APPLICATION NOTE

Technical guide on efficient sample preparation for clinical diagnosis

Authors: Romana Hinz, Application Specialist, Thermo Fisher Scientific; Markus Boehm, Technical Writer, Research and Development, Thermo Fisher Scientific; Edith Cheung, Product Manager, Micro and Small Bench Centrifuges, Thermo Fisher Scientific

Key words: *Centrifuges, blood tubes, urine tubes, specimens*

INTRODUCTION

Clinical laboratory testing plays a crucial role in the detection, diagnosis, and treatment of disease. It includes the examination and analysis of biological specimens, such as blood, urine, or other body fluids, to obtain information about a patient´s health condition. Some tests provide general information, while some convey more detailed data about specific health issues. A common goal of most clinical laboratories is to provide high-quality, efficient, and accurate testing. Therefore, the handling of the biological specimen, from sample collection through preparation and analysis, is one of the most essential prerequisites for accurate test results.

Tube selection is an important part of the sample collection process. Depending on the purpose of the testing to be performed, the tubes used for collecting biological specimens vary in size, color, and additives contained in the tubes. The correct centrifuge and rotor selection is an important aspect of the analysis process so as to ensure the best sample processing. There are several factors to consider when selecting the correct centrifuge and rotor.

These include:

- Relative centrifugal force required
- Sample volume
- Tube capacity/size
- Temperature for separation
- Biocontainment needs

As a result, choosing a suitable centrifuge system based on application needs can ensure good separation, which can then optimize testing results.

ThermoFisher SCIENTIFIC

…the handling of the biological specimen is one of the most essential prerequisites for accurate test results.

To assist in the best and most efficient processing of the various clinical samples in the lab, the goal of this article is to provide practical information which may help clinical laboratories and hospitals in selecting the best centrifuge, rotor, and accessories for their diagnostic samples. In addition, this article will also illustrate some of the most frequent, but improper, sample handling issues which laboratories should avoid throughout the pre-analytical stages of clinical tests. This article is not only appropriate for clinical and hospital laboratories, but any laboratory setting where blood, urine, and other body fluid samples are collected and processed.

SPECIMENS

Blood specimens

Some of the most common blood tests are:

- Complete blood count (CBC)
- Blood chemistry tests
- Blood enzyme tests
- Lipid blood tests
- Coagulation tests

A **CBC** is done to detect blood diseases and disorders such as infections, clotting problems, blood cancers, and immune system disorders. This test measures red blood cells (RBC), white blood cells (WBC), platelets, hemoglobin, and hematocrit.

Blood chemistry tests measure different chemicals in the blood such as glucose, calcium, and electrolytes like potassium and sodium. The tests can give information about the muscles, bones, and organs.

Blood enzyme tests are done to evaluate the levels and activity of certain enzymes that control the chemical reactions in the body. There are two main blood enzyme tests: troponin and creatine kinase, both of which are used as indicators of a potential heart attack or damaged heart muscles.

Lipid blood tests are used to determine the levels of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol and triglycerides, which are other indicators of a risk for heart disease and other problems caused by narrowed or blocked arteries.

Coagulation tests are used to diagnose clotting disorders and indicate a risk of bleeding or developing clots in blood vessels[1].

Other tests include trace metal tests, which determine the amount of metals such as Aluminum (Al), Copper (Cu), Manganese (Mn), Nickel (Ni), Zinc (Zn), Arsenic (As), Bismuth (Bi), Cadmium (Cd), Magnesium (Mg), and Mercury (Hg) in the blood.

Tests are performed on whole blood, plasma, or serum for a variety of reasons. Whole blood (WB) is human blood from a standard blood collection. Plasma is the liquid component of blood, in which cells are suspended, and the serum is the plasma after coagulation factors have been removed.

Blood collection tubes contain either a clot activator or an anticoagulant. A clot activator, which accelerates clotting of blood, creates a serum sample while an anticoagulant, which prevents the blood from clotting, produces a plasma sample.

Table 1. Laboratory tests of blood samples.

