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The Myths Behind DOAC Measurement: Analyses of Prescribing Information from Different Regulatory Bodies and a Call for Harmonization

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Abstract

For more than a decade, US laboratories have failed to implement solutions to help their clinicians in managing complex situations or patients on DOACs. The problem may find different origins among which the position of the Food and Drug Administration, which categorized these drugs as monitoring- and measurement-free, while other regulatory bodies like the European Medicines Agency or the Therapeutic Goods Administration in Australia were more conservative on the principle that the absence of proof (of monitoring/measurement benefits) is not proof of an absence (of monitoring/measurement needs). Pivotal clinical studies which led to the approval of DOACs were presented as devoid of such testing although some companies considered monitoring as a solution to improve their benefit/risk ratio. In this JTH In Clinics issue, we report more than a decade of development which has permitted the activation of smart laboratory solutions to qualify or quantify DOACs and discuss on myths and misconceptions around technical and regulatory requirements that support the current reluctance of implementing these technologies in most US laboratories. Use of DOACs is ever expanding, with DOAC prescriptions now exceeding those of other anticoagulants, including VKA, in some geographies. As this use increases, the likely need to measure DOAC exposure will also increase. Measurement of DOACs does not represent any technical difficulty. That these laboratory tests are not available in some geographies suggests disparities in patient care, and we suggest it is time to address such disparities.

Keywords:

DOAC, anti-Xa, anti-thrombin, monitoring, prescribing information, regulatory bodies

Introduction

For hemostasis testing, there are documents related to technical practice standards or guidelines developed by organizations such as British Society for Haematology (BSH), Clinical and Laboratory Standards Institute (CLSI), and International Council for Standardization in Haematology (ICSH) to name a few, but there is no real guidance for laboratories treating the *why* or *when* to implement a clinical test that will assist the clinician for the management of a patient. Historically the decision to implement laboratory services was based on clinician demand, changes in the standard of care, or technological advances that increase diagnostic sensitivity, specificity and related (positive/negative) predictive values. A common benchmark used by administrators for determining new test needs is by searching for past year number of requests for a given test. However, that benchmark may not be an accurate assessment of clinical need, especially if the test turnaround-time result is not sufficient for acute management decisions. Practice guidelines related to disciplines (e.g., surgery, trauma, etc.) may also provide recommendations for hemostasis testing for which the local/institutional stakeholders would request these assays or methods be available for their patient management. A less favorable or successful approach to the laboratory would be citing case reports, or research methods that may provide insufficient evidence for implementation of a new test or method. Cost considerations are often a primary issue for the clinical laboratory, as well as required instrumentation to perform any new services, where the use of existing equipment would likely accelerate a new test implementation rather than new equipment purchase requirements.

Specific to this manuscript is *why* or *when* should a laboratory consider providing services for patients receiving anticoagulation therapy that do not require routine monitoring. For decades we have monitored unfractionated heparin (UFH) infusions, with dose adjustment based on the reported activated partial thromboplastin time (aPTT) and in some institutions, the anti-FXa activity.[1-4] Likewise, vitamin K antagonists (VKA) have been monitored using the prothrombin time (PT)/International Normalized Ratio (INR) for nearly 30 years in the US, and longer elsewhere.[5] Adjustment of daily VKA dose was primarily based on the patient's INR result with dietary intake, and other factors also considered. When low molecular weight heparin (LMWH) became available for clinical use (1993 in the US), the drug prescribing information (PI) indicated, "*There is usually no need for daily monitoring of the effect of Lovenox® in patients with normal presurgical coagulation parameters*."[6] However, notable in more recent enoxaparin labeling are pharmacokinetic (drug level) data, and subsequent iterations suggesting monitoring of anti-FXa may be warranted in certain populations including renal insufficiency, abnormal

laboratory coagulation results or bleeding.[7] When LMWH anticoagulation was available for the pediatric population, guidance suggesting the use of anti-FXa monitoring and dose adjustment algorithms soon followed.[8]

Today the clinical laboratory is faced with a familiar dilemma with the direct oral anticoagulants (DOACs) comprising a direct thrombin inhibitor, dabigatran etexilate and 3 direct factor Xa (FXa) inhibitors, apixaban, edoxaban and rivaroxaban. DOACs have predictable pharmacokinetics and pharmacodynamic properties at fixed doses for approved indications, and do not require routine monitoring to the same context as previous oral anticoagulants.[9] However, soon after their approval, it became evident that having some capacity to measure (quantify) or detect (qualify) DOACs could be beneficial to address acute situations such as trauma, emergent surgery, neuraxial anesthesia, acute stroke, and others.[10] Certain patient populations such as the frail elderly, renal impairment, extreme body weight, drug overdose or interactions may also benefit for such assessment.[11]

What is the Role of the Laboratory in DOAC Therapy?

The US FDA definition of a laboratory is a facility that provides, "….*information for the diagnosis, prevention, or treatment of any disease or the impairment of, or assessment of the health of human beings*."[12] Similarly, International Organization for Standards (ISO) 15189:2012 defines the role of a clinical laboratory as providing, "…*examination of materials derived from the human body for the purpose of providing information for the diagnosis, management, prevention and treatment of disease…".*[13] Clearly, one laboratory responsibility would be to provide the necessary tests for patient management and treatment, the salient question being what are the necessary tests?

