Recommendations for harmonization of the coagulation screening tests laboratory report

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ABSTRACT

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are important screening tests that are used in the laboratory evaluation of patients suspected of having congenital or acquired alterations of the coagulative phase of hemostasis, including the presence of coagulation inhibitors. PT explores the extrinsic pathway and the common pathway of the coagulation system and is also the test of choice to monitor the efficacy and safety of oral anticoagulant therapy with anti-vitamin K (VKA) drugs. APTT explores the intrinsic pathway and the common pathway of coagulation and is also used in monitoring unfractionated heparin therapy. How to communicate the results of these tests in the laboratory report is still a source of debate today and many possibilities have been proposed: seconds, prothrombin activity, ratio [the ratio between the coagulation time of the plasma under test and the Mean Normal Prothrombin Time (MNPT)] and INR (International Normalized Ratio) for PT, seconds and ratio for APTT. As with all laboratory tests, it is necessary that the results of these tests are communicated clearly, with a single value, to allow a correct and unambiguous interpretation of the tests by the requesting clinicians. The use of multiple results for both tests can be confusing and contradictory in some cases and can lead to incorrect interpretations of the tests. Aim of this document is to make proposals to harmonize the laboratory report of these tests. For PT, it is recommended to use the ratio for patients who are not on anticoagulant treatment with VKA and the INR for patients who are on VKA treatment. For APTT, there is only one correct way of reporting the test results, which is represented by the ratio. Both tests, PT and APTT, must not be used to monitor the Direct Oral Anti-Coagulants (DOACs) therapy; for these tests, the use of specific tests is necessary.

Key words: *coagulation screening tests, laboratory report, harmonization*

INTRODUCTION

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are the most frequently prescribed coagulation tests in general and specialized laboratories for:

- pre-operative screening;
- monitoring of oral anticoagulant therapy with vitamin K antagonist (VKAs) drugs (Coumadin and Sintrom);
- identification of congenital coagulation factor deficiencies (with the exception of FXIII);
- identification of acquired deficiencies [e.g. reduced intake or absorption of vitamin K, liver damage Disseminated Intravascular Coagulopathy (DIC)].

Actually, as for the first indication, the sensitivity of screening tests to the presence of bleeding disorders, regardless of the reagents used, is very low (1.0-2.1%) (1-4) and the International Society on Thrombosis and Haemostasis - Bleeding Assessment Tool (ISTH-BAT), a score based on clinical criteria and medical history (5-8), should always be used by clinicians to assess hemorrhagic risk. Actually, this score has an excellent sensitivity to the presence of hemorrhagic diathesis, much higher than the coagulation screening tests.

Furthermore, PT and APTT show variable sensitivity to the presence of circulating anticoagulants directed against specific coagulation factors or against negatively charged antiphospholipid antibodies, such as lupus anticoagulant (LAC) (9).

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APTT is also sensitive to the presence of unfractionated heparin (UFH), while PT is not substantially affected by the presence of this drug as commercial thromboplastins (i.e. the reagents used to perform the PT) are spiked with exogenous inhibitors of heparin (e.g. polybrene).

The presence of exogenous heparin inhibitors in commercial thromboplastin allows the measurement of INR during co-administration of VKA and UFH in the treatment of acute venous thromboembolism (VTE). Low molecular weight heparins (LMWH) do not affect PT; on the other hand, contrary to widespread opinions, APTT can be variously prolonged, depending on the type and dose of LMWH used (9).

Aim of this document is to provide recommendations for the harmonization for the laboratory report of hemostasis assessment screening tests, expressing the results of these tests in an univocal way, with a single result and a single unit of measurement, allowing a correct and unambiguous interpretation of the tests by the requesting clinicians. The use of multiple reporting modalities for these tests can in some cases, generate, confusion to the point of leading to incorrect interpretations of the results by clinicians.

PROTHROMBIN TIME

Since the original description by Quick in 1935 (10), PT has represented and still represents an important screening test in the laboratory evaluation of patients with suspected hemostasis disorders. It is the most frequently requested coagulation test in clinical laboratories. Although PT was originally described as a specific method to measure prothrombin (coagulation factor II), it is actually sensitive to the presence of quantitative and/or qualitative abnormalities of any of the factors involved in the extrinsic and the common pathways of the hemostatic system (factors II, V, VII, X and fibrinogen), as well as to the presence of inhibitors of these factors. Moreover, it is used as an early marker of liver disease, from moderate to severe, or of chronic liver disease, with a sensitivity equal to that of gamma glutamyl transpeptidase (γ-GT). PT is also the most commonly used test for monitoring the efficacy and safety of VKAs therapy (Coumadin and Sintrom).

