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ORIGINAL ARTICLE

Detection of Serum Bisphenol A with Enzyme-linked

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Abstract: To establish an indirect competitive enzyme-linked immune assay (IC-ELISA) usingMonoclonal Antibody of bisphenol A (BPA) for the Detection of Serum BPA.MethodsTotally 176 puils aged 8-10 years were selected from three primary schools in suburban area of Nanchang city of Jiangxi Province and Serum bpa of the puils were determined with IC-ELISA established.ResultsThe 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 puils than the girl puils, but the detection rate was not significant difference (P= 0.195).ConclusionThe 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 contactsource of BPA.

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

1. Materials and Methods

1.1 Main instruments and reagentsBPAStandard (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/mLSkim milk; washing liquid: containing0.05%(V: V) Twain-20(Tween-20)PBSNamelyPbst; Standard DilutionFund 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],37Number-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 in50 mlIn volumetric bottles, dimethyl formamide (Dimethyl formamide,DMF) Constant Volume Concentration1.0 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)EppendorfCompany);Milli-QUltrapure Water System ;96Hole enzyme labeled plate . 1.2 Object2014According 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 beBPAAfter centrifugation, the serum is

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.COvernight packagePbstWash Plate4.Times, add each hole320 μL 0.05 g/mLThe 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_{450}. Determination of each concentration

3. Take the average. Calculated binding rate= B/B_0X 100%,B_0Do not addBPAOfOd_{450}Value,BYūgaBPAOfOd_{450}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 concentrationsBPASerum spiked solution, respectively3 mLEther Extraction, vortex Oscillation3 min, Static1 min,

Separate ether layer and add2 mLEther Extraction, combination2.Secondary extraction of ether with nitrogen at room temperature (N_2.) Drying, residue plus500 μLUltra pure water dissolved. Indirect CompetitionElisaThe recovery rate was calculated. (2.) Trichloroacetic acid Precipitated protein extraction0.5Serum 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]Draw0.5Serum spiked solution, add1.5Acetonitrile, Shake well,40CStatic30 min,

Remove the mixture in4.C,10 000 r/minCentrifugal20 min, Collect supernatant. Join again1 mLAcetonitrile in precipitation, mixed oscillation,4.C,

000 r/minCentrifugal10 min, Collect supernatant. Combine two times in clean10 mlGlass test tubes for use at room temperatureN_2.Dry blow, residue plus500 μLUltra pure water dissolved. Indirect CompetitionElisaThe recovery rate was calculated.

1.3.3 SerumBPADetermination of recovery rate (1.) SerumBPAThe recovery rate was determined with fetal bovine serum as the spiked sample. Preparation of ultra-Pure Water100 ng/mlOfBPASpiked solution. Draw separatelyBPASpiked solution (100 ng/ml)1.25,2.5,5.,12.5

25 mlIn the volumetric flask, a series of different mass concentrationsBPASpiked solution, respectively, absorb0.5After adding the labeled serum, according1.3.2After the optimized method is processed, the indirect competition isElisaMethod for Determination, parallel determination of each concentration4.The recovery rate was calculated.

(2.) SampleBPAContent detection, removal and preservation in-20CThe serum was thawed and shaken at room temperature.0.5Yu5 mLIn the test tube of the stick glass, according to the extraction method (3.) Step for pretreatment of serum. Get handled well176Samples, in a linear range, in accordance with indirect CompetitionElisaDetermination, parallel determination of each sample4.Take the average to calculate the sampleBPAConcentration.

1.4 Statistical AnalysisUseSPSS 13.0Analyze data. UseK-SInspection determined in the sampleBPAWhether the concentration distribution is normal. UseChi2.Testing to determine age and genderBPADetection rate difference. Using Rank Sum test to determineBPAConcentrations 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 000The working

concentration of enzyme labeled second antibody was1: 500For the bestElisaIndirect created by analysis conditionsElisaThe linear range of standard curves is

1.~50 ng/mlThe linear equation isY =-0.339x + 1.116,R^2= 0.995, Detection limit is0.43 ng/ml,50%Inhibitory concentration (50%Exhibition concentration,IC_{50})6.56 ng/ml.

