

Hemostasis Preanalytical Guidelines



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Recommendation



Anticoagulant

Recommended Sodium citrate 3,2 % (109 mmol/l) Acceptable Sodium Citrate 3,8% (0,129 M) CTAD acceptable in some circumstances



Sufficient volume. Respect the required ratio of sodium citrate to whole blood (1:9) Fill volume: ≥90 % Do not transfer from 1 tube to another

Check expiry date of the tube

Potential Risk

Serum or any other anticoagulant can lead to an incorrect result

Sample dilution (excess CaCl2-Citratre binding)⁹

False results



Tube filling order : Before filling citrate tube, discard the first tube (neutral or citrate) Citrate tube : before tubes with additives Sample contamination Sample dilution (excess CaCl2-Citratre binding)⁹

Tube filling order during venous sampling

NEEDLE TYPE	: 1	2	3	4	5	6	7
Vacuum tube holder	Without neutral tube (If swift puncture & regular blood flow)	Citrate tube	Serum tube	Heparin tube	EDTA tube	Fluoride tube	Others
Winged collection butterfly	Without Blood culture neutral tube (line air purge) With Blood culture 1 2 Aerobic Anaerobic vial vial						(ESR, Aprotinin)

Proper Tube Identification Label / patient demographic and collection date and time

The diameter of the needle recommended should preferably be between 19 and 22 gauge¹¹

Acceptable: 23 (pediatric, compromised veins, geriatrics, oncology, ...)¹¹



Vacuum tube holder : the venipuncture must be swift and the blood flow, regular¹¹



Winged collection butterfly : before filling citrate tube, discard the first tube (neutral or citrate)¹¹



Release the tourniquet immediately when the first tube starts to fill (<1 mn)

Avoid traumatic phlebotomy (draw) Avoid drip lines Avoid wet alcohol carryover

Immediately mix **gently** by 3 to 6 complete end-over-end inversions to ensure adequate mixing with anticoagulant and to prevent clotting



Room temperature $(15 - 25 \ ^{\circ}C)$: **should be maintained** Keep the tube vertical during transport Prohibits transport on ice or refrigerate transport (2°C - 8°C)

Sample stability

Fresh sample: Room temperature $(15 - 25 \text{ °C})^{13} = 4$ hours for most tests NOTE : this specification is true for most routine tests, for details per parameter, refer to GFHT ² Wrong result

Hemolysis^{1,6}

Risk of contamination from tissue thromboplastin and hemolysis.

Risk of under filling due to the air from sampling line.

Hemolysis^{1,6} Fibrinolysis activation Acidosis (pH <7,3) PT prolonged

Coagulation activation^{4,7} Hemolysis¹ Interferences, sample dilution

Factor activation, Hemolysis¹ Sample clotting^{4,7} (partial) Variable anticoagulant gradient (gradient of sample with different citrate buffering)

False results : activation of some coagulation factors

False results : activation of some coagulation factors



Centrifugation

Standard recommendation : 1500 g, 15 minutes Centrifugal conditions must be established and validated by the laboratory. Maximum time for centrifugation after sampling is 2 hours Room temperature (15 – 25 °C)¹³ "Rapid centrifugation" may be used (higher speed, shorter duration) under lab validation

Double centrifugation recommended before freezing.

Transfer the plasma to a non-activating plastic centrifuge tube using a plastic pipette, then re-centrifuging the sample for an additional 10 minutes. When transferring to a secondary tube, take care to not include any residual platelets that may have collected at the bottom of the centrifuge tube.

Storage (depending on the parameter) Room temperature (15 – 25 °C)^{12,13}.4 hours for most of tests Minus 20°C = Maximum storage time : 2 weeks Minus 70° C = 6 month to 12 month

NOTE : these specifications are true for most of tests, for details per parameter, refer to GFHT²

Defreezing must be done at 37°C in bain-marie during 5 to 10 minutes maximum1.

False results due to contamination by phospholipid from platelets. Lupus and Heparin Assays particularly affected 10. PT, APTT, TT not affected up to Platelet count=200 000/µl¹

False results Lupus and Heparin Assays affected¹⁰.

FVII activation, platelet disruption, Loss of coagulation components, Hemolysis¹

False results due to the release of phospholipid



Literature

1 - Clinical Laboratory Standard Institute

Avoid clotted samples

CLSI. Collection t, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays; approved guideline. 5e edition. CLSI Document H21-A5. Ed. Wayne: PCaLSI, 2008

CLSI. Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline – Fourth Edition, CLSI document GP44-A4, Wayne, PA : CLSI, 2010, 57 p.

CLSI. Laboratory Testing for the Lupus Anticoagulant; Approved Guideline. CLSI Document H60-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2014 CLSI. Quantitative D-dimer for the exclusion of venous thromboembolic disease; approved guideline. 1e edition. CLSI Document H59-A. Ed. Wayne: PCaLSI, 2016

CLSI. Collection of Diagnostic Venous Blood Specimens; Approved Standard- Seventh Edition, CLSI document GP41-A7, Wayne, PA: CLSI, 2017, 86 p.

2 - GFHT Recommandations pré-analytiques en hémostase : Stabilité des paramètres d'hémostase générale et délais de réalisation des examens - Mai 2017

- 3 Töpfer et al. J Lab Med 2000: 24 : 514-20
- 4 Lippi et al., Blood Coagul Fibrinolysis 2005; 16: 453-9

Check samples for Hemolysis, lipid and icterus

- 5 Lippi et al., Clin Lab Haematol 2006; 28: 332-7
- 6 Interference of Blood cell lysis on routine coagulation testing, G.Lippi et al, Arch pathol Lab Med, 2006
- 7 Preanalytical Variables in Coagulation Testing Associated with diagnostic errors in Hemostasis, E.J. Favaloro, D. Funk, G.Lippi, Labmedicine, 2012
- 8 Cattaneo M et al. J Thromb Haemost 2013; 11: 1183-9

9 - Preanalytical and Post analytical Variables: the leading causes of diagnostic error in Hemostasis?, E.J. Favaloro, G.Lippi, D.Adcock, Seminars in Thrombosis and Hemostasis Volume 34 Number 7, 2008 10 - Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. J Thromb Haemost. 2009; 7(10):1737-1740.

11 - Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories A. Magnette, M. Chatelain, B. Chatelain, H. Ten Cate, and F. Mullier Thromb J. 2016; 14:49

12 - Toulon P et al. Impact of different storage times at room temperature of unspun citrated blood samples on routine coagulation tests results. Results of a bicenter study and review of the literature. Int J Lab Hematol 2017; 39:458-468. doi:10.1111/ijlh.12660

13 - Pharmacopée Européenne







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