Evaluation of a Rapid Method for Measuring Theophylline in a Clinical Setting

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The need to determine rapidly the theophylline levels of patients in ambulatory settings such as family practice offices has led to the development of instruments purported to be fast, reliable, and cost-effective. This study evaluated one such instrument, the Ames Seralyzer, and compared the findings with those of the Du Pont aca. Forty-six patient samples were split and run on both instruments by trained laboratory personnel. Validation studies yielded a correlation coefficient of r = .9680 (N = 46).

Precision assays showed the 27.5 and 82.5 μmol/L (5 and 15 mg/L) levels of theophylline found in control samples produced day-to-day coefficients of variation of 8.8% (n=43) and 5.8% (n=35), respectively. After initial evaluation of the Seralyzer, duplicate assays were performed because of erratic results. The evaluators felt that a major source of error was in the diluting and pipetting steps involved in the procedure. The Seralyzer was easy to run but did require some technique-dependent skills. **J Fam Pract 1990; 30:665-669.**

Theophylline (1,3-dimethylxanthine) is a bronchodilator that exhibits pharmacological actions including stimulation of respiration, augmentation of cardiac function, diuresis, and relaxation of smooth muscle.[1–3] Asthmatic symptoms often result from constriction of the bronchial smooth muscle, and theophylline functions to relax this muscle.[2]

Analysis of theophylline is necessary for maximum beneficial use as a bronchodilator and for its respiratory stimulant effect for asthma. Measurement of theophylline levels is also necessary to prevent toxic effects such as various gastrointestinal symptoms (vomiting, nausea), seizures, and possible cardiac and respiratory arrests. These toxic effects can result from drug overdose or when the theophylline level is out of the therapeutic range, 55 to 110 μmol/L (10 to 20 mg/L).[3] In a recent study,[4] it was found that some asymptomatic patients had toxic levels; the toxicity levels of the patients varied according to their individual metabolic rates and any other underlying disease state. Toxicity could occur over a wide range of serum concentrations.

The need for rapid results in clinical settings where physicians can prescribe immediate dose changes prompted this clinical study of the Ames Seralyzer system (Seralyzer Reflectance Photometer, Ames Division, Miles Laboratories, Elkhart, Ind). The system uses a dry chemistry technology that provides results 80 seconds after application of diluted serum. This study evaluated the accuracy and precision of the Seralyzer system and compared patient results with those obtained from a Du Pont aca. Specimens that did not agree were analyzed by a high-pressure liquid chromatograph[5] method for verification.

METHODS

Five medical technologists and one medical laboratory technician, all certified by the American Society for Clinical Pathology, performed the assays using the Seralyzer. Each laboratorian had at least 3 years of experience. The comparison studies performed on the Du Pont aca (Du Pont aca Discrete Clinical Analyzer, E.I. du Pont de Nemours and Company, Inc, Wilmington, Del) were also

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performed by medical technologists or medical laboratory technicians.

The Ames Seralyzer reaction involved an apoenzyme reactivation immunoassay system[6] reaction based on competitive binding of patient theophylline and theophylline–flavin-adenine dinucleotide conjugate for monoclonal antibodies. The unbound conjugate activated several other enzyme reactions that later oxidized tetramethylbenzidine (TMB). The intensity of the color produced by oxidized TMB is proportional to the amount of theophylline present in the patient sample, which was measured at 740 nm by the reflectance photometer of the Seralyzer.[6–8]

The Ames Seralyzer system included the Seralyzer Reflectance Photometer and MLA and Dipstat diluting pipettes with rack. Module inserts were used in the instrument for theophylline measurement in the test system. Other tests may be performed if modules for that specific test were purchased for the system.

