variance with the observations of Hendriks et al.

On occasion the RA-1000 prints no result; this happens for ACE activities at the lower end of the reference interval. Machine malfunction was ruled out, because the instrument was checked for optimal performance, including lamp efficiency. With problems such as poor precision, no print, and inappropriate low results, we cannot recommend measurement of ACE in the RA-1000.

References

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Two Types of Error Found with the Seralyzer® ARIS Assay of Theophylline

To the Editor:

Results with the Seralyzer ARIS reagent strip test (Ames Div., Miles Labs., Elkhart, IN), recently introduced as a convenient method for determination of theophylline in the emergency room, small laboratory, or physician’s office (1), reportedly correlate well with those by liquid chromatography (1–5), fluorescence polarization immunoassay (6–8), fluorimunoassay (1, 4), and enzyme immunoassay (1). Nevertheless, a proportional positive error of 8–12% with the ARIS test has been frequently noted (1, 2, 4, 5). A physicians’ group-practice laboratory participating in the New York State drug-monitoring proficiency-testing program has consistently reported survey results 10–20% higher than the target concentrations. We have identified the cause of this analytical error and have found another, potentially more serious, error with this method.

The ARIS analyses were performed according to the instructions in the manufacturer’s operation manual and reagent insert. The liquid-chromatographic method was performed as previously described for determination of acetaminophen in serum (9).

The ARIS Drug Assay Calibrators were assayed by liquid chromatography, and the theophylline concentrations assigned by the manufacturer were determined to be accurate. However, the calibrators also contained, per liter, 1 g of sodium azide, a compound that inhibits peroxidase activity (10). Peroxidase (EC 1.11.1.7) is used in the ARIS test to catalyze the oxidation of tetramethylbenzidine. It seemed likely that any inhibition of peroxidase activity by azide in the calibrator would cause a positive bias in assay results for specimens not containing azide.

To evaluate the sensitivity of the ARIS reagent system to azide, we added incremental amounts of sodium azide to aliquots of a serum pool containing 17 mg of theophylline per liter. The aliquots contained 0, 1, 2, 4, or 8 g of sodium azide per liter. The means of quadruplicate ARIS assays of each aliquot decreased with increasing sodium azide concentration. The apparent mean theophylline concentrations (and SD) were 18.9 (0.4), 17.4 (0.7), 16.9 (0.4), 15.0 (0.5), and 12.7 (0.6) mg/L, respectively—clearly demonstrating the adverse effect of azide on the ARIS test.

For a second evaluation, we assayed 35 samples of patients’ sera obtained from a local medical center that had quantified their theophylline content by the Abbott "TDx" test (Abbott Labs., North Chicago, IL). The samples, stored at −20°C for a week or less, were then assayed in our laboratory by both the ARIS and liquid-chromatographic methods. Immediate before each analysis we added to each sample 10 µL of an aqueous 50 g/L solution of sodium azide. Treated and untreated specimens were assayed by each method, in duplicate where sodium azide was added, the results were corrected for the 2% dilution.

The liquid-chromatographic (LA) and the TDx results agreed well; TL = 0.98 (±0.02)LC + 0.15 (±0.33), r = 0.99, SP = 0.77. Linear regression analysis of ARIS and liquid-chromatographic data for untreated specimens revealed a proportional error of -8% at a constant bias of 1.0 mg of theophylline per liter (Figure 1). The systemat error for azide-treated specimens were statistically insignificant. The addition of azide had little effect on the very high bias found for five samples (a–e Figure 1).

We analyzed the specimens for various xanthine derivatives and evaluated the sensitivity of the ARIS system to these compounds. Caffeine, 8-chlorotheophylline, theobromine, 1,7-dimethylxanthine, 3-methylxanthine, and 7-methylxanthine were not found by liquid chromatography or were present in only trace amounts. We were unable to screen the samples for the theophylline metabolite 1,3-dimethyluric acid, a compound that is not extracted in the sample preparation procedure. As in other immunoassays (11), this metabolite cross reacts in the ARIS test (a), the apparent theophylline concentration being 9.6 mg/L in a drug-free serum supplemented with 1,3-dimethyluric acid at 20 mg/L.

