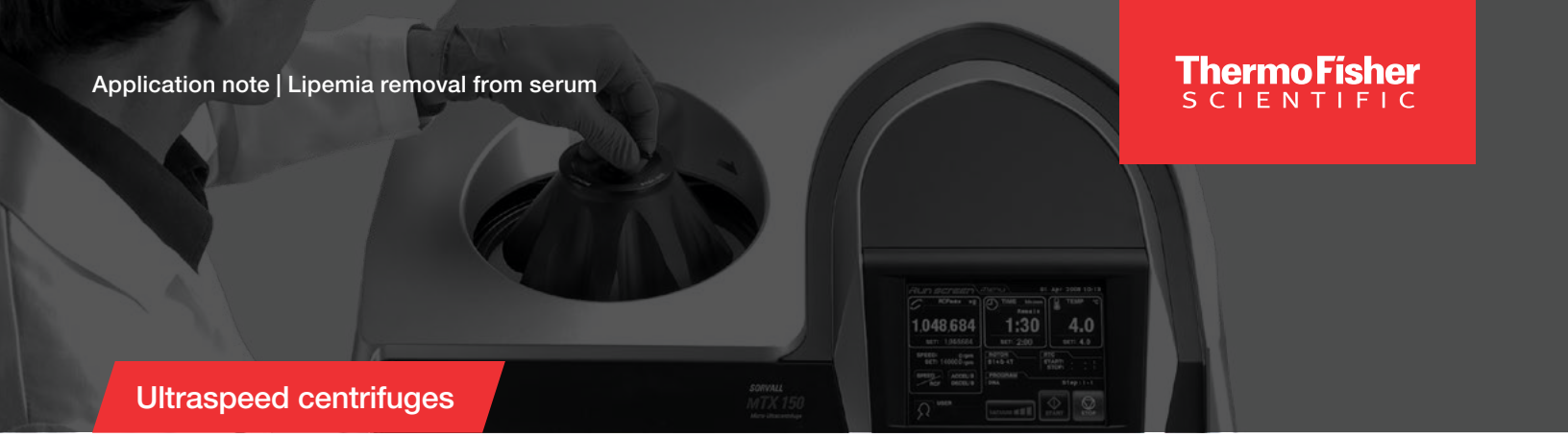

Ultraspeed centrifuges

A comprehensive protocol for lipemia removal from serum using an ultra microcentrifuge

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Keywords

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Introduction

A lipemic sample refers to a serum or plasma sample that has abnormally high concentrations of lipoproteins. This condition, known as lipemia, is caused by factors that lead to hypertriglyceridemia and other lipid-related disorders. Lipemia can be encountered in approximately 0.5–2.5% of samples, with the frequency varying depending on the characteristics of the hospital and the inpatient-to-outpatient ratio. Lipemia interference is a significant source of laboratory errors, despite its relatively low frequency. It can lead to inaccurate test results, affecting patient care and treatment decisions. To mitigate this issue, laboratories should adopt standardized procedures for detecting and managing lipemic samples. [1,2,3].

Fig 1: Lipemic sample image that shows the cloudiness of serum/plasma.

Processing lipemic samples aims to reduce lipid content to minimize interference in clinical tests. Effective management of lipemic samples enhances diagnostic quality and improves patient care [4,5]. Ultracentrifugation is recognized as the recommended preanalytical method to separate chylomicrons and VLDLs from serum or plasma.

In this application note, in collaboration with the Mayo Clinic, we demonstrate the efficacy of the ultracentrifuge technique in eliminating lipemia from serum without compromising analyte measurements. Additionally, we provide a comprehensive protocol that can be adopted by clinical laboratories for the treatment of lipemic samples.

