# RAPID ANALYSIS METHODS FOR PARALYTIC SHELLFISH POISONING TOXINS (PSTS) MONITORING IN RAW WATERS

## SNABBMETODER FÖR ANALYS AV PARALYTISKT SKALDJURSTOXIN (PST) I RÅVATTEN


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**Abstract**
The presence of paralytic shellfish poisoning toxins (PSTs) in drinking water resources has urged the development of simple and fast screening tools for drinking water surveillance. This article aims to validate a quick test kit for freshwater PSTs detection. Preliminary estimates of the limit of detection (LODs) were done for 5 PSPs variants, namely C1 toxin (a "C" toxin); GTX5(a Gonyautoxin); and neosaxitoxin, dcSaxitoxin and saxitoxin (STX) and a mixture of C1 and saxitoxin. Duplicate samples and Blank/Control are used. In total 50 samples were tested plus 7 negative controls. Results show that clear estimated LOD levels for all variants except for C1. Further validation for lower detection limits is recommended if a lower concentration is of concern. Owing to cross-reactivity within the different derivatives, water operators/managers should be aware of certain false-negative risks to certain variants. Therefore, a thorough understanding of the toxin profile in source water is necessary for its application.

*Key words:* Saxitoxin, LFA, detection limit, validation, quick test kit

**Sammanfattning**
Utvecklingen av enkla och snabba screeningsverktyg för övervakning av dricksvatten har uppmärksammats p.g.a förekomsten av paralytiskt skaldjurstoxin (PSTs) i råvatten. Denna artikel syftar till att validera ett snabbt testpaket för PSTs i sötvatten. Preliminära tester av testpaketens minimumdetektionsnivå (Limit Of Detection, LODs) gjordes för 5 PST-varianter, nämligen C1-toxin (ett "C" -toxin); GTX5 (ett Gonyautoxin); och Neosaxitoxin, dcSaxitoxin och Saxitoxin (STX) och en blandning av C l och Saxitoxin. Duplicerade prover och blank/kontroll användes. Totalt testades 50 prover plus 7 negativa kontroller. Resultaten visar att tydliga LOD-nivåer uppmättes för alla varianter utom C1. Ytterligare validering för lägre detektionsgränser rekommenderas om lägre koncentration kan förekomma. Enkla och snabba screeningverktyg är användbara för att indikera PST-risker i råvatten. Förbehandling av prover för att öka toxinkoncentrationen kan behövas vid låga PST-halter. På grund av korsreaktivitet inom olika derivat bör kontrollansvariga vara medvetna om risken för falska negativa resultat. Därför är en grundlig förståelse av toxinprofil i råvatten nödvändig vid val av screeningverktyg.

## Introduction

Saxitoxins which are well known as Paralytic Shellfish Poisoning Toxins (PSTs) is a group of neurotoxins, mostly produced by marine dinoflagellates and freshwater cyanobacteria. Since the first PST (saxitoxin) was discovered in 1957 called saxitoxin isolated from the mussel species Saxidomus giganteus, more than 60 analogues have been found in this group. The word saxitoxin can thus partly represents the 'original toxin' itself while is sometimes applied for the whole group of structurally related toxins. In order to avoid misunderstanding, the term PSTs is used instead of the word 'saxitoxins' in this article. PSTs poisoned sea food such as shellfish, molluscs and crustaceans may attack the nerve system after they are consumed by people. Symptoms of poisoning can vary from sensations of tingling, loss of sensation around the mouth, general weakness to total paralysis and even death.

The high incidences of PSTs in drinking water sources showed another route of potential human exposure beside consuming seafood (Dorantes-Aranda et al., 2017). Drinking water resources are prone to paralytic shellfish poisoning toxins (PSTs) risk worldwide (Faber, 2012). Together with other cyanotoxins, PSTs are threatening our freshwater resources and drinking water safety. Several PSTs producing species in freshwater are identified and reported for example, Aphanizomenon spp. (Ballot et al., 2010; Pereira et al., 2000), Cylindrospermopsis raciborskii (Nostocales) (Lagos et al., 1999; Molica et al., 2002), Planktothrix sp. (Oscillatoriales) (Pomati et al., 2000), Dolichospermum spp. (Grachev et al., 2018; Rapala et al., 2005), Lyngbya wollei (Carmichael et al., 1997)), Scytonema agardh (Smith et al., 2011) and more (Li and Persson, 2020).