Before analysis, the instrument was calibrated, when necessary, and controls were run. The Ames theophylline calibrators used for the instrument were 27.5 and 137.5 μmol/L (5.0 and 25.0 mg/L). Instrument calibration was performed whenever a new bottle of reagent strips was opened, when control material failed to meet acceptable criteria after second analysis, every 2 weeks, when the instrument was turned on, and when indicated on the instrument by "CAL" or "LO" display.[8]

Calibrators, controls, and patient serum specimens were all analyzed by the following procedure: 30 μL of the sample was diluted with 800 μL distilled water; 30 μL of this dilution was placed onto a reagent pad previously loaded onto the movable reaction tray. As soon as the diluted sample was pipetted onto the reagent pad, a start button was pressed on the instrument, and the reaction tray was inserted into the instrument. In 80 seconds the results were displayed. Values were extrapolated from a two-point calibration curve previously constructed from the two calibrators and stored in the instrument module.

For this study, the controls used were American Dade Stratus TDM Control (American Dade, Division of America Hospital Supply Corporation, Miami, Fla), level I approximately 27.5 μmol/L (5 mg/L) and level II approximately 82.5 μmol/L (15 mg/L). The controls were run after each instrument calibration or each day patient samples were analyzed. Within-day control runs (control runs repeated on the same day) and day-to-day control runs (controls run on a daily basis) were also analyzed.

Although the manufacturer-stated instrument linearity is from 16.5 to 165 μmol/L (3.0 to 30.0 mg/L),[8] the evaluators considered linearity to be from 27.5 to 137.5 μmol/L (5.0 to 25.0 mg/L) based on calibrator values. Samples with values less than the lowest calibrator were reported as less than 27.5 μmol/L (5.0 mg/L). Samples greater than 137.5 μmol/L (25.0 mg/L) were further diluted according to the manufacturer's recommendations as follows: 100 μL of the previously diluted sample with 200 μL of distilled water. Pipettes for this dilution were also provided with the system. To test this second diluted sample, the "dil" button was pressed on the instrument display, and the further diluted sample was run as previously stated. The instrument then calculated the correct value using the dilution factor.[8]

The accuracy of the test was measured by comparing the mean value of Stratus level I and level II controls with the target values for all laboratories and all methods stated by the manufacturer.

Over a 4-month period, 46 samples were analyzed on patients from a pediatric clinic. The samples were collected by venipuncture in nonanticoagulated tubes or in microtainers, allowed to clot, and then immediately centrifuged. The serum was removed from the clotted cells and analyzed immediately in the outpatient laboratory on the Seralyzer.

The specimens were frozen at −20°C. Batches of these specimens were analyzed on the Du Pont aca in the main hospital clinical chemistry laboratory.

Because of variability of results in preliminary studies, the 46 samples were run in duplicate on the Seralyzer and singly on the aca. In the preliminary study, 7 samples were analyzed by the high-pressure liquid chromatograph method for verification; none had to be verified by this method once samples were assayed in duplicate on the Seralyzer.

A linear regression analysis and a correlation coefficient for the methods were determined. These statistical values for the Seralyzer were based on the mean of the duplicate analysis of each sample. Seralyzer samples with values less than 27.5 μmol/L (5 mg/L) were disqualified in this study. Sample values of 137.5 μmol/L (25 mg/L) or higher were diluted as stated and used in this analysis.

The Du Pont aca linearity range was from 11 to 220 μmol/L (2 to 40 mg/L). Stratus TDM Control levels I, II, and III were run at the beginning of each shift or as needed.

RESULTS

In the initial comparison of results, variations were found in the results of controls as well as patients. The initial coefficient of variation on Stratus I was 10.97%, which denoted too large a variation in results from the true value for that control. The coefficient of variation is a statistical analysis used to describe the precision and reproducibility of a laboratory test and is defined as the standard deviation of a group of results divided by the mean value multiplied by 100. The coefficient of variation is used to

TABLE 1. ACCURACY AND PRECISION STUDIES OF SERALYZER STUDIES

Material	Target Value (mg/L)	No.	Mean (mg/L)	Within-run % CV	Day-to-Day % CV
Stratus TDM					
Level I	4.71	22	5.18	7.14	
Level II	14.56	20	15.5	7.14	
Level I	4.71	43	4.98		8.75
Level II	14.56	35	15.47		5.76