In DOACs, there is a chasm between intended use of the drugs with the advertised lack of requiring continuous or episodic monitoring and the reality of needing to measure DOAC levels in acute clinical settings.[14-19] Routine coagulation tests that have historically been useful and successful for assessing anticoagulation, such as the PT and aPTT no longer serve as a warning beacon in the age of DOACs as their insensitivity or variable sensitivity to DOAC exposure limits their utility.[20-24] Other routine, albeit perhaps less commonly available, coagulation tests available for clinicians would be the thrombin time (TT) or anti-FXa.[25, 26] The TT is highly sensitive to dabigatran levels and while a normal TT can exclude the presence of dabigatran, the TT cannot be reliably used to estimate or measure drug concentration unless the test is modified.[27] Anti-Xa testing using LMWH calibration curves can provide both an indication of FXa DOAC presence, and if properly assessed by the laboratory can be used to estimate direct

FXa inhibitor anticoagulant intensity, although difference between kits were noted.[26-29] Therefore, TT and anti-FXa could be used to indicate DOAC presence or absence if drug-specific testing is not locally available. This information may be of value in emergent cases where clinical history is not readily available and acute intervention is required (e.g. trauma, surgery, acute stroke).

The additional issue is whether the coagulation laboratory should provide DOAC PK (i.e. drug concentration) measurements. The current absence of strong evidence provided by clinical studies to support the monitoring or measurement of DOAC levels may seem a limiting factor for test implementation, even in off-label uses of these drugs or drug use in special populations such as the frail elderly, morbidly obese, and others.[30] Nevertheless, the absence of evidence is not the same as the evidence of absence and more and more scientific data support the assumption that there is room for improvement in decreasing the number of bleeds (and herewith associated mortality, comorbidity, and health-care costs) among patients who are anticoagulated with DOACs.[17, 31, 32]

Another point to consider is the reversal of these anticoagulant agents. Reversing DOACs using specific reversal therapies in acutely bleeding patients are based on time from last dose [33] but estimating drug levels may be useful in some indications to determine the need for a reversal.[34, 35] A single dose of Praxbind® neutralizes approximately 1,000 ng/mL of dabigatran but several reports showed that this may not be sufficient in certain cases, suggesting that measurement may be of interest should the standard dose be inadequate to reverse the entire effect of dabigatran.[36, 37] Andexanet alfa is a potential reversal agent for anti-Xa DOACs. In the ANNEXA-4 trial, subjects with acute bleeding events with rivaroxaban or apixaban levels of >75 ng/mL were eligible for enrollment, suggesting lower DOACs exposure would not require this reversal agent.[38] Nevertheless, the andexanet alfa PI does not advocate pretreatment FXa DOAC levels, even if noted exceptions for drug efficacy was reported for select patients with high baseline values.[39-41]

Such situations are not isolated and as mentioned, several cases reports mentioned dabigatran exposure as high as 3000 ng/mL and rivaroxaban exposure as high as 2500 ng/mL.[36, 42] This is not so occasional, in our own laboratories, levels close to 2500 ng/mL have also been measured in some patients. Thus, in patients with high DOAC exposure, additional reversal doses may be required as suggested in the PI of Praxbind®.[43] A cautionary note is warranted about post-reversal DOAC measurements, as rebound of dabigatran concentration after Praxbind® administration has been described [44] and factitious (falsely increased FXa DOAC level) due to in vitro dissociation of andexanet alfa with anti-FXa methods that utilize

a high pre-dilution of the sample.[45] Post-treatment anti-FXa measurements as a surrogate of hemostatic efficacy has not been demonstrated.[39]

Drug Prescribing/Labeling Information – Is This a Valuable Resource for Clinicians and Laboratorians?

There are noted differences in the regional DOAC PI despite obtaining the data from the same clinical trials (**Supplemental Tables 1-4**). The US PI has laboratory testing addressed in sections 5.2 or 5.3 (Risk of Bleeding), 8.6 (renal impairment), 10 (Overdose), 12.2 (Pharmacodynamics) or 12.3 (Pharmacokinetics) for Factor Xa DOACs, whereas section 2.4 (Dosage adjustments), section 10 (Overdose) and section 12.2 (Pharmacodynamics) have laboratory information related to dabigatran. Ironically for some recommended laboratory tests, there is no Food & Drug Administration (FDA) approved methods for those cited measurands, thus requiring laboratories to develop in-house or laboratory developed tests (LDT). The EMA provides the healthcare providers with highly detailed and specific laboratory information related to coagulation testing and DOACs via the European Summary of Product Characteristics (Eu-SmPC). This information can be found in section 4.4 (Special Warnings and Precautions of Use), section 4.5 (Interaction with Other Medicine), section 5.1 (Pharmacodynamic Properties) and section 5.2 (Pharmacokinetic Properties). The approval procedure has been centralized for the European market and thus national competent authorities must provide their healthcare professionals with the latest information provided by the EMA ensuring a common distribution of the knowledge in the European Union. Additional information is also easily accessible in the different assessment reports available on the EMA website. Unique to the United Kingdom DOAC PI are the listing of National Health Service drug prices defined as "basic costs" listed by dose and tablet quantity per carton. These PIs also referred to the SmPC, available on the website "www.medicines.org.uk/emc". The Canadian monographs serve as the source of PI, which contains 3 parts: I Health Professional Information; Part II Scientific Information and Part III: Consumer Information. Parts I and II contain subsections that are represented by capitalized, bold-faced headers without numeric assignments. For all DOAC monographs, there is a dedicated section entitled Monitoring and Laboratory Test under the Warnings and Precautions subsection of Part I, with additional information sprinkled throughout sections related to Adverse Events, Dosing and Administration, Drug Interaction, Action and Clinical Pharmacology or Detailed Pharmacology. The Australian PI is derived from the information reviewed in the Australian Public Assessment Report (AusPAR), approved by the Australian Therapeutic Goods Administration (TGA), and provided by through the Monthly Index of Medical Specialties (MIMS). The AusPAR is equivalent to Eu-SmPC and provides laboratory details in

sections Precautions, Interactions with Other Medicines and Pharmacology. The MIMS PI is similar to other regional prescribing information with laboratory information provided in Pharmacology sections 4.4 (Special Warnings and Precautions), 4.5 (Interaction with Other Medicines), 5.1 (Pharmacodynamic Properties) or 5.2 (Pharmacokinetic Properties).

