safety in Europe. The method would have an impact on several levels by increasing the availability of safer seafood on the market, improving consumer trust and seafood consumption habits, and contributing to an improved health profile of the European population. The method could support an overall increase in seafood sector growth, sustainability and profitability.

**Contributes to the UN Sustainable Development Goal 12:** Responsible consumption and production.



## END-USERS & APPLICATIONS

- ➔ **Research and Development community:** scientists, researchers and academics working in the field of seafood safety can use the validated datasets and new knowledge in this novel method to support further research on TTX, including its regulation, presence in marine organisms and distribution in different geographical areas.
- ➔ **Shellfish industry figures:** further development at industrial level is needed to develop a commercial kit that would allow producers to apply this method and to ensure the production of safer shellfish. This knowledge could also be applied by production site managers to control harvesting sites and manage stocks.
- ➔ **EU food safety authorities and policy makers:** new knowledge generated by this knowledge output can be used to support near-future EU regulation on TTXs, including the development of a monitoring programme, ensuring the production and sale of safer seafood.



## INNOVATION STATUS

Technology Readiness Level 6 – 7: the technology and a system prototype have been demonstrated, but further stability testing of the reagents and seafood industry beta tests are required.

**IPR:** Licensing agreement for use of antibodies in the test kit may be required – discussions are underway.

**Other:** Working towards potential commercialisation of the kit, Biorex Food Diagnostics are in the process of manufacturing a small Research and Development batch of the reagents for evaluation.

## DISSEMINATION AND EXPLOITATION

### Exploitation activities for policy makers:

- Monitoring agencies, regulators and policy makers will be reached through the final **SEAFOOD<sup>TOMORROW</sup>** event, info session, and a dedicated EU policy event.

### Exploitation activities for seafood industry:

- Tetrodotoxin Detection Assay Protocol – video and flyer (developed by Biorex Food Diagnostics, IRTA): [seafoodtomorrow.eu/media](http://seafoodtomorrow.eu/media)
- Demonstration of the strategy at **SEAFOOD<sup>TOMORROW</sup>** demonstration workshops, in France and Spain (February – March 2021) to support uptake and use of the method.
- Horizon Results Platform: [seafoodtomorrow.eu/horizon-results-platform](http://seafoodtomorrow.eu/horizon-results-platform)

### Dissemination activities for scientific community:

- Campàs M, Reverté J, Rambla-Alegre M, Campbell K, Gerssen A, Diogène J. (2020). A fast magnetic bead-based colorimetric immunoassay for the detection of tetrodotoxins in shellfish. *Food and Chemical Toxicology*, 139, 111315. DOI: 10.1016/j.fct.2020.111315
- Open access version of publication: [repositori.irta.cat/handle/20.500.12327/744](http://repositori.irta.cat/handle/20.500.12327/744)
- Open access validated data sets: please see [seafoodtomorrow.eu](http://seafoodtomorrow.eu)
- Website news article: [seafoodtomorrow.eu/irta-qub-and-bfd-researchers-develop-a-fast-method-for-the-screening-of-tetrodotoxins-in-shellfish](http://seafoodtomorrow.eu/irta-qub-and-bfd-researchers-develop-a-fast-method-for-the-screening-of-tetrodotoxins-in-shellfish)



## FUTURE RESEARCH

Beta testing in the industry should be completed before commercialisation of the kit can occur. Further Research and Development batches (x3) by a manufacturer are required to validate each for precision, accuracy, robustness, shipping stability, interferences, recovery and sensitivity. The supply of raw materials must also be evaluated to ensure that ISO accreditation for the development and manufacture areas of the certification can be adhered to.

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