Detection of Serum Bisphenol A with Enzyme-linked

Yinyin Lan
Technology normalUniversity,Nanchang,Jiangxi Province330013, China

Abstract: To establish an indirect competitive enzyme-linked immune assay (IC-ELISA) using Monoclonal Antibody of bisphenol A (BPA) for the Detection of Serum BPA. Methods Totally 176 pupils aged 8-10 years were selected from three primary schools in suburban area of Nanchang city of Jiangxi Province and Serum bpa of the pupils were determined with IC-ELISA established. Results The detection limit of the established IC-ELISA method was 0.43 ng/ml. The recovery rates of BPA in serum spiked at 4 different levels were between 70.1% And 87.8% With the variation coefficients from 4.79% To 9.41%. BPA was detected in 95 of the 176 serum samples and the detection rate was 54%. The Serum BPA content ranging from non-detectable to 26.48 ng/ml for all the samples. The detection rate of Serum BPA was higher along the boy pupils than the girl pupils, but the detection rate was not significant difference (P = 0.195 ). Conclusion The established IC-ELISA could meet the requirement of Serum BPA detection along children. BPA was detectable in serum of Children in Nanchang city of Jiangxi Province and relatively higher Serum BPA content was detected in some of the children, suggesting studies are needed to explore the contact source of BPA.

KeyWords: Enzyme-linked immune assay; serum bisphenol A; Children

1. Materials and Methods

1.1 Main instruments and reagents
BPA Standard (purity)≥99% (And sheep anti-mouse enzyme labeled second antibody (USA?SigmaCompany); acetonitrile (chromatographic purity) (xilong Chemical Co., Ltd );N, N-Dimethyl formamide (analytical purity) (Tianjin damao chemical reagent factory) (Tetramethylbenzidine,TMB) (Shanghai Biological Engineering Co., Ltd);BPAMonoclonal Antibody Detection Antigen[9](Laboratory of Life Sciences College, Jiangxi Normal University of Science and Technology ). Coated Liquid: 0.01 mol/L Phosphate buffer (Phosphate buffer solution,PBS,The pH 7.4); Closed liquid: 0.05 g/mL skim milk; washing liquid: containing0.05%(V: V) Twain-20(Tween-20)PBSNamelyPbst; Standard Dilution Fund projects; National Natural Science Foundation of China (81360429); Jiangxi Provincial university science and technology Landing Plan (Gjj13573/13574); Nanchang Science and Technology Plan Project[2012]37 Number-23) Author's unit: 1.College of Life sciences, Jiangxi Normal University of Science and Technology, Nanchang, Jiangxi, China330013; 2.Nanchang Center for Disease Control and Prevention 50 mg, Put in 50 ml In volumetric bottles, dimethyl formamide (Dimethyl formamide,DMF) Constant Volume Concentration 1.0 mg/ml Diluted into a series of standard working solutions of different concentrations with ultra-pure water before analysis. Multikan mk3Enzyme marker,Wellwash versaWashing machines 5804rCentrifuge (Germany)Eppendorf Company ); Milli-QUltrapure Water System ; 96 Hole enzyme labeled plate . 1.2 Object 2014 According to the principle of informed consent and voluntary consent of the children and their parents3. The primary school8.–10CCO176Children as the research object. Venous Blood4 mLTo clean glass test tubes (plastic pipes were not used during operation as plastic pipes may beBPA After centrifugation, the serum is

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placed-20CSave in the fridge.

1.3 Method

1.3.1 Indirect CompetitionElisaBuildPBSWillBPADetection antigen diluted1 g/ml?Package in96On the enzyme labeled plate,120 µL/Kong,4.COvmmakePBSWash Plate4.Times, add each hole320 µL 0.05 g/mL The skim milk in37CWen Yu2 hWashing Plate4.Times, add ultra pure water dilutedBPAStandard50 µlAnd50 µlThe appropriate dilution ratio of the antibody dilution,37CWen Yu40 minWashing Plate4.Times, add each hole500 µL 1:2 100The enzyme labeled second antibody,37CWen Yu40 minWashing Plate4.Time, joinTMBChromogenic Liquid100 µl/Hole, avoid light color8 minRemove each hole and add50 L 2 mol/LSulfuric acid termination reaction, determination by enzyme-labeled Instrument450 nmAbsorbanceOd

\[ \text{Od}_{450} \]

3. Take the average. Calculated binding rate= \( \frac{B}{B_0} \times 100\% \), \( B_0 \)Do not addBPAOfOd Value,BYūgaBPAOfOd Value), and10TimesBPAThe logarithm of the standard concentration is abscissa and the binding rate is ordinate, and the standard curve is drawn. And select the inhibition rate10%The corresponding concentration is the minimum detection limit.

