

Using a Fisher Scientific accuSpin 24C Centrifuge for the Effective and Efficient Processing of Clinical Samples

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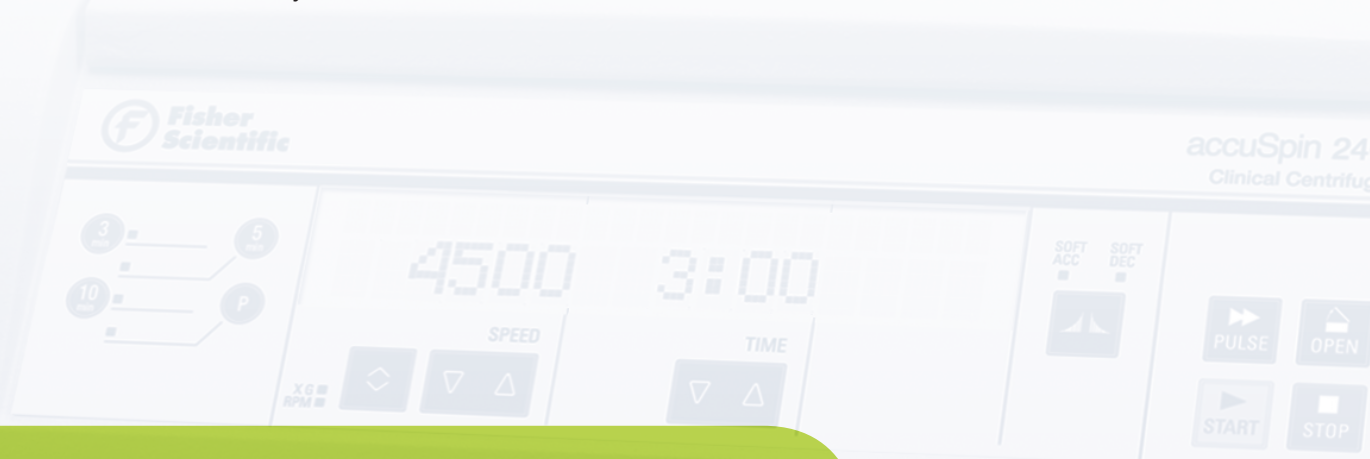




Figure 1. Fisher Scientific accuSpin 24C Centrifuge.



INTRODUCTION

Clinical laboratory testing plays an important role in the detection, diagnosis and treatment of disease. The use of centrifugal force for separation of blood and urine is a very crucial step within the process. It is very important not only to perform this step correctly, but also efficiently so as to derive the best results for the patient sample.

Blood is typically separated to yield platelet-poor plasma (PPP), which in turn is used for most coagulation tests. Ideally, PPP should have a platelet count of less than 10×10^3 per μL ¹.

Similarly, the analysis of urine is also very important in the clinical laboratory. Information derived from this testing can predict many important medical concerns, one of which is the function of kidneys or any associated infections. This common test measures various compounds such as white blood cells, red blood cells, epithelial cells, bacteria, calcium oxalate crystals, mucous threads, yeast, and hyaline casts that pass through the urine^{2,3}.

Finally, blood chemistry analysis is also a very common test in the clinical laboratory. These results are used to evaluate a variety of components such as potassium, aspartate aminotransferase (AST), phosphorus, and lactate dehydrogenase (LDH)⁴.

The objective of this note is to illustrate the efficiency and effectiveness of the Fisher Scientific accuSpin 24C clinical centrifuge in critical centrifugation steps of the following clinical testing: (A) platelet-poor plasma (PPP) preparation, (B) urinalysis, and (C) blood chemistry analysis.

The Fisher Scientific accuSpin 24C is a clinical centrifuge supplied with a swinging bucket rotor (TX-150) for high-speed horizontal processing. The TX-150 rotor accommodates up to $24 \times 5/7$ mL blood collection tubes or 8×50 mL conical tubes at 4500 rpm ($3260 \times g$) but when retrofitted with the HIGHConic rotor, it can achieve up to 8,700 rpm ($10,155 \times g$) with 6 tubes. While a lower capacity than the standard swinging bucket rotor, this rotor's higher speed does make it a very important rotor in efficiently and effectively processing PPP⁵.

The accuSpin 24C centrifuge offers sample protection with the ClickSeal™ biocontainment lid system certified

by Public Health England, Porton Down, UK. It also provides Auto-Lock™ rotor exchange which allows rotors to be installed and removed from the chamber of the centrifuge with the push of a button – no tools required. Finally, this centrifuge is equipped with three fixed programs standardizing the procedures being discussed in this applications note⁵.