2.2 Extraction Method Selection (figure2.)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 extractionBPAThe recovery rate is23.6%~43.2%. The protein in serum was precipitated by trifluoroacetic acid.BPARecovery rate is30.3%~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,0.5Adding serum samples1.5Acetonitrile40CExtraction30 min,BPARecovery Rate≥70%.

2.3 Serum mediumBPADetermination of spiked recoveries (Table1.) Will tim

With different concentrationsBPASerum Samples of standard samples were precipitated with acetonitrile

After protein extraction, the indirect CompetitionElisaMethod detection BPAContent in serum samples were measured.BPAThe recovery rate is70.1%~ 87.8%The coefficient of variation is4.79%~9.41%. Indirect competition after processingElisaExperiment. UseSPSS 13.0Analyze data,K-SThe test showed that the frequency distribution did not follow the normal distribution.176Child serum,81Not DetectedBPA,95DetectedBPA. All serum samplesBPAConcentration range is unchecked26.48 ng/ml.95Example check-outBPAThe lowest content is0.44 ng/ml, Highest26.48 ng/ml.

2.4.2 Serum levels of children of different ages and gendersBPAConcentration and DetectionRate (Table2.,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).71Of the boys, there are44Name DetectionBPA,27Mingwei

Check out. The age-level analysis showed that there was no significant difference in the detection rate among different age groups (P= 0.066).105Of the girls, there are51Name check outBPA54Without detection.

3 Please On

AddBPAOf 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 containsBPA.2003Years worldBPAYears production total have more200Tons its demand rate to every year6%~10%Of rate growth[16-17]. At present not only in air, water, sewage sludge, soil and food and environment samples in foundBPAAnd 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,BPAHas estrogen role it can simulation endogenous hormone, estrogen and male sex hormone influence and animal of central neural system and Reproductive System[21]. ZhengJieSuch.[22]Study foundBPAWill reduce male mice sperm quantity and activity increase sperm of deformity rate. A large number of long-term pick up

BPAWill the kidney, liver, spleen, pancreas and lung and other A

Officer system damage[23]. In, China from2011Years6Month1Day banned the productionBPAOf infant bottle,9Month1Day the prohibited import and salesBPAOf infant bottle[24]. So for effective controlBPAThe Environment

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

This study use Laboratory Preparation of-BPASingle cloning Antibody3H1Established the indirect CompetitionELISAMethods The determination of serum inBPAContent. Detection range1~50 ng/mLIC_{50}For6.56

ng/mLMinimum detection limit0.43 ng/mL. The study display Jiangxi Nanchang Area Children serum inBPAResidual all serumBPAConcentration range for not detected26.48 ng/mLIndividual content is high may and long-term of contact and cumulative about need to pay close attention to its source. For prevent Plastic Products inBPADissolution 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 onBPAOf detection no influence. Due to serum in protein content is high will andBPAHappen hydrophobic role and Hydrogen Bond role[25]InterferenceELISAOf detection so in established methods of process in select the with acetonitrile precipitation protein of methods extraction serum inBPAReduce the matrix interference to the is good extraction effect. Study show that,BPAIn> 60℃Of temperature under oxidation rate will speed up obviously. Extraction process in for makeBPACan rapid dissolved in acetonitrile and don't oxidation select the temperature40℃.

This paper established indirect CompetitionELISAMethod detectionBPAAnd get

BPAIn Nanchang Area Children blood in residual level for further of in the study on the basis. Next will on serum inBPAContent is high Children Investigation know its the contact to the food, food packaging and environment pollutants such. At the same time onBPAThe migration rule of study for people put forward valuable of recommendations protection people of health.

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