1.3.2 Extraction Method Establishment (1.) Ether Extraction Draw

0.5 mLSeries of different mass concentrationsBPA Serum spiked solution, respectively3 mL Ether Extraction, vortex Oscillation3 min, Static1 min,
Separate ether layer and add2 mL Ether Extraction, combination2.Secondary extraction of ether with nitrogen at room temperature (N2.) Drying, residue plus500 µL Ultra pure water dissolved. Indirect CompetitionElisaThe recovery rate was calculated. (2.) Trichloroacetic acid Precipitated protein extraction0.5 Serum spiked solution, add150 L 10% Trichloroacetic acid, vortex oscillation,4.C, 10 000 r/minCentrifugal20 min. Take supernatant, adjustPHTo7.4,
Indirect CompetitionElisaThe recovery rate was calculated. (3.) Acetonitrile precipitation protein extraction[10]Draw0.5 Serum spiked solution, add1.5Acetonitrile, Shake well,40C Static30 min,
Remove the mixture in4.C, 10 000 r/minCentrifugal20 min, Collect supernatant. Join again1 mL Acetonitrile in precipitation, mixed oscillation,4.C,
000 r/minCentrifugal10 min, Collect supernatant. Combine two times in clean10 mL Glass test tubes for use at room temperatureN2. Dry blow, residue plus500 µL Ultra pure water dissolved. Indirect CompetitionElisaThe recovery rate was calculated.

1.3.3 SerumBPADetermination of recovery rate (1.) SerumBPA The recovery rate was determined with fetal bovine serum as the spiked sample. Preparation of ultra-Pure Water100 ng/ml Of BPASpiked solution. Draw separatelyBPASpiked solution (100 ng/ml) 1.25, 2.5, 5.0, 12.5
25 mLIn the volumetric flask, a series of different mass concentrationsBPASpiked solution, respectively, absorb0.5.After adding the labeled serum, according1.3.2.After the optimized method is processed, the indirect competition is ElisaMethod for Determination, parallel determination of each concentration4.The recovery rate was calculated.
(2.) SampleBPAContent detection, removal and preservation in-20C The serum was thawed and shaken at room temperature.0.5Yu5 mL In the test tube of the stick glass, according to the extraction method (3.) Step for pretreatment of serum. Get handled well176 Samples, in a linear range, in accordance with indirect CompetitionElisa Determination, parallel determination of each sample4.Take the average to calculate the sampleBPAConcentration.

1.4 Statistical AnalysisUseSPSS 13.0 Analyze data. UseK-SInspection determined in the sampleBPA Whether the concentration distribution is normal. UseChi2.Testing to determine age and genderBPADetection rate difference. Using Rank Sum test to determine BPAConcentrations vary between gender and age groups.

2. Knot Guo

2.1 Indirect CompetitionElisaStandard Curve creation (figure1.)

The coating antigen concentration was1 g/ml?Antibody working concentration1: 512 000 The working
concentration of enzyme labeled second antibody was 1: 500. For the best ElisaIndirect created by analysis conditionsElisaThe linear range of standard curves is

1.~50 ng/mlThe linear equation is y = -0.339x + 1.116, R² = 0.995. Detection limit is 0.43 ng/ml, 50% Inhibitory concentration (50% IC₅₀) 6.56 ng/ml.

2.2 Extraction Method Selection (figure 2). BPASlightly soluble in water, soluble in methanol, ethanol, ether and other organic solvents. When the extraction method was established, ether was used as the extraction agent and the serum was mixed before extraction. BPA. After extraction BPA The recovery rate is 23.6% ~ 43.2%. The protein in serum was precipitated by trifluoroacetic acid. BPA Recovery rate is 30.3% ~ 34.6%. Replaced with acetonitrile

As the extraction solvent, because of the high protein content in serum samples, the protein in serum should be removed as much as possible in the extraction process, and the amount of acetonitrile needed to be optimized, the added acetonitrile volume was the serum volume. 3. Protein precipitation is more complete. The extraction time and temperature were optimized, 0.5 Adding serum samples. 1.5 Acetonitrile 40C Extraction 30 min, BPA Recovery Rate ≥ 70%.

