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**RESEARCH PAPER**

# Rapid Detection of Ochratoxin A in Malt by Cytometric Bead Array Based on Indirect Competition Principle

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**Abstract:** A cytometric bead array (CBA) method based on indirect competition principle was developed for the sensitive and rapid detection of ochratoxin A (OTA) in malt. The malt samples were extracted by 60% methanol/PBS and the extracts were diluted five times with 20% methanol/PBS. After centrifugation, the supernatant was collected to prepare sample solution for analysis. The fluorescence-encoded microsphere surface was labeled with bovine serum albumin OTA (BSA-OTA) to compete with OTA in samples for anti-OTA monoclonal antibody (mAb). Then, FITC-IgG was added to bind with the captured mAb on the microsphere. After centrifugation and washing, the mean fluorescence intensity from FITC on the surface of microsphere was detected by a BD FACSCalibur analyzer for accurately qualitative and quantitative analysis of OTA. The results showed that the half inhibitory concentration ($IC_{50}$) was 1.20 ng $mL^{-1}$ with the correlation coefficient ($R^2$) of 0.989, and the limit of detection (LOD) for OTA was 0.12 ng $mL^{-1}$. The average recovery rates in malt samples were 93.9%–97.4% with relative standard deviations (RSDs) less than 3.6% at three spiking levels. Sixteen malt samples were analyzed and OTA was found in two samples with the contents less than 3.83 μg $kg^{-1}$ which was lower than the maximum permitted residue level (5 μg $kg^{-1}$) proposed by the European Union. All the positive samples were confirmed by LC-MS/MS. In this study, the CBA technique based on indirect competition principle was developed for the first time for successful detection of OTA in malt samples. The method was easy, rapid, sensitive and reliable with high potential for the qualitative and quantitative of multiple mycotoxins in other complex matrices.

**Key Words:** Cytometric bead array; Indirect competition; Malt; Ochratoxin A; Rapid detection

## 1 Introduction

Ochratoxin A (OTA) is the secondary metabolite of Aspergillus and Penicillium[1], and has diverse toxicological effects, including carcinogenicity, teratogenicity, mutagenicity, hepatotoxicity, immunosuppression and reproductive disorders and so on[2]. It is considered as a possible carcinogen (group IIB) by the International Agency for Research on Cancer (IARC)[3], as well as the most likely carcinogenic substance to human by the US National Toxicology Program[4]. OTA is the main exogenous harmful pollutant of food, agricultural products and crops, medicinal plants, which would not only affect the quality of related products, but also be a potential threat to human health and safety[5,6]. In view of the serious harmfulness of OTA, many countries and international organizations set strict limit standard for OTA. The European Union (EU) rules that the concentration limit value of OTA in cereals is 5 μg $kg^{-1}$, and in the counterpart products is 3 μg $kg^{-1}$. The maximum permitted residue level of OTA in wine and grape juice beverages is 2.0 μg $kg^{-1}$ [7]. So, it is vital to develop a simple, rapid, precise, sensitive and specific method for the detection of OTA to ensure the safety of foods, agricultural products, medical plants and human health[8].

Most of current methods for OTA are based on laboratory instrumental analyses[9–11], such as liquid chromatography

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coupled with fluorescence detection (LC-FLD) and tandem mass spectrometry (LC-MS/MS). Although these methods have obvious advantages and a wide range of applications, there are also some limitations and disadvantages. Heavy and expensive equipment, complex and time-consuming operations make the instrumental analyses inconvenient, especially for onsite real-time analysis. Usually, the detection of OTA in foods, agricultural products, Chinese herbal medicines and other samples needs strict pretreatment and purification steps due to their complex matrix compositions, which brings many difficulties to the actual operation. Because of the advantages including high specificity, high selectivity, less demanding on purity and high throughput, enzyme-linked immunosorbent assay (ELISA) is gradually being developed and applied for the rapid detection of OTA in many complex matrices[12,13], but there are some disadvantages including low sensitivity, poor repeatability, and so on[14].

