Editorial

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On the complexity of hemostasis and the need for harmonization of test practice

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Hemostasis=Love. Everyone talks about it, but no one understands it.

Hemostasis, a homeostatic process that is meant to prevent blood loss, is achieved by the interaction of various systems, sometimes opposing (or acting to balance pro- and antihemostatic forces) [1]. Although hemostasis is sometimes also called “coagulation” by some people, this term actually reflects only a small part of hemostasis. Hemostasis comprises four main “systems”, although it is impossible to fully separate any from another. The four systems are “primary hemostasis”, “secondary hemostasis”, “anticoagulation system” and “fibrinolytic system” [1, 2]. The “primary hemostasis” system is mostly driven by the interaction of blood platelets, adhesive plasma proteins including von Willebrand factor (VWF) and the (damaged) vessel wall comprising endothelium and sub-endothelial matrix components. The “secondary hemostasis” system is mostly driven by “sequential” activation of procoagulant proteins (the so-called “coagulation cascade”) causing generation of thrombin and eventual conversion of fibrinogen to fibrin (the so-called “clot”). The “anticoagulation system” aims to control the process of “secondary hemostasis”, so that it does not progress irrevocably, in which case a thrombosis may occur and lead (for example) to deep vein thrombosis (DVT) or pulmonary embolism (PE). The “fibrinolytic system” acts to further control “secondary hemostasis”, and also to dissolve any clot formed to enable tissue repair. A breakdown in any of these systems will predispose to either bleeding or thrombosis, depending on what element of the process has failed.

As mentioned, “coagulation” is only a part of hemostasis, and essentially only reflective of the “secondary hemostasis” system. This system comprises many coagulation proteins, perhaps most well-known being factors VIII (FVIII) and IX (FIX), the deficiency of which will, respectively, cause hemophilia A and B, thus reflecting the most common inherited bleeding disorders related

to secondary hemostasis [3]. The term “coagulation” (or “coag”) laboratory usually refers to laboratories that are capable of performing (a limited number of) “routine” tests of hemostasis, as listed in Table 1.

The four hemostasis systems mentioned are not independent of each other, and all processes intertwine. For example, although VWF is primarily involved in “primary hemostasis”, because it binds to, and protects, and also delivers FVIII to sites of vascular damage, it is also integral to the success of “secondary hemostasis”. This is why some patients with deficiency or defect in VWF, thereby suffering von Willebrand disease (VWD), may experience bleeding considered due to defects in “primary hemostasis” as well as “secondary hemostasis” [4]. Similarly, fibrinogen is primarily involved in “secondary hemostasis”, generating the clot after conversion to fibrin. However, fibrinogen can also bind to activated platelets and, thus, contribute to “primary hemostasis”.

The modern hemostasis laboratory has an arsenal of tests it can perform for investigation of hemostasis (Table 1). With these tests, laboratories can help diagnose bleeding disorders such as hemophilia or VWD, explain the occurrence of familial thrombophilia (e.g. deficiency in proteins of the anticoagulant system) or manage patients under therapy (e.g. patients being treated for a DVT with vitamin K antagonist [VKA] therapy). However, the ability of laboratories to help clinicians diagnose hemostatic “disease” or better manage patients is compromised by lack of standardization and harmonization. Different laboratories may have the appearance of having the “same tests” in their repertoire, but given the high variety of test reagents and methods available for any given test, it is unlikely they will all perform these test in the same way. This variability will compromise patient management because different test results can arise from testing the same sample. As test result will typically direct further clinical management, different data may lead to different clinical actions.

Moreover, even using the same test reagents and instruments can still lead to differences in test results.

Table 1: A summary of hemostasis tests, variables, and why harmonization is required.

