

Hemostasis: Pre-Analytical Conditions

Introduction

The pre-analytical phase is a critical step in hemostasis. Failure to comply with sampling, specimen handling, or transport conditions may lead to erroneous results and impact patient management.

These recommendations are based on GEHT guidelines (updated May 2017).

1. Tube and Anticoagulant Selection

The recommended tube is a sterile vacuum tube, made of airtight PET or polypropylene for citrate tubes, with a residual air volume $\leq 20\%$.

The reference anticoagulant is 3.2% **sodium citrate**.

2. Sampling Conditions

Blood collection must be performed via venipuncture, using a needle of 19 to 22 gauge (23 gauge acceptable in cases of difficult veins, pediatrics, geriatrics, or oncology).

The tourniquet must be applied for less than one minute and should not be too tight. Beyond 3 minutes or if overly tight, the sample is considered non-compliant.

The patient's hematocrit must be between 20% and 55%. Outside this range, notification is required.

Adjustment of the anticoagulant volume may be considered according to CLSI recommendations.

The hemostasis tube should ideally be drawn **second**, after a discard tube or blood culture bottles.

Filling must reach at least 90% of the nominal volume (80% acceptable).

3. Mixing and Transport

Immediately after filling, the tube must be gently mixed by slow and complete inversions.

Whole blood transport must be carried out at room temperature (15–25°C), without refrigeration. Transport at 2–8°C, on ice, or above 37°C is non-compliant.

Shock and vibrations must be minimized. Transport via pneumatic systems requires specific validation depending on network characteristics.

4. Centrifugation: Double Centrifugation

Standard centrifugation conditions are:

- 1,500–2,000 g for at least 15 minutes, or
- 2,000–2,500 g for at least 10 minutes

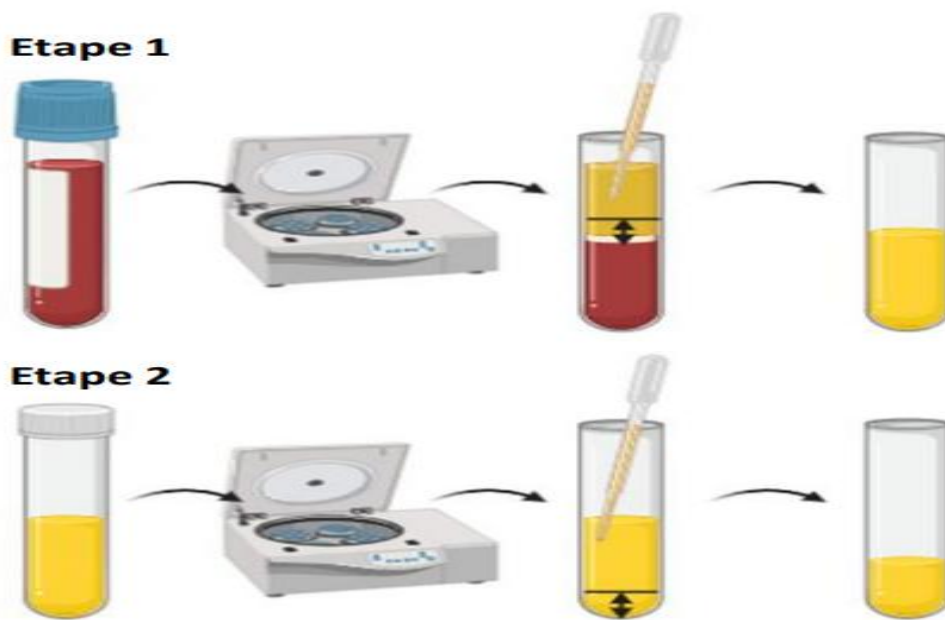
Double centrifugation, required for specialized assays, aims to obtain plasma with <10 G/L residual platelets.

It consists of two successive standard centrifugations with decanting between them.

After the first centrifugation, plasma is removed using a pipette, ensuring that the buffy coat (leukocyte-platelet layer) above the red cell pellet is not aspirated. It is recommended to leave at least 0.5 cm of plasma above the cell pellet.

The plasma is then transferred into another tube and subjected to a second centrifugation under standard conditions.

Finally, plasma is carefully collected and transferred into a new tube, avoiding aspiration of residual platelets at the bottom.



Centrifugation must be performed at a controlled temperature of 15–25°C, using a swinging-bucket rotor with the brake disabled.

Centrifuges must undergo metrological control at least once per year, with a target of <10 G/L platelets in at least 6 consecutive samples.

According to CLSI, pooling plasma from different primary tubes is discouraged.

5. Freezing, Storage, and Thawing

If analysis is delayed, freezing must be rapid at –20°C. Storage must not exceed 15 days.

Transport of frozen samples must be carried out on dry ice.

Pre-Analytical Recommendations in Hemostasis

GEHT — Revision October 2015 (updated May 2017)

Parameter	Recommended	Acceptable
Tube	Sterile vacuum tube Citrate tube: airtight PET, polypropylene CTAD tube: PET or siliconized glass Residual air ≤ 20%	Siliconized glass
Anticoagulant	3.2% citrate CTAD (including 3.2% citrate)	3.8% citrate
Hematocrit	0.20 (20%) to 0.55 (55%)	If >0.55: notify prescriber/reference laboratory If <0.20: notify prescriber/reference laboratory Adjustment possible (laboratory discretion)
Needle gauge	19 to 22 gauge	23 gauge (difficult veins, pediatrics, geriatrics)
Collection material	Inert polymer, sterile, pyrogen-free Winged needles allowed	—
Tourniquet	< 1 min, not tight	1 to 3 min
Puncture site	Venous	Arterial From catheter (after discarding 5–10 mL)
Tube order	2nd tube after discard tube (neutral) or blood cultures	—
Filling	≥ 90%	≥ 80%
Mixing	Immediately after filling, slow and complete inversions	—
Whole blood transport	Not refrigerated, 15–25°C Minimize shocks and vibrations	Intermediate temperatures (with risk assessment)
Standard centrifugation	1,500–2,000 g AND ≥ 15 min or 2,000–2,500 g AND ≥ 10 min	—
Double centrifugation (Specialized tests)	Two successive standard centrifugations (with decanting between)	—

Parameter	Recommended	Acceptable
Centrifuge temperature	15–25°C (controlled temperature)	No cooling if <25°C guaranteed
Rotor	Swinging-bucket	Fixed-angle (if absence of contamination verified)
Centrifuge brake	Brake off	Minimum braking
Centrifuge control	≥ once/year Criterion: platelets < 10 G/L in ≥ 6 samples	—
Freezing	Rapid at ≤ -70°C	Rapid at ≤ -20°C
Frozen storage	≤ -70°C Non-wettable tube, screw cap	≤ -20°C (< 15 days)
Frozen transport	Dry ice (compliance checked upon receipt)	Ice or eutectic cold packs