Test	Analytes	Specimen type
Complete blood count	RBC, WBC	Plasma
	Hemoglobin	Plasma
Blood chemistry	Glucose	Plasma
	Ca, K, Na	Plasma/serum
	Creatinine	Plasma/serum
Blood enzyme	Troponin	Serum
	Creatine kinase	Serum
Lipid blood	HDL/LDL	Serum
	Triglyceride	Serum
Coagulation	Prothrompin	Plasma/WB
Trace metal	Al, Cu, Mn, Ni, Zn	Serum
	Cd, Mg, As, Hg, Bi	Plasma/WB

These tubes are usually color-coded and labeled with the type of additive.

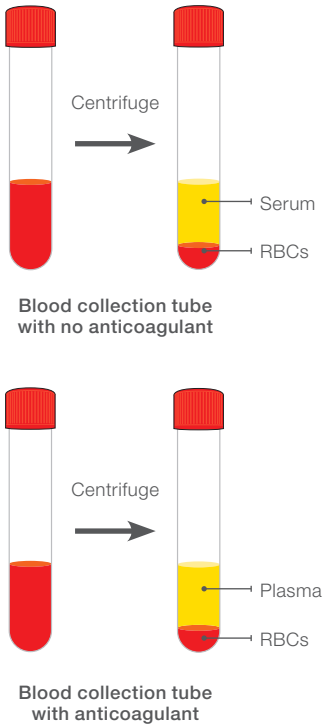

Figure 1. Blood component separation.

Urine specimens

Urine sediment is separated from urine to detect and measure compounds such as RBC, WBC, epithelial cells, bacteria, calcium oxalate crystals, mucous threads, yeast, and hyaline casts that pass through the urine. A high number of WBC, epithelial cells, bacteria, and yeast are typical indicators of an infection, while RBC, casts and crystals are typical signs of a kidney disease[2], [3].

Urine used for testing is usually classified by the collection procedure and also the different collection times and durations (amongst others): random, first morning, timed, and midstream clean catch specimen.

Random specimens are collected at any time and suitable for most screening purposes.

The **first morning specimen** is collected in the morning immediately after waking up. It is the most concentrated specimen, due to the duration of time it remained in the body. It consists of a high amount of analytes. This specimen type is preferred for examination of chemical and microscopic components.

There are generally 2 types of **timed specimens**. One is taken for 2 hours and the other for 24 hours. A 2-hour collection is taken 2 hours after a meal in order to analyze the amount of glucose. The 24-hour collection is required in order to average the amount of substances analyzed in the urine, due to variation of substances during a day.

Midstream clean catch specimen is collected at any time for culture and sensitivity testing because of the reduced incidence of cellular and microbial contamination.

Evacuated tubes, similar to the blood collection tubes, are used for the analysis of urine samples. Tubes with screw or snap-on caps are also used.

Other specimens

Specimens other than blood and urine, such as amniotic fluid and saliva, are used in limited clinical settings and are tested for only a few special analytes.

TUBES

Biological specimens are collected in specific tubes, including BD's Vacutainer™, Greiner's Vacuette™, Sarstedt's Monovette™ and Thermo Fisher's Sterilin™ collection tubes, and are available in various sizes, colors and materials[4], [5], [6], [7].

Vacutainer, Vacuette and Sterilin tubes provide evacuated systems, while Monovette tubes provide a combination of evacuated and aspiration systems. All are also available for pediatric applications.

Table 2. Common tests for specimens other than blood or urine.

Specimen	Tests
Amniotic fluid	Bilirubin
Saliva	Hormones, drugs
Pleural fluid	Protein, LDH, glucose, pH
Cerebrospinal fluid (CSF)	Glucose, bilirubin, protein
Pericardial fluid	Protein, LDH
Sputum	Tuberculosis germs

Figure 2. Vacutainer tubes by size.

The blood tube caps are usually color-coded with reference to the specimens to be used or additives contained inside the tubes. Table 3 (right) lists the tube cap colors of different blood collection tubes while Table 4 and 5 list the popular blood tubes with dimensions stated.

Table 3. Color-coded caps of various blood tubes.

Tube type	Vacutainer	Vacuette	Monovette
Serum	Red	Red	White
Serum - gel	-	-	Brown
Citrate	Blue	Blue	Green
EDTA	Purple	Purple	Red
Li-heparin	Green	Green	Brown

Table 4. Vacutainer, Vacuette and Sterilin blood/urine sample tubes with dimensions[4], [5], [7].