No clinical histories were available but the chemistry profiles of the tw

Fig. 1. Correlation of theophylline results obtained by ARIS and chromatography (HPLC)

To one portion of each patient’s serum was added sodium azide (1 g/L). Data for specimens a–e were not included in the regression analysis.
specimens for which data were available documented renal impairment (sample a: urea 1.02 g/L, creatinine 91 mg/L; sample b: urea 490 mg/L, creatinine 44 mg/L). It thus is likely that an increased concentration of 1,3-dimethyluric acid in serum was responsible for the discrepancy in theophylline concentrations.

We conclude that the azide in the Aries calibrators is responsible for the systematic error reported in several evaluations of this analytical technique. Correcting for the azide effect made the Aries results accurate and precise and thus confirmed the need for a different bacteriostatic agent. As reliable as the Aries performance is for most specimens, we believe that 1,3-dimethyluric acid was responsible for a bias of 57% or more in 14% of the samples assayed. Until this source of error has been eliminated, we recommend that physicians routinely evaluate the patient's renal function; when function is determined to be compromised, they should use a method specific for theophylline.

We thank Ames for the donation of reagents and the loan of the Seralyzer equipment. We also thank John Meola (Albany Medical Center) for providing clinical samples.

References


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A researcher from Ames responds: To the Editor:

Dr. Jenny, communicating his concerns with the Seralyzer Aries Theophylline Reagent Strip test, has kindly provided us with samples from the New York State Proficiency Program and, more notably, specimens A and B described above. Jenny and Jackson describe two problems: a small proportional bias that apparently results from the presence of azide (1 g/L) in the calibrators, and a very significant positive bias in five specimens, attributed to the presence of 1,3-dimethyluric acid.

Inhibition of strip reactivity by azide is clearly demonstrated by Jenny and Jackson, although their results show azide at 1 g/L in the serum pool containing 17 mg of theophylline per liter did not inhibit the reaction. The addition of this concentration of azide to two clinical specimens (theophylline content, approximately 9 and 18 mg/L) changed the apparent concentrations by 1.5% and 3.0%, respectively, as assayed in our laboratory with the same lot of test strips as used by these authors. Calculation of an average regression equation from data produced in 25 independent studies (12 TDx and nine HPLC comparisons, many of which are published) yielded a slope of 1.0366 (SD 0.085) and an intercept of -0.042 (SD 0.048). While azide in the calibrators may contribute some bias, the bias is not as high as reported in the Letter. Also, this error is clinically insignificant. Nonetheless, a theophylline calibrator containing 0.1 g of thimerosal per liter rather than 1 g of azide as a preservative is in the final phases of development and will replace the current product. The calibrators and controls for the Seralyzer Aries Phentoin and Phenobarbital tests also contain thimerosal, 0.1 g/L.

Interference by 1,3-dimethyluric acid has been specifically noted in the product labeling since the theophylline strip was introduced to the market. The product insert also indicated that high concentrations of this metabolite had been reported in the serum or plasma of uremic patients and that such results must be interpreted "with caution." The labeling was later revised to strengthen the language and warn the customer not to use the product to measure theophylline in uremic patients. This change was noted by Volch RB et al. (1). Of the patient specimens Dr. Jenny sent us, we confirm his results with the theophylline strip and liquid chromatography, and also with the monoclonal Ames TDA® Theophylline test, which contains a more specific antibody (2, 3) than does the strip. The appearance of a large peak coincident with 1,3-dimethyluric acid in specimen a, as analyzed by liquid chromatography, makes it unlikely that the interference is due to anything other than this metabolite.

References


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SP1—Really a Tumor Marker for Lymphoid Malignancy?

To the Editor:

Fagnart et al., using PACIA, reported (1) a high frequency of detectable pregnancy-specific β1-glycoprotein (SP1, reviewed in 2) in serum of patients with malignant hemopathies. To evaluate these data, we screened retrospectively for SP1 (Enzygnost SP1; Behringwerke AG, Marburg, F.R.G.) 68 sera of 40 patients with neoplasia of lymphoid origin. All samples had been originally sent to our laboratory for determination of β2-microglobulin (Pharmacia Diagnostics,