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REVIEW

Immunoassay of chemical contaminants in milk: A review

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Abstract

The detection of chemical contaminants is critical to ensure dairy safety. These contaminants include veterinary medicines, antibiotics, pesticides, heavy metals, mycotoxins, and persistent organic pollutants (POPs). Immunoassays have recently been used to detect contaminants in milk because of their simple operation, high speed, and low cost. This article describes the latest developments in the most important component of immunoassays—antibodies, and then reviews the four major substrates used for immunoassays (i.e., microplates, membranes, gels, and chips) as well as their use in the detection of milk contaminants. The paper concludes with prospects for further applications of these immunoassays.

Keywords: milk, chemical contaminants, immunoassay, antibodies

1. Introduction

Milk is an indispensable source of high quality protein and is especially important to infants, children, the elderly, and the sick. Global milk production reached 782 million tones or 109.6 kg per capita in 2013 (International Dairy Federation 2014). In addition, milk is a raw material for many dairy products such as yogurt, butter, cheese, ice cream, candy, etc. Before getting to consumers, milk goes through production, processing, and circulation. Each step involves potentially unsafe factors such as chemical contamination that can affect milk quality.

The potential chemical contaminants in milk products include veterinary medicines, antibiotics, pesticides, heavy metals, mycotoxins, persistent organic pollutants (POPs), radionuclides, nitrates or nitrites, as well as packaging contaminants or adulterants (Griffiths 2010). The source of this problem of these main problems are farmland contaminated with medication, forage pollution, drinking water contamination, illegal additions as well as transportation and processing pollution. Table 1 lists some of the major milk chemical contaminants and their maximum residue limits in the EU and China (MOA 2002; Council Regulation (EU) 2010).

Researchers have focused on the detection of chemical contaminants in milk. The EU, the U.S., China, and others have enacted many validated methods for monitoring milk safety including gas chromatography (GC) (Hernandes *et al.* 2014; Meneghini *et al.* 2014), high-performance liquid chromatography (HPLC) (Karami-Osboo *et al.* 2014), gas

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Table 1 Primary chemical containments in milk

Category	Contamination	Pathway	MRLs (μg L^{-1})	
			EU	China
Veterinary drugs	Ampicillin	Drug abuse in the process of breeding or failure to follow milk withdrawal time	4	10
	Cefalexin		100	100
	Enrofloxacin		100	100
	Gentamicin		100	200
	Streptomycin		200	200
	Sulfonamides		100	100
	Tetracyclines		100	100
Pesticides	DDT	Feed stuff (forage or cereal) pollution	40	20
	HCH		Forbidden	20
Heavy metal	Pb	Environment (soil or water) pollution	20	50
	Cd		Forbidden	300
	As		Forbidden	50
	Hg		Forbidden	10
Mycotoxin	Aflatoxin M1	Feed stuff stored improperly and added illegally	0.05	0.5
Others	Melamine		2 500	2 500

chromatography-mass spectrometry (GC/MS) (Liu *et al.* 2014; Zheng *et al.* 2014), liquid chromatography tandem mass spectrometry (LC-MS/MS) (Freitas *et al.* 2014; Sniegocki *et al.* 2014; Young *et al.* 2014), differential pulse anodic stripping voltammetry (DPASV) (Sadeghi *et al.* 2014), etc. These technologies are highly sensitive, specific, and reliable, but their application was limited by costly instrumentation and complicated sample preparation.

In contrast, immunoassays based on specific antibody recognition are a rapid method of screening samples (Wild 2013). Unlike chromatography-based methods, they are simple, specific, easy to use, high-throughput, and do not require costly instrumentation. This makes them suitable for on-site detection. Therefore, a combination strategy with fast immunoassay screening followed by instrument-based confirmation has been widely considered. Moreover, immunoassay is also suitable for the analysis of milk. Because milk is a fluid, milk samples can be analyzed after only simple pretreatment such as dilution or protein precipitation. They can even be analyzed without sample preparation. For this reason, immunoassays have become the subject of increasing research attention in the detection of chemical contaminants in milk.

Immunoassays include three major elements: (1) preparation of the target-specific antibody; (2) antigen-antibody recognition based on a specific carrier; and (3) acquisition of the detection signals. This review describes recent developments in antibodies and then details the four major carriers widely used in milk contaminant immunoassays.

2. Antibodies and antibody substitutes

Antibodies are a key factor for a successful immunoassay. In addition to the continuous improvements in traditional antibody production, the discovery and production of novel antibodies or antibody substitutes with specific properties has been reported including single-chain antibody fragments (Ahmad *et al.* 2012), aptamers (Toh *et al.* 2015), receptor proteins (Beltrán *et al.* 2014), and molecularly-imprinted polymers (Song *et al.* 2014) (Fig. 1).

2.1. Traditional antibodies

Polyclonal and monoclonal antibodies are generated from immunized animals (rabbits, mice, or goats). Polyclonal antibodies can recognize multiple epitopes on any one antigen, and are inexpensive to produce. However, they are not as popular as monoclonal antibodies because they have high batch-to-batch variability and non-specificity. Monoclonal antibody technology is based on secreted antibodies from a hybridoma amplified *in vitro*. It was invented by British scientists Köhler and Milstein (1975) and was a major breakthrough in the field of immunology. In recent decades, monoclonal antibody preparation technology has gradually matured and mass production is now possible. Several companies, such as Biopharm (Germany), IDEXX (U.S.), and Randox (U.K.), have produced rapid screening products (kits and strips) for the assessment of milk contamination using monoclonal antibodies. Traditional antibodies are inferior in stability to many of their alternatives considered below because of the long time required for preparation and ethical concerns associated with the use of animals.

Another term of “nanoantibody” or “nanobody” was given more attention these years. They are single-domain variable fragments of special type of antibodies, which naturally exist in blood of Camelidae family animals and in some chondrichthyan fishes (Tillib 2011). It is interesting that only a single variable domain of this antibody (without the first CH1