Test name	Abbreviation	What test measures	Test variables	Examples of why standardization and harmonization may improve test reporting
Prothrombin time	PT	Tissue factor pathway of coagulation; sensitive to factors II, V, VII, X, II, I	Different reagents and instruments will yield different test times. Also, variable sensitivity to certain factors	Will help standardize test results between instruments and reagents and thus across different laboratories. Most common process is to create a PT ratio (e.g. as part of liver disease MELD score) or convert to an INR (for monitoring of VKA therapy). Such modifications provide a more standard means of prioritizing patients for liver transplant MELD score or reduce variability in VKA therapy (INR)
Activated partial thromboplastin time	APTT	Contact factor pathway of coagulation; sensitive to factors XII, XI, IX, VIII, V, X, II, I	Different reagents and instruments will yield different test times. Also, variable sensitivity to certain factors, heparin and lupus anticoagulant (LA)	Will help standardize test results between instruments and reagents and thus across different laboratories. One means to help standardization is to create an APTT ratio. Another is to test and group reagents according to various sensitivities. For example, LA sensitive vs. LA insensitive reagents can be defined and partnered for investigation of LA
International normalized ratio	INR	PT conversion using mathematical formula (INR = [patient PT/MNPT] ^{ISI})	PT, mean normal PT (MNPT) and international sensitivity index (ISI)	The INR system represents a harmonization process for the PT, which takes into consideration the test reagent and instrument used. However, different laboratories identify different MNPT and ISI values for the same reagent/instrument used because they use different ways of deriving these values. Thus, further harmonization of practice is needed to further reduce INR variation and improve VKA therapy
Fibrinogen	fib	Fibrinogen. Potentially sensitive to afibrinogenemia dysfibrinogenemia hypofibrinogenemia	Test methodology – von Clauss, derived or antigen	Functional assays using a von Clauss procedure are the most relevant assays, and thus harmonization to such assays is preferred. Derived assays are cheaper, and so may be offered instead. Antigenic assays only useful in select situations (e.g. to identify dysfibrinogenemia)
D-dimer	D-D	Fibrin breakdown products containing D-D domains. Potentially applicable to investigation of DVT, PE, DIC	Test methodology (reagents) and reporting units	Different test reagents measure D-dimers differently (i.e. variably sensitive to D-dimer fragments), thereby yielding different values for the same sample, and potentially different conclusions regarding “positive” findings (e.g. suggestive of thrombosis or not), and therefore leading to different clinical interventions. Reporting of different units complicates comparison between research studies and some clinicians may misunderstand whether a sample is positive or not. Harmonization will improve clinical management as it will decrease variability of measurement and response to test results
Thrombin time	TT	Fibrinogen conversion to fibrin. Potentially sensitive to afibrinogenemia dysfibrinogenemia hypofibrinogenemia and anticoagulants, including unfractionated heparin and dabigatran	Different reagents and instruments will yield different test times	Will help standardize test results between instruments and reagents and thus across different laboratories. This will improve clinical patient management
Protein C	PC	Congenital deficiencies in PC	Functional clotting and/or chromogenic assays. Chromogenic assays are preferred. Clotting assays may be sensitive to factors other than PC	Will help standardize test results between across different laboratories, and improve identification/exclusion of PC deficiency

Table 1 (continued)