Seralyzer Reflectance Photometer, Ames Division, Miles Laboratories, Elkhart, Ind.
Stratus TDM Control, American Dade, Division of America Hospital Supply Corporation, Miami, Fla.
CV—coefficient of variation.

determine the 95% confidence limits for reported values. For example, if a patient's theophylline is 110 μmol/L (20 mg/L), then 95% of the values obtained from repeated testing of the same specimen would fall in the range between 88 and 132 μmol/L (16 and 24 mg/L) with a coefficient of variation of 10% on this method. Since this wide range also includes some values that may be toxic to some patients, determining the coefficient of variation in evaluating method variations is extremely important. With lower coefficient of variation values, the 95% confidence range would be smaller and provide more accurate and reliable results.

The results of later precision studies are shown in Table 1. The Stratus controls were compared within run (controls run on the same day) by four technologists, each testing each control five times. Coefficients of variation for both levels were 7.14%.

Results for these two controls were also compared for day-to-day precision (controls run on a daily basis) over a 3-month period. These assays were run one to three times per week. At the end of this period, coefficients of variation were calculated at 8.75% for level I and 5.76% for level II.

The results of the comparative study (N = 46) performed on the Seralyzer system and Du Pont aca are shown in Figure 1. The correlation coefficient was found to be $r = .9680$.

The paired t test showed a value of 0.361 ($P < .13$), which is not statistically significant. The paired t test can be used to compare paired results when methodologies are being compared to determine whether a statistically significant difference exists between them. The paired t test was run by comparing patient values obtained on the Seralyzer with those on the Du Pont aca, using the statistical formula.

DISCUSSION

The coefficient of variation of 10.97% obtained during the initial study was unacceptable, resulting in poor precision and reproducibility. For theophylline measurements, coefficients of variation up to 8% were considered to be clinically acceptable.

The comparative study showed a good correlation ($r = .968$, $P < .13$) between the rapid Seralyzer method and the Du Pont aca. The correlation coefficient ($r = .9680$) was similar to that found in other studies using trained laboratory personnel that compared the Seralyzer with the high-pressure liquid chromatograph or other systems.[9] Other studies have shown lower correlations when non-laboratory-trained personnel performed the assay.[9,10]

Several studies have found the Seralyzer to be accurate and reproducible.[6,7,9,10] One study[10] suggested that physicians who purchase this system for use in physician office laboratories or clinics should periodically send out portions of specimens for confirmation; also, if significant errors randomly occur, assays should be performed in duplicate. This problem with reproducibility was also

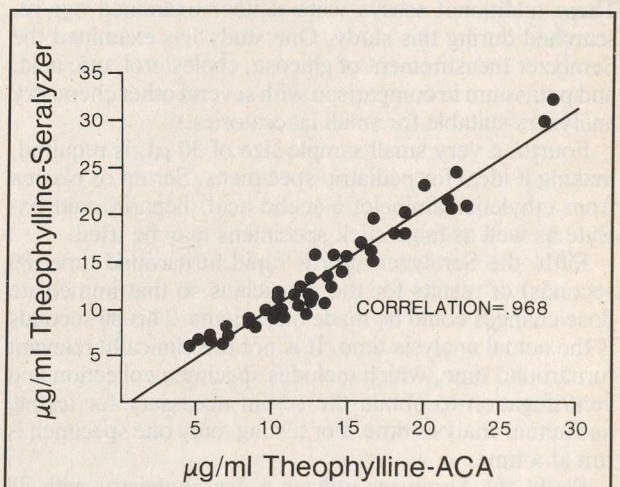

Figure 1. Theophylline correlation study between the Ames Seralyzer and Du Pont aca.

