Standardization of thrombin generation assays

Lupus anticoagulant / aPL

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Description Abstract

Background: Functional assays are promising tools for diagnosing a patient having the Antiphospholipid Syndrome (APS). It has been shown that by applying thrombin generation the prediction of thrombotic episodes can be improved. Several attempts have been made to standardize thrombin generation, predominantly the pre-analytical variables. The efforts to standardize pre-analytical variables have improved the lab-to-lab variability. In contrast, differences between manufacturers and reagents still exist and are responsible for a large part of the lab-to-lab variability.

Long term objective: In this project, we aim to investigate which TG assays are used and how they are used to clarify the lab-to-lab variability and ultimately to decrease the variability.

Specific aims:

(1) To address this question, we have first set up a survey to inventory the methods that are currently used, as well as the reagent sources and concentration and the experimental protocols used.

(2) To send a set of samples for TG measurement to different labs to determine the variation in TG measurements. The labs will use their own protocol and a pre-specified protocol to investigate whether this improves the variability of TG measurements by using a standardized protocol and standardized reagents.

(3) To investigate in which clinical settings TG is used for detection and prediction of diseases or monitoring treatment.

Design and methodology and Study population

For the initial survey (see attachment), we aim to include data collected from approximately 100 laboratories experienced in thrombin generation measurements. We will inventory the methods used and propose a protocol for the second and third aim in which we will invite as many labs as possible that also took part in the first aim to measure thrombin generation in shipped samples with the lab-specific protocol and the newly proposed standardized protocol.

Expected timeline:

Project stage/set up	01-10-2017
Launch	01-03-2018
Duration	01-09-2020

Finalization/analysis	01-03-2021
Reporting	01-09-2021

Expected outcomes (ie. publications):

Every aim will result in a publication and a recommendation manuscript.

Description of project set/up and management, needed infrastructure and resources (summary):

For the surveys (aims 1 and 3), we require SSC support for survey development, distribution, and result summarization. For the shipment of test and validation samples (aims 2 and 3) we would like to request financial support from the SSC and we will also try to obtain financial support for this specific aim via other sources.

SSC working party on thrombin generation

A survey

Thrombin generation is widely used for years in research, hemostasis phenomena understanding and sometimes in the clinical practice. The test is now gradually moving into the clinical lab as fully automated analyzers are becoming available.

Several commercials methods exist for the measurement of thrombin generation and in-house protocols are being used as well. This survey aims at making an inventory of the current (pre-analytical and analytical) practices used to measure TG, with the final goal being the development of recommendations for the standardized measurement of thrombin generation.

PART 1: The participant

1. Which type of laboratory do you run?
   A. Research lab
   B. Diagnostic lab
   C. Manufacturers lab
   D. Other (please specify)

2. In how many samples do you measure thrombin generation each year?
   A. <100
   B. 100-300
   C. 300-500
   D. 500-1000
   E. >1000

PART 2: Commercial methods

Several assay methods for the measurement of thrombin generation are commercially available and several labs use in house methods. This first section of the questionnaire aims at identifying which methods are currently used across labs.

3. Which method is used in your laboratory?
   A. Siemens Healthcare Diagnostics – Endogenous Thrombin Potential
   B. Thrombinoscope/Diagnostica Stago - Calibrated Automated Thrombography
   C. Stago – ST Genesia
   D. Technoclone – Technothrombin thrombin generation assay
   E. Technoclone – Ceveron Alpha

F. In-house method
G. Other, please specify

4. Do you use this method with commercial or home-made reagents?
   A. Commercial
   B. Home-made
   C. Both

5. Which brand of commercial reagents do you use?
   A. Siemens Healthcare Diagnostics – Endogenous Thrombin Potential
   B. Thrombinoscope/Diagnostica Stago - Calibrated Automated Thrombography
   C. Stago – ST Genesia
   D. Technoclone – Technothrombin thrombin generation assay/Ceveron Alpha
   E. Other, please specify

6. Do you use the commercial reagents according to the manufacturer's protocol?
   A. Yes
   B. No – please specify what is changed and why (also see part 3 of this questionnaire)

PART 3: Samples

Thrombin generation can be measured in platelet poor plasma, platelet rich plasma and has also been described in whole blood. The pre-analytic handling of the blood samples is known to affect thrombin generation. This section inventories which type of samples are used across labs and how samples are currently being prepared and stored.

