# Innovative rapid solid-phase immunobead assay for the detection of okadaic acid and related DSP toxins in shellfish

## *Test immunologique pour la détection rapide en phase solide de l'acide okadaïque et toxines DSP apparentées dans les coquillages*

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**Abstract**

The solid-phase immunobead assay (Ciguatect™) has been optimized to detect toxins associated with diarrheic shellfish poisoning (DSP). The Ciguatect™ test kit utilizes a membrane to bind the toxins and a monoclonal antibody-latex bead solution as the determination step. The presence or absence of okadaïc acid (OA) and related toxins is determined by binding the toxins to a membrane attached to a plastic strip and exposing the toxins ladened membrane to a monoclonal antibody-colored latex bead complex which has a high specificity for OA and related polyether compounds. The intensity of the color on the membrane denotes the presence of the toxins. A rapid extraction method (REM™) has been developed to allow for confirmation of positive test results in less than 30 minutes. The application of the Ciguatect™ test kit has been evaluated through analysis of toxic and non-toxic mussels obtained from DSP outbreaks, and also from shellfish harvesting areas in France with and without evidence of *Dinophysis sp.* blooms. Results have been compared with those obtained using the HPLC procedure. The Ciguatect™ test kit is more sensitive and rapid than the HPLC method, and is applicable to field: market place screening and depuration monitoring of toxic shellfish.

**Keywords**: Diarrheic toxins, Shellfish, Detection, Immuno-assay

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**Résumé**

Le test de détection en phase solide par voie immunologique (Ciguatect ™) pour la mise en évidence rapide de toxines diarrhéiques (DSP) utilise une membrane pour lier les toxines et un anticorps monoclonal sur support latex lors de l'étape de détermination. La présence ou l'absence de l'acide okadaïque et des toxines apparentées sont mises en évidence en liant les toxines à une membrane attachée à une bandelette de plastique et en exposant les toxines fixées à un complexe anticlonal coloré sur support latex. Ce com-

plexe possède une très forte spécificité pour l'acide okadaïque et les composés proches. L'intensité de la couleur de la membrane révèle la présence de toxine dans l'extrait. Une méthode rapide d'extraction (REM ™) a été mise au point pour permettre la confirmation des résultats en moins de 30 minutes. L'applicabilité du test Ciguated ™ a été évaluée par une analyse de moules toxiques et non-toxiques obtenues provenant de zones de production française. Lors d'épisodes à DSP les résultats ont été comparés avec ceux obtenus en utilisant la procédure d'analyse par HPLC. Le test Ciguatect ™ est plus sensible et plus rapide que la méthode HPLC et applicable sur le terrain pour les contrôles à la vente et le suivi de la décontamination des coquillages toxiques.

## INTRODUCTION

Okadaic acid (OA), dinophysistoxins 1 and 3 (DTX1 and DTX3) are the principal phycotoxins responsible for diarrheic shellfish poisoning (DSP) outbreaks (Murata *et al.*, 1982). In addition, OA and DTX1 have been recognized as powerful tumor promotors (Nishiwaki *et al.*, 1990). DSP has been associated primarily with toxic shellfish, particularly mussels, harvested from temperate regions of the world such as Japan and Europe (World Health Organization, 1984). The last reported cases of DSP outbreaks in France were caused by the consumption of cooked mussels imported from Denmark in 1990. Monitoring programs for screening harvesting areas is one of the most efficient approches to prevent DSP. The mouse bio-assay has been used traditionally to monitor suspect shellfish (Yasumoto *et al.*, 1984 ; Yasumoto, 1985). Unfortunately, it involves a time consuming process of extracting the toxins and lacks specificity. An innovative rapid solid-phase immuno-bead assay (S-PIA, Ciguatect™) for the detection of toxins associated with Ciguatera Fish Poisoning (CFP) has been recently developed (Park and Goldsmith, 1991). The monoclonal antibody used as a basis in the development of the Ciguatect™ shows a high specificity for OA and related polyether compounds (Hokama *et al.*, 1992). The present paper describes the first attempt of the application of the Ciguatect™ test kit to evaluate DSP potential in mussels collected along the Atlantic coastline of France during dinoflagellate blooms and depuration operations, and in imported mussels from Denmark involved or not in recent DSP outbreaks in France. The High Performance Liquid Chromatography procedure developed by Lee *et al.* (1987) was used as the reference method for OA identification and quantification.

