Acetic Acid Interference with Determination of 5-Hydroxyindoleacetic Acid Obviated

To the Editor:

Reportedly (1), acetic acid interferes with the Goldberg (2) procedure for quantitation of 5-hydroxyindoleacetic acid in urine. We also encountered this problem, and resolved it so that we could continue using acetic acid as a preservative.

Acetic acid is extracted into ether in the first extraction. In the second extraction, the acetic acid back extracts into the phosphate buffer, pH 7.0. The concentration of this phosphate buffer is only 0.1 mol/L, so the acetic acid can lower the pH to the extent that the buffer is no longer alkaline enough to extract the 5-HIAA into the buffer quantitatively. Our solution was to increase the buffer molarity to 0.4. The HCl concentration in the nitrous acid can be increased slightly to compensate for the more concentrated buffer. We routinely run a patient's sample as a double check on recovery. A check of the buffer pH after the second extraction would detect any significant change.

References

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Use of Glass Beads to Defibrinate Serum in “Microtainers”

To the Editor:

Recently we examined the problem of fibrin formation in serum analyzed in our “stat” chemistry laboratory, because fibrin strands were adversely affecting the performance of our mechanized analyzer. Extensive clot formation in the sample cups was affecting analyses for sodium, potassium, chloride, and creatinine. Our solution to the problem will be of general interest.

Blood specimens for this laboratory are collected in B-D Microtainers (Becton-Dickinson and Co., Rutherford, NJ 07070), which are well suited for the small volumes of blood ordinarily collected from pediatric patients. The silicone serum separator in this tube increases the yield of serum. Because our “stat” laboratory is adjacent to the Intensive Care Unit it services, specimens are received promptly after collection, and a prompt analysis is essential. Specimens were usually centrifuged in the Microtainers within 20 min after collection. Coagulation was incomplete and the supernatant fluid contained large fibrin clots.

We assessed this problem by observing the formation of fibrin in sera from whole blood from adult volunteers with no known coagulation disorders. Specimens were allowed to clot in Microtainers and centrifuged (Microcentrifuge, Model 3200; Brinkmann Instruments, Westbury, NY 11590) for 90 s at timed intervals after the blood was placed in the tubes. We found that whole blood took 50 min to clot in the Microtainers and that fibrin formed in the serum of specimens centrifuged earlier than this. This interval is unacceptable in a “stat” laboratory.

As noted by others (1), blood clotting is retarded in Microtainers, owing to the polypropylene surface. To counteract this, we added one to four 3-mm Pyrex glass beads (Corning Glassworks, Corning, NY 14830) to Microtainers, which were centrifuged at intervals up to 20 min after blood was placed in the tube and then observed for fibrin formation. After centrifugation, we removed with a wooden applicator stick any fibrin that adhered to the walls of the Microtainers, and transferred the sera to sample cups. Some fibrin was found as late as 17 min when three beads or fewer were used, and when we used four beads and waited 8 min for centrifugation, fibrin formation was so much reduced that operation of the analyzer was greatly improved.

We conclude that adding glass beads accelerates the clotting of whole blood in Microtainers, presumably owing to activation of the Hageman factor (factor XII) and initiation of the intrinsic pathway of blood coagulation in vitro (2). Glass beads seemed to be of benefit even with samples presumably contaminated with some heparin from intravenous lines.

Better to expose blood to the glass beads, we routinely rock blood specimens on a hematoloy blood rocker (Ames Co., Elkhart, IN 46516) for 2 min before centrifugation. The exposure to glass beads and the rocking do not promote hemolysis or leakage of potassium into serum from erythrocytes.

References

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