PT is defined as the time (in seconds) needed to coagulate the platelet poor plasma, obtained by an appropriate centrifugation (9), after the addition of coagulation triggering factors, such as tissue factor complexed with phospholipids and calcium ions (thromboplastin). Thromboplastin is therefore the reagent used to perform the PT; numerous varieties of thromboplastin preparations of human or animal origin are commercially available, obtained either by extraction or recombinant techniques. The different origin (animal or human) and the different method used for their preparation give thromboplastins important differences in terms of sensitivity to congenital or induced by VKAs, deficiencies. Further significant differences in the PT results are also attributable to the method used to determine the clot formation which involves the use of photo-optical or mechanical coagulometers.

The use of different types of thromboplastins coupled with different analytical analyzers make available a considerable number of systems, each potentially capable of providing significantly different PT results. This led in the '80s to the creation of a standardization system, the International Normalized Ratio (INR), based to the statistical approach of Kirkwood TB (11), in the wake of earlier observations by Biggs et al. (12). The INR system allows, utilizing codified procedures, the calibration of commercial thromboplastins against an international standard and the calculation of a sensitivity index, the International Sensitivity Index (ISI). ISI is the link between the thromboplastin to be calibrated and the international standard. ISI values of 1 denote a sensitivity of the commercial reagent equal to that of the international standard, while ISI values >1 demonstrate a lower sensitivity and vice versa. When ISI is known, it is possible to transform the PT value into the INR scale (13-16).

The INR value is obtained by dividing the coagulation time of the plasma under examination by the Mean Normal Prothrombin Time (MNPT) and raising the ratio thus obtained (ratio) to a power equal to the ISI value of the reagent in use. The MNPT represents the geometric mean of the PTs of 20 (or more) apparently healthy subjects, obtained under the same experimental conditions as those of the tests performed in the patients (17-18). INR, on the other hand, represents the value of the PT that would have been obtained if the international standard had been used. Since the ISI has an exponential effect on the INR result, thus amplifying even small differences, it is recommended to use reagents with ISI values as close as possible to the unit. The thromboplastins currently on the market in Italy show ISI values between 0.93 and 1.05 for thromboplastins obtained with recombinant technique and between 1.01 and 1.33 for thromboplastins of extraction origin.

Different units to report Prothrombin Time results

Seconds

Seconds are the unit absolutely NOT to be used as they are strongly affected by the reagent and the instrument utilized (19); there is, moreover, a significant intra- and inter-day variability linked both to the reagent's reconstitution and its stability. The only exception to the above applies when the test is required for the DIC diagnosis and monitoring: in this case the DIC score of ISTH (20-21) recommends, up to now, only the use of seconds, assigning a different score depending on the prolonged PT (<3 seconds, 0 points; between 3 and 6 seconds, 1 point; >6 seconds, 2 points).

Prothrombin activity

The prothrombin activity (or percentage activity) has some important limitations related particularly to the type of the curve that is used for calibration. The shape of the calibration curve (hyperbolic) tends to parallel the axes at low and high dilutions and therefore makes the percentage activity not very sensitive to the variation of

clotting times at low percentage activity and excessively reactive at high percentage activity, respectively. This effect implies that relatively large changes in clotting time in patients with VKAs may result in relatively small percentage changes in activity, thus complicating the dose adjustment of patients using these drugs.

In contrast, small changes in clotting time in healthy subjects result in large variations in percentage activity, which may occasionally lead to values much higher than 100% (corresponding to the highest reference limit), which could be misinterpreted as an marker of hypercoagulability. In addition, percentage activity does not take into account the different sensitivity of commercial thromboplastin to the VKA-induced defect: therefore, percentage activity of PT cannot be used to monitor patients taking VKAs (22).

Ratio

At the beginning of the '60s, the use of the ratio was proposed as a replacement of the percentage activity; this parameter is rather intuitive as it is evident that ratios lower or higher than 1 respectively indicate increased or defective hemostasis. Although the PT-ratio is a more robust parameter than the percentage activity, it is however not able to take into account the different sensitivity of commercial thromboplastins to the deficit induced by VKAs and therefore cannot be used for monitoring efficacy and safety of VKA therapy. Its use should therefore be reserved only for patients who are not treated with VKAs.

As reported above (17,18), the ratio must be obtained from the geometric mean of the PTs of 20 (or more) apparently healthy subjects, obtained under the same experimental conditions as those in which the tests are performed in patients.