Test name	Abbreviation	What test measures	Test variables	Examples of why standardization and harmonization may improve test reporting
Protein S	PS	Congenital deficiencies in PS	Functional clotting and/or antigenic assays. Antigenic assays for Free PS are preferred. Clotting assays may be sensitive to factors other than PS	Will help standardize test results between across different laboratories, and improve identification/exclusion of PS deficiency
Antithrombin	AT	Congenital deficiencies AT	Chromogenic or antigenic assays. Chromogenic assays are preferred. Antigenic assays may not identify functional defects in AT	Will help standardize test results between across different laboratories, and improve identification/exclusion of AT deficiency
Activated protein C resistance	APCR	Congenital or acquired thrombophilia (APCR, FVL)	APTT or RVVT based assays. These have different sensitivities to APCR and FVL	Will help standardize test results between across different laboratories, and improve identification of thrombophilia
Anti-Factor Xa	Anti-Xa	Monitoring LMWH, or measuring the level of apixaban and rivaroxaban	Chromogenic assay using assay specific calibrators	Will help standardize test results between across different laboratories, and improve clinical patient management
Dilute thrombin time	dTT	Measuring the level of dabigatran	Functional clotting assay	Will help standardize test results between across different laboratories, and improve clinical patient management
Dilute Russell's Vipervenom time (screen and/or confirm)	dRVVTsc, dRVVTcon	Identification of LA	Functional clotting	Will help standardize test results between across different laboratories, and improve identification of thrombophilia
Silica clotting time	SCTsc, SCTcon	Identification of LA	Functional clotting	Will help standardize test results between across different laboratories, and improve identification of thrombophilia
VWF:Antigen	VWF:Ag	Measures the quantity of VWF protein (not its functionality)	ELISA, LIA, or CLIA assay	Will help standardize test results between across different laboratories, and improve the diagnosis and management of VWD
VWF:Ristocetin co-factor	VWF:RCO	Identification of Type 2 VWD; sensitive to the loss of the HMW forms of VWF; sensitive to VWF mutations affecting GPIb binding	Assesses the ability of VWF to bind to platelets (via GPIb) in the presence of ristocetin. Usually platelet aggregation or LIA assay	Will help standardize test results between across different laboratories, and improve the diagnosis and management of VWD
VWF:Collagen binding	VWF:CB	Identification of Type 2 VWD; sensitive to the loss of the HMW forms of VWF; sensitive to VWF mutations affecting collagen binding	Assesses the ability of VWF to bind to sub-endothelial matrix proteins (namely collagen). ELISA or CLIA assay	Will help standardize test results between across different laboratories, and improve the diagnosis and management of VWD
VWF:Activity	VWF:Act	Identification of Type 2 VWD; may be sensitive to loss of HMW forms of VWF; may be sensitive to various VWF mutations affecting VWF function	Generic term for measurement of VWF "activity" that otherwise not defined by specific activity. Usually ELISA or LIA assay	Will help standardize test results between across different laboratories, and improve the diagnosis and management of VWD

Table 1 (continued)

Test name	Abbreviation	What test measures	Test variables	Examples of why standardization and harmonization may improve test reporting
VWF:FVIII binding	VWF:FVIIIIB	Identification or exclusion of Type 2N VWD; sensitive to loss of VWF-FVIII binding function	Usually an ELISA based assay	Will help standardize test results between across different laboratories, and improve the diagnosis and management of VWD
VWF multimers	NA	Assesses structural composition/ defects of the VWF molecule	Immunological assay (complex assay consisting of several steps). Typically gel electrophoresis	Will help standardize test results between across different laboratories, and improve the diagnosis and management of VWD
Platelet function screen	PFA-100/200	Identification of VWD, platelet function defects or antiplatelet medications	PFA-100 or PFA-200 instrument	Will help standardize test results between across different laboratories, and improve the identification/exclusion of VWD and platelet dysfunction
Platelet function testing	NA	Identification and characterization of VWD, platelet function defects or antiplatelet medications	Light transmission or whole blood aggregometry	Will help standardize test results between across different laboratories, and improve the identification/exclusion of platelet disorders
Factor assays	FII, FV, FVII, FVIII, FIX, FX, FXI, FXII	Identification of hemophilia (FVIII, FIX), or other specific factor deficiencies	Clotting assays (modification of PT or APTT assays); chromogenic assays for FVIII and FIX	Will help standardize test results between across different laboratories, and improve the identification/exclusion of hemophilia and other bleeding disorders/factor deficiencies
Factor inhibitor	NA	FVIII or FIX (or other factor) inhibitors	PT/APTT based Bethesda or Nijmegen modification assays	Will help standardize test results between across different laboratories, and improve the identification/exclusion of acquired hemophilia

CLIA, chemiluminescence assay; DIC, disseminated intravascular coagulation; DVT, deep vein thrombosis; ELISA, enzyme-linked immunosorbent assay; FVL, factor V Leiden; HMW, high molecular weight; ISI, international normalized ratio; LIA, latex immune assay; LMWH, low molecular weight heparin; MNPT, mean normal prothrombin time; PE, pulmonary embolism; UFH, unfractionated heparin; VKA, vitamin K antagonist.