In Swedish freshwater, a nationwide 108 monitoring lakes' study showed that the most frequent cyanobacteria species are Dolichospermum (Anabaena) and Aphanizomenon followed by Microcystis and Woronichinia and Planktothrix agardhii (Li et al., 2020). It might indicate that Swedish freshwater bodies are prone to contain PSPs. It has also been shown in a national project of methods for early warning and emergency preparedness for toxins from cyanobacteria in drinking water" (SOFÄ 24-12) that saxitoxin was present in a cyanobacteria bloom in Långasjön, Blekinge. Luckily, no such toxin was found in the treated drinking water. Therefore, a risk assessment regarding saxitoxin in drinking water was provided (Swedish National Food Authority, 2018). Simple and fast screening tools for drinking water surveillance both in source water and drinking water are needed.

Thus, what to measure and the corresponding guideline values become important. As saxitoxin is the most potent toxin among the PSTs found in lakes and streams, EFSA (European Food Safety Authority) has provided equivalent factors to convert other PSPs analogues to saxitoxin, named saxitoxin equivalent (EFSA, 2009). Equivalence factors refer to (EFSA, 2009) and (Swedish National Food Authority, 2018). As there is only limited toxicological information on PSTs, and the available data are insufficient to determine a Tolerable Daily Intake (TDI). EFSA has calculated an ARfD (acute reference dose) which is 0.5 micrograms saxitoxin equivalents/kg body weight. ARfD is the highest amount of a substance a person can take during a day without any health risk.

As mentioned by Swedish National Food Authority that there is no data that can give an idea of whether people in Sweden have been exposed to PSTs via drinking water, or which can give an idea of how great the risk is for this to happen (Swedish National Food Authority, 2018). Based on ARfD 0.5 micrograms of saxitoxin equivalents/kg body weight and that 100% of intake of PSTs allocated to drinking water, a target value of 3 microg saxitoxin equivalents/L water can be calculated. The calculation is based on the most sensitive population group, i.e. 3 weeks' infants that of weight 4.2 kg with 700 ml drinking water per day.

To support monitoring PSTs in drinking water resources, this paper aims to roughly estimate limits of detections (LOD) for a few selected PSTs variants and validate a quick test kit's application for PSTs risk screening. Future study direction on the Limit of Detection (LOD) of PSTs are recommended and a multistep monitoring system is suggested for practical application for PSTs monitoring in source water.

## Materials and Methods

*Principle of the Method*

LFA (Lateral Flow Immunoassay) gives qualitative results by indicating positive/negative or yes/no signal. Pre-made strips of carrier material that contain regions where antibodies and toxin have been bound are used (Fig. 1). After the extract is pipetted in the Sample Pad, it flows downstream over to an adjacent reagent pad containing labeled antibodies (colored). Any toxin in the extract competes with toxin bound on the test line for the colored antibody. The toxin bound on the test line only attach the colored antibodies that not attached to the toxin in the sample. So that, the more toxin in the sample, more antibodies will bound with free toxins in the water sample and leave the test line colorless. There is a control line downstream, which has bound antibodies that always bind the labeled antibody, which yields intense color, confirming the assay's proper functionality. There are several commercially available LFA-based products for some of the major algal toxin groups such as diarrheic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP) and PSP. They are commercially available.

A commercial quick test kit was applied for the validation test. Each supplier of such quick test kits provides detailed instructions of how to interpret the results. To reduce subjectivity when reading result test lines it is possible to use a reader, for example, the Scanex system consisting of scanner and function of saving pictures (Turner et al., 2015).