For dabigatran, the US PI provides aPTT, and ecarin clotting time (ECT) trough PK data in patients treated with NVAF and expected trough levels in adult and pediatric populations for VTE treatment (**Supplemental Table 1**).[46] The US PI does not list any PD test associated with bleeding risk. The Canadian monograph provides peak and trough PK levels for 2 dose treatment in NVAF.[47] The Eu-SmPC provides peak and trough PK levels (and some PD values) for VTE prevention, NVAF, VTE treatment, and pediatric populations.[48] Additionally, the AusPAR, Canadian monograph and Eu-SmPC all indicate bleeding risks associated with dTT, ECT and aPTT (**Supplemental Table 2**).[47-49]

For rivaroxaban the AusPAR provides expected peak PD using PT for VTE prevention in THR and TKR, NVAF and DVT treatment and prevention of recurrent DVT and PE,[50] whereas the Canadian monograph provides both expected PD and PK values for the same dose regimens and indications [51] and the Eu-SmPC [52] reports 3 expected peak and trough PK and PD levels for stated doses and indications. It also reports plasma concentration in pediatric population (**Supplemental Table 3**).[52]

For apixaban, peak and trough PD (anti-FXa measurements using the Rotachrom® Heparin Chromogenic Assay, no longer commercialized) and PK (ng/mL measurements) information are available with Canadian monograph [53], AusPAR [54], and Eu-SmPC [55] for the prevention of VTE in THR and TKR, for NVAF and for the treatment DVT and the prevention of recurrent DVT and PE (**Supplemental table 4**).

For edoxaban, the Canadian monograph provides trough and peak levels observed for ENGAGE-AF and HOKUSAI VTE trials (**Supplemental Table 5**), as well trough levels in select subpopulations (renal function, weight, concomitant use of P-gp inhibitors, age, study locale, fragile patients) from ENGAGE-AF TIMI 48 clinical trial (**Supplemental Table 6**).[56] The Eu-SmPC of Lixiana® provided peak and trough anti-FXa measurements for 2 doses used for stroke prevention in NVAF and VTE prophylaxis and treatment (**Supplemental Table 7**).[57] Results are reported in IU/mL, as measured by the Rotachrom® Heparin Chromogenic Assay, if we refer to the published data of Ruff *et al.* for the NVAF indication.[58] No information is provided by the manufacturer in the Eu-SmPC for the anti-FXa kits that have been used in both the NVAF and the treatment and prevention of recurrent PE/DVT. Knowing the inter-kit variability,[28] this information is not relevant for the clinical practice. Edoxaban is not approved for use in Australia.

In contrast, the US PI does not provide any PD or PK data for rivaroxaban, apixaban or edoxaban.[59-61]

Does DOAC Label Influence a Laboratory Test Menu for DOACs?

It is unclear whether the information in drug PI (or lack thereof) represent drivers for clinicians to seek DOAC measurements. As previously noted, the decision for a clinical laboratory to implement new testing may be predicated on numerous factors. For anticoagulation monitoring, the PI, or sometimes referred to as drug labeling, may provide details about PK, PD, and impact on laboratory tests, and/or if monitoring is indicated or other precautions, but likely the clinical laboratory will respond to local clinicians needs. As seen with Lovenox® PI, when the PI started providing indications for drug monitoring (anti-FXa activity) and guidance documents reported same, there an increasing number of laboratories began reporting these assays.[62, 63]

There is a noted regional difference in DOAC testing availability for clinicians, and perhaps such testing availability is linked to regional DOAC PI. One indicator of testing availability in a given region is assessing External Quality Assurance (EQA) programs. EQA programs represent laboratory quality tools that assure the local laboratory meets performance expectations when testing blinded samples containing particular analytes and where the locally reported results are compared to peer groups. EQA programs detail the number of enrolled participants, the test methodology used, and statistical thresholds for performance for acceptability (pass) or not (fail). Comparing the enrolled participants for DOAC measurements, the US laboratories are lagging their Australian, Canadian, and European colleagues, in that approximately 1% of all the laboratories performing hemostasis testing (rivaroxaban reporting labs/PT reporting labs) are performing quantitative DOAC testing and ± 4% of the laboratories that perform anti-FXa testing are quantifying Factor Xa (FXa) DOACs (**Table 1**). In comparison, ± 29% of British laboratories and ± 65-70% of Australian and European laboratories that perform heparin anti-FXa testing also perform quantitative FXa DOAC measurements, when compared to methods that are existing for potentially measuring this class of drugs. To be clear, the only modification required to quantify FXa DOACs using existing anti-FXa test kits used for UFH or LMWH reporting would be a change in calibrators and controls.