As a pragmatic example, consider the routine tests “prothrombin time” (PT) and “activated partial thromboplastin time” (APTT). The PT is used for several reasons, including evaluation of the tissue factor pathway of coagulation, for prioritizing liver transplantation in end stage liver disease (usually as a PT ratio of patient PT/normal PT) and for monitoring patients on VKA therapy (usually as an international normalized ratio [INR]) (Table 1). The APTT is also used for several reasons, including evaluation of the contact factor pathway of coagulation, as a screen for hemophilia, for identification of lupus anticoagulant (LA; a prothrombotic disorder) and for monitoring of anticoagulant therapy with unfractionated heparin. First, different reagents and different instruments, and the arising various combinations, can yield vastly different PT and APTT values among laboratories. Second, although normalizing test values by use of test ratios (as a form of test harmonization) or producing an INR will help reduce this variability, it will not completely eliminate it. Indeed, laboratories using the same instrument and the same test reagent (even the same lot of reagent) may still generate highly disparate INR values, simply because they have calculated the components of the INR equation (namely, mean normal prothrombin time [MNPT] and international sensitivity index [ISI]) in different ways [5, 6]. For patients on VKA therapy, the recommended INR is usually around 2.0–3.0 for most indications. In some cases, as identified in external quality assessment (EQA) surveys, different laboratories can yield differences in INR that can range from 2.4 to over 6.0 for the sample homogenous sample [5],

as also shown in Figure 1. A value of 2.4 would be considered therapeutic, and no clinical intervention would be needed (the patient would likely continue the same dose of VKA until next testing). However, an INR value of 6 is typically considered an alert (critical) value [7] and would suggest clinical intervention – reducing dosage and possibly close monitoring for potential bleed events. Although the wide range of reported INRs is influenced by outlier INRs, most INRs fall in the region of 3.3–4.5 for this example, which may still represent different clinical responses. As explanation for the wide INR range, it can be reflected that participant reported ISI and MNPT values were also widely variable and could have theoretically led to the wide INR values, as highlighted in Figure 1. Similarly, for the APTT, a heparin therapeutic range can be exceeded or not for the same sample as tested in different laboratories [8]. Moreover, the new direct oral anticoagulants may also (variably) affect routine coagulation tests such as PT and APTT, and/or these tests may be used to help identify the presence or absence of such drugs [9, 10].

As another example, D-dimer testing is plagued not only by differences in test reagents and methods, leading to differences in test results for the same sample, but also by inconsistency in reporting units [11, 12]. In fact, 28 different combinations of units are feasible, thereby acting to considerably confuse clinicians who order these tests and thereafter manage patients. Notably then, based on differing reagents, the same sample may be identified as “positive” or “negative” for D-dimer based on different test values. However, even with the same reagent,

Figure 1: An example of external quality assessment (EQA) for an individual sample as assessed in a local EQA program in Australia (the RCPAQAP Hematology [7]).

A wide range of INR values was reported by laboratories for the same homogeneous sample.

the same sample may also be interpreted as “positive” or “negative” by different clinicians for the same numerical value, depending on their experience/inexperience with the reported unit [13].

Another final example is VWF testing for VWD diagnosis/exclusion. VWF is a large and complex protein with multiple functionalities, including binding to platelets, subendothelial matrix components such as collagen, and binding to FVIII (thereby protecting it from degradation and also delivering it to sites of injury). VWD can arise from defects in any of these activities. Thus, identification/exclusion/characterization of VWD requires the use of multiple assays [14]. This may not seem a problem because indeed an arsenal of potential assays exist in laboratories (Table 1). However, each of these assays measures different aspects of VWF (level/different activities), and laboratories may use many different combinations of assays as test panels or may not understand strengths and limitations of the assays they use individually or in composite, thereby leading to many (potentially avoidable) errors in VWD diagnosis [15]. Moreover, these same assays may be used to monitor therapy in VWD, with the same limitations applied in terms of current knowledge, and corresponding adverse outcomes in regards to differential therapies [4, 16].

Standardization and harmonization is intended to reduce the variability of laboratory test practice and, thus, to provide more consistent standards of health care. In this issue of the journal, there are several papers describing various initiatives that attempt to drive standardization and harmonization of hemostasis test practice at local, regional or international levels [17, 18].

Hemostasis is complex. Standardization and harmonization aims to make understanding hemostasis less complex and tests of hemostasis more useful. We hope readers enjoy this issue of the journal, and in particular, the papers related to harmonization of hemostasis test practice.

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