# Material and methods

## Shellfish samples

Selected samples of mussels (*Mytilus sp.*) were collected from shellfish growing zones during known *Dinophysis sp.* blooms (figure 1) and imported cooked frozen specimens implicated in human diarrheic shellfish poisoning outbreaks. Samples also included imported non-toxic cooked specimens. All analyses were performed on homogenized hepatopancreas gland tissue. Initial sample selection was based on HPLC analytical results. Samples were stored at – 20°C until analysis. All analyses including HPLC re-analysis were conducted during the same time period.

### HPLC methodology

The Hight Performance Liquid Chromatographic (HPLC) procedure developed by Lee *et al.* (1987) was used as the reference method for OA identification and quantification in the collected samples and can be summarized briefly as follows : Extraction and purification of OA was performed on 1g of digestive gland (hepato-pancreas) homogenate from each sample. 500 µl of chloroformic extract was derivatized overnight directly without evaporation using the 9 anthroil-di-azo-methane (ADAM) purchased from SIGMA. The derivatized isolate was further purified with a silicagel SEP-PAK mini-column (MILLIPORE-WAATERS). The purified derivatized extract was injected on the HPLC system (MERCK-France) with the following parameters : oven temperature, 35°C ; pre-column Lichrocart 4,4 RP-8 (5 µm); column, Lichrocart 250-4 Superspher 100 RP-18 ; mobile phase, acetonitrile+water (80+20) ; flow rate, 1 ml/min. A fluorimeter F-1000 (MERCK-France) set at 365 nm excitation and 412 nm emission was used for final detection.

### Immuno-bead assay methodology

The principle of the Ciguatect™ test kit recently developed by Park and Goldsmith (1991) can be described briefly as follow: the presence or absence of the toxins is determined by binding the toxins to a membrane attached to a plastic strip and exposing the toxin ladened to a monoclonal antibody-colored latex bead complex which has a high specificity for the toxins of interest. The intensity of color on the membrane denotes the presence of the toxins. DSP toxicity potential can be determined directly on edible tissue or following specific extraction procedures. A rapid extraction procedure (REM™) was recently developed where toxins are extracted with a chloroform: methanol: water mixture and partitioned into selected phases by varying polarity of the extraction/isolation system (Park *et al.*, 1992).

## Results and discussion

Comparative results for the determination of okadaic acid and related compounds using HPLC and the solid-phase immunobead assay (S-PIA, Ciguatect™) are presented in table I. These samples of mussels (*Mytilus sp.*) were collected during shellfish monitoring operations conducted in 1986, 1987, and 1988. The Ciguatect™ test kit was evaluated for possible application in shellfish safety monitoring programs for diarrheic shellfish poisoning (DSP). This assay provides qualitative results, a pass/fail response, which can be modified depending of the level of sensitivity desired. This method would be useful as an initial screening tool since the test can be performed under non-laboratory conditions. This procedure also uses a rapid extraction method (REM™) which can be used for HPLC analyses. The direct method and REM™ were used in this study. HPLC methods for OA, although avalaible for several years, suffer from the inability to detect and quantify OA analogs. Good global agreement was observed between HPLC and Ciguatect™ test results for OA levels in the mussel digestive gland tissue. Although not reported in this study, the test kit can analyze other mussel tissue either directly or following the

REM where problems with interfering subtances are evident with HPLC analysis.

Samples collected during the depuration operations were also tested using the same methodology (table II). The Ciguatect™ test kit was easy to run, rapid and did not require specialized training or instrumentation. Its role in monitoring and/or depuration programs appears to be an excellent tool for initial screening for DSP-related toxins.

Analysis of imported cooked frozen specimens implicated in human DSP outbreaks are presented in table III. These data demonstrate the varied composition of DSP related toxins in mussel specimens, i.e., not all samples contained detectable levels of OA or OA analogs by HPLC. Ciguatect™ test results on the other hand demonstrated greater sensitivity and specificity for okadaic-like compounds. The antibody used in the test kit cross reacts with the spiroketal east spere of OA as well as ciguatoxin (Hokama *et al.*, 1992). The hepatopancreas gland contained higher levels of toxins than the remaining mussel tissue. The REM™ procedure showed increased values suggesting concentration of the toxins and potential use as an extraction procedure for HPLC analysis. This extraction procedure utilizes varied concentration of chloroform, methanol, and water to extract and partition the toxins. The Ciguatect™ test kit is more sensitive and rapid than HPLC method and will be applicable to field/market place sceening and depuration monitoring of toxic shellfish.