Type of samples

7. Which type of samples do you use for thrombin generation? (multiple answers possible)
   A. Platelet poor plasma (PPP)
   B. Platelet rich plasma (PRP)
   C. Whole blood

Sample collection

8. Which blood drawing system does your lab use? (please specify if possible)
   A. Straight needle draw through system: (please specify brand and needle diameter)
   B. Butterfly needle draw through system: (please specify brand and needle diameter)
   C. Intravenous catheter draw: (please specify brand and diameter)
   D. Intra-arterial catheter draw: (please specify brand and diameter)
   E. Other (please specify)

9. Which blood tubes does your lab use? (please specify if possible; multiple answers possible)
    A. Glass tube: (please specify brand and blood volume)
    B. Plastic tube (<5 ml): (please specify brand and blood volume)
    C. Plastic tube (>5 ml): (please specify brand and blood volume)
    D. Other (please specify)

10. Which type of anticoagulant do you use in the blood tubes for thrombin generation?
    A. Citrate: (please specify concentration)
    B. Citrate and corn trypsin inhibitor (CTI): (please specify concentrations)
    C. CTAD: (please specify concentrations)
    D. Other (please specify type and concentration)

11. Do you discard the first tube of blood or first milliliters?
    A. Yes
    B. No

Sample preparation

12. How is platelet poor plasma (PPP) prepared for thrombin generation methods in your lab?
    A. One centrifugation step (indicate amount of g, temperature, and duration)
    B. Two centrifugation steps (indicate amount of g, temperature, and duration)
    C. Other, please specify:

13. Do you use an ultracentrifugation step for PPP preparation?
    A. Yes (indicate amount of g, temperature, and duration)
    B. No

14. How is platelet rich plasma (PRP) prepared for thrombin generation methods in your lab?
    A. One centrifugation step (indicate amount of g, temperature, and duration)
    B. Other, please specify:
    C. Not applicable (lab does not use PRP)

15. Do you adjust for platelet number in PRP?
    A. Yes (please specify the targeted platelet platelet count)
    B. No

Sample storage

16. How long do you keep whole blood samples minimally before use or processing?
    A. 15 minutes
    B. 30 minutes

C. 1 hour
D. 2 hours
E. Other (please specify)

17. How long do you keep whole blood samples maximally before use or processing?
F. 1 hour
G. 2 hours
H. 3 hours
I. 4 hours
J. Other (please specify)

18. At which temperature do you store whole blood samples?
A. Room temperature
B. 37 °C
C. Other (please specify)

19. How long do you keep plasma samples maximally before use or freezing?
A. Less than 1 hour
B. 1 hour
C. 2 hours
D. 3 hours
E. 4 hours
F. More than 4 hours (please specify)

20. At which temperature do you store plasma samples before testing them (if not frozen)?
A. 4 °C
B. Room temperature
C. 37 °C
D. Other (please specify)

21. How do you freeze fresh plasma samples?
A. Snap freeze
B. Place directly in freezer
C. Other (please specify)

22. How do you store frozen plasma samples?
A. -20 °C
B. -80 °C
C. Liquid nitrogen
D. Other (please specify)

23. For how long do you maximally store frozen samples?
A. 1 month
B. 6 months
C. 1 year
D. 2 years

E. 5 years
F. Other (please specify)

24. How do you thaw samples?

A. Room temperature
B. 37 °C
C. Dry heating block (please specify temperature)
D. Warm water bath (please specify temperature)
E. Other (please specify)

25. For how long do you thaw samples?

A. Certain amount of time (please specify)
B. Not standardized
C. Other (please specify)

PART 4: Additional questions for in-house or adapted commercial methods

Please only fill out this part of the questionnaire if you use an in house method or have adapted a commercial method.

As many labs use an in house TG method or have adapted a commercial method, this section focuses on the reagents and protocols used in adapted TG assays.

Triggers | Source and concentration

26. Which type of triggers do you use in your lab (multiple answers possible)?

A. Tissue factor
B. Tissue factor + phospholipids
C. Phospholipids
D. Kaolin
E. FIXa
F. FXIa
G. Other (please specify:_____)

27. Which source of tissue factor does your lab use?

A. Human
B. Bovine
C. Recombinant
D. Other (please specify:_____)

28. Which source of phospholipids does your lab use?

A. Synthetic
B. Soy
C. Other (please specify:_____)

29. Which concentration of tissue factor does your lab use (please indicate final concentrations)?

30. Which concentration of phospholipids does your lab use (please indicate final concentrations)?

31. Which composition of phospholipids does your lab use (multiple answers possible)?
    A. PS-PC-PE (20%-60%-20%)
    B. Other (please specify:_____)