Note that the External quality Control of diagnostic Assays and Test (ECAT) EQA program is primarily directed towards specialized coagulation laboratories and thus may not accurately reflect the percentage of laboratories performing DOAC testing in a restricted area like Europe for example. It is possible that some participants for these EQA programs lie outside their geographic region, thus biasing the data. For example, most US laboratories participate in the College of American Pathologist (CAP) EQA program for PT given the convenience but are likely enrolled in a different EQA program to satisfy other US regulatory requirements related to EQA testing.

The information provided in the US PI for rivaroxaban states, "*Monitoring for the anticoagulation effect of rivaroxaban using a clotting test (PT, INR or aPTT) or anti-factor Xa (FXa) activity is not recommended*."[60] For US PI of apixaban, similar verbiage is indicated with "…*monitoring for the anticoagulation effect of apixaban using a clotting test (PT, INR, or aPTT) or anti-factor Xa (FXa) activity is not useful and is not recommended*.",[59] although the PI does describe a linear relationship between anti-FXa measurements and drug concentration. For US PI of edoxaban, "*Changes observed in PT, INR, and aPTT at the expected therapeutic dose, however, are small, subject to a high degree of variability and not useful in monitoring the anticoagulant effect of edoxaban*."[61] but later indicates peak edoxaban concentration can be observed 1-2 hours after ingestion, but no PK data is provided. Searching US direct FXa inhibitors PIs for "ng/mL" or "mcg/L" yields no results. The lack of information provided by US PIs, in contrast to PIs from other regions, may be a confounder for the relative low proportion of US clinical laboratories providing quantitative DOAC measurements (**Table 1**).

For dabigatran, the only oral direct thrombin inhibitor, there is no comparable existing test that can be transitioned to quantifying this drug without significant modifications to the test profile or sample conditions. That said, approximately 1% (dabigatran reporting labs/aPTT reporting labs) of US laboratories provide dabigatran measurements, which is lower than that of UK and Australasia (both approximately 7%) (**Table 1**). Nearly 40% of the participants that report aPTT in the ECAT survey are also reporting dabigatran measurement, a sharp contrast with US laboratories, although this may reflect the bias of high numbers of specialised laboratories. Interestingly, dabigatran and edoxaban are less frequently selected by the specialized laboratories participating in the ECAT survey, probably reflecting the lesser demand for these measurements by the clinicians due to lower use of dabigatran and edoxaban in these regions. In the US PI for dabigatran, the laboratory-related information is limited. Section 2.4 (Dosage Adjustments) suggests, "*using the aPTT or ECT, but not INR for assessing dabigatran exposure*". For section 12 (Overdose), "*the measurement of aPTT or ECT may help guide therapy*." but provides no target ECT.

Section 12.2 (Pharmacodynamics) provides graphic representation of aPTT time course based on renal function, but further indicates the median aPTT in patients receiving 150 mg dose was 52 seconds (10^{th} – 90^{th} percentile aPTT of 40 – 76 seconds). Doing a more extensive investigation to the aPTT method used in the RE-LY trial, the reagent was described as containing "*cephalin and microcrystalline kieselguhr*", none of which are directly related to US available aPTT reagents.[67] The PI notes the aPTT test can provide "*an approximation of Pradaxa's anticoagulant effect*" and there may be "*quantitative differences between various established methods for aPTT*".[46] Section 12.2 (Pharmacodynamics) also describes prolongation of aPTT, ECT, TT and dTT, with the INR relatively insensitive to dabigatran exposure, with the indication the ECT is a more specific measure of dabigatran effect with expected, "*median (10^{th} to 90^{th} percentile) trough ECT in patients receiving the 150 mg dose was 63 (44 to 103) seconds*".[46] Other verbiage includes generalized concepts such as "*relationships*", "*linear proportion*", and "*increases in non-linear fashion*", but devoid of numeric values.[46] Only in section 12.3 (Pharmacokinetics), under specific populations, does the PI provide quantitative values for trough (with 10^{th} – 90^{th} percentile) concentrations for pediatric and adult populations with DVT/PE.[46]

PIs from the other regulatory agencies report much more information, which can help the clinician and the laboratory in the management of their patients.[47-49] Nevertheless, there is currently no evidence that clinicians in these countries provide better patient management based on the availability of this information, nor that these measurements lead to better patient outcomes. To this aspect, a survey comparing the practice in the different region of the globe could provide more insight on how the information provided in the region-specific PI and the availability of methods for DOAC testing impact the patient's management.

DOAC Testing Myth #1 – Methods for DOAC Testing Are Time Consuming and Result Turn-Around-Times (TATs) Too Long for Acute Clinical Use or Need

Specific tests for screening or quantifying DOACs are not time-consuming as compared to other traditional hemostasis assays (**Table 2**).[10, 11, 68-70] When looking at the stepwise protocols for performing testing related to dabigatran, the dTT and ECT are clot-based assays that have the same testing time as a fibrinogen or thrombin time test.[71] Ecarin chromogenic assay (ECA) methods can also be automated, with this test requiring 3 different reagents, although the first reagent is a diluent and subsequent two test reagents (ecarin and substrate) for a total of 3 independent steps. This is equivalent to performing a UFH or LMWH level using anti-FXa testing. For FXa DOACs, the only difference between a UFH/LMWH