*Certified toxin standards*

Validation test covered PSTs variants with a board variants and toxicity. Standard solutions of saxitoxin (STX), neosaxitoxin, dcSaxitoxin, GTX5 (a Gonyautoxin) and C1 toxin (a "C" toxin) were obtained from the National Research Council of Canada (NRCC, Halifax, Canada) for the validation test. The standard solution of the toxins mixed with raw water (PSTs free) were used as samples and the same raw water was used as Blank/Control. According to (Wiese et al., 2010), the order of toxicity of the main PSTs groups are saxitoxin (STX), neosaxitoxin (NSTX), gonyautoxin (GTX), decarbamoylated toxins (dcSTX,dcNeoSTX,dcGTX1-4) and C-toxines (C1-4).

*Experiment design*

The design of the experiment is shown in Table 1. Duplicate samples are prepared: 1) four levels of concentrations for each standard solution; 2) four levels of concentrations of C1; 3) a mixed solution with C1100 µg/l and STX 5µg/l.

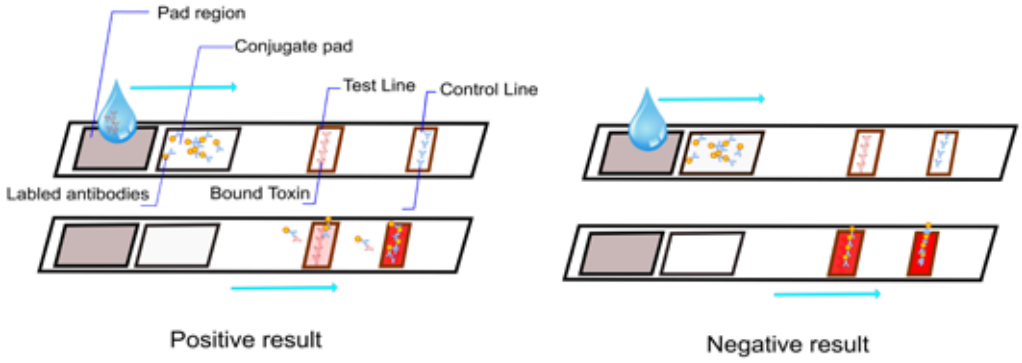


**Figure 1.** *Example of LFA principle, the left picture shows that the toxin in the sample competes with the toxin bound on the test line for the labelled antibodies and produces less color on the test line than in the right figure where all labelled antibodies are bound to the test line toxin, showing intense color.*

**Table 1.** *The experiment design for the validation test. In total 50 samples were analysed, and each series of test contains one Blank/Control.*

| | Concentration Series | | | |
|---|---|---|---|---|
| Type | 1 µg/l | 5 µg/l | 25 µg/l | 50 µg/l |
| Saxitoxin | Duplicate | Duplicate | Duplicate | Duplicate |
| Neosaxitoxin | Duplicate | Duplicate | Duplicate | Duplicate |
| dxSaxitoxin | Duplicate | Duplicate | Duplicate | Duplicate |
| GTX5 | Duplicate | Duplicate | Duplicate | Duplicate |
| C1 | Duplicate | Duplicate | Duplicate | Duplicate |
| C1 | C1 75 µg/l Duplicate | C1 100 µg/l Duplicate | C1 150 µg/l Duplicate | C1 200 µg/l Duplicate |
| C1 100 µg/l and STX 5µg/l | Duplicate | | | |

## Results

The validation results demonstrate clear LOD levels for all toxins except C1, as shown in Table 2. More specifically saxitoxin has been assigned an estimated limit close to 5 µg/l, which is the same as the declared estimated value by the company. Neosaxitoxin LOD has been estimated to be close to 25 µg/l, which is much lower than the declared estimated value 71 µg/l. The estimated LODs for dcSaxitoxin lies between 5 and 25 µg/l and for GTX5 between 5 and 25 µg/l.

