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SUMMARY

Reported is the evaluation of a new centrifugation method, Statspin®, that addresses both time and sample separation integrity. The method can successfully separate the plasma fraction from the cellular material in 2 minutes as compared to 20 minutes for the conventional centrifuge method. The Statspin, combined with the ACS:180 system, can generate test results in less than 30 minutes, exclusive of transport to the laboratory. This study demonstrated that the combined technologies offer timing-saving improvements for clinical laboratories offering STAT immunoassays for cardiac markers, endocrine molecules, and therapeutic drugs. (Clin. Lab. 2000;46:157-160)

KEY WORDS

Sample processing, STAT analysis, Statspin, ACS:180 assays,

ABBREVIATIONS

TAT, turnaround time, cardiac troponin-I (cTnI), myoglobin (Myo), creatinine kinase MB isoform (CK-MB), digoxin(DIG), digitoxin (Digit), and human chorionic gonadotropin (hCG).

INTRODUCTION

Rapid turnaround time (TAT) in the biochemical testing of patient samples is critical for delivery to the clinician for the decision making process. With the advent of high-speed diagnostic assay systems, total testing times can be as short as 15-20 minutes. The transport of blood to the central laboratory and the centrifugation of blood to prepare serum or plasma prior to analysis on the automated system often become the rate limiting steps in the ability of centralized laboratories to provide these rapid results to their clinicians. In standard blood sample preparation procedures, serum is collected in a gel barrier tube and allowed to clot for 10-15 minutes followed by centrifugation for 15-20 minutes at 3000 rpm ( ~1500 x g) in order to allow the formation of the gel barrier. Including clotting time, centrifugation and separation of samples in preparation for running on the instrument adds up to 40 additional minutes to the TAT. Using this standard preparation procedure, TAT can easily exceed 1 hour, exclusive of sample transport and labeling. The National Academy of Clinical Biochemistry recently published its recommendations for standard laboratory practice for cardiac markers and coronary artery disease. One of those recommendations is that STAT cardiac marker testing should have a turnaround time of 1 hour or less (1).

We performed an evaluation of the StatSpin® Express Primary Tube Centrifuge (Statspin Inc, Norwood MA), a recently introduced high speed centrifuge that claims equivalent separation of plasma in 2.5 minutes compared to the conventional 15-20 minute centrifugation. Using heparinized plasma as the sample of choice and this rapid preparation centrifuge we investigated the possibility of reducing sample preanalytical time, exclusive of transport to the laboratory, to 5 minutes or less. We evaluated six ACS: 180 assays that are often requested in the emergency room: three cardiac assays; cTnI, myoglobin, CK-MB, two digitalis drugs, digoxin (DIG) and digitoxin (Digit), and human chorionic gonadotropin (hCG). The three cardiac markers are used for the detection and monitoring of acute myocardial infarction (AMI) (2). The digitalis drugs are extensively used in congestive heart failure. They have a rather narrow therapeutic indexes, and must be monitored...
during treatment (3,4). The levels of hCG are used for any female patient to ascertain pregnancy, so that any treatment may be carefully modified as needed under pregnancy (5).

MATERIALS AND METHODS

Sample collection and spiking with the analytes
Samples were obtained by standard phlebotomy procedures from 19 apparently healthy individuals. For each volunteer, 2 x 4 mL lithium heparin plasma separation tubes (PST® Plasma Separation Tubes, Becton-Dickinson® VACUTAINER® Systems, Franklin Lakes, NJ) were drawn using a multi-sample needle and Vacutainer holder. Following collection, each tube was spiked with a specific level of three cardiac markers (cTnI, Myo, CK-MB) as well as with two digitalis drugs (DIG and Digit) and hCG. Concentrations of the analyte spikes were varied to investigate analyte recovery over the range of concentrations that the analyte would be monitored. Following spiking, stoppers were reinserted, the tubes were centrifuged and plasma was removed as described below.

Separation of Blood Samples:
Standard Approach/reference sample: One PST tube from each volunteer was prepared by conventional centrifugation in a standard tabletop clinical centrifuge (Beckman TJ-6 Centrifuge, Beckman Corporation, Fullerton CA). Samples were centrifuged for 20 minutes at 2700 rpm (~1500 x g). Ramp time to full speed was 3 minutes and brake time to full stop was 1.5 minutes. The total centrifugation process time was 22 minutes. The plasma fraction was then removed from the gel interface by pipetting, using a disposable plastic transfer pipette.

StatSpin Approach:
One PST gel tube from each volunteer was centrifuged in the StatSpin Express using the two minute automatic cycle. This centrifuge reaches 8500 rpm (4440 x g) in 25 seconds and decelerates to a full stop in 20 seconds. Total centrifugation process time is ~2.5 minutes. The plasma fraction was then removed from the gel interface by pipetting, using a disposable plastic transfer pipette.