reported result and a DOAC reported result is the calibrator source, otherwise, the stepwise test protocols are equivalent, and therefore the test TAT would be the same as for heparin levels. Thus, the time required to calibrate DOAC-specific testing is no longer than the time required for a heparin chromogenic assay and represents approximately the time required to finalise five tests. Nevertheless, most analyzers are able to run several samples at a time, enabling fast calibration procedure, i.e. around 10-15 minutes once the reagent and the calibrators are on-board. Note that the calibrators (as per calibrators used in most hemostasis tests) and some reagents may need to be reconstituted, and this can represent 30 additional minutes, since calibrators need to stabilize after reconstitution. However, the calibration is not a procedure that needs to be done on a daily basis, since it is usually valid for the entire batch of reagents, providing controls are within range, although some regional regulatory requirements may require a higher calibration frequency. Also, the caveat being for those tests that require calibration that additional procedure step is required and verified to be valid using calibration curve-statistical algorithms and quality control assessment. However, calibration requirements are a common requirement for many hemostasis tests, and calibrated tests are indicated by the reporting units (e.g. IU/mL, % activity, etc.) which quantify the measurand. Nearly any automated coagulation analyzer purchased from 2000 onward, that is an open system (programmable) can be modified to perform dabigatran specific tests (dTT, ECT, ECA) and nearly all automated instruments have a default (UFH/LMWH) anti-FXa method available that can be copied, then modified to use FXa DOAC calibrators and controls. Modifications to instrumentation or existing methods on instrumentation may be considered a laboratory-developed test (LDT) with regional regulatory requirements for method validation (see below). Some groups reported the possibility to use LWMH calibration to report results in terms of anti-Xa activity (IU/mL) [26, 72, 73]; however, this approach should be validated by each laboratory, since there is an important inter-reagent and inter-kit variability which precludes current international standardization of anti-Xa measurement, including the establishment of any harmonized anti-Xa cut-off for clinical decision making [28, 74].

DOAC Testing Myth #2 – Methods for Rapid DOAC Testing Are Too Expensive for Implementation

Defining what is expensive is subjective in any organization and also depends on reimbursement and regional policies. The basic premise is defining a "cost-per-reportable" which indicates the expenses associated with performing a testing, which may include direct costs (reagents, supplies, maintenance contracts, technical time/labor, etc., related to the specific measurand testing) and indirect costs (phlebotomy cost, supplies, administrative costs, etc.). In the hemostasis laboratory, the more tests you perform, the lower the cost-per-reportable, as limitations for direct costs would include QC testing, reagent stability, number of tests per vial and laboratory scientist time, which may be the same for performing 1 test or 100 tests. High volume tests such as PT/INR and aPTT tend to have a lower cost-per-reportable than lower volume tests such as anti-FXa measurements. As previously mentioned, indications for measuring DOACs would include acute or emergent management, that may comprise the use of reversal agents. When looking at the cost-per-reportable at a single site (RCG) for quantitative DOAC testing, the annual cost for these specialized tests is significantly lower than the cost for a single dose of DOAC reversal therapies (**Table 3**). For direct FXa inhibitors, it must be noted that the same anti-FXa kit can be used to measure any direct FXa inhibitor with drug specific calibrators/controls. However, it must be emphasized this anti-FXa measuring method cannot differentiate between anti-FXa drugs, and there will generally be additive effects with more than one anti-FXa drug exposure (e.g. LMWH + direct FXa inhibitor).[75, 76]

DOAC specific tests are slightly more expensive that basic chronometric/clot-based assays like PT and aPTT. Chromogenic kits for measuring dabigatran or anti-FXa are approximately $500USD per kit, with the number of tests that can be run per kit estimated to be around 40 – 50 tests. Clot-based assays run approximately $200USD per kit, with number of tests that can be performed ~20 – 30 tests. Reagents for either chromogenic or chronometric methods can be purchased separately to reduce costs but may incur more variability between reagent lost than with commercial providers of kits. Calibrator and control sets run ~$200USD each but both can be reconstituted, aliquoted and frozen for longer stability according to the instruction for use (IFU). The limiting factor for most "kits" will be either tests per kit or stability of the kit after reagent reconstitution. The reagent stability for most coagulation reagents range between 1-5 days on board of a coagulation analyzer. However, the stability of reconstituted reagents can be extended when using storage conditions (e.g., refrigeration or frozen) that can prolong reagent stability to weeks or months. While there is certainly convenience for having reagents on-board for 24/7 testing, loading

reagents on-board a coagulation analyzer takes less than 5 minutes and therefore could easily accommodate emergency testing with the acceptable 15 – 30 min TATs.[10, 77, 78]

US Specific DOAC Myth: A Laboratory Cannot Implement a Laboratory Developed Test (LDT)

Since a 2014 FDA guidance document related to implementing and overseeing LDTs, there appears to be a reluctance for US clinical laboratories to implement these required tests. The FDA indicates LDTs are those in vitro assays that are they are "*designed, manufactured, and used within a single laboratory*."[81] The FDA does not consider diagnostic devices to be LDTs if they are designed or manufactured completely, or partly, outside of the laboratory that offers and uses them.[82] LDTs often fill a gap between clinical need and regulatory approved test methods. The key element in the 2014 guidance document was more related to those LDTs that are not used within a single laboratory or healthcare institution and sought to obtain regulatory oversight for same. However, in 2017 the FDA published a discussion paper to detail FDA position on LDTs based on risks and oversight with a continued desire for future discussions with appropriate stakeholders, but in no case forbade the use or implementation of LDTs so long as these methods were validated appropriately. Ironically, the hemostasis laboratory has performed LDTs for decades. The practice of mixing studies, for example, where unexpected prolonged PT or aPTT samples are mixed with normal plasma and the testing repeated and the result used to differentiate the prolongation due to factor deficiency(ies) or inhibitor. That test is "manufactured" in the local laboratory as that method is not described in the reagent manufacturer IFU. In the same category, coagulation instrumentation that has received FDA approval for "adult use" would suggest that any pediatric testing on these instruments may constitute an LDT.

