We evaluated the analytical performance of the Abbott "Vision" analyzer for theophylline measurement. The within-day precision (CV) was 1.8% and 3.1% at theophylline concentrations of 15.2 and 25.2 mg/L, respectively; between-day precision was 3.5% and 4.8% at 14.9 and 24.4 mg/L, respectively. Bilirubin (143 mg/L) and triglyceride (7.4 g/L) did not interfere, but hemoglobin caused lower values for apparent theophylline, the magnitude of the decrease being proportional to the hemoglobin concentration. At the cutoff concentration of 1 g/L programmed into the instrument by the manufacturer, hemoglobin reduced the theophylline value by <10%. Results by the Vision method (y) compared well with those by the "TDX" procedure (x): \( r = 0.98, y = 0.978x - 0.270 \) mg/L. The Vision method gave comparable theophylline values for serum, plasma, and whole-blood samples. We also validated analytically and clinically that capillary blood samples collected by finger stick can be used interchangeably with blood samples collected by venipuncture for monitoring theophylline therapy.

Additional Keyphrases: enzyme inhibitor immunoassay, serum, plasma, and whole-blood samples compared, two-dimensional centrifugation, physician's office testing, fluorescence polarization immunoassay compared

Theophylline is a bronchodilator, potent in relieving acute asthmatic symptoms or to control bronchospasm (1). Many clinical studies show that measurement of the theophylline concentration in serum is important in managing patients who are receiving this drug (3–6).

Recently, Abbott introduced a new system for theophylline analysis, the "Vision System," in which centrifugal force is used to separate the sample, measure the reagent and sample volumes, and complete all steps required for homogeneous immunoassay (4). The assay is a homogeneous enzyme-inhibitor immunoassay. In this test, the theophylline in the sample competes with an enzyme inhibitor (a theophylline conjugate), which is structurally similar to theophylline, for binding sites on a specific antibody. The amount of free theophylline conjugate present is proportional to the concentration of theophylline in the specimen. Because only the free but not the antibody-bound theophylline conjugate is a potent inhibitor of acetylcholinesterase (EC 3.1.1.7), the enzyme in the assay, the theophylline concentration in the sample is inversely proportional to the activity of the enzyme:

- Antibody-(theophylline conjugate) + acetylcholinesterase
- → active enzyme
- Free theophylline conjugate + acetylcholinesterase
- → inactive enzyme

The activity of acetylcholinesterase is measured by the enzymatic hydrolysis of acetyl-\( \beta \)-(methylthio)choline iodide to the free thioph product, \( \beta \)-(methylthio)choline. Reaction of this thio product with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) will generate a highly colored thionitrobenzoate anion, which absorbs at 417 nm:

\[
\text{Acetyl-} \beta \text{-}(\text{methylthio})\text{choline + active enzyme} \rightarrow \beta \text{-}(\text{methylthio})\text{choline}
\]

\[
\beta \text{-}(\text{Methylthio})\text{choline + DTNB} \rightarrow \text{thionitrobenzoate}
\]

In this evaluation we compared the Vision enzyme inhibitor immunoassay with the Abbott TDX fluorescence polarization immunoassay for theophylline. We also validated this method for measuring theophylline in whole blood, plasma, serum, and in capillary blood sampled from a finger stick.

Materials and Methods

Reagents. Theophylline was obtained from Sigma Chemical Co., St. Louis, MO 63178; unconjugated bilirubin from the National Bureau of Standards, Washington, DC 20234; and intralipid (10% solution) from KabiVitrum Inc., Alameda, CA 94501.

Samples. We used 200 serum or plasma samples, collected from hospitalized patients receiving theophylline. These samples were either assayed immediately or stored at \(-20^\circ \text{C}\) for later analyses. In addition, 36 paired blood and serum specimens collected from patients taking theophylline were used to examine the relationship among theophylline concentrations in blood, serum, and plasma. Samples for serum theophylline measurements were collected into evacuated tubes without anticoagulant, promptly centrifuged, and the sera transferred to another set of tubes. The samples for theophylline measurements in whole blood and plasma were collected into Vacutainer Tubes (Becton Dickinson, Rutherford, NJ 07070) containing lithium heparin. After withdrawing an aliquot of whole blood, we centrifuged the heparin-treated samples to obtain plasma.