**Table I:** Comparative results between HPLC and Ciguatect™ for okadaic acid and related polyether compounds in mussels *(Mytilus sp.)* monitored along French coastline

| Sampling location | Date year/month/day | µg OA/g HG[a] HPLC | ng OAE[b] Ciguatect ™ |
|---|---|---|---|
| Groix | 86/6 | 5.0 | 4.0 |
| Bois Cise | 87/5/21 | <0.5 | 0.5 |
| Douarnenez | 88/3/14<br>88/6/12<br>88/8/23 | <0.5<br>11.5<br><0.5 | 0.0-2.0<br>>100.0<br>0.0-1.0 |
| Baie Vilaine | 88/3/21<br>88/6/06<br>88/6/27<br>88/8/17 | <0.5<br>3.7<br>2.0<br><0.5 | 2.0<br>100.0<br>10.0<br>0.0-1.0 |

(a) HG = Hepatopancreas Gland; Method of Lee *et al.*, 1987
(b) OAE = Okadaic Acid Equivalence; color intensity on test strip assigned a value between 0-5 where 0 = non-detectable and 5 = color intensity equal to 5ng okadaic acid.

**Table II**: Comparative results between HPLC an Ciguatect™ test kit for okadaic acid and related compounds in mussels during depuration operations (hepatopancreas gland)

| Sample | Time (days) | HPLC[a] (µg OA/g) | Ciguatect™ [b] OAE[c] |
|---|---|---|---|
| June 7 | 0 | 4.0 | 2 |
| | 12 | 2.2 | 0 |
| June 26 | 0 | 2.0 | 5 |
| | 3 | 1.9 | 2 |
| | 12 | 1.4 | 2 |
| | 15 | 1.6 | 2 |

(a) Method of Lee *et al.*, 1987.
(b) Solid phase immunobead assay, Hawaii Chemtect International, Pasadena, California, USA.
(c) OAE : Okadaic Acid Equivalents; color intensity on the test strip assigned a value between 0-5, where 0 = non detectable and 5 color intensity equal to 5ng of okadaic acid on the test strip.

**Table III**: Comparative results between HPLC and Ciguatect™ test kit for okadaic acid and related compounds in mussels implicated in diarrheic shellfish poisoning outbreaks (hepatopancreas and remaining mussel tissue)

| Sample | HPLC[a] | | Ciguatect™[b] (OAE)[c] | | |
|---|---|---|---|---|---|
| | (µgOA/g) | DSP-related | Direct method | REM | Aqueous extact |
| 1 - Hepatopancreas analysis | | | | | |
| 02 | 2.0 | negative | 3 | ND[d] | 0 |
| 12 | <0.4 | negative | 5 | ND | 2 |
| 14 | 3.0 | positive | 3 | ND | 2 |
| 20 | <0.4 | negative | 5 | ND | ND |
| 22 | 3.0 | positive | 3 | ND | 3 |
| 23 | ND | ND | 5 | ND | 5 |
| 2 - Remaining mussel tissue | | | | | |
| 03 | ND | ND | 0 | 4 | 0 |
| 12 | ND | ND | 1 | 6 | 2 |
| 14 | ND | ND | 0 | 3 | 1 |
| 23 | ND | ND | 1 | 6 | 2 |

(a) Method of Lee *et al.*, 1987.
(b) Solid phase immunobead assay, Hawaii Chemtect International, Pasadena, California, USA.
(c) OAE : Okadaic Acid Equivalents ; color intensity on the test strip assigned a value between 0-5, where 0 = non detectable and 5 color intensity equal to 5ng of okadaic acid on the test strip.
(d) ND : not determined.

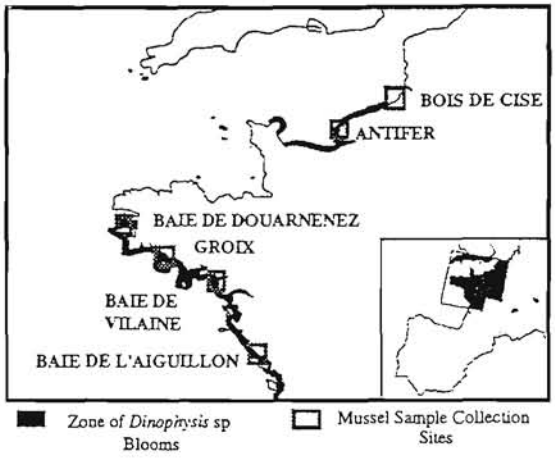


**Figure 1**: Mussel *(Mytilus sp.)* collection sites and *Dinophysis sp.* bloom locations along French coastline

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