Aluminum determination in serum is included in the regular surveillance program of patients with chronic renal failure. The most commonly used analytical technique is graphite furnace atomic absorption spectrophotometry with Zeeman background correction.

Kaneko et al. (1) reported a sensitive and simple method based on ion-pair reversed-phase HPLC with spectrophotometric detection after serum protein precipitation and aluminum chelation by 2,2'-dihydroxyazobenzene (DHAB).

We propose modifying the chelating reaction to decrease contamination with external aluminum. The DHAB is dissolved in acetonitrile, thus obviating the use of the surfactant PONPE (1), and the reaction is processed at room temperature. After the reaction has proceeded, we stop it by extracting the excess DHAB with an organic solvent. Thus the Al-DHAB chelate stays in an aqueous phase, which will not react with any contamination. The use of ammonia, an aluminum-poor base, instead of choline and the suppression of methanol and Tris buffer lower the absorbance of the blank.

To 500 μL of serum in a 5-mL polystyrene tube, add 25 μL of 70% perchloric acid (Merck ACS-ISO, Darmstadt, Germany), pipetting this into the middle of the tube while vortex-mixing. Heat for 5 min in a 70°C water bath and centrifuge for 10 min at 1500g. Pipet 100 μL of the supernate into a 5 mL acid-washed polystyrene tube containing 300 μL of ammonia M (Merck Suprapur), and then add 30 μL DHAB (Aldrich, Milwaukee, WI) solution (5.6 mmol/L in acetonitrile; Merck) and let stand for 20 min at room temperature. Add 400 μL of organic solvent (carbon tetrachloride:chloroform, 3:1 by vol.; Merck) and vortex-mix for 20 s. After centrifugation for 3 min at 600g, transfer the supernate to autosampler vials (plastic or glass), which are kept at 4°C, and inject a 50-μL aliquot.

The HPLC procedure (1) is not modified, except for changing the mobile phase pH from 8 to 8.3. We used HPLC apparatus from Thermo Separation Products (Fremont, CA): solvent conditioning module SCM 400, P 4000 pump, AS 3000 autosampler, and UV 2000 spectrophotometer. The 125 × 4.6 mm column of Spherisorb ODS2 (5-μm particles) is from Hichrom (Berks, UK).

DHAB solubility is favored by alkaline medium. The organic solvent extracts >95% of the excess DHAB, whereas >90% of the Al-DHAB chelate stays in the aqueous phase. The chelates are stable at 4°C for at least 4 h (standards in water) and 24 h (serum extracts).

The detection limit, defined as 2 SD of the blank, was 1.7 μg/L in serum, the mean blank value in serum being 7.5 μg/L. The linear range extended to 1000 μg/L. The within-run CV for serum samples was 9.1% at 8.5 μg/L, and 5.5% at 137 μg/L (n = 10 each). The recovery of 100 μg/L Al added to five serum samples was 103% ± 7.1% (Table 1). The reference interval for Al in 10 healthy subjects was 5.5 ± 3.7 μg/L.

To assess interference from desferrioxamine, we added to serum 10 and 100 mg of this compound (Sigma Chemical Co., St Louis, MO) and 100 μg/L Al; the amount of aluminum recovered was respectively 96% and 50% of that added. Thus desferrioxamine interference is negligible at a plasma concentration of 10 mg/L; at that concentration, the assay reaction tube will contain 2.3 mg/L desferrioxamine and 83.7 mg/L DHAB. According to the manufacturer's literature on desferrioxamine (Desferal; Ciba-Geigy, Basle, Switzerland), 30 mg intramuscular injection of 10 mg/kg desferrioxamine, plasma concentrations peak at 15.5 μmol/L (8.7 mg/L) in healthy volunteers, the half-life in serum being 1 h. Generally, for test or treatment, this compound is administered during the last 60 min of a hemodialysis session; the blood samples for aluminum determination are taken at the start of the next session (44 h later), by which time the desferrioxamine concentrations will be <10 mg/L.

The method is simple, sensitive, reproducible, and convenient for clinical laboratories that have HPLC apparatus.

Reference


The ability to detect DNA sequence alterations has had a major impact on molecular genetics by facilitating the identification of specific disease-causing mutations and by identifying polymorphisms that can be used as genetic linkage markers. Ataxia telangiectasia (A-T) is an auto-

<p>| Table 1. Recovery of added aluminum, 100 μg/L. |</p>
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<th>Al, μg/L</th>
<th>Original</th>
<th>Measured</th>
<th>Recovery, %</th>
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mal recessive disorder characterized by progressive cerebellar degeneration, predisposition to cancer, immunodeficiency, chromosomal instability, and radiosensitivity (1). A-T heterozygotes, whose frequency in the general population is estimated at 1.4%, show moderate radiosensitivity and an increased susceptibility to cancer (2, 3). The A-T gene has been localized to a region tightly linked to the locus D11S384 by Lench et al. (4). A Taq1 restriction fragment length polymorphism (RFLP), derived from the cosmid CJ52.193 (D11S384), which showed zero recombination with the A-T locus, was originally used to haplotype the A-T gene locus with allele 1 of 8.5 kb and allele 2 of 4.3 kb (5). The allele frequency was estimated from 160 chromosomes of unrelated family parents. In normal or unaffected chromosomes, the frequency of allele 1 (−) was 35% and of allele 2 (+) was 65%, whereas among the affected or A-T chromosomes, the frequency of allele 1 was 23% and of allele 2 was 77%. Significant linkage disequilibrium was not observed between this RFLP marker and the A-T locus (P = 0.0807).

To increase the throughput and decrease the complexity of the assay, we isolated, subcloned, and sequenced a 1.3-kb HindIII-XbaI fragment from the P1 clone to determine the location of the Taq1 polymorphic site. We subsequently designed oligonucleotide primers for PCR amplification of a 1008-bp fragment containing the Taq1 RFLP site.

To amplify this specific 1008-bp DNA region containing polymorphic Taq1 site, we used oligonucleotides Taq1 (5'-GAGACAAGATGCTTGCCTCTG-3') and Taq5 (5'-CATGTTTCTGAGGGCAGG-3') as primers. PCR was performed with 100 ng of genomic DNA, 10 pmol of each primer, and 200 μmol/L dNTP in 50 μL of a buffer containing 10 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 1.5 mmol/L MgCl2, and 1.32 μg of Taqstart antibody (Clontech, Palo Alto, CA) and 6 U of Taq DNA polymerase (Boehringer Mannheim, Indianapolis, IN). Amplification proceeded with an initial denaturation cycle of 99°C for 5 min, followed by 30 cycles each of 94°C for 5 min, 56°C for 1 min, and 72°C for 1 min. The PCR products were analyzed on 3% NuSieve agarose gels (FMC BioProducts, Rockland, ME). If the polymorphic site is present, the amplification product can be cleaved by Taq1 into 662-bp and 346-bp fragments (Fig. 1).

The use of DNA amplification, followed by restriction enzyme cleavage, forms a reliable, nonradioactive diagnostic assay of all known RFLPs. We conclude that this PCR-based approach, which is a cost- and labor-efficient assay, can be adapted to other disease loci to speed up prenatal diagnosis/linkage studies without the use of radioactively labeled probes.

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References