For analytical validation of the Vision theophylline method for capillary samples, we collected 22 whole-blood samples in lithium heparin-containing tubes, thoroughly mixed each sample, and withdrew portions of the samples into heparinized capillary tubes. We measured theophylline concentrations in both the lithium heparin-containing tubes and the capillary tubes. To validate the Vision method with clinical samples, we collected, by venipuncture into lithium heparin-containing tubes and by finger stick into heparinized capillary tubes, 14 paired blood samples from patients on theophylline therapy.

Samples for interference study. Samples for the studies of potential interferences were prepared from pooled plasma supplemented with theophylline to a final concentration of 15 mg/L. We assessed the effects of various amounts of added hemolysate prepared according to Chan et al. (5), unconjugated bilirubin prepared according to the method of Doumas et al. (6), or lipids (Intralipid). In order for the instrument to print out the apparent theophylline values rather than the comment "Interfering substance" when
hemoglobin exceeds 1 g/L, we had to set the minimum interval at 0.000.

**Calibration, standards, and controls.** The Vision and TDX analyzers were calibrated with the calibrators supplied by Abbott Laboratories, according to the manufacturer’s protocol and also whenever we used a new lot of test packs or whenever two consecutive quality-control values were out of range. During the evaluation, we analyzed two to four times daily each of the Vision I and Vision II quality-control sera. In the determination of within-day and between-day precision, we used Vision I and Vision II quality-control sera for the Vision analyzer and Lyphocheck 2 and Lyphocheck 3 (Bio-Rad Laboratories, Richmond, CA 94804) quality-control sera for the TDX analyzer. The assay curve was linear up to at least 300 mg/L.

**Results and Discussion**

**Precision**

The within-day (n = 20) coefficients of variation (CVs) for the Vision assay were 1.8% for a theophylline concentration of 15.2 mg/L and 3.1% for 25.2 mg/L. The day-to-day CVs for theophylline, measured in 80 samples over a 20-day period, were 3.5% and 4.8% at theophylline concentrations of 14.9 and 24.4 mg/L, respectively.

**Correlation Studies**

**TDX vs Vision.** Vision-determined theophylline values (y) for 200 serum and plasma samples from patients receiving the drug correlated well with the TDX-measured theophylline values (x): r = 0.978, y = 0.978x - 0.270 mg/L (S, y = 1.000). Repeated comparison between the Vision and TDX analyzers after the Vision was calibrated with a different lot of calibrators showed similar correlation (r = 0.989; y = 0.987x + 0.363 mg/L).

**Serum vs plasma and whole blood.** Although Ferron et al. (7), in a separate study with use of “high-performance” liquid chromatography, noticed that the values for theophylline in plasma and whole blood were 7–8% and 20–23% lower than serum values, respectively, and recommended using linear regression to convert the theophylline values measured in one matrix to values in another matrix, we found no such differences. The theophylline values in serum, plasma, and whole-blood samples from 36 patients correlated well: r = 0.994 for serum (S) and plasma (P) or whole blood (B):

- P = 1.015 S - 0.037 mg/L (S, y = 0.564) and
- B = 0.980 S + 0.282 mg/L (S, y = 0.628)

The mean theophylline values were insignificantly different between S (11.49 mg/L) and P (11.63 mg/L) (p = 0.92) or between S and B (11.54 mg/L) (p = 0.97).

**Whole blood by venipuncture vs capillary-blood samples.** Portions of well-mixed whole-blood samples collected by venipuncture were drawn into capillary tubes and introduced into the reagent packs by either adding the whole-blood sample into the sample well or inserting the capillary tube directly into the capillary slot. The theophylline values for 22 whole-blood (x) and capillary-blood samples (y) correlated well: r = 0.993, y = 1.013x - 0.230 mg/L (S, y = 0.599). The mean theophylline values for capillary (11.81 mg/L) and whole-blood samples (11.89 mg/L) were not significantly different (p = 0.96).

We also addressed whether theophylline values in whole-blood samples collected in capillary tubes via finger stick could be used interchangeably with those collected in heparinized tubes via venipuncture. Results from 14 patients showed that the two kinds of samples gave comparable theophylline values (r = 0.989). The mean theophylline values for the capillary and venipuncture blood samples were 11.43 and 11.01 mg/L, respectively (p = 0.86).

**Interference Study**

Triglyceride concentrations as great as 7.4 g/L and unconjugated bilirubin concentrations as great as 143 mg/L had little or no effect on theophylline measurement in the Vision Analyzer for a theophylline concentration of 19 mg/L.

