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Two Phase Monoclonal Antibody-Based Testing Protocol for Zearalenone Screening of Food Review

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Abstract

Screening for contaminants in food and feed is of vital importance. A method has been developed for Zearalenone (Zen) testing by combined enzyme-linked immunosorbent assay (ELISA) and column immunoaffinity chromatography. Zen is a highly toxic mycotoxin present in food and feed. The authors generated a monoclonal antibody (mAb) with high specificity for Zen. The mAbs developed had no cross-reactivity with other mycotoxins. The mAbs did recognize related Zen structures. Column immunoaffinity chromatography was then developed using the mAbs. The dual testing format was used to test for Zen artificially treated, in a dose dependent manner, food and feed samples. The successful results allowed the authors to then use their testing strategy on food and feed samples from a local market. Again positive results were obtained.

Abbreviations: ELISA: Enzyme-linked Immunosorbent Assay; mAb: Monoclonal Antibody; ZEN: Zearalenone.

Introduction

In the following review Ting Wang, et al. [1] and colleagues present an enhanced screening method for identifying zearalenone. The method is a combined monoclonal antibody utilizing (ELISA) enzyme-linked immunosorbent assay, and affinity purification. Zearalenone (ZEN) is a mycotoxin common in human and animal food stuffs. Some countries publish guidelines for allowable amounts of Zen in food. Due to the adverse physiological effects of this mycotoxin in both humans and animals it is important to have reliable screening methods.

There are mass spectrometry-based methods for ZEN screening. Though sensitive, these methods are expensive and need well-trained technicians. Immunoassays are another option for ZEN screening. Methods such as ELISA require an antibody specific for a given antigen. They also have many advantages, among them are specificity, speed, cost and the format being high throughput. Success of the method is highly dependent on antibody specificity to the target antigen.

Purified ZEN is too small to elicit an immune response, so it is covalently linked to BSA (bovine serum albumin) and validated by mass spectrometry. This complex was used as an immunogen. After immunizations and titer determination, a fusion experiment was carried out to generate hybridomas [2]. The monoclonal antibody (mAb) with the highest determined binding affinity for ZEN was then used for affinity column chromatography. ZEN from artificially treated food samples was able to be column extracted and further tested by ELISA. When the results were confirmed by HPLC, this method was used successfully on samples from a nearby marketplace.

Ramping up the production of the mAb clones was performed in nude mice generating ascites fluid. Such ramping up may have just as easily been done utilizing a hollow-fiber cell culture apparatus [3]. This method would have produced a high mAb concentration without sacrificing mice. In addition, media from a hollow-fiber apparatus can easily be purified by column chromatography.

MAbs have high specificity to the antigen to which they are immunized. However, they can cross react with compounds of similar structure. The presence of an olefinic bond in the Zen structure is an indication of specificity. Extensive testing was done along this line to ensure specificity. In addition, assay optimization for ELISA was also performed.

The previously mentioned artificially ZEN treated food samples were of known concentrations. The ZEN was then extracted by the immunoaffinity column and then run on an ELISA platform. Results showed the combined testing format provided additional accuracy to their results. It also gave them a standard curve to use against testing for samples of unknown ZEN concentration. The ELISA results were then validated by HPLC. The data showed high accuracy for the artificially treated food samples.

When the authors used their dual testing format with food samples from a nearby marketplace, the results were again highly accurate. Such data shows the usefulness of first performing column chromatography followed by ELISA for ZEN testing.

In conclusion, the author's ZEN testing ELISA format was highly accurate. Their results showed specificity with no cross reactivity. This ZEN testing method is a valuable tool for protecting human and animal food.

References

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