Methods⁶:

- Platelet-poor plasma (PPP) preparation was done by performing centrifugation of whole blood using 2.7 mL BD Vacutainer™ Plus plastic citrate tubes. The initial platelet concentrations from whole blood as well as for each PPP preparation were determined with the aid of a hematology analyzer.
- Urinalysis is composed of two main steps - In the first step urine was centrifuged using 12 mL KOVA™ tubes in the TX-150 swinging bucket rotor to separate sediment from urine which was then examined in the second step for crystals, casts, cells, yeast, and bacteria under a microscope.
- Blood chemistry analysis was performed by collecting blood into 7 mL BD Vacutainer SST Plus tubes, followed by centrifugation (TX-150 and HIGHConic rotor). The serum was tested for potassium, aspartate aminotransferase (AST), phosphorus, and lactate dehydrogenase (LDH) using a chemistry analyzer.

Results

Clinically acceptable results were derived for all centrifugation steps performed using the accuSpin 24C centrifuge with TX-150 swinging bucket rotor or HIGHConic fixed angle rotor. Acceptable results for PPP were accomplished at 4,500 rpm for 10 minutes using the TX-150 rotor and 1 minute at 8,700 rpm using the HIGHConic rotor. Similarly, it was at 1,700 rpm for 4 minutes using the TX-150 rotor, whereby acceptable results were achieved for the Urinalysis

testing. And finally, a 2-minute centrifugation time at 4,500 rpm using the TX-150 rotor for blood samples were the centrifugation parameters suitable for stat clinical blood chemistry testing. Subsequently, centrifugation of peripheral blood was also successfully performed at 7,200 rpm for 1 minute using the HIGHConic rotor.

I. METHODS AND MATERIALS

Table 1. Rotor Buckets & Adapter

Clinical Testing	Rotor	Buckets	Adapter
PPP; Blood chemistry analysis	TX 150 rotor (PN 75005701)	Round buckets (PN 75005702)	5/7 mL blood collection tube adapter (PN 75005739) Rubber pads (PN 76003266)
	HIGH Conic rotor (PN 75005709)	–	5/7 mL adapter (PN 75005804) Rubber pads (PN 005700F)
Urinalysis	TX 150 rotor (PN 75005701)	Round buckets (PN 75005702)	14 mL urine tube adapter (PN 75005738)

Table 2. Centrifugation Conditions^a

Purpose ^a	Rotor	RPM	RCF [x g]	Time ^b [min]
PPP	TX 150	4500	3260	10
	HIGHConic	8700	10155	1
Urinalysis	TX 150	1700	465	4
Blood chemistry analysis	TX 150	4500	3260	2
	HIGHConic	7200	6967	1

^a Room temperature

^b Acceleration time/Deceleration time: Standard

A. Platelet-poor plasma (PPP)

Blood was obtained from 10 unknown donors by venipuncture collection at the same day of the testing. 2.7 mL of whole blood was collected into BD Vacutainer Plus plastic citrate tubes and after 30 minutes each sample was tested to get the initial platelet count before processing. The tubes were processed using the accuSpin 24C centrifuge. The rotors and centrifugation conditions are summarized in Table 2. Platelet counts were performed on the whole blood and PPP samples using a hematology analyzer.

B. Urinalysis

Urine specimens were pooled from 10 unknown patients and collected at the same day of the testing into 12 mL KOVA tubes. The tubes were processed using the accuSpin 24C centrifuge until a moderately cohesive button was formed at the bottom of the tubes (see Table 2). The supernatant was decanted and a volume of 1 mL was left inside the tube. After resuspending the sediment a drop was poured onto a glass slide, covered with a coverslip and examined under a microscope at 10x (low power field) and 40x magnifications (high power field).

C. Blood chemistry analysis

Blood was collected into BD Vacutainer SST Plus tubes from 6 random donors (2 tubes per donor). After waiting 30 minutes for samples to clot, the tubes were centrifuged using the accuSpin 24C centrifuge. The rotors and spin conditions are listed in Table 2. The serum was tested for potassium, aspartate aminotransferase (AST), phosphorus, and lactate dehydrogenase (LDH) using a chemistry analyzer.

II. DATA ANALYSIS

A. Platelet-poor plasma

The values were reported as the mean and the standard deviation (SD) of the Platelet concentrations from whole blood and PPP preparation.

B. Urinalysis

The white blood cells (WBCs), red blood cells (RBCs), and epithelial cells counts were reported as number of cells/HPF (red blood cells per high power field); the counts of casts (cylindrical particles sometimes found in urine that are formed from coagulated protein secreted by kidney cells) were reported as number of casts/10 LPF (low power field). Bacteria, calcium oxalate crystals, uric acid crystals, mucous threads and yeast were all examined at LPF and estimated as “rare”, “few”, “moderate”, or “many”.