For the US, there is only one FDA approved method for measuring DOACs. Instrumentation Laboratory (IL) received an FDA reclassification order for HemosIL® Liquid Anti-Xa for measuring apixaban in bleeding patients or patients at risk for bleeding.[83] However, under the "labeling" section of this document the FDA requires that the manufacturer must include "*A prominent statement that the device is not intended for use in monitoring patients taking heparin or direct oral factor Xa inhibitors*.". There are two existing IFUs for this reagent, one without apixaban information (*HemosIL Liquid Anti-Xa, product 0020302602, Insert revision 06/2017*) and the other including apixaban information (*HemosIL Liquid Anti-Xa, product 0020302602, Insert revision 12/2020*). For the IL reagent including apixaban information, the intended use statement indicates, "…*the following situations where measurement of apixaban levels could be useful to have as additional information: - Patients at risk for major bleeding - Patients experiencing a bleeding episode. The assay is not a stand-alone test, and the results should be used in conjunction with other clinical and laboratory findings.*" Noted that the use of this kit is specific to a certain class of instruments,

therefore using this kit on other instruments would be considered a modification to the IFU and thus an LDT.

Laboratories should follow regulatory recommendations or guidance documents when implementing an LDT to assure adequate method validation. A method "validation" is a robust assessment of the test characteristics with similar elements evaluated as a regulatory agency requires from a manufacturer. This is not the same as a method "verification" of test performance, in which the local laboratory verifies some operational characteristics of an 'approved' instrument or test method. Verification of performance requires the assessment of precision, confirmation or determination of referent interval, method comparison to a regulatory approved device or method, and in some cases, linearity confirmation. The local laboratory will "verify" the IFU provided performance characteristics of the instrument or reagent which were part of the validation and approval process by regional regulatory authorities. When local validation of a method is required, additional testing characteristics are required. Such considerations would include the verification elements but may also include assessing sample and/or reagent carryover (automated instrumentation), lower limit of quantitation (LLOQ), additional precision studies (between-run, between instruments), assessment of interferences (sample conditions such as lipemia or other drugs), reagent stability (if maintained on the coagulation analyzer) and possibly sample carryover (if not already assessed).

In our experience, the most common difficulties with validating LDTs for low volume or emerging tests have been the limited availability of patient samples required for method comparison analysis. What constitutes the minimum requirements for LDT validation and what are considered acceptable test characteristics remain elusive. Each local laboratory should engage in a discussion with local clinical stakeholders to assess and address their clinical needs. The clinician query as to "what is the question" would start the conversation about whether a sensitive test is required for screening DOAC exposure, or whether a quantitative DOAC measurement is more desirable. Based on good laboratory practice, federal regulatory requirements, decades of laboratory experience, previous acceptance criteria used for manufacturer 510k submissions, the performance characteristics, and recommendations for DOAC test validation are established (**Table 4**). Of note, not all listed elements may be required for validation of an LDT. Whether all elements of validation are required are likely dependent on reagent status, as modifying a regulatory approved reagent would not require reagent carryover studies. Readership should consult local and regional regulatory requirements for additional guidance prior to method validation and clinical implementation.

The laboratory should have a plan or strategy to aid clinicians if concomitant anticoagulation exposure is present (e.g., direct FXa inhibitors and heparin) to assure accurate monitoring.[9-11, 27, 77, 78] The laboratory should have a plan or strategy to mitigate DOAC effect on diagnostic assays (e.g., lupus anticoagulant) or provide alternative test methods.[84] For both scenarios, the neutralization of DOAC using in vitro products such as activated charcoal or filtering mechanisms have been described [85-87] and use of these products may be characterized as an LDT as the patient sample has been modified. Consideration of which neutralization method to consider may be predicated on regional approvals, sample volume requirements and residual volume after treatment process. To validate the use of these DOAC neutralizing products, we would recommend post-neutralization precision studies and method comparison analysis comparing baseline results (i.e., anti-FXa, lupus anticoagulant test) to post-neutralization treatment results using DOAC naïve samples to assure minimal effect of these devices on test accuracy.

Recommendations for Laboratories Screening or Quantifying DOACs.

If the laboratory is providing screening tests that are sufficiently sensitive to detect around 25-30 ng/mL of DOACs, there should be accompanying information with test result to indicate laboratory verified test sensitivity. For laboratories that are using LMWH calibrated anti-FXa to screen for direct FXa inhibitors, we suggest that the test be reported as "not detected" or "detected", with information provided that the LLOQ associated with LMWH is "estimated" to be a given concentration of FXa DOAC based on local laboratory findings.[74, 88]

Prior to implementing a DOAC quantitative assay, we strongly recommend the laboratory consult clinical stakeholders for their feedback. Early recommendations suggest trough time collections be used although more recent studies suggested peak values may be associated with bleeding risks.[9-11, 16, 77, 78, 88-90] For laboratories that provide quantitative DOAC measurements, we recommend that: 1) each DOAC be a separate orderable test to assure proper drug calibrated test is used, 2) each result is accompanied by an expected 'within therapy' range traceable to clinical study or peer-reviewed publication based on collection time (peak or trough), and 3) results reported in ng/mL when properly drug calibrated. DOAC levels that are required for acute or emergent clinical needs should be reported within 30 minutes of receipt in the testing laboratory.[10]