From the above results, it can be noted that all estimated LODs vary with the saxitoxin being the lowest. If lower LODs are of concern, further validation tests need to be done. For example, to test if it is applicable for detecting saxitoxin in the treated water, where the safety level is 3 µg STX-eq/L for most of countries, saxitoxin concentrations of 1 to 3 µg/L are suggested to be tested. Validation protocol refers to validation criteria for LOD determination, validation methods for screening purpose and validation methods for qualitative methods.

For qualitative method LOD: EU Reference Lab. 2015: "The limit of detection (LOD) for a qualitative method can be determined by testing low levels of fortified blanks until a concentration

**Table 2.** *Summary of validation test and rough estimation of LOD for targeted PSPs congeners (for details we refer to the validation report).*

| Toxin Type | Conclusion |
|---|---|
| Saxitoxin | At 25 µg/l, the result is clearly positive. Difficult to evaluate at 5µg/l, the detection limit might be close to 5µg/l. |
| Neosaxitoxin | At 50 µg/l, the result is clearly positive. Difficult to evaluate at 25 µg/l, the detection limit might be close to 25 µg/l. |
| dcSaxitoxin | At 25 µg/l, the result is clearly positive. Difficult to evaluate at 5 µg/l, the detection limit might be in between 5 and 25µg/l. |
| GTX5 | At 25 µg/l, the result is clearly positive. Difficult to evaluate at 5 µg/l, the detection limit might be in between 5 and 25 µg/l. |
| C1 | Has been tested up to 200 µg/l, the strips still show a negative result. |
| C1 100 µg/l and STX 5µg/l | The result is clearly positive |

is reached where replicate samples test say 25% negative, 75% positive. A cut-off limit for that method will be set above the LOD at a concentration where the false negative rate reaches a stated low probability. The selection of the levels of analyte for the SLV (Single Lab Validation) and ILS (Inter-laboratory study) are obviously crucial to obtaining a good estimate of the LOD and establishing a cut-off for the method."

For screening purpose: Due to the increase use of screening method, the Codex Committee on Fish and Fishery Products (CCFFP) in 2012 developed performance criteria for screening methods that were to be used by competent authorities to select methods that are adequate to support routine toxin monitoring programmes. Unfortunately, no international agreement was achieved. A draft was made and a decision regarding the performance was included that:

- Cross reactivity to the toxin congeners should be investigated and well understood;
- Sensitivity to all relevant congeners should be known;
- Blank matrix fortified with other toxins that could possibly be found in samples should be tested to establish negative response;
- Preference given to methods that have undergone ILS;
- False negative rates should be less than 5% at a level equating to half the maximum allowable level, and no false negatives at the maximum level;
- The detection limit should enable detection of biotoxins at half the maximum level.

The EU Reference lab note that SLV is not enough for methods that are to be used within the EU to support shellfish safety decisions and that inter-laboratory validation studies are required (Personal Communication, Ana Gago Martinez, December 2014) (Mcleod et al., 2015).

AOAC have set minimum criteria for collaborative study of qualitative analyses. Ten laboratories reporting on two analyte levels per matrix; six test samples per level; and six negative controls per matrix ((AOAC International, 2002)

It can be concluded that the core criteria are to get false negative rates < 5% at a level equating to half the maximum allowable level, and no false negatives at the maximum level; and the detection limit should enable detection of biotoxins at half the maximum level.

Another option is to pre-treat samples to higher concentration prior test such as concentrating samples. One option is to apply passive sampling device such as Solid Phase Adsorption Toxin Tracking (SPATT) Technology (Roué et al., 2018).

## Discussion

From current study, such type of quick analysis tool for screening the most toxic PSTs variants in source water is applicable, particularly when saxitoxin is the main concern. However, it should be used with concern of the nature property of the immunoassay regarding cross-reactivity and sensitivity.