International Normalized Ratio

As previously described, ISI is a reliable measure of the sensitivity of various commercial thromboplastin to VKA-induced deficits compared to the international standard and can be used to convert the PT-ratio to INR according to the equation *(23-25)*:

$$INR=(PT\text{-}ratio)^{ISI}.$$

By definition, the INR represents the PT value that would have been obtained if the patient's plasma had been tested with the international reference standard rather than with the commercial thromboplastin used in the laboratory. In 1983 the calibration model of thromboplastins proposed by Kirkwood TB (11) was approved by the Committee of experts on biological standardization of the World Health Organization (WHO), which issued Guidelines (GL) for its application (13); over the years improvements have been proposed up to the current model, detailed in the latest GL issued by WHO in 2013 (14). Commercial thromboplastin manufacturers have been asked to report the ISI of their thromboplastin by calibrating them to existing international standards on a similar basis (same species) for all instruments of their production. The cornerstone of these GL is the preparation and supply of thromboplastin standards from different species, which are interconnected by iterative calibration (predecessor-successor) by means of international collaborative studies, which ensure continuity of the system over time. Currently, there are two international standards made available by the WHO: RBT/05 from rabbit brain and rTF/09 from recombinant human re-lipidated tissue factor. By definition, the INR (as a harmonization scale) is only valid for patients in the stable phase of treatment with VKAs. In all other circumstances, i.e. outside the VKA treatment, from a semantic point of view, INR could not be used for reporting of results. It is necessary, however, underline that it is used more and more often to express PT values in the recent literature, even in scientific journals of international prestige, such as Journal of Thrombosis and Haemostasis (26), PLOS One (27), Journal of Pediatric and Neonatal Individualized Medicine (28) and International Journal of Laboratory Hematology (29). Other authors argue, however, that INR fails to harmonize the results between different thromboplastin when it is used to express the PT results in patients with chronic liver disease, in patients with acquired factor deficiency of pro-coagulants in DIC or in treatment with Direct Oral Anti Coagulants (DOAC). This is due to the fact that ISI (which is the cornerstone of the calibration model) is determined using plasma from patients in stable phase of VKA therapy, and is therefore dependent only on the hemostasis defects induced by these drugs. VKA-induced coagulation abnormalities show characteristics different from other defects that may prolong PT (e.g. chronic liver disease, DIC, hypocoagulability due to a single factor deficiency, some DOAC). In the case of end-stage liver disease, the Model for End-stage Liver Disease (MELD) score (which includes bilirubin, creatinine and INR) is used to evaluate the priority for liver transplantation (30): some authors (31) have proposed the use of another model of calibration (INR-LIVER) to replace INR-VKA.

When reporting the INR instead of the ratio, for ratio values >1.00 (scope of interest), the resulting relative error, is given by the formulas

$$error=(INR–ratio)/ratio$$

or

$$error=(ratio–INR)/ratio$$

respectively for ISI >1.00 or ISI <1.00.

The definition of the maximum acceptable error can be based on various clinical or laboratory criteria. A possible solution, to avoid the arbitrariness of this choice, is to use one of the three models presented at the EFLM strategic conference in Milan in 2014 (outcome, biological variability, state of the art) (32-34). According to the biological variability model, the total error (TE) derives from the linear combination of imprecision (I) and bias (B):

$$TE=1.65xI+B,$$

where I and B are calculated from the intra-individual (CVi) and inter-individual (CVg) biological variability.

Since meta-analytic estimates of the PT biological variability are not currently available in the EFLM database (https://biologicalvariation.eu/), Table 1 shows the biological variability data from two recent studies and from the Westgard 2014 online database (https://www.westgard.com/biodatabase1.htm). Table 1 also reports estimates of desirable bias, imprecision, and TE (35-37). It seems logical for the maximum acceptable error, resulting from considering the INR instead of the ratio, not to use TE, since it is also determined by the contribution of imprecision, but rather the bias alone. According to that, it is therefore possible to calculate, for any ISI value, the maximum allowable INR at which the error (due to reporting INR instead of ratio) does not exceed the chosen bias of 2.00% (0.02). To this aim, for ratios >1.00, the following formula are used:

$$INR=(error+1)^{ISI/(ISI-1)}, \text{ when ISI } >1.00$$

and

$$INR=(1-error)^{ISI/(ISI-1)}, \text{ when ISI } <1.00$$

For example, when the ISI in use in the laboratory is 1.05, the maximum allowable INR which can be reported (corresponding to a maximum acceptable error of 2.00% due to reporting INR instead of PT-ratio) is equal to:o

$$INR=(0.02+1)^{[1.05/(1.05-1)]}=1.52.$$

Using the formulas indicated above, respectively for ISI values >1.00 or <1.00, Table 2 shows the maximum reportable INR, for ISI ranging between 0.95 and 1.05, at which the error done, due to having used INR instead of the ratio, does not exceed the chosen 2.00%.