Sample container	Tube dimensions Ø x L (mm)				
	13 x 75	13 x 100	16 x 100	16 x 125	24 x 90
Vacutainer	Serum 3.0 / 4.0 mL	Serum 5.0 / 6.0 mL	Serum 10.0 mL		
	Citrate 1.8 / 2.7 / 4.5 mL				
	CTAD 4.5 mL				
	SST 3.5 mL	SST 4.0 / 5.0 mL	SST 7.5 / 8.5 mL	SST 10.0 mL	
		RST 5.0 mL			
	EDTA 2.0 / 3.0 / 4.0 mL	EDTA 6.0 / 7.0 mL	EDTA 10.0 mL		
	Heparin 2.0 / 3.0 / 4.0 mL	Heparin 6.0 mL	Heparin 10.0 mL		
	PST 3.0 mL	PST 3.5 / 4.5 mL	PST 8.0 mL		
	Fluoride 2.0 / 4.0 mL	Fluoride 6.0 / 7.0 mL	Fluoride 10.0 mL		
		ACD 6.0 mL	ACD 8.5 mL		
		PPT 5.0 mL	PPT 8.5 mL		
		CPT 4.0 mL		CPT 8.0 mL	
	Urine 4.0 mL	Urine 6.0 mL	Urine 8.0 mL		
Vacuette	CTAD 1.0 / 2.0 / 3.0 / 3.5 mL				
	Serum 1.0 / 2.0 / 3.0 / 3.5 / 4.0 / 4.5 mL	Serum 5.0 / 6.0 mL	Serum 7.0 / 8.0 / 9.0 mL		
	Heparin 2.0 / 2.5 / 3.0 / 4.0 / 4.5 mL	Heparin 6.0 mL	Heparin 9.0 mL		
	K2E E2EDTA Sep 4.0 mL	K2E E2EDTA Sep 5.0 mL	K2E E2EDTA Sep 8.0 mL		
	EDTA 1.0 / 2.0 / 3.0 / 4.0 / 4.5 mL	EDTA 6.0 mL	EDTA 9.0 mL		
	Sodium Fluoride 2.0 / 3.0 / 4.0 mL	Sodium Fluoride 6.0 mL			
		ACD 6.0 mL	ACD 9.0 mL		
	CPDA 4.0 mL	CPDA 6.0 mL	CPDA 9.0 mL		
		Trace element 6.0 mL	Trace element 9.0 mL		
		Crossmatch 6.0 mL	Crossmatch 9.0 mL		
	Urine 4.0 mL	Urine 6.0 / 6.5 mL	Urine 9.5 / 10.5 mL	Urine 4.0 mL	
Sterilin			Urine 13 mL		Universal container 30.0 mL

Table 5. Monovette blood/urine sample tubes with dimensions[6].

Sample container	Tube dimensions Ø x L (mm)										
	8 x 66	11 x 66	11 x 92	13 x 65	13 x 75	13 x 90	13 x 100	15 x 75	15 x 92	15 x 100	16 x 92
Monovette	Serum 1.2 mL	Serum 2.7 mL		Serum 2.6 mL	Serum 2.7 / 4.0 mL	Serum 4.9 mL		Serum 5.5 mL	Serum 7.5 mL		Serum 9.0 mL
	Serum-Gel 1.1 mL			Serum-Gel 2.6 mL		Serum-Gel 4.9 mL		Serum-Gel 4.7 mL	Serum-Gel 7.5 mL		Serum-Gel 9.0 mL
	Plasma 1.2 mL	Plasma 2.7 mL	Plasma 4.5 mL	Plasma 2.6 mL	Plasma 2.7 mL	Plasma 4.9 mL		Plasma 5.5 mL	Plasma 7.5 mL		Plasma 9.0 mL
	Plasma-Gel 1.1 mL			Plasma-Gel 2.6 mL	Plasma-Gel 4.0 mL	Plasma-Gel 4.9 mL		Plasma-Gel 4.7 mL	Plasma-Gel 7.5 mL		
	Haematology (EDTA K_3) 1.2 mL	Haematology (EDTA K_3) 2.7 mL		Haematology (EDTA K_3) 2.6 / 3.4 mL	Haematology (EDTA K_3) 2.7 mL	Haematology (EDTA K_3) 4.9 mL		Haematology (EDTA K_3) 4.0 mL	Haematology (EDTA K_3) 7.5 mL		Haematology (EDTA K_3) 9.0 mL
	Glucose (Fluoride) 1.2 mL	Glucose (Fluoride) 2.7 mL		Glucose (Fluoride) 2.6 mL	Glucose (Fluoride) 2.7 mL						
	Coagulation (Citrate) 1.4 mL	Coagulation (Citrate) 3.0 mL	Coagulation (Citrate) 5.0 mL	Coagulation (Citrate) 2.9 mL	Coagulation (Citrate) 3.0 / 4.3 mL				Coagulation (Citrate) 8.2 mL		Coagulation (Citrate) 10.0 mL
					Urine 4.0 mL		Urine 6.0 mL			Urine 9.5 / 10.0 mL	

CENTRIFUGES

Centrifuges are one of the most important and widely used devices in laboratories. A centrifuge uses centrifugal force for the separation of heterogeneous mixtures based on the different physical properties between particles, such as size and density.

The separation efficacy of the centrifugation process depends on four parameters: time, speed or g-force, temperature, and acceleration and deceleration profiles. Thermo Scientific™ benchtop centrifuges are designed for use in laboratories with the versatility of a wide range of rotor options, capacities, and speeds.

With so many variations and sizes of tubes to spin, rotor selection is extremely important for the application and, as such, it is directly related to the sample tube type, the desired capacity, and desired quality of separation.

Table 6: Thermo Scientific benchtop centrifuge rotor capacities.

Thermo Scientific™ rotors	Rotor capacity
TX-1000	4 x 1000 mL
TX-750	4 x 750 mL
TX-400	4 x 400 mL
BIOShield™ 1000A	4 x 250 mL
TX-200	4 x 180 mL
BIOShield 720	4 x 180 mL
TX-150	4 x 145 mL
CLINIConic™	30 x 15 mL
8 x 50 sealed	8 x 50 mL
HIGHConic™ III	6 x 50 mL
DualSpin™	8 x 15 mL

The primary rotor types are swinging bucket (SW) and fixed angle (FA) rotors.

Figure 3. Left: TX-1000 swinging bucket rotor. Right: 8 x 50 sealed fixed angle rotor.

Table 7. Sample capacities for different blood/urine tubes in various rotors[4], [5], [6], [7].

Thermo Scientific centrifuge	Thermo Scientific rotor	Thermo Scientific adapter part number	Tube dimensions Ø x L (mm)									
			Vacutainer/Vacuette				Monovette					Sterilin
			13 x 75	13 x 100	16 x 100	16 x 125	13 x 75	15 x 75	15 x 92	15 x 100	16 x 92	24 x 90
General Purpose 4 L	TX-1000	75003671	196[1]	196[1]	-	-	196	-	-	-	-	-
		75003709	164[2]	164[2]	-	-	164	-	-	-	-	-
		75003697	-	-	-	-	-	100	100	100	100	-
		75003672	-	-	148	76/92[3]	-	148	148	148	148	-
	TX-750	75003723	108	108	-	-	108	-	-	-	-	-
		75003716	-	-	-	-	-	-	-	-	-	28
		75003768	80	80	-	-	80	-	-	-	-	-
		75003755	-	-	-	-	-	-	-	-	-	16
		75003719	-	-	84	32	-	84	84	84	84	-
		75003767	-	-	64	-	-	64	64	64	64	-
	BIOShield 1000A	75003768	80	80	-	-	80	-	-	-	-	-
		75003755	-	-	-	-	-	-	-	-	-	16
		75003767	-	-	64	-	-	64	64	64	64	-
General Purpose 1.6 L	TX-400	75003681	-	-	56	16	-	56	56	56	56	-
		75003680	76[1]	76[1]	-	-	76	-	-	-	-	-
		75003706	-	-	-	-	-	-	-	-	-	12
		75003794	-	-	16	-	-	16	16	16	16	-
		75003825	64[2]	64[2]	-	-	64	-	-	-	-	-
	BIOShield 720	75003818	-	-	-	-	-	-	-	-	-	16
		75003821	48	48	-	-	48	-	-	-	-	-
		75003701	-	-	48	-		48	48	48	48	-
	TX-200	75003785	32	32	-	-	32	-	-	-	-	-
		75003804	-	-	-	-	-	-	-	-	-	4
		75003808	-	-	28		-	28	28	28	28	-
Compact	TX-150	75005739	24	24	-	-	24	-	-	-	-	-
		75005744	-	-	-	-	-	-	-	-	-	4
		75003504	-	-	16	-	-	16	16	16	16	-
	TX-100/TX-100S	Direct fit	-	-	16/8	-	-	-	-	16/8	16/8	-
		11172596	16/8	16/8	-	-	16/8	-	-	-	-	-
		11172595	16/8	-	-	-	16/8	-	-	-	-	-
	8x50 Sealed	75005804	-	-	8	-	-	8	8	8	8	-
		75005805	8	8	-	-	8	-	-	-	-	-
	CLINIConic	Direct fit	-	-	30	30	-	-	-	30	-	-
		11172595	30	30	-	-	30	-	-	-	-	-
		11172596	30	30	-	-	30	-	-	-	-	-
Small Clinical	DualSpin (Fixed angle)	Direct fit	-	8	8	8	-	-	-	8	-	-
		Green	-	-	-	-	-	-	8	-	8	-
		Yellow	8	-	-	-	8	8	-	-	-	-
	DualSpin (Swinging bucket)	Direct fit	-	8	8	-	-	-	8	8	8	-
		Green	8	-	-	-	8	8	-	-	-	-

1 only applied to Vacutainer tubes
2 only applied to Vacuette tubes
3 without cap

Accessories may also be added as needed to tailor the rotor to a specific application or sample type. For example, swinging bucket rotors usually offer a wide variety of adapters specifically designed for the proper support of specific tubes and their sizes. Additionally, biocontainment lids, which are designed to keep hazardous samples contained in the buckets rather than in the chamber of the centrifuge or the laboratory environment in the event of a tube leak or breakage, are an option for laboratories where biosafety is a concern.

SELECTING THE RIGHT SYSTEM

Several factors, such as the fit of the particular tube in the rotor or the sample volume, must be considered in order to ensure optimal separation, a high sample recovery, and no risk for potential damage to the tube and rotor. The necessary relative centrifugal force, time, and temperature for sample separation are provided by the blood collection tube manufacturers [4], [5], [6].

Table 7 provides guidance for selecting the proper rotor for various blood and urine collection tubes according to different throughput needs.

SOURCES OF ERROR

One of the sources of testing error in laboratories is found in the handling and processing of samples. The following section reviews some of the causes of errors and suggests ways to minimize them.

Due to the significant heterogeneity in the blood tube cap colors, tubes should be double checked before using.

Tubes should be properly labeled. Mislabeled tubes may lead to a serious diagnostic error with patients.

Blood should be drawn in the proper order. Otherwise, this could result in a contamination from anticoagulants.

The proper order is shown in the following:

a) Blood culture tube

b) Sodium citrate tube

c) Serum tube with or without clot activator or gel separator

d) Heparin tube

e) EDTA tube

f) Oxalate/fluoride tube

A correct positioning of blood tubes during clotting is important for the centrifugation result. Figure 4 shows a sample coagulated in a horizontal position.

A good separation is achieved by putting blood tubes in an upright position during clotting.

Figure 4. Samples coagulated in a horizontal orientation.

The period of time during clotting is also of great importance[8]. The time between sample collection and separation of serum from the clot should be long enough to allow coagulation, but should be shorter than the time in which diffusion of certain analytes occur.

Variation in centrifugation time, speed, and temperature affects the separation result. Choose the correct centrifugation conditions according to the instruction of blood tube manufacturers.

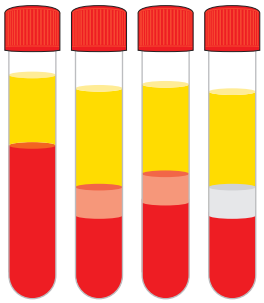

Figure 5. Impact of g-force on separation, increasing from left to right. Tube at the right representing the highest g-force has the best separation.

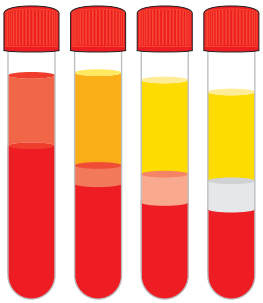

Figure 6. Impact of centrifugation time on separation, increasing from left to right. Tube at the right representing the longest time has the best separation.

Extreme cooling down or warming up might lead to a release of hemoglobin from RBC, called hemolysis, which is indicated by a reddish discoloring in the serum. Results of all follow-up laboratory tests might be affected. For instance, a false positive may result in potassium or lactate dehydrogenase tests.

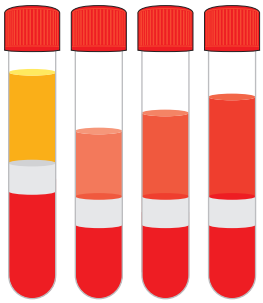

Figure 7. Samples in various strengths of hemolysis. Tube at the right representing the most serious case of hemolysis.

Tube manufacturers recommend a temperature range of 15 to 25 °C for effective separation. Since ventilated centrifuges heat up during the run, refrigerated centrifuges are recommended in order to get a better temperature-controlled separation.

The type of rotor being used can influence the separation. For example, a swinging bucket rotor is highly recommended for gel tube separation to achieve a stable gel barrier. Figure 8 compares the gel tube separation using swinging bucket and fixed angle rotors[9].

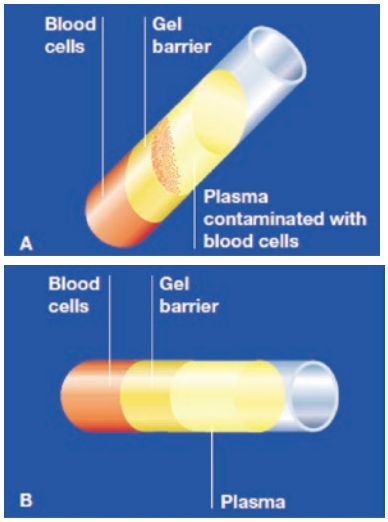

Figure 8: Gel tube separation. A. Using a fixed angle rotor with gel barrier blood collection tubes can result in plasma contamination. **B.** The swinging bucket rotor purifies plasma with no red blood contamination.

The tubes need to be removed carefully from the centrifuge after run in order not to disturb the separation layers.

SUMMARY

It is important for clinical laboratories and hospitals to understand the factors that help in selecting a suitable centrifuge, rotor, and accessories for their sample separation. In addition, it is critical to understand common errors in sample preparation, so they can be avoided. Selecting the right centrifuge system and following proper protocols may help laboratories achieve efficient and accurate diagnosis for patients.

REFERENCES

1. Shahangian S. et al. (2005), Results of 2001 survey of hospital coagulation laboratories in the United States, *Archives of pathology and laboratory medicine*, 129, 47 - 60.
2. Fogazzi GB, Garigali G (2003), The clinical art and science of urine microscopy, *Curr Opin Nephrol Hypertens*, 12, 625 - 32.
3. Graham JC, Galloway A (2001), ACP best practice no. 167, the laboratory diagnosis of urinary tract infection. *J Clin Pathol.*, 54, 911 - 9.
4. www.bd.com [Online] 21.01.2016
5. www.greinerbioone.com [Online] 21.01.2016
6. www.sarstedt.com [Online] 21.01.2016
7. www.thermofisher.com [Online] 21.01.2016
8. Dongbo J. Zhang, R.K. Elswick, W. Greg Miller, and Jimmy L. Bailey; effect of serum-clot contact time on clinical chemistry laboratory results; *Clinical Chemistry* 44, 6 1325 – 1333 (1998).
9. Centrifuge Rotor Selection and Maintenance Ms. Goodman, Sample Preparation and Separations Applications Product Manager, Thermo Fisher Scientific Inc., 275 Aiken Rd., Asheville, NC 28804, U.S.A.

Find out more at **thermofisher.com/clinicalcentrifuges**

ThermoFisher SCIENTIFIC