For screening purpose, on one hand, it is important to be able to represent a boarder variety of PSTs analogues (stronger cross-activity). The cross-reactivities in relation to STX varies for this type test kits (Jawaid et al., 2015; Li and Persson, 2020). Our study also shows that this specific type of LFA can detect all type of selected analogues except for C1. Up to 200 µg/l of C1, the kit still shows a negative result. As C toxins are regarded less toxic compared to other analogues (Swedish National Food Authority, 2018), it might not be harmful when it comes to false negative results. While for false negative regarding analogues of higher toxicity, low cross-reactivity might cause withdraw the application from routine monitoring program, such as GTX1&4 in UK (Turner et al., 2015). This is because that the antibodies are designed to attach the structurally similar analogues not to toxicity. Sample preparation with acid hydrolysis was used to avoid false negatives caused by C-toxins (relatively high levels in Australian freshwater samples with Anabaena circinalis) (Humpage et al., 2010).

For achieving higher sensitivity (lower LODs), on the other hand, might be achieved by changing the amount of serum applied (Laycock et al., 2010). While it is also lowering cross-reactivities

(Laycock et al., 2010). Therefore, to increase the detection limit is necessary to sacrifice the detection of other congeners. Furthermore, LOD depends on the toxin composition of the sample, i.e. what kind of toxins are present and the sensitivity of the test (Laycock et al., 2010).

Besides cross-reactivity challenge, matrix effects compromise their measurement reliability (Bratakou et al., 2017). Matrix effect is the effect on an analytical method, which is caused by all other components of the sample except the specific compound to be quantified. Matrix effects and selectivity issues have been associated with bioanalytical techniques for long (Smeraglia et al., 2002).

Although LFA is facing great challenges for further development, its simplicity and ease for onsite use is of great benefits for industrial and water managers. Lateral flow immunoassay (LFA) is regarded as a leading immunoanalytical technique for onsite analysis, particularly after LFA architecture adapted to multiplexing, and may therefore be a possible answer to the demand of multiplexing point-of-need analysis with new types of magnetic, fluorescent, and colored labels (Anfossi et al., 2018)

On this context, practical suggestions of PSPs monitoring in drinking water resources are to measure cell densities of potential PSPs producing cyanobacteria as early warning indicator; collaborate with advanced analytical laboratory and understand the toxin profile in the raw water before select a proper onsite screening tool. For clear positive results, it is recommended to confirm the concentration by advanced analytical equipment such as LC-MS/MS.

## Conclusions

The validation test estimated a rough limit of detection for saxitoxin: around 5µg/l, neosaxitoxin around 25 µg/l, dxSaxitoxin: between 5 to 25 µg/l, GTX5: between 5 to 25 µg/l and C1 > 200 µg/l. To assess whether this method can reach even lower limits to meet drinking water standards, further study needs to be done including focusing on the low false negative rate at 3 µg/l and reaching the LOD at 1.5 µg/l. An international inter lab study can be proposed for improving LAF for screening purpose validation for PSPs in freshwater and drinking water. Another option is to pre-treat samples to higher concentration prior test such as concentrating samples.

From validation test, such type of quick analysis tool for screening the most toxic PSTs variants in source water is applicable, particularly when saxitoxin is the main concern. However, it should be used with concern of the nature property of the immunoassay regarding cross-reactivity and matrix effect. Prior knowledge of toxin profile for quick test tool selection and collaboration with advanced analytical laboratories are necessary for PSTs drinking water surveillance. Certain variants of concern in source water might consider applying specific variant detection tool such as for neosaxitoxin.

Rapid testing methods for screening of saxitoxins could enhance public safety and phytoplankton monitoring could provide early warnings of PSTs in freshwaters. Practical suggestions are for drinking water PSPs monitoring are to measure cell densities of potential PSPs producing cyanobacteria as early warning indicator; to select possible quick analysis tools based on raw water toxin profiles; when clear positive result occur, it is recommended to confirm concentration and toxin profile with advanced analytical equipment such as LC-MS/MS.

## Acknowledgement

Many thanks to Heidi Pekar and Paolo Cappelli for assisting this experiment. This project is part of a project funded by MSB (Swedish Civil Contingencies Agency), entitled ”Enhanced capacity of drinking water producers for hazard analysis and risk management in toxic algal blooms in water cover” (2016 – 2018).

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