Conclusion

For more than a decade, US laboratories have failed to implement solutions to help their clinicians in managing complex situations or patients on DOACs. The problem may find different origins among which the position of the FDA, which categorized these drugs as monitoring- and measurement-free, while other regulatory bodies were more conservative on the principle that the absence of proof (of monitoring/measurement benefits) is not proof of an absence (of monitoring/measurement needs). Pivotal clinical studies which led to the approval of DOACs were presented as devoid of such testing although some companies considered monitoring as a solution to improve their benefit/risk ratio.[32] Key opinion leaders and early guidelines on DOAC management also spread the message that these tests were not useful (if not harmful). Nevertheless, numerous groups of experts in hemostasis laboratory practice have contributed to the general knowledge around DOACs by providing independent laboratory data on test method development, evaluation of drug levels in real-life and their association with clinical events. If we all agree that "monitoring" is not the adequate term to qualify the nature of the clinical need in the era of DOAC, "point measurement" is certainly more appropriated as almost all clinicians dealing with DOAC-treated patients may have needed to evaluate the residual anticoagulant activity in certain situations. More than a decade of development has also permitted the activation of smart laboratory solutions to qualify or quantify DOACs and current reluctance of implementing these technologies in the laboratory relies on myths and misconceptions around technical and regulatory requirements.

Laboratories in Canada, UK, Australasia and Europe have demonstrated that these tests are easily implementable, show adequate analytical and clinical performance (according to their regional regulations), and are helpful for clinicians as demonstrated by the numerous studies arising from these parts of the globe. In the US, there is no need to wait for FDA approval of DOAC dedicated methods in order to develop these methods, since almost all laboratories have the minimum material requirements for performing these analyses. In centers dealing with DOAC reversal agents, offering these product-dedicated methods may even be extremely cost-effective since it may help rationalizing the administration of andexanet alfa or idarucizumab for direct factor Xa inhibitors and dabigatran, respectively.

Use of DOACs is ever expanding, with DOAC prescriptions now exceeding those of other anticoagulants, including VKA, in some geographies.[91, 92] As this use increases, the likely need to measure DOAC exposure will also increase. Measurement of DOACs does not represent any technical difficulty. That these

laboratory tests are not available in some geographies suggests disparities in patient care, and we suggest it is time to address such disparities.

Author Contribution:

RCG wrote the first draft of the manuscript. RCG and JD compiled the information from prescribing information. EJF performed review of the initial draft. EJF, JD and RCG were responsible for the final version of the manuscript.

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▶ Table 1: 2021 External Quality Assurance programs and sample size of respective measurand reported results.[63-66]

	CAP	ECAT	UKNEQAS	RCPAQAP
PT	4309	239	920	807
aPTT	4132	249	942	777
LMWH Anti-Xa	1273[§]	394	383[*]	127
Dabigatran	26	98	66	56
Rivaroxaban	51	293	111	83
Apixaban	48	274	106	66
Edoxaban	ND	84	57	ND

*[§]includes Hybrid and Low Molecular Weight Heparin calibration data; *2021 registered participants for heparin assay.*

Abbreviations: *aPTT, activated partial thromboplastin time; CAP: College of American Pathologist, US; ECAT, External quality Control of diagnostic Assays and Test, Netherlands; LMWH, low molecular weight heparin, ND: Not done, No data; PT, prothrombin time; RCPAQAP, Royal College of Pathologists of Australasia Quality Assurance Programs; UKNEQAS, United Kingdom National External Quality Assessment Service, United Kingdom;*

Table 2: Common hemostasis test protocols (automated platforms).[71]

Measurand	Reagent 1	Reagent 2	Reagent 3	Average Time to test result[§]
PT/INR	TF or equivalent, PL + $CaCl_2$	None	None	3 – 5 mins
aPTT	Activator + Phospholipids	$CaCl_2$	None	5 – 7 mins
FBG[#]	Sample Diluent	Thrombin	None	3 – 5 mins
TT	(Sample Diluent)	Dilute thrombin	None	3 – 5 mins
Anti-Xa[#]	Sample Diluent	Substrate	Factor Xa	3 – 4 mins
dTT*	PPP	Dilute thrombin	None	3 – 5 mins
ECT*	Ecarin	None	None	3 – 5 mins
ECA[#]	Prothrombin buffer	Substrate	Ecarin	5 – 7 mins

[§]These do not reflect test result turn-around-times, only test time on automated analyzer, with manual testing likely associated with increased time to test reporting. Average time based on presumption reagents are on board instruments and ready for use, the number of testing steps, test incubation periods, and maximum clotting times using automated platforms with eventual result generation and transmittance to electronic laboratory information system or medical record.

**These tests may be used for raw data reporting (seconds) or require drug calibration for quantitative measurement reporting.*

[#] These tests require calibration for quantitative measurement reporting.

***Abbreviations:** aPTT, activated partial thromboplastin time; dTT, dilute thrombin time; ECA, ecarin chromogenic assay; ECT, ecarin clotting time; FBG, Fibrinogen; INR, International Normalized Ratio; PL, phospholipids; PPP, Platelet Poor Plasma; PT, prothrombin time; TF, tissue factor; TT, thrombin time.*

▶ Table 3: Cost per reportable for a single result from single site (RCG) compared to cost for a single DOAC reversal dose.

Measurand	Number of tests per annum	Cost per reportable	DOAC Reversal Agent	Per Dose Reversal USD Cost	Alternative Reversal Agent per dose (USD cost)
Heparin (UFH/LMWH)	1136	$13[§]	NA	NA	NA
Dabigatran	43	$55	Praxbind	~$5,000	NA
Apixaban	13	$197[§]	Andexanet alfa	± $26,000 low dose ± $52,000 high dose [79][†]	PCCs ± $5500 [79]
Rivaroxaban	72	$23[§]	Andexanet alfa	± $26,000 low dose ± $52,000 high dose [79][†]	PCCs ± $5500 [79]

[§] Cost per reportable represents cost for a calendar year at a single testing site (RCG) for calibrator and control costs, with relative anti-FXa kit cost distributed based on percentage of use for a single commercial source. When combining the cost to perform all FXa assays, the cost per reportable is $16. NA: Not applicable; PCC, Prothrombin Complex Concentrates.

[†] Recent data reports price for andexanet alfa around $12,000 for 4 vials of 200 mg.[80]

***Abbreviations:** DOAC, direct oral anticoagulants; LMWH, low molecular weight heparin; UFH, unfractionated heparin; USD, US dollar.*

Table 4: Provisional guidance for desirable test characteristics when validating a DOAC LDT. Each element must be performed for each DOAC even if reagent kit is the same.

Element	Suggested Testing Criteria Additional comments	Desirable Characteristics Additional comments
Precision	• Within-run: N=10 replicates, assessing at ± 40 ng/mL and ± 200 ng/mL or lower and upper AMR • Alternatively, for two levels of control material: 2 runs per day in triplicate for 5 days is another suitable precision assessment. • Between-run: N=10 days minimum, N=20 days optimal	• Within-run: ≤10% CV • Between-run: ≤15% CV
Limit of detection	• Drug naïve sample testing. Minimum N=10, optimal N=20 samples. • Not applicable for chronometric or clot-based assays reporting in seconds.	• <5 ng/mL or <LLOQ • This element addresses analytical specificity
Reference Interval	• Not required for quantitative drug measurements. • Required for dTT and ECT testing if these tests are not dabigatran calibrated and report results in seconds or ratio • At a minimum, N=20, optimally N=40, ostensibly healthy adults.	• If normally distributed, then mean ± 2*SD would acceptably range. • If not normally distributed, then 10^{th} – 90^{th} percentile should be used.
Lower Limit of Quantitation (LLOQ) **Linearity**	• Predicated on calibration. Commercial DOAC calibrator sets may not provide a 0 ng/mL concentration. • Consider 0 ng/mL calibration point to improve LLOQ if performance criteria are acceptable. • Linearity not required for chronometric or clot-based methods reporting in seconds. • Assessing the level of dabigatran required to elevate the ECT or dTT beyond the upper limit of the RI would be desirable to indicate test sensitivity threshold.	• LLOQ: Desirable to be ± 20 ng/mL or less. • Linearity within 10% of theoretical (recovery) values. • This element addresses analytical sensitivity and reportable range • This element will address whether extended measurement interval can be applied.
Method Comparison	• At least 20 samples, optimally 40 samples, from DOAC treated patients spanning the measurement range. Ideally comparator method is mass spectrophotometer measurements considered the gold standard.[47] • Use of other commercial calibrators, controls or other assayed material for method comparison may be acceptable in lieu of patient samples.	• Correlation coefficient >0.90; slope 1.0 ± 0.15; • Bias ≤15% between paired results. Recommend Bland-Altman bias plots • This element addresses analytical sensitivity
Carryover	• Reagent carryover required if new automated platform is used. Reagent carryover is not required if kit method approved for other indication (e.g., anti-FXa kit used for heparin). • Sample carryover – may be required unless published studies on local instrument has already been assessed.	No carryover detected
Stability	• If reagent is to be maintained on-board for extended periods of time, then on-board stability must be assessed. Use of longitudinal material (e.g., controls) can be used and measured at defined frequency (0, 4, 8, 16 hours intervals, etc.).	Recovery of longitudinal material should be within 15%CV (between-run precision acceptability criteria)

	• Not required when modifying regulatory approved methods for intended use on an IFU listed instrument with stated stability limits.	
Interfering substances	• For dabigatran testing – assess whether heparins or other direct thrombin inhibitors affect the assay (likely) • For FXa DOACs – assess effect of UFH, LMWH and/or pentasaccharide will affect the assay (likely). • Assess test method interferences such lipemia, icterus or hemolysis on result. Not required when modifying regulatory approved methods. • Determine if ultracentrifugation will alter reported results when used for clarifying lipemic samples.	• Concomitant drug effect is expected, and findings shared with clinical team. • Method interferences are expected, especially with chromogenic assays (ECA, anti-FXa). Consider seeking other IFUs to aid in method interferences. • This element addresses some aspects of analytical specificity
Other	• Highly desirable: Obtain external quality assurance (EQA) material for DOAC to assure between-laboratory precision.	Within EQA acceptability limits as determined by either peer group or method.

Abbreviations: *AMR: analytical measurement range; CV, coefficient of variation; LLOQ, lower limit of quantitation; dTT, dilute thrombin time, ECT, ecarin clotting time; RI, reference interval; SD, Standard deviation; DOAC, direct oral anticoagulant; IFU, instructions for use; UFH, unfractionated heparin; LMWH, low molecular weight heparin; ECA, ecarin chromogenic assay.*