2.3 Serum medium BPA Determination of spiked recoveries (Table 1.) Will tim

With different concentrations BPASerum Samples of standard samples were precipitated with acetonitrile

After protein extraction, the indirect Competition ElisaMethod detection BPA Content in serum samples were measured. BPA The recovery rate is 70.1% ~ 87.8% The coefficient of variation is 4.79% ~ 9.41%. Indirect competition after processing Elisa Experiment. Use SPSS 13.0 Analyze data, K-Sthe test showed that the frequency distribution did not follow the normal distribution. 176 Child serum, 81 Not Detected BPA. 95 Detected BPA. All serum samples BPA Concentration range is unchecked 26.48 ng/ml. 95 Example check-out BPA The lowest content is 0.44 ng/ml, Highest 26.48 ng/ml.

2.4.2 Serum levels of children of different ages and genders BPAConcentration and Detection Rate (Table 2,3.) BPAThere were certain differences in concentrations among different age groups, but the difference was not significant (P = 0.195). The detection rate of all samples was analyzed, and the detection rate of boys was higher than that of girls. The detection rate was different among different age groups, but not significant (P = 0.16).71 Of the boys, there are 44 Name Detection BPA. 27 Mingwei

Check out. The age-level analysis showed that there was no significant difference in the detection rate among different age groups (P = 0.066). 105 Of the girls, there are 51 Name check out BPA 54 Without detection.

3 Please On

Add BPAO plastic products has colorless transparent, heat-resistant, resistance hit and lightweight characteristics widely used in baby bottle, water bottle, Plastic Tableware and food packaging material and various commodity in[11-14]. Wang Dan[15] Study found that in addition to plastic products the some shower gel, shampoo, skin care products also contain BPA. 2003 Years world BPA Years production total have more 200 Tons its demand rate to every year 6% ~ 10% of rate growth[16-17]. At present not only in air, water, sewage sludge, soil and food and environment samples in found BPA And in human body fluid sample such as blood, urine, saliva, amniotic fluid and breast milk also found its residual [18-Natural 20]. Animal Experimental and in vitro study show that, BPA Has estrogen role it can simulation endogenous hormone, estrogen and male sex hormone influence and animal of central neural system and Reproductive System[21]. Zheng JieSuch.[22] Study found BPA Will reduce male mice sperm quantity and activity increase sperm of deformity rate. A large number of long-term pick up

BPA Will the kidney, liver, spleen, pancreas and lung and other A

Officer system damage[23]. In, China from 2011 Years 6 Month 1 Day banned the production BPA Of infant bottle, 9 Month 1 Day the prohibited import and sales BPA Of infant bottle[24]. So for effective control BPA The Environment

And human body of harm fast effective of detection methods will not less.

This study use Laboratory Preparation of BPA Single cloning Antibody 3H1 Established the indirect Competition ELISAMethods The determination of serum in BPA Content. Detection range 1 ~ 50 ng/ml IC₅₀ For 6.56
ng/mL. Minimum detection limit 0.43 ng/mL. The study display Jiangxi Nanchang Area Children serum in BPA Residual all serum BPA Concentration range for not detected 26.48 ng/mL Individually content is high may and long-term of contact and cumulative about need to pay close attention to its source. For prevent Plastic Products in BPA Dissolution pollution samples processing process in try to "with glass container and must be after strict cleaning. The experimental results show that, this study in with the ELISA plate on BPA detection no influence. Due to serum in protein content is high will and BPA Happen hydrophobic role and Hydrogen Bond role interfere ELISA detection so in established methods of process in select the with acetonitrile precipitation protein of methods extraction serum in BPA Reduce the matrix interference to the is good extraction effect. Study show that, BPA In > 60 °C Of temperature under oxidation rate will speed up obviously. Extraction process in for make BPA Can rapid dissolved in acetonitrile and don't oxidation select the temperature 40°C.

This paper established indirect Competition ELISA Method detection BPA And get BPA In Nanchang Area Children blood in residual level for further of in the study on the basis. Next will on serum in BPA Content is high Children Investigation know its the contact to the food, food packaging and environment pollutants such. At the same time on BPA The migration rule of study for people put forward valuable of recommendations protection people of health.

References


25. Thank you xiao yun.Several Typical endocrine interference of and people serum albumin of each other role research[D].Gansu:Lanzhou University2011