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Establishment of a lateral flow colloidal gold immunoassay strip for the rapid detection of estradiol in milk samples

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ABSTRACT

It is a fact that the level of estradiol (E2) in powdered milk has been increased manyfold with growing concerns on dairy safety. Our study produced a panel of monoclonal antibodies (mAb) specific for E2 screening and established a lateral flow colloidal gold immunoassay strip for determining E2 in milk samples with the E2 mAb. The sensitivity of the test strip was found to be 180.42 ± 3.17 pg/mL and the lower detection limit (LDL) value was 37.14 pg/mL. For negative milk samples spiked with 400, 800, and 1200 pg/mL, the recovery range was 86.7–93.5% and the coefficient of variation scope [CV (%)] was 4.8–6.2%. The results of comparative studies between test strips and HPLC were relatively identical while the strip test only required less than 8 min. In the study, the test strip was a practical screening technology for quantitative, semi-quantitative, or qualitative detection of E2 residues.

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1. Introduction

Estradiol, also called E2 (known as endocrine disrupting chemicals, 17β-estradiol) is a category of steroid hormone derived from cholesterol (Chambers, Casey, Hakk, DeSutter, & Shappell, 2014). The physiological actions of the endocrine disruptor mainly exert on regulating re-productive system function and maintaining underlying hormone metabolism in human and many other animal species (Batrinos, 2013). In light of its effective impacts on reproduction, E2 has widely been applied in stock farming industries (Shi et al., 2011). It surely accelerates cattle production and shortens fattening period for lower cost (Pinheiro et al., 2013). But, there are environmental scientists concerns that the bio-accumulation effect of E2 in biota will eventually load on top of the food chains – human beings who continuously consume massive amount of cattle originated commodities (typically dairy products) (Suzuki et al., 2009). In china, the illegal use of E2 in dairy cattle lack of control and monitor. The level of E2 in powdered milk has been actually a considerable number which has been increased manyfold with dairy industry development. Steroid hormones in animal food source are still not considered by food safety monitor system in the worldwide scale (Chen et al., 2014).

E2 has been suspicious to generate unpleasant effects in human body. In last decades, extensive studies have reported that E2 potentially play a role of carcinogen to trigger and exacerbate tumor formation (Wegner et al., 2014). Between recent studies, E2 was proved to up-regulate the secretion of cytokine tumor necrosis factor-α in some breast cancer cell lines and regarded as a key feedback signal within estrogen receptor-positive tumor microenvironments (To, Cheung, Lazarus, Knower, & Clyne, 2014). Another team reported that E2 was also responsible for tumor cell invasion and migration of nuclear estrogen receptor-negative breast cancer cells through cross-talk (Jiang et al., 2013). Based on the reports, steroid hormones like E2 in dairy products should be concerned for human health within food safety field.

Nowadays, the food safety administration and research towards commercial dairy products is primarily concentrated on the residues detection of microbial contaminants (Jin et al., 2013), heavy metal (Ping, Wang, Wu, & Ying, 2014), pesticide (Shaker & Elsharkawy, 2015) or antibiotics (Romero, Beltrán, Pérez-Baena, Rodríguez, & Molina, 2014), and little is on the milk of steroid

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hormone (Sciascia, Pacheco, & McCoard, 2013; Zheng, Zou, Li, & Machesky, 2013). Classical analytical methods for the determination of E2 in powdered milk were mainly gas chromatography mass spectrometry (GC–MS) (Chen et al., 2014), high performance liquid chromatography (HPLC) (Shi et al., 2011), and liquid chromatography-mass spectrometry LC–MS (Pape-Zambito, Roberts, & Kensinger, 2010; Zhao, Lin, Li, & Ying, 2006). But these sophisticated methods are not suitable for detecting large quantity of samples in real time administration. As the important fact, several studies have conducted immunoassays in environmental E2 detection, but most of them circumscribed the scope of E2 detection in aquatic environment, such as waste water, sewage effluent and natural water bodies (Lee, Cho, Kim, & Kim, 2011; Stumpe & Marschner, 2007; Sun & Zhou, 2014). Hintemann et al. demonstrated that the levels of E2 in the environmental water samples were determined by using two immunoassays, and the detection limits reached at 0.05 ng/L for E2 with a 50-fold enrichment treatment (Hintemann, Schneider, Schöler, & Schneider, 2006). Chaisuwan et al. recently conducted indirect competitive immunoassay by CdSe quantum dots conjugation with bovine serum albumin-E2, and the detecting limits of E2 were 52.56 ± 0.125 pg/mL for tap water and 51.42 ± 0.453 pg/mL for waste water (Chaisuwan, Xu, Wu, & Liu, 2013). Currently reported immunoassays of determination of E2 were based on the highly specific molecular recognition of the immune-reaction, however, professional operation and expensive equipment are still required during the assays and not suitable for on-site examination of food safety administration. So far, rapid detection of E2 in powdered milk is still an ignored subject.