Measurement of Myo, cTnI, CK-MB, DIG, Digit, and hCG on the ACS:180 System:
All samples were evaluated in the following ACS:180 assays: cTnI, Myo, CK-MB, DIG, Digit, and hCG. The assays were run on the automated random-access chemiluminescent system, ACS:180, following the manufacturer’s protocol. All samples were tested in triplicate for each assay. Recovery in the StatSpin-prepared samples was compared to samples prepared by the conventional centrifugation separation method. Student’s t-test was used to determine whether statistical differences existed between the two centrifugation methods.

Figure 1: Recovery of Statspin Prepared Samples compared to the Standard Centrifugation Method. Results with Cardiac Markers.
Recoveries of samples prepared by the StatSpin Centrifugation method were compared and calculated from the conventional centrifugation method for the three cardiac markers (cTnI, Myo, CK-MB). In each case, the correlation coefficient equaled 0.99, demonstrating excellent correlation of the StatSpin method with the conventional centrifugation method.

RESULTS

The results of the study are shown in Figures 1 and 2. All markers were tested with analyte spiked across the range of each individual assay. Figure 1 shows the data from the ACS:180 cardiac marker assays (cTnI, Myo, and CK-MB), comparing the analyte concentrations in samples centrifuged in the routine centrifuge method versus the StatSpin method. Compared to the reference tube centrifuged for 22 minutes, the mean recoveries of cardiac markers (cTnI,
Digit, hCG assays were all $R = 0.99$. Student’s t-test analysis revealed no significant difference in the recoveries of each analyte between the Statspin centrifugation method and the conventional centrifugation method.

**DISCUSSION**

The advent of rapid, random access, automated analyzers permits faster and better care of the emergency room patients. Faster TAT on the analysis requested by the emergency room allows reduction of costs. As a result there is a constant demand on the laboratory to reduce the TAT. While the demand for faster TAT has recently pushed the demand of ‘bed-side’ or Physician’s Office Center (POC) tests for emergency marker, quality control and regulatory issues remain with many of these tests. The other movement in clinical laboratory is towards a central ‘core laboratory’, whose services may be preserved with these markers, if TAT can be improved. Today’s automated immunochemistry analyzers make most analyses possible in less than 20 min. Sample transport has been made faster through bar-coding and automated transport systems. Sample processing, mainly centrifugation, on the other hand, remains the step where processing time could be reduced. The new Statspin centrifuge system allows for this time reduction.

Our results demonstrate that by combining a fast centrifuge with a rapid automated immunoassay analyzer TAT is reduced to less than 30 minutes. Our results demonstrate that the Statspin method gives recoveries that are equivalent to the conventional centrifugation method. These results demonstrate that the Statspin method offers a quick and reliable alternative to traditional means of blood processing. The Statspin method considerably shortens the turnaround time of blood fractionation. Compared to conventional centrifugation, about 20 minutes are saved in total processing time. The use of plasma instead of serum for these tests saves an additional 10-15 minutes. By switching to plasma and a high-speed centrifuge, at least 30 minutes can be saved in the preanalytical aspects of sample testing. By using the ACS:180 system in conjunction with the Statspin centrifuge, TAT ranges from 17.5 to 22.5 minutes, exclusive of sample transport and labeling. Laboratories with bar code generators at the site of sample draw, LIS sample entry and result reporting, and pneumatic tube transport should be able to achieve a TAT of 30-45 minutes from sample draw to result.

In our studies, the accuracy of results of six different assays with the Statspin method has been shown to be the same as the conventional separation method. For these reasons the Statspin method may be considered as a positive addition to any laboratory that is concerned about rapid turnaround time of blood processing. This method can be of great utility not only for samples where STAT answers are required but also for samples where the analyte may be especially labile and where sample handling is critical.

Figure 2: Recovery of Statspin Prepared Samples compared to the Standard Centrifugation Method. Results with DIG, Digit, hCG Markers.

Recoveries of samples prepared by the Statspin Centrifugation method were compared and calculated from the conventional centrifugation method for the DIG, Digit, hCG markers. In each case, the correlation coefficient equaled 0.99, demonstrating excellent correlation of the Statspin method with the conventional centrifugation method.

Myo and CK-MB) from samples centrifuged for only 2.5 minutes were 98%, 100%, 100%, respectively. The correlation coefficients for all three cardiac markers were $R = 0.99$. Some variation in the recovery of cTnI was observed at the upper end. Since correlation across the rest of the assay range was good we believe that this difference must be due to an error during the sample pipetting. Figure 2 shows comparable data for ACS:180 DIG, Digit, and hCG assays. The mean recoveries for these three assays were 100%, 99%, 101%, respectively. Like the three cardiac markers, the correlation coefficients for the DIG,
References:


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