Hemostasis Preanalytical Guidelines
Sample tube

**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-Citrate binding)
- False results

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Sample collection

**Tube filling order**

Before filling citrate tube, discard the first tube (neutral or citrate)

Citrate tube: before tubes with additives

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**Tube filling order during venous sampling**

<table>
<thead>
<tr>
<th>NEEDLE TYPE</th>
<th>1</th>
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<th>4</th>
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<th>7</th>
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</thead>
<tbody>
<tr>
<td><strong>Vacuum tube holder</strong></td>
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<td><strong>Without neutral tube</strong> (If swift puncture &amp; regular blood flow)</td>
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<td><strong>Winged collection butterfly</strong></td>
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<td><strong>Without Blood culture neutral tube</strong> (line air purge)</td>
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<td><strong>With Blood culture</strong></td>
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<td><strong>Citrate tube</strong></td>
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<td><strong>Serum tube</strong></td>
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<td><strong>Heparin tube</strong></td>
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<td><strong>EDTA tube</strong></td>
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<td><strong>Fluoride tube</strong></td>
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<td><strong>Others</strong></td>
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<td>(ESR, Aprotinin ...)</td>
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</table>
Proper Tube Identification Label / patient demographic and collection date and time

The diameter of the needle recommended should preferably be between 19 and 22 gauge\(^{11}\)
Acceptable: 23 (pediatric, compromised veins, geriatrics, oncology, …)\(^{11}\)

Vacuum tube holder: the venipuncture must be swift and the blood flow, regular\(^{11}\)

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

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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\(^{4,7}\)**
Hemolysis\(^{1}\)
Interferences, sample dilution

**Factor activation, Hemolysis\(^{1}\)**
Sample clotting\(^{4,7}\) (partial)
Variable anticoagulant gradient (gradient of sample with different citrate buffering)

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**Sample transport**

Room temperature (15 – 25 °C) : **should be maintained**
Keep the tube vertical during transport
Prohibits transport on ice or refrigerate transport (2°C - 8°C)

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**Sample stability**

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

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 GFHT7

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

Pre-analytical Sample Integrity

Avoid clotted samples
Check samples for Hemolysis, lipid and icterus

Centrifugation and Storage

False results due to contamination by phospholipid from platelets.
Lupus and Heparin Assays particularly affected10.
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

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

Pre-analytical Sample Integrity

Avoid clotted samples
Check samples for Hemolysis, lipid and icterus

False results
Avoid clotted samples
Coagulation activation4,6,7
(micro clots could be not visible)
False results

Pre-analytical Sample Integrity

Avoid clotted samples
Check samples for Hemolysis, lipid and icterus

False results
Avoid clotted samples
Coagulation activation4,6,7
(micro clots could be not visible)
False results

Literature
1 – Clinical Laboratory Standard Institute
2 – GFHT Recommandations pré-analytiques en hémostase : Stabilité des paramètres d’hémostase générale et délais de réalisation des examens - Mai 2017
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
11 – Pre-analytical issues in the haemostasis laboratory: guidance for the clinical laboratories. A. Magnette, M. Chatelain, B. Chatelain, H. Ten Cate, and F. Muller Thromb J. 2016; 14: 49
13 – Pharmacopée Européenne