noted during the initial comparison in this study. Further comparisons using patient specimens on the Seralyzer were performed in duplicate. A major source of error was thought to be the diluting and pipetting steps required on all specimens. One study cited specific sources of error using the Seralyzer as determined by the evaluators of that study: diluent, specimen, and dilution pipetting; specimen and reagent applications.[11] This study was performed by certified laboratory personnel with many years of experience but the theophylline assay was not specifically performed.[11] Others doing theophylline studies, however, have pointed out lower correlations with untrained personnel.[9,10] In these studies, the problem was attributed to either poor test performance or lack of properly trained laboratory personnel.[9,10]

Instruments such as the Seralyzer have advantages for small laboratory settings, for example, urgent care centers, group practices, and some physician office laboratories. First, the initial instrument cost is approximately $3750; each theophylline test strip is approximately $3. Instrumentation such as the Du Pont aca costs $100,000, making it too costly for a small laboratory setting. The cost per test for theophylline on the aca is approximately $1.50.

Second, the instrument and pipette system require a small counter area, whereas a large floor area is required by the Du Pont aca.

Third, the Seralyzer has the capability to do 16 other assays including glucose, cholesterol, triglyceride, other drugs, and some enzymes. To perform these assays, specific test modules, calibrators, and controls must be purchased. Most assays are performed on initially diluted specimens, and assay times vary from 30 to 240 seconds. These additional assays were neither examined nor researched during this study. One study has examined the Seralyzer measurement of glucose, cholesterol, uric acid, and potassium in comparison with several other chemistry analyzers suitable for small laboratories.[11]

Fourth, a very small sample size of 30 μL is required, making it ideal for pediatric specimens. Serum or plasma from ethylenediaminetetra-acetic acid, heparin, and oxalate as well as fingerstick specimens may be used.

Fifth, the Seralyzer affords rapid turnaround time (80 seconds) of results for the physicians so that immediate dose changes could be made on patients. This 80 seconds is the actual analysis time. It is not the clinically relevant turnaround time, which includes specimen collection and centrifugation to obtain the serum necessary for testing and actual analysis time. For testing, only one specimen is run at a time.

Sixth, the Seralyzer utilizes a dry chemistry with all reagents on a disposable dipstick. The only mixing or diluting with reagents occurs during the initial dilution of the patient sample with distilled water or subsequent dilution, if the value is greater than 165 μmol/L (>30 mg/L). Diluting (×3) extends the range of the instrument up to 495 μmol/L (90 mg/L), as stated by the manufacturer.

Seventh, in the clinically important therapeutic ranges of 55 to 110 μmol/L (10 to 20 mg/L), this comparison showed the Seralyzer to be reliable.

Eighth, cross-reaction with other theophylline metabolites such as caffeine do not significantly interfere with the test[10,12] unless patients are uremic. The presence of 1,3-dimethylaric acid in uremic patients does interfere with the test results, giving a higher value.[8] Patients with renal dysfunction should have theophylline measured by an alternate method.[8,10]

The Seralyzer system has some inherent disadvantages, the most important of which is that the test system is dependent on consistent operator technique[6,9] and does require technique-dependent skills. This disadvantage was found in this study. A study comparing four chemistry analyzers suitable for physician office and clinic laboratories also showed this most important disadvantage.[11] Although theophylline was not studied, other available tests on the Seralyzer, such as glucose and cholesterol, were analyzed. Their results showed, as did this theophylline study, that the Seralyzer system is dependent on operator technique and skills in a wide variety of tests.[11]

The second disadvantage found is the frequency of calibration. The instrument must be calibrated every 2 weeks, each time a bottle of strips is opened, or as previously stated in the Methods section. The strips are supplied in bottles of 25 and are moisture sensitive. In the duplicate analysis of samples in this study, recalibration occurred every 2 to 3 days, depending on the number of specimens tested. Each calibration required two strips for low and high calibrators plus two for Stratus I and II controls. Even though reagent strips may come from the same lot, recalibration of a new bottle is extremely important.

In conclusion, the Seralyzer is easy to operate in a setting with trained technologists. The rapid turnaround time and sample size make it suitable for clinical measurement of theophylline, especially in pediatric patients. This study also found, as others[6,9,11] have, that some technique-dependent skills are required for accurate results.

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