Substrates | Type and concentration

32. Which type of substrate does your lab use?
    A. Z-Gly-Gly-Arg-AMC
    B. H-β-Ala-Gly-Arg-pNA
    C. Other (please specify:_____)

33. Which substrate concentration does your lab use (final concentration)?
    A. 417 µM
    B. 500 µM
    C. 1 mM
    D. Other (please specify:_____)

Plasma | volume and dilution

34. Do you predilute plasma samples before measuring thrombin generation?
    A. Yes (please indicate dilution ratio: _____)
    B. No

35. Which is the final plasma dilution in the assay?
    A. 40 %v/v plasma
    B. 52 %v/v plasma
    C. 67 %v/v plasma
    D. Other (please specify:_____)

Calibration method

36. Which calibration methods does your lab use?
    A. Calibration with α2M-thrombin (CAT Thrombin Calibrator)
    B. Calibration by adding a fixed amount of fluorophore
    C. Calibration by a fixed amount of thrombin in buffer
    D. Other (please specify:_____)

37. In how many replicates do you measure TG?
    A. Duplicate
    B. Triplicate
    C. Other (please specify)

Temperature control

38. Do you perform thrombin generation measurements at 37 °C?
    A. Yes
    B. No

39. Do you preheat the sample and trigger solution before the start of the TG measurement?
    A. Yes
    B. No

40. How do you preheat sample and trigger solution before the start of the TG measurement?
    A. In the measuring device
    B. Other equipment (please specify)
    C. Not applicable

Equipment

41. Which device is used in your lab to measure thrombin generation?
    A. Behring Coagulation System
    B. Ceveron Alpha
    C. Fluoroskan Ascent (CAT method)
    D. ST Genesia
    E. Microplate reader (Technothrombin TGA), please specify: _____
    F. Other (please specify:_____)

PART 5: Analysis and interpretation

This section inventories whether data analysis is done using commercial software or manual calculation methods, and which TG parameter(s) are most often used in the research setting.

42. Which software do you use to analyze thrombin generation data?
    A. BCS embedded software
    B. Ceveron Alpha embedded software
    C. Thrombinoscope software
    D. ST Genesia embedded software
    E. Technothrombin spreadsheet
    F. In house method
    G. Other (please specify:_____)

43. Which TG parameters do you generally use for further analysis (multiple answers possible)?
    A. Lag time / T_{lag}
    B. Time-to-peak / T_{max}
    C. Velocity index
    D. Peak height / C_{max}
    E. Endogenous thrombin potential/Area Under The Curve
    F. Start tail
    G. Other (please specify:_____)

44. Do you calculate reference values?
    A. Yes
    B. No

45. How many samples do you use to determine reference values? (please indicate number of samples)

46. How do you calculate reference values? (please describe statistical method used)

PART 6: Normalization and quality control

This section concerns the use of data normalization, for example using a reference plasma, and the performance of quality control.

47. Do you normalize the obtained TG data?
    A. No
    B. Yes, we compare the results to results in a normal plasma measured in the same experiment
    C. Yes, we compare the results to results in a normal plasma measured in another experiment
    D. Other normalization method (please specify:_____)

48. Which type of reference plasma do you use to normalize data?
    A. None
    B. Healthy donor sample
    C. In house frozen pooled normal plasma
    D. Commercial frozen pooled normal plasma (please specify brand)
    E. Commercial freeze-dried sample (please specify brand)

49. Do you participate in external quality assessment for thrombin generation measurements?
    A. Yes (please specify which program)
    B. No

PART 7: Clinical use and parameter of choice in specific conditions

This section concerns the use of thrombin generation and specific TG parameters in the clinical setting.

50. Do you use thrombin generation as an aid in the management of patients (multiple answers possible)?
    A. No
    B. Yes, for bleeding management
    C. Yes, for thrombotic disorder management
    D. Yes, for anticoagulant management
    E. Yes, other situations (please specify the clinical situation and how TG results are used)

51. Which TG parameters do you use in the management of patients (multiple answers possible)?

	Lag time / T_{lag}	Time-to-peak / T_{max}	Peak height / C_{max}	Endogenous thrombin potential/Area Under The Curve	Velocity index	Start tail	Other (please specify)
Bleeding Management							
Thrombotic disorder management							
Anticoagulation management							
Other situation (please specify)							

52. Do you compare thrombin generation with other assays?
    A. No
    B. Yes, with thromboelastography
    C. Yes, with other assays (please specify)