C. Blood chemistry analysis

The mean and standard deviation were calculated.





III. RESULTS

A. Platelet-poor plasma (PPP)

Table 3. Platelet Count In Whole Blood And PPP Preparation From 10 Unknown Donors

Rotor	WB platelet count ^{a,b} [K/ μ L] (Before spin)	Platelet count ^{a,b} [K/ μ L] (After spin)
TX150	222.9 \pm 94.6	2.5 \pm 2.2
HIGH Conic	178.7 \pm 105.8	4.3 \pm 1.3

^a Centrifugation conditions shown in Table 2
^b Mean \pm SD

B. Urinalysis

The number of WBCs, RBCs, epithelial cells, casts, crystals and yeast are shown in Table 4 and 5.

Table 4. Urinalysis From 10 Unknown Patients

	WBC ^{a,b}	RBC ^{a,b}	Epithelial cells ^{a,b}	Bacteria ^{a,c}	Calcium oxalate crystals ^{a,c}	Uric acid crystals ^{a,c}
1	6 - 10	> 50	1 - 5	rare	none	none
2	1 - 5	> 50	6 - 25	moderate	none	none
3	> 50	6 - 10	1 - 5	moderate	none	none
4	0 - 1	0 - 1	1 - 5	none	moderate	none
5	1 - 5	0 - 1	6 - 25	moderate	none	rare
6	1 - 5	0 - 1	1 - 5	none	none	–
7	10 - 25	6 - 10	6 - 25	many	none	–
8	10 - 25	1 - 5	1 - 5	rare	moderate	–
9	10 - 25	1 - 5	1 - 5	moderate	none	–
10	10 - 25	1 - 5	6 - 25	moderate	none	–

^a Centrifugation conditions shown in Table 2
^b hpf, high power field
^c lpf, low power field

C. Blood chemistry analysis

The mean and SD values of potassium, AST, phosphorus, and LDH in serum are summarized in Table 6.

IV. CONCLUSION

With a spin of 10 minutes at 4500 rpm using the TX-150 swinging bucket rotor, the accuSpin 24C centrifuge achieved a clinically acceptable PPP yield per laboratory standards. In addition, when this centrifuge was retrofitted with its HIGHConic fixed angle rotor, acceptable PPP yield were achieved within 1 minute when spun at 8700 rpm. With both rotor options the residual platelet counts in the final PPP sample were definitely < 10000/ μ L commensurate of an acceptable platelet count the CLSI guidelines for PPP used for coagulation tests⁷. Therefore, this study has illustrated the effectiveness and efficiency by which the accuSpin 24C centrifuge generates not only a clinically acceptable PPP separation, but also reduces the time required for routine and prompt results.

Similarly, with urinalysis, the sample was also effectively and efficiently processed at 1700 rpm in 4 minutes using the TX-150 swinging bucket rotor and at the end, the acceptable results were comparable with values typically obtained in the lab using other small clinical centrifuges. In addition, this same success was also seen with the results from the blood chemistry samples. With a 2 minute run at 4500 rpm using the TX-150 swinging bucket rotor or a 1 minute run



Table 5. Urinalysis From 10 Unknown Patients

	Mucous threads ^{a,b}	Yeast ^{a,b}	Hyaline casts ^{a,b}	Coarse granular casts ^{a,b}	WBC casts ^{a,b}	Amorphous sediment ^{a,b}
1	none	few	none	–	–	rare
2	moderate	few	none	–	–	none
3	none	none	none	–	–	none
4	moderate	none	1 - 5	–	–	rare
5	few	few	0 - 1	–	–	rare
6	none	none	none	none	none	none
7	none	few	none	0 - 2	none	none
8	none	none	1 - 5	none	none	none
9	moderate	few	1 - 5	none	0 - 2	none
10	many	none	none	none	none	none

^a Centrifugation conditions shown in Table 2

^b lpf, low power field

Table 6. Blood Chemistry Analysis From 6 Unknown Donors

Rotor	K [mmol/L] ^a	AST [IU/L] ^a	PHOS [mg/dL] ^a	LDH [IU/L] ^a
TX-150	3.85 ± 0.19	19.83 ± 7.83	2.87 ± 0.27	172.17 ± 33.90
HIGH Conic	4.35 ± 0.23	20.50 ± 4.32	3.90 ± 0.38	172.33 ± 27.98

^a Mean ± SD

when the HIGHConic fixed angle rotor was spun at 7200 rpm, the results were also commensurate with successful results traditionally seen by clinicians when using other clinical centrifuges.

The versatility of the accuSpin 24C centrifuge enables it to be effective and efficient in performing the multiple centrifugation steps required in the various applications within a clinical lab.

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