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Partner in Research

Development of a dipstick immunoassay for the detection of algae toxins

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Introduction

Over the past decade, the growth of membrane-based rapid-test technologies has created a vast market for gold-linked immunological reagents. Gold labels have a great potential stability, an accurately defined size and proteins can be coupled with an enormous strenght to gold surface via covalent bonds.
The used format is the lateral-flow or dipstick design, which has become more important in in-vitro diagnostic applications. Dipstick sensors are used in test systems for allergies, infectious diseases, environmental contaminants (e.g. toxins), drugs, fertility and veterinary applications.
Such test system shows many advantages in relation to analytical labor-based methods such as ELISA or HPLC. A dipstick test is an inexpensive, disposable, convenient membrane-based assay which is usually complete within 2-5 minutes. No instrumentation is required and also inexperienced personnel can perform such tests.

Problem

The need for specific, reliable and fast analytics to determine toxic compounds from algae as well in control of bathing water quality as for food control systems is steadily increasing. The toxins formed by various algae such as cyano bacteria accumulate in case of algae blossoms in maritime organisms and in the waterbodies. These algae blossoms (see Fig. 1) arise regularly depending on climate effects.
The accumulated toxins can lead to various diseases when incorporated by humans. Therefore the WHO has set an upper limit of toxin content in bathing water at 1µg/L.

Fig. 1: Typical view of an algae blossom

Algae toxin microcystin

The toxin investigated in this study was microcystin, a highly toxic hepatotoxin derived from cyano bacteria. Microcystins are a group of cyclic non-ribosomal peptides, varying marginally in the peptide sequence. They consist of several uncommon non-proteinogenic amino acids, including dehydroalanine derivatives and the uncommon β-amino acid ADDA ((all-S,all-E)-3-Amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-diene acid).
The best investigated and for this study chosen microcystin is microcystin LR (MC-LR).

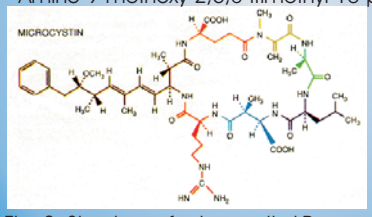

Fig. 2: Structure of microcystin LR

Dipstick immunoassay for toxin analysis

The developed analytical system for microcystin is based on a sandwich-ELISA dipstick assay, similary to common pregnancy tests. This used test system is based on a competitive test format. Here, MC-LR is covalently attachted to a further protein (bovine serum albumin) because of the shortness of the toxin for direct immobilization on the membrane. Coupling of BSA does not result in blockage of the epitope. The principle is based on the reaction of MC-LR in the liquid sample with its corresponding gold labelled antibody. These antibodies were blocked by MC-LR. Only the non-reacted labelled antibodies can bind to the immobilized antigens at the test line and form a sharp red line. Thereby, the signal intensity is anti-proportional in relation to the antigen concentration and can be evaluated by eye.

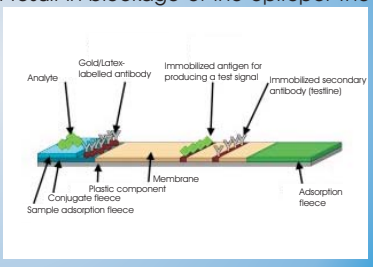

Fig. 3: Schematical setup of a dipstick immuno assay

Results

Premilary tests were performed with a model mouse-IgG system to find out optimal conditions for antibody labelling. Stable conjugates without agglomeration can be produced with an antibody concentration of 4.35 µg/mL at pH 9. The stability of prepared conjugate was proven by spectrophotometrical assay and transmission electron microscopy analyses (TEM) (see Fig. 4).

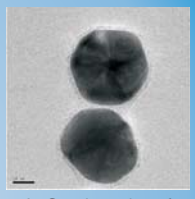

Fig. 4: Conjugate of antibody bound to gold-particle

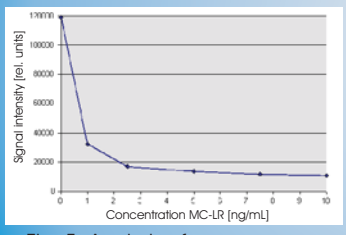

Fig. 5: Analysis of a concentration series of MC-LR

Investigation of natural samples in the form of water samples with different toxin contents, shell meat extracts and fish entrails were performed. The samples from maritime organisms were artificially contaminated with MC-LR. Respectively, detection limits of 90 and 150 ng / g fresh weight shell meat and fish entrails were achieved (see Fig. 5). Even saltwater samples or unfiltrated cloudy samples from freshwater bodies could be analyzed by the developed system .

Summary

The achieved results lie under the detection limits of the WHO guide lines and are therefore sufficient for all named applications. The long term stability of the liquid conjugate was increased by addition of trehalose up to 60 days enabling a direct use by inexperienced personnel in the field.
Further improvements can be achieved e.g. by modifying support materials. These investigations are currently performed.
The developed system shows higher accuracy then the established methods for toxin control (e.g. HPLC) and are therefore a prospective alternative for toxin monitoring as well in bathing water control as in food control.

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