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Influence of Hematocrit on Quantitative Analysis of “Blood Spots” on Filter Paper

To the Editor:

Peripheral blood “spotted” onto special filter paper offers an economical method for collecting samples in mass screening programs. Uniform punches removed from the dried blood spots are extracted for analysis. The precise volume of blood accommodated by these punches can be preestimated for individual batches of paper by using blood labeled with \(^{125}\text{I}\)thyroxine (1). Factors that contribute to imprecision include the paper batch (2), the volume of blood added, and the location of the punch in the blood spot (3). Arends (3) also noted that a decreasing area of paper was covered by the same volume of blood as the hematocrit increased. This “spreadability factor” was shown to influence the estimation of thyrotropin (TSH) from paper punches, TSH being exclusively associated with the serum.

Our study of hematocrit effects on the distribution of whole blood in paper was prompted by the desire not only to measure hemoglobin but also to develop blood-spot assays for growth factors such as folates, which are concentrated in the erythrocytes. A fresh normal blood sample (25 mL) was collected in acid–citrate–dextrose. Plasma was separated and then recombined with cells in different proportions to give four simulated “blood samples” having a range of hematocrit values (Coulter Electronics, Hialeah, FL). The mean cell volume of each sample was also noted as an index of sufficient mixing of cells. \(^{125}\text{I}\)Thyroxine (Amersham, Bucks, UK) was added to each sample. Samples were mixed and spotted onto S&S Grade 903 filter paper (Schleicher and Schuell, Keene, NH) in 75- and 150-μL aliquots (n = 20 each). A portion of each sample was retained as a control. The paper was air-dried overnight, and the gamma radiation in the 1/4-in. (6.35-mm)-diameter punches taken from the center of each spot was counted. The radioactivity in measured volumes of blood (100 μL) from each mixed control sample was also counted and used to calculate the volume of blood in the punches. For hematocrit values of 0.503, 0.456, 0.36, and 0.237 these calculated punch volumes were 10.8, 10.5, 8.8, and 8.2 μL, respectively (CV <5.6%) for 75-μL applications and 11.9, 10.3, 10.5, and 8.8 μL (CV <7%) for 100-μL ones. These results demonstrate how low hematocrit and volume added may influence the absorbancy of blood on paper.

Similar normal blood samples (n = 3) were dual-labeled, each in separate experiments. Plasma was separated and the erythrocytes were labeled with sodium chromate (\(^{51}\text{Cr};\) Amersham) by a Reference Method (4). Sodium chromate binds predominantly to hemoglobin (5). Iodinated human serum albumin (Amersham) was mixed with the plasma. The cells and plasma for each sample were then recombined to give “blood samples” with a range of hematocrit values. These samples were spotted (100 μL) onto plain S&S paper as described. In this case, the punches (1/4-in. diameter), again from the center of the spots, and a control for each were counted for both \(^{51}\text{Cr}\) and \(^{125}\text{I}\) radioactivity. The volumes of blood in the punches were calculated for each sample from its own control, based on the counts of both isotopes (Figure 1).

The results (Figure 1) confirm that the calculated volume of blood in a 1/4-in. punch will vary according to the sample hematocrit. If both isotopes were distributed evenly throughout the blood spot, then the total blood volumes as calculated by either isotope would be similar and independent of the hematocrit. In fact, however, the serum absorbancy of the paper (\(^{125}\text{I}\) counts) became less as the sample hematocrit was decreased, whereas the erythrocyte absorbancy (\(^{51}\text{Cr}\) counts) remained relatively constant under the same conditions (Figure 1).

Of course, these experiments cannot compare with blood collection from donors in the field, where clotting and other biological changes may influence the spreading of blood components. However, they do allow us to attempt to examine the effect of hematocrit in isolation. Given that the results (Figure 1) show variability among the three samples under similar study conditions, further studies will be necessary to determine whether hematocrit influence is predictable, or whether factors such as plasma viscosity are also significant.

Dried blood spots are used primarily for screening tests on selected populations, and a considerable degree of imprecision is allowed for. The identification of the individual factors contributing to this imprecision is essential. At present, however, hematocrit could have a significant influence on precision in screening populations at risk of hematocrit deficiencies.

Although estimations of analytes
associated with erythrocytes may be less influenced by hematocrit than are serum analytes, specific studies on the distributions of individual analytes of interest are important. Such studies would be an essential prerequisite to the utilization of filter paper as a matrix for collecting blood samples for any quantitative analysis.