Notably, immunochromatographic lateral flow test strips have been accepted as a popular diagnostic tool for detecting low quantity of analytes such as viruses (Li et al., 2013), bacteria (Pengsuk, Chaivisuthangkura, Longyant, & Sithigorngul, 2013), and parasite antigens (Fu et al., 2013), with the sandwich format. Its unique advantages over instrumentally based methods are well known as low-cost instrumentation requirements, easy operation, and real-time result display within 5–10 min (Sajid, Kawde, & Daud, 2014). Up to now, lateral flow colloidal gold immunoassay strip for determining E2 in milk samples have not been reported yet. In this study, we produced a panel of monoclonal antibodies (mAb) specific for E2 screening and established a lateral flow immunoassay strip with the E2 mAb, which was effectively applied for the rapid detection of E2 residues in milk samples.

2. Materials and methods

2.1. Reagents and materials

E2 (Sigma USA); N-Hydroxy succinimide (NHS), N,N′-Dicyclohexylcarbodiimide (DCC) (Fluka, China); bovine serum albumin (BSA), ovalbumin (OVA), Freund's complete adjuvant FCA and Freund's Incomplete Adjuvant FIA, (Pierce USA); a mouse monoclonal antibody isotyping kit were from Sigma (USA); whole molecule of goat anti-mouse IgG antibody (GaMIgG-HRP) and 96-well culture plates (Luoyang, China); 1% Tris-BSA solution (PH7.0) (Shanghai, China); RPMI-1640, PEG1500, HAT, and HT medium were purchased from Gibco; nitrocellulose membrane, glass fiber, and absorbent pad were purchased from Millipore; eight-week-old female BALB/c mice were obtained from the SPF standard Laboratory Animal Center, Zhengzhou University, China; GS-NS0 myeloma cells were donated by institute for animal health of Great British; Double deionized H_2O was made by Millipore system. The assay buffer consisted of 0.01 mol/L phosphate-buffered saline (PBS) (pH 7.4), containing 145 mmol/L NaCl. The washing buffer (PBST) consisted of assay buffer containing 0.1% (v/v) Tween-20. All applied reagents and solvents were of analytical grade or higher in this study.

2.2. Apparatus

U-3000 UV scanner (Shimadzu, Japan); gel imaging system (Syngene UK); electrophoresis system JM-250 (Jiemai, China); microplate Readers 450/550 (Bio-Rad USA); microplate mixer (Xinjingke Biotechnology, China) was employed to blend the solutions in microwells; XYZ Biostrip Dispenser, CM4000 Cutter, and TSR3000 membrane strip reader (Bio-Dot USA); Ultrasonic cell disrupter system was from Ningbo Scientz Biotechnology Co., Ltd; Rotational Vacuum Concentrator RC1010z (Jouan, France); high speed freezing centrifuge 3K-18 (Sigma Germany).

2.3. Preparation of immunogens and coating antigens

E2 conjugate preparation was carried out according to the instruction described by Monnet et al. (Monnet et al., 2002). 300 mg E2 was dissolved in 6 mL of DMSO (Dimethyl sulfoxide) with 1 g KOH for 5 min of mixing. 300 mg bromoacetic acid was added for another 2 hours' mixing with a 50 mL ice cold water treatment. Then ethyl acetate extraction collected unreacted E2. Aqueous phase was acidized by 2 mol/L HCl, and produced white precipitate. Filtered white precipitate was rinsed with distilled water for reaching PH value 7 following with a vacuum drying step. Transparent color of crystal E2–CME (carboxymethyl ether) was obtained by methanol-chloroform recrystallization protocol (Monnet et al., 2002). 6.6 mg of E2–CME was mixed with 2.9 mg NHS, 5.2 mg DCC and 1 mL DMSO in 2 h. Then E2-BSA and E2-OVA conjugates were prepared by an active ester method which has been successfully applied in our following studies. The conjugation ratio was calculated through UV scanning according to derivation of Lambert–Beer law.

2.4. Production of mAb against E2

2.4.1. Immunization of mice

Six 8-week-old BALB/c female mice were immunized with E2-BSA conjugates. The first dose for subcutaneous injection consisted of 100 μg E2-BSA and equal amount of FCA as an emulsion. After 30 days, four subsequent injections were given at 20 days' intervals with the same dosage of immunogen which was emulsified with the same amount of FIA. Antisera were collected at the tenth day after the fourth immunization and were screened for anti-E2 activity by ciELISA. The fifth intraperitoneal injection was carried out on the mouse with the highest anti-E2 activity. Three days later, the spleen of the injected mouse was removed for hybridoma production.

2.4.2. Cell fusion and hybridoma screening

Hybridomas secreting anti-E2 antibodies were generated by a standard protocol (Song et al., 2011). The spleen was firstly removed from the immunized mouse; the isolated splenocytes were sampled from the removed spleen and fused with NS0 cells by using PEG1500. The fused cells were then distributed into 96-well culture plates with mouse peritoneal macrophages which had been spread on the day before the fusion. The plates with fused cells were incubated with the selective HAT medium. Ten days after fusion, supernatants of hybridoma colonies were collected and screened by indirect ELISA for secretion of mAb binding to E2. And the selected clones were subcloned by limiting dilution. Ascites fluids were generated in paraffin primed BALB/c mice. The subclass of the isotypes of the antibody was determined by using a commercialized mouse monoclonal antibody isotyping kit

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