Coagulation Assay

Praveen Papareddy*, Martina Kalle, Artur Schmidtchen

Division of Dermatology and Venereology, Department of Clinical Sciences, Lund University Biomedical Center, Lund, Sweden
*For correspondence: praveen.papareddy@med.lu.se

[Abstract] Clotting times can be measured by using citrate plasma. The intrinsic pathway of coagulation is measured by the activated partial thromboplastin time (aPTT), the extrinsic pathway of coagulation, monitored by measuring the prothrombin time (PT), and thrombin-induced fibrin-network formation (thrombin clotting time; TCT).

Materials and Reagents

1. Citrated plasma (fresh or frozen)
2. Eppendorf tubes
3. Coagulation reagents
   a. Thrombin reagent (Technoclone, catalog number: 5100005)
   b. TriniCLOT PT Excel reagent (Trinity Biotech, catalog number: T1105/T1106)
   c. DAPTTIN TC (Technoclone, catalog number: 5035060)
4. 30 mM $CaCl_2$ (freshly made)
5. Test agent (*e.g.* antimicrobial peptide LL-37)

Equipment

1. BD vacutainer® plus blood collection tubes (BD, catalog number: 364305)
2. Coagulometer (Mc10 Plus merlin medical)
3. Cuvettes and balls macro (merlin medical, catalog number: Z05100)

Procedure

1. Warm up the coagulation machine (at least 10 min before you begin the experiment switch on the machine).
2. Prepare all reagents (should have room temperature before use).
3. First place the special cuvette with a steel ball on the measuring positions in instrument related racks.

Principle behind this technique: Once the cuvette is kept in the rack it starts rotating and due to gravity the metal ball inside the cuvette always remains. When the plasma/blood is in solution the ball remains in the position and if the plasma/blood starts clotting the clot pulls the ball out of the basic position and the sensor detects the disturbance and measures the clotting time.

4. In order to measure the clotting times, add the reagents to the cuvette according to the schedule:
   a. For whole blood assay
      i. Add 100 µl citrate blood to the coagulometer and press incubation.
      ii. After 60 sec incubation (helps to bings the sample to 37 °C).
      iii. Add 100 µl of 30 mM $CaCl_2$ → then immediately press manual start button to measure coagulation.
   b. Prothrombin time (PT)
      i. Add 100 µl citrate plasma to the coagulometer and press incubation.
      ii. After 60 sec incubation.
      iv. Add 100 µl TriniCLOT PT Excel → then immediately press manual start button to measure coagulation.
   c. Thrombin time (TT)
      i. Add 100 µl citrate plasma to the coagulometer and press incubation.
      ii. After 60 sec incubation, add 100 µl Thrombin reagent → then immediately press manual start button to measure coagulation.
   d. Activated partial thromboplastin time (APTT).
      i. Add 100 µl citrate plasma to the coagulometer and press incubation.
      ii. After 60 sec incubation.
      iii. Add 100 µl DAPTTIN TC.
      iv. Alter 200 sec incubation, add 100 µl 30 mM $CaCl_2$→ then immediately press manual start button to measure coagulation.

Notes

1. This assay is very sensitive, so precise sample pipetting and timing of each step is crucial!
2. Prior testing coagulation time, test agent should be mixed with plasma or blood in an Eppendorf tube and incubate for a desired time.
3. 50 µl citrate plasma is the minimal volume to use or the optimum in 100 µl.
4. Use 1:1 volume ratio of the reagents or samples *e.g.* 100 µl plasma and 100 µl reagent/$CaCl_2$.

5. For coagulometer operation manual visit this link: http://www.merlinmedical.net/fileadmin/media/pdf/anleitungen/MC_10_OPERATION_MANUAL_ENGLISH.pdf

Acknowledgments

This protocol is adapted from Papareddy *et al*. (2013).

References

1. Papareddy, P., Kalle, M., Sorensen, O. E., Malmsten, M., Morgelin, M. and Schmidtchen, A. (2013). The TFPI-2 derived peptide EDC34 improves outcome of gram-negative sepsis. *PLoS Pathog* 9(12): e1003803.