References

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False-Positive Serum Tricyclic Antidepressant Screen with Cyproheptadine

To the Editor:

A 14-year-old girl was admitted to our Emergency Department after an intentional ingestion of ~30 4-mg tablets of cyproheptadine (Periactin; Zenith Laboratories, Inc., Northvale, NJ). The patient was mentally confused, ataxic, and had a history of atypical migraine headaches. The toxicology requisition submitted with this patient's urine and blood specimens indicated symptoms consistent with a hallucinogenic or anticholinergic pattern and current drug therapy consisting of cyproheptadine and Midrin (isomethuphenene muate, dichloralphenazone, and acetaminophen; Duramed Pharmaceuticals, Cincinnati, OH). Cyproheptadine is an antihistamine and a serotonin antagonist with anticholinergic and sedative effects and, reportedly, calcium-channel blocking activity (1, 2). We performed a drug screen on both the urine and blood specimens submitted from this patient.

The results of all chemical spot tests for salicylates (urine and serum) (3), acetylsalicylic acid (urine) (4), and phenothiazines (urine) (5); EMIT drug d.a.u. (Syva Co., Palo Alto, CA) tests performed with the ETS instrument (Syva Co.); and EMIT st tests performed with the Qstq/Qst instrument system (Syva Co.) were negative except for the EMIT st serum assay for tricyclic antidepressants. Confirmatory testing by thin-layer chromatography (Toxi-Lab A; Toxi-Lab, Inc., Irvine, CA) and by gas chromatography–mass spectrometry (GC-MS; 5890 Series II gas chromatograph equipped with an Ultra-1 capillary column and a 5971 Mass Selective Detector, all from Hewlett-Packard, Palo Alto, CA) of a sample prepared from a Toxi-Tube A (Toxi-Lab, Inc.) extract of the patient's urine specimen did not confirm the presence of a tricyclic antidepressant. The GC–MS spectrum of the principal peak observed on the total ion chromatogram of this patient's urine extract was consistent, however, with the presence of cyproheptadine.

To determine the minimum concentration of cyproheptadine necessary to produce a reading equivalent to 200 µg/L for nortriptyline, the calibrator used in the EMIT st serum tricyclic antidepressant assay, we tested 10 samples of pure cyproheptadine (Sigma Chemical Co., St. Louis, MO; cat. no. C-6022) prepared in drug-free serum at concentrations ranging from 100 to 1000 µg/L in 100 µg/L increments. All samples with apparent cyproheptadine concentration <400 µg/L were negative, while all samples with cyproheptadine ≥400 µg/L were positive. Quantitative GC–MS analysis of the sample containing cyproheptadine at 400 µg/L indicated the actual concentration was 390 µg/L. After a single oral dose of cyproheptadine hydrochloride in healthy adults, ~30% of the dose is excreted as conjugated glucuronized and sulfated metabolites in urine within 24 h, ~50% within 48 h, and ~65–75% within 6 days; the remainder of the dose is excreted in feces (1). Peak plasma concentrations of metabolites are detected ~6–9 h after oral administration of parent drug (2).

To determine if therapeutic concentrations of cyproheptadine produce a positive response in the EMIT st serum tricyclic antidepressant assay, a healthy, drug-free volunteer ingested orally one 4-mg cyproheptadine tablet three times per day for 3 days. Serum obtained from blood samples taken from this volunteer before ingestion of the cyproheptadine, just before administration of the last dose; and 1, 2, and 4 h after the last dose of cyproheptadine were all negative when tested with the EMIT st tricyclic antidepressant assay.

Because immunoassays are typically affected by cross-reactants (6), knowledge of potential sources of false positives is important in the interpretation of drug-screening results and in the application of appropriate drugspecific therapy (7, 8). Fortunately, only concentrations of cyproheptadine ≥400 µg/L, an amount not likely to be observed in a therapeutic dosing regimen with cyproheptadine, provide a positive response in the EMIT st serum tricyclic antidepressant assay. Thus, cyproheptadine at ≥400 µg/L may be added to the current list of structurally related compounds that produce a positive result using this assay. Currently, the manufacturer of the EMIT st serum tricyclic antidepressant assay does not list cyproheptadine in their table of “Compounds Detected” by this assay (Syva Co., package insert, EMIT st serum tricyclic antidepressant assay, January 1987).

References
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