In the presence of hemoglobin, on the other hand, the Vision method gave falsely lower theophylline values, the effect being proportional to the hemoglobin concentration. Hemoglobin concentrations of ~2 g/L caused a 10 to 15% decrease in apparent theophylline, and at a hemoglobin concentration of 9 g/L the theophylline result was only 46% of that in the absence of hemoglobin (Figure 1). The source of this negative interference by hemolysis was probably spectrophotometric as well as chemical. The theophylline assay involves measurement at 417 nm, a wavelength at which hemoglobin also absorbs. In addition, the assay involves use of the enzyme acetylcholinesterase (EC 3.1.1.7), an enzyme that is present in erythrocytes and that would be released into serum on hemolysis (8). An increase of acetylcholinesterase activity in this inhibitor immunoassay in turn reflects a lower theophylline concentration. When hemoglobin concentration in the sample is above the cutoff concentration of 1 g/L programmed into the instrument by the manufacturer, an error message, “Interference Substance,” will be printed instead of the results.

Falsely increased theophylline values measured with the IMIT (Syva Co.) or TDX procedures in samples from uremic patients have also been reported (9–12). The nature of the interfering substance is not known, but its extent depends on the specificity of the antibody that is being used. We also measured serum theophylline with the Abbott Vision and TDX analyzers in 26 patients whose serum creatinine was between 25 and 150 mg/L. The difference in theophylline values obtained with Vision and TDX analyzers was less than ±2 mg/L for all samples regardless of serum creatinine concentration (data not shown), indicating that there was no correlation between higher theophylline values and increased serum creatinine values.
In conclusion, we find the Vision procedure for theophylline procedure suitable for clinical use. It is sufficiently precise and produces results reliably and rapidly, and we encountered few problems during this evaluation. Our finding that capillary samples collected by finger stick and whole-blood samples obtained by venipuncture have similar theophylline values demonstrates that blood samples obtained via finger stick in clinics, emergency rooms, or physicians’ offices can provide valid, transferable results for theophylline.

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References

Quantification of Desferrioxamine in Blood Plasma by Inductively Coupled Plasma Atomic Emission Spectrometry

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A sensitive method, inductively coupled plasma atomic emission spectroscopy, is used to measure desferrioxamine in blood plasma. The desferrioxamine is transformed into its iron chelate, ferrioxamine, which is extracted into benzyl alcohol, then re-extracted into HCl (0.5 mol/L), which is used as the sample for the spectroscopy. For a 0.5-mL plasma sample, the detection limit (1 µg/mL) suffices for following the concentration of desferrioxamine in plasma after its subcutaneous or intramuscular injection (40 mg per kg of body weight). Neither blood pigments nor trace metals interfere.

Additional Keyphrases: thalassemia • Al encephalopathy • colorimetry compared • pediatric chemistry • chronic renal failure

Described as a chelating agent (1–2), desferrioxamine is commonly used in the treatment of major thalassemia and aluminum encephalopathy, to increase the removal of iron and aluminum (2–9). Despite much work on optimal conditions for its use, monitoring desferrioxamine remains difficult, mainly because of the poor sensitivity (in blood plasma) of reported methods (10–11).

We attempted to establish a sensitive method for use with small-volume samples obtained after injection of desferrioxamine into children with thalassemia major and into adults with chronic renal failure.

The following technique is based on the formation and extraction of ferrioxamine, with subsequent measurement of the chelate by inductively coupled plasma atomic emission spectroscopy (ICP AES). The assay's sensitivity permits measurement of desferrioxamine in a plasma sample as small as 200 µL, if necessary. The same technique is easily applied to urine, but in most cases the sensitivity of the colorimetric method is sufficient for use with urine.

Materials and Methods

Instrumentation

We used:
• an IPS 1500 spectrometer (Sopra, 68, Rue Pierre Joignieux, 92270—Bois Colombes, France);
• a plasma torch argon (Philips, 105, Rue de Paris, 93002—Bobigny, France);
• a cross-flow pneumatic nebulizer fed by a peristaltic (minipulse) pump (Gilson, 72, Rue Gambetta, 95400—Villiers le Bel, France) at a flow rate of 600 µL/min (Table 1).

Reagents

Ferric reagent, 40 mmol/L: To 800 mL of distilled water add 7.75 g of nitrotriacetic acid and 10.80 g of ferric chloride (FeCl₃·6H₂O); adjust the pH to 6–7 with ammonia; dilute to 1000 mL with distilled water.

Perchloric acid (d = 1.67), diluted 10-fold. Sodium hydroxide solution (d = 1.33), diluted 10-fold.

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