It is opportune to add some clarifications to the reasoning:

- the relevant studies have fairly overlapping estimates; in the absence of meta-analytical estimates values of 2.00% for bias and of 5.00% for TE, respectively, should be considered;
- the biological variability model is calculated on healthy subjects, with ratio values in the range of 0.8-1.2. In this context, the bias, based on CVi and CVg, is <2.00%; however, for higher ratio values, a higher bias value is probable and therefore the 2.00% estimate considered is conservative;

Table 1

Estimates of the intra- (CVi), and inter-individual (CVg) biological variability and the desirable performances for imprecision (I), bias (B) and total error (TE) are presented according to the different source of data.

Source	CVi	CVg	I	B	TE
Falay et al. 2018 (35)	2.78	5.07	1.39	1.45	3.74
Online database 2014 (36)	4.00	6.80	2.00	1.97	5.27
Carobene. et al. 2021 (37)	2.60	5.10	1.30	1.43	3.58

Table 2

For ISI values between 0.95 and 1.05, the INR values at which the maximum error obtained is lower than the chosen limit of 2.00% are reported.

ISI	maximum accettable INR
0.95	1.47
0.96	1.62
0.97	1.92
0.98	2.69
0.99	7.39
1.01	7.39
1.02	2.75
1.03	1.97
1.04	1.67
1.05	1.52

- the error “budget” attributable to the bias in the previous reasoning was completely “spent” on the error deriving from the mathematical transformation from ratio to INR, implying an analytical bias equal to 0.00%. In the presence of analytical bias, the maximum acceptable error for the transformation to INR would be less than 2.00%, with INR values lower than those reported in Table 2.

As can be observed in Table 2, if the PT was incorrectly reported as INR rather than as ratio (incorrect inclusion of the diagnostic question or lack of knowledge of a possible treatment with VKA), the resulting error would be tolerable within certain limits of the INR, more or less restrictive depending on the ISI in use in the laboratory.

For patients treated with DOAC, the PT results have to be espressed as ratio; reporting INR in these patients is strictly not recommended. In any case, to measure the DOAC plasma concentration and then evaluate the intensity of the anticoagulation achieved in these patients, only the tests suggested by the available GL, Position Papers and documents of the Scientific Societies (38-42) should be used.

RECOMMENDATION FOR THE PROTHROMBIN TIME LABORATORY REPORT

It is highly recommended that any PT request always be accompanied by indications of any ongoing anticoagulant treatments, thus facilitating not only the interpretation of the results, but also the choice of the correct unit of measurement to use in the report

We recommend the use of only one unit of measurement:

- Ratio for patients who are NOT on vitamin K antagonists treatment

or

- INR for patients treated with vitamin K antagonists

ACTIVATED PARTIAL THROMBOPLASTIN TIME

APTT is a comprehensive coagulation test that explores the intrinsic pathway of hemostasis and is sensitive to factors of the intrinsic pathway and the common pathway and to factors of the contact phase. Actually, the APTT is altered in case of deficit of the factors of the phase of contact: prekallikrein (PK), high molecular weight kininogen (HMWK) and factor XII, which do not give clinical hemorrhagic manifestations. It is also prolonged in the deficiency of intrinsic pathway factors XI, IX and VIII and, together with PT, in the deficiency of the common pathway factors X, V and II and fibrinogen (9,43-49).

APTT is commonly required by clinicians to evaluate the intrinsic pathway of the coagulation system and then to assess the possible deficiency of one of the factors of the intrinsic pathway (VIII, IX, and XI). It is important to note, however, that although prolonged APTT is commonly present in patients with severe/moderate hemophilia, normal APTT does not exclude mild hemophilia, due to the different sensitivity of APTT reagents to different factor deficiencies (50-53).

Most APTT reagents (defined as cephalin or partial thromboplastin, as they do not contain tissue factor) are also sensitive to contact factor deficiency (XII, PK and HMWK); these deficits are not clinically relevant, as they are not associated with hemorrhagic diathesis.

APTT can also be altered in other acquired conditions such as liver disease, severe vitamin K deficiency and in DIC. In the latter case, the alteration also affects the primary hