Determination of Theophylline in Serum with the Seralyzer® Aris Reagent Strip Test Evaluated

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We evaluated a new Seralyzer® Aris reagent strip test (Ames Div., Miles Labs.) for the determination of theophylline in human serum. The method is based on the monoclonal enzyme immunoassay with dry reagent chemistry. The analysis is rapid and simple to perform: results are available only 5–10 min after receipt of the sample. Intra-assay precision (CV) was 2.2–3.3% (n = 15) for theophylline concentrations of 5–25 mg/L; interassay CV was 5.9% (n = 19) at 15 mg/L. The results (y) agreed well with those by liquid chromatography (x): r = 0.949 (p < 0.001), and y = 0.967x + 0.214. We conclude the method is useful for rapid evaluation of theophylline concentrations in asthmatic patients.

Additional Keyphrases: asthma • enzyme immunoassay • monoclonal antibodies • "kit" methods

Monitoring the concentration of theophylline in serum is important for securing a therapeutically effective and nontoxic steady-state concentration of the drug in the treatment of bronchial asthma (1–3). Among the analytical techniques available for the measurement of theophylline in serum (4) are gas chromatography, "high-performance" liquid chromatography (HPLC), radioimmunoassay, and enzyme immunoassay methods. Of these only enzyme immunoassay is available for the vast majority of laboratories and suitable for rapid determinations of theophylline.

Recently, a new commercial version of a monoclonal enzyme immunoassay based on dry-reagent chemistry has become available for theophylline (Seralyzer® Aris, Theo; Ames Division, Miles Laboratories, Elkhart, IN). The development of dry-reagent chemistries offers several advantages for clinical chemical analyses (5, 6), including improved storage of reagents.

The objective of our study was to evaluate the performance of this new theophylline reagent strip test by using it to analyze serum samples from asthmatic patients. The results were compared with those obtained with the HPLC technique (7) routinely used in our laboratory for monitoring the theophylline concentration in serum samples.

Materials and Methods

Instrumentation. We used a Seralyzer® reflectance photometer (Model 5110) equipped with Seralyzer Aris theophylline test module (Ames Division, Miles Laboratories) for the analyses. For the HPLC method, we used a Model SP 8700 solvent delivery system with an SP 8750 organizer and an SP 8300 ultraviolet detector (Spectra-Physics, Santa Clara, CA), a Rheodyne injector with a 10-μL sample loop, and a 30 cm x 3.9 mm (i.d.) μBondapak C18 column (Waters Assoc., Milford, MA). The absorbance of effluent was monitored at 254 nm and the chromatograms were recorded with a potentiometric recorder.

Reagents. Ames provided bottles of reagent strips and low- and high-concentration theophylline calibrators. Theophylline, theobromine, β-hydroxyethyltheophylline, and caffeine were purchased from Sigma Chemical Co., St. Louis, MO, and 8-chlorotheophylline chloride from Boehringer Mannheim, Mannheim, F.R.G. 1-Methylxanthine was a gift from ICN Pharmaceuticals, Inc. (Life Sciences Group, Plainview, NY) and 3-methylxanthine from Fluka AG, Buchs SG, Switzerland. Analytical-reagent grade methanol was from E. Merck, Darmstadt, F.R.G.

The standards (range 5 to 30 mg/L) were prepared by adding to blank control serum appropriate microliter volumes of working drug solution (theophylline 1 g/L in distilled water).

Procedure. We calibrated the Seralyzer according to the instructions in the operating manual, then assayed serum samples according to the package-insert instructions. One dilutes 30 μL of sample with 800 μL of distilled water, then assay 30 μL of this dilution.

Serum samples. We used 48 blood specimens received from asthmatic patients for the routine determination of theophylline. We separated the sera without delay and divided them into two portions, for determination by each method. The sera were stored frozen at −20 °C until analysis.

Results and Discussion

Figure 1 shows the correlation between the Seralyzer Aris and the HPLC method for the determination of theophylline in 48 serum samples. The concentration of theophylline varied from 3.7 to 25.4 mg/L. The equation of the linear regression line shown is y = 0.967x + 0.214 (r = 0.949; p < 0.001).

The precision of the Seralyzer Aris method was assessed by multiple analyses of pooled serum containing 5, 15, or 25 mg of theophylline per liter. The intraassay CVs were 2.8, 2.2, and 3.3% (n = 15), respectively; the precision of the

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Amniotic Fluid Acetylcholinesterase Activity and Alpha-Fetoprotein in Chromosomal Anomalies and Neural Tube Defects

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Measurement of alpha-fetoprotein concentration and acetylcholinesterase activity in amniotic fluid can be used to identify chromosomal defects as well as neural tube defects. In seven cases of trisomy 21 and one case of partial trisomy 3, alpha-fetoprotein concentrations were below the reference range but values for acetylcholinesterase activity were normal for the appropriate gestational age. One case of trisomy 13 had an increase in acetylcholinesterase activity and normal alpha-fetoprotein concentration.

Additional Keyphrases: chromosomal anomalies • neural tube defects • heritable disorders • antenatal screening

We have previously demonstrated that in trisomy 21 the acetylcholinesterase (AChE, EC 3.1.1.7) isoenzyme pattern in amniotic fluid may be identical to that found in cases of neural tube defects, and thus it may be necessary to determine the fetal karyotype of amniotic-fluid cells to differentiate the two (1). The longer incubation period required to demonstrate the AChE isoenzyme pattern in chromosomal anomalies (Buamah, unpublished observation) suggests that the total AChE activity in these cases is within normal limits.

Concentrations of alpha-fetoprotein (AFP) in maternal serum have been shown to be lower when the fetus has a chromosomal abnormality (2, 3). We have attempted to determine a relationship between the total AChE activity and the concentrations of AFP in amniotic fluid in cases of chromosomal anomalies and neural tube defects. The results for these analytes may be useful in antenatal screening for chromosomal anomalies, especially for trisomy 21, which occurs about once per 1000 live births.

Patients and Methods

Samples of amniotic fluid were obtained by percutaneous amniocentesis from 29 normal pregnancies, 12 pregnancies associated with neural tube defects, and nine pregnancies resulting in chromosomal abnormalities. The samples were obtained at known gestational ages during the second trimester of pregnancy and they were stored at −20 °C until analyzed. The procedure was performed on patients in hospitals in the geographical areas served by the Department of Human Genetics, University of Newcastle upon Tyne. The trisomies were determined from fetal karyotypes.

References