As a novel analytical tool, cytometric bead array (CBA) technique[15–20] based on a series of different intensities of fluorescence-encoded microspheres can achieve sensitive, accurate and rapid detection of multiple targets in complex matrices in one run only needing a small amount of samples, with the advantages such as good repeatability and stability, short analysis time and low false-positive results. For this detection, a flow cytometer was used to collect and analyze the fluorescence signal of microspheres for quantitation of target analytes. This technique was applied for high-throughput detection of various macromolecules and small molecule compounds in many kinds of matrices[21–25]. As a small molecule compound, OTA has semi-antigenicity, small molecular weight and single epitope, and does not express immunogenicity[26]. Therefore, the CBA immunoassay technique based on indirect competitive principle and "antibody-antigen" specific recognition is usually used to detect OTA because the microsphere is not damaged in the coupling process, and the antibody-antigen recognition sites are not interfered[27,28].

Malt is a widely used Chinese herbal medicine (CHM) in clinic with high medical and edible values. It is beneficial for spleen and stomach and has important functions of promoting digestion and removing breast swelling[29]. Modern pharmacological studies have also shown the antibacterial, antiviral and antineoplastic properties of malt. In addition, malts can inhibit the increase of cholesterol and blood lipids, prevent cardiovascular disease and enhance immunity[30]. Malt not only has important medical values, also is an important raw material for foods, feeds and wines. In the process of preparation, malt is easy to be mouldy to produce mycotoxins such as OTA[31]. So, development of a sensitive and reliable method for the objective and accurate detection of mycotoxins in malt is important for the quality and safety control of this medical and edible CHM[32,33].

In this study, a cytometric bead array (CBA) method based on indirect competition principle and "antibody-antigen" specific recognition was established for rapid detection of OTA in malt. The results showed that the developed CBA method had the advantages such as simple operation, high-throughput analysis, low detection limit, high sensitivity, good specificity and small matrix interference for OTA at trace level, which was generalized for detection of more mycotoxins in other complex matrices.

## 2 Experimental

### 2.1 Instruments and reagents

The following instruments were used: BD FACS Calibur Flow Cytometry (Becton, Dickinson and Company, USA), LC-20AD XR HPLC (Shimadzu, Japan), 5500 QTRAP® mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA), TUS-200P constant temperature oscillation metal bath (Yiheng Company, China), KQ-500 ultrasonic apparatus (Kunshan Ultrasonic Instruments Co. Ltd, Jiangshu, China), ANKE TGL-16C high speed centrifuge (Anting Company, China), AB-135-Selectronic analytical balance (Mettler TOLEDO Company, Switzerland), and pH Meter (Sartorius Company, Germany).

FC05F Carboxylated red fluorescent microspheres (particle size 4.95 μm; excitation wavelength 630 nm and emission wavelength 690 nm) were purchased from Bangs Laboratories (Fishers, IN, USA). OTA reference substance (1 mg $mL^{-1}$) was from Pribolab Company (Singapore). Bovine Serum Albumin (BSA), morpholine ethyl sulfonic acid (MES), *N*-hydroxysulfosuccinimide (NHS) and carbodiimide (EDC) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). BSA-OTA (7.8 mg/mL) and OTA Monoclonal antibodies (mAb) (7.2 mg $mL^{-1}$) were purchased from Shandong Green Biological Engineering Co. Ltd. (China). Fluorescein isothiocyanate (FITC) labeled Goat anti-Mouse IgG (H+L) (1.5 mg $mL^{-1}$) was from Fitzgerald Company (Birmingham, UK). Other reagents were all of analytical grade.

### 2.2 Preparation of buffer solution

A 10 mM PBS (pH 7.4) solution was prepared by dissolving 8.0 g of NaCl, 1.2 g of $Na_2HPO_4$, 0.2 g of $KH_2PO_4$ and 0.2 g of KCl in a certain amount of distilled water to the volume of 1000 mL. PBSBT blocking buffer (pH 7.4, including 1% BSA and 0.1% Tween 20), PBST washing buffer (pH 7.4, including 0.1% Tween 20), PBSBTN preservation buffer (pH 7.4, including 0.1% BSA, 0.01% Tween 20 and 0.05% $NaN_3$), 50 mM MES activation buffer (pH 5.0), 50 mM MES coupling buffer (pH 6.0), coupling agent of 0.32 M EDC and 0.33 M NHS, sample extract solvent of methanol/PBS (60:40, *V/V*), and sample diluting solvent of methanol/PBS (20:80, *V/V*) were used. All these reagents were