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Equipment

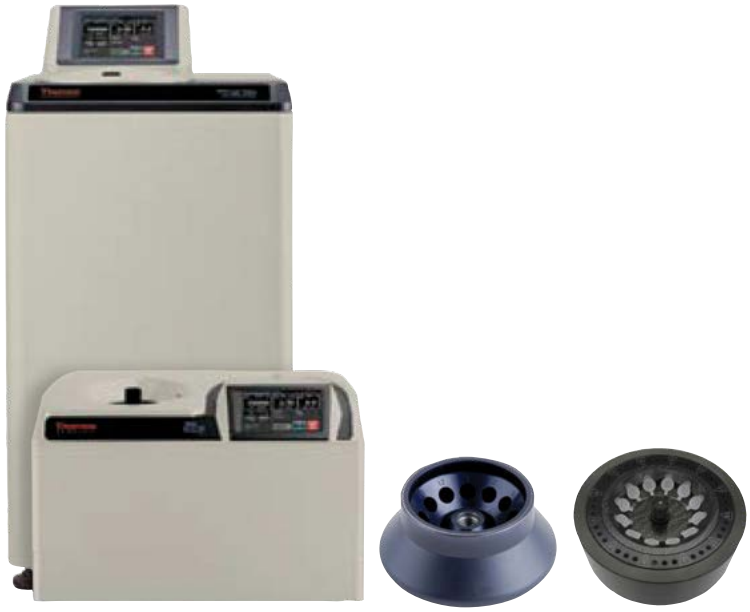


Fig 2: Thermo Scientific™ MX+ (floor-standing) and MTX (benchtop) Micro Ultracentrifuges with S55-A2 and F55-12x1.5 MUC Rotor

Comprehensive protocol for lipemic samples

Preparation steps before diagnostic and clinical analysis

1. Collect blood serum according to clinical lab procedures.
2. Transfer the lipemic serum into the High Performance Microtube, Cat No. 314352H01. Ensure that the tubes are balanced in terms of weight for proper centrifugation.
3. Place the centrifuge tubes into the Thermo Scientific S55-A2 rotor, Cat No. 45865, or the Thermo Scientific Fiberlite F55-12x1.5 MUC rotor, Cat No. 096-127022. Ensure the rotor's lid is securely fastened.
4. Set the speed on the touchscreen to 55,000 rpm. Ensure that the centrifuge is operating correctly, and that the rotor is securely attached.
5. Set the centrifugation time to 6 minutes. Note: Ensure the centrifugation is set at the correct speed. For more information, refer to the Ultracentrifuge MTX/MX + instruction manual.
6. In addition to the proposed 6-minute centrifugation spin, spin durations of 4, 5, 7, and 8 minutes have been tested to evaluate their effectiveness in achieving successful clearance of lipemia.
7. Set the temperature to 20°C and set the acceleration and deceleration to 9.
8. Once the centrifugation is complete, carefully remove the tubes from the centrifuge. There should be a clear separation of the lipids from the rest of the sample.
9. Carefully collect the infranatant (the solution below the lipid layer) using a fine pipette. This is the part of the sample that is now free of lipids.
10. The preparation is finished. The infranatant is now ready for further processing or analysis.

Analyte measurement methodology*

- Lipemia was estimated by measuring the L-index (a measure of sample turbidity).
- Residual serum samples with varying extents of lipemia near the current L-index thresholds for electrolyte measurement (L-index = 80-250) were obtained.
- L-indices were measured before and after ultracentrifugation.
- All analytes were measured using the Roche Modular Chemistry Analyzer (D or P modular) and Immunoassay (e601, Indianapolis, IN) instruments.
- Residual serum samples with L-indices below the interference threshold for each assay were analyzed pre- and post-ultracentrifugation.
- Bias between pre- and post-ultracentrifugation results was calculated

Results

From the data presented in table 1, it was agreed that a 7-minute centrifugation spin provides the most consistent and effective clearance of lipemia from the samples. This duration was found to achieve optimal separation, resulting in clear supernatant and minimal residual lipemic content. Although the 4-minute spin also showed some degree of lipemia clearance, it was not as consistent or thorough as the 7-minute duration. The shorter spin time often left behind a higher amount of lipemic interference, which could affect subsequent analyses and results.

Table 1. Lipemic index before and after ultracentrifugation

Lipemia (L index)						
		Minutes of spin at 55k RPM				
Sample #	Neat	4	5	6	7	8
1	247	19	59	28	14	16
2	85	8	23	16	8	5
3	57	11	14	17	4	7
4	45	12	11	12	10	7
5	54	7	10	21	11	8
Total	488	57	117	94	47	43
% Clearance	N/A	88	76	81	90	91

As can be seen from table 1, after spin time for 7 minutes, lipemia post-treatment was reduced to an L-index of <18. For each test (n=5 samples or n=3 for immunoassays), maximum bias (absolute or percent) between results from pre- and post-ultracentrifuged samples was as follows (analyte, mean bias (range)) as demonstrated in table 2.

Table 2. Analyte measurements results* – provided by Mayo Clinic

Test	Acronym	Avg. % diff	Avg. # diff	Acceptable bias limit
Sodium	Na	1.0	1.4	+/- 5 mmol/L
Potassium	K	0.9	0.0	+/- 0.3 mmol/L
Chloride	Cl	1.2	1.2	+/- 5 mmol/L
Bicarbonate	CO2	0.8	0.2	+/- 4 mmol/L
Creatinine	CREA	5.7	0.1	+/- 0.3 mg/dL
Blood Urea Nitrogen	BUN	0.8	0.2	+/- 5.0 mg/dL
Calcium	Ca	-1.3	-0.1	+/- 0.3 mg/dL
Aspartate Aminotransferase	AST	3.7	1.2	+/-5 U/L or +/- 10%

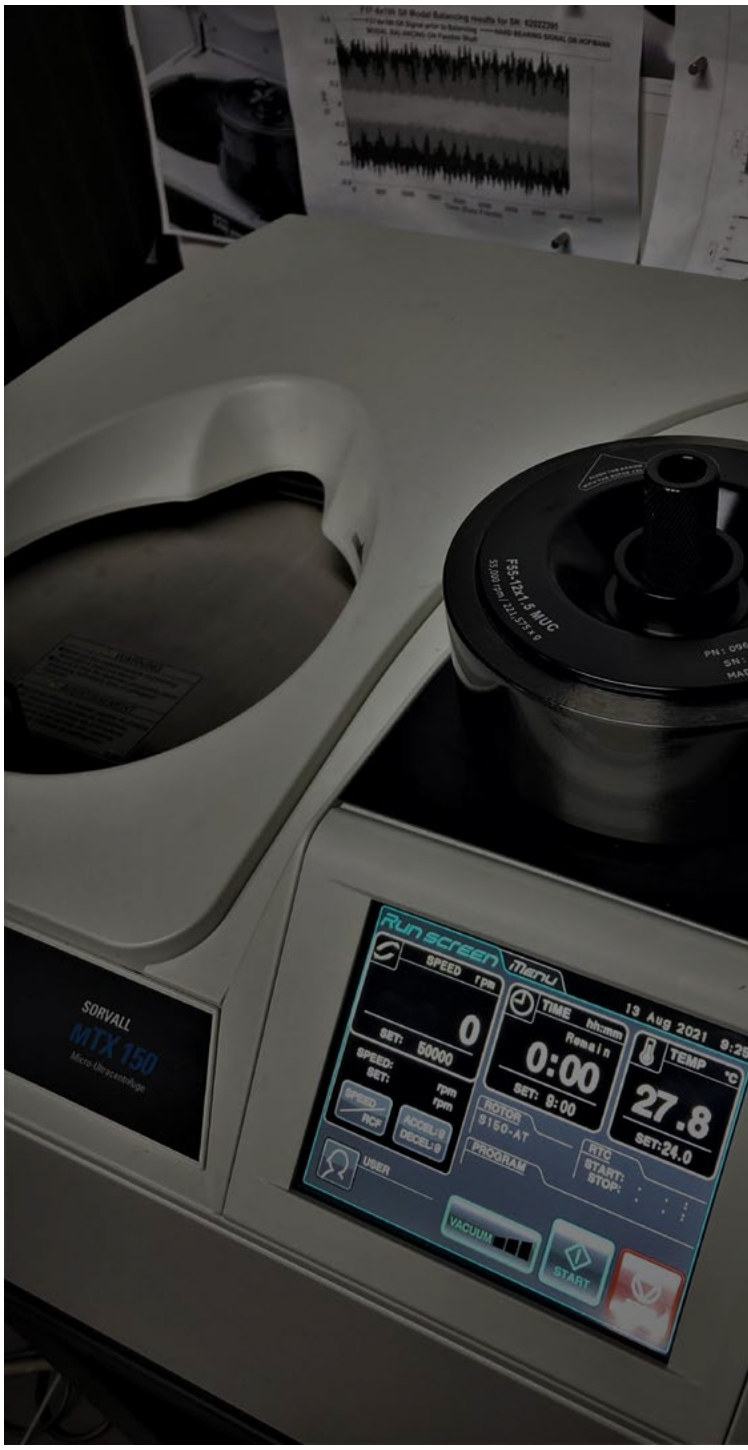
*This section is supported not by ultracentrifugation, but by analytical instruments.

Table 2. Continued

Test	Acronym	Avg. % diff	Avg. # diff	Acceptable bias limit
Alkaline phosphatase	ALP	1.3	1.4	+/- 10%
Creatine kinase	CK	2.3	2.6	+/- 10 U/L or 10%
Alanine Aminotransferase	ALT	-0.9	-1.4	+/- 5 U/L or +/- 10%
Gamma-Glutamyl Transferase	GGT	-1.1	-1.0	+/- 5 or 10%
Phosphorus	Phos	0.2	0.0	+/- 0.3 mg/dL
Uric Acid	Uric	-0.3	0.0	+/- 0.4 mg/dL
Albumin	Alb	5.2	0.2	+/- 0.3 mg/dL
Glucose	GLU	1.3	2.2	+/- 5%
Total protein	TP	4.2	0.3	+/- 0.4 mg/dL
Lactate Dehydrogenase	LD	0.7	0.8	+/- 10%
Total bilirubin	TBIL	1.0	-0.2	+/- 0.3 mg/dL
Direct bilirubin	DBIL	-3.7	-0.2	+/- 0.2 mg/dL
Magnesium	Mg	1.1	0.0	+/- 0.3 mg/dL
Amylase	AMYL	2.3	1.4	+/- 5 or 10%
Lipase	LPS	-2.8	-1.2	+/- 10 or 10%
Fructosamine	FRUC	0	1.0	+/- 10%
Soluble Transferrin Receptor	STFR	5.5	0.2	+/- 0.3 mg/L or 10%
C-Reactive Protein	CRP	0.7	0.1	+/-10%
Beta-Hydroxybutyrate	BHYD	0	0.0	+/- 0.3 mmol/L
Digoxin	DIG	-5.9	0.0	+/-20%
N-terminal pro b-type Natriuretic Peptide	PROBNP	0.3	-0.7	+/-20%
Troponin T	TPNT	-0.3	0.0	+/- 0.029 ng/mL or 10%
Thyroid-Stimulating Hormone	TSH	2.4	0.5	+/-20%
Prostate-Specific Antigen	PSA	3.6	0.4	+/- 0.10 ng/mL or 10%
Free Prostate-Specific Antigen	FPSA	1.3	0.0	+/- 0.10 ng/mL or 10%
Human Chorionic Gonadotropin	HCG	0.1	178.3	+/-10%
Estradiol	ESTS	3.1	21.3	Results <100 +/-10, results >100 +/- 15%
Parathyroid Hormone	PTH	0.2	-0.4	+/-10 ng/mL or 10%
Insulin	INS	2.4	4.3	Results <25 +/- 5, results >25 +/- 10%
C-peptide	CPTD	4.4	0.7	+/- 0.5 or 5%

Conclusion

In conclusion, the comprehensive protocol for lipemia removal from serum using the Ultra Microcentrifuge demonstrates a significant reduction in lipemia, as evidenced by the marked decrease in L-index values post-ultracentrifugation. The study showed that a 7-minute spin time was effective in reducing lipemia to an L-index of less than 18, ensuring minimal interference in subsequent analyte measurements. The bias between pre- and post-ultracentrifugation results for various analytes was within acceptable bias limits, indicating the reliability and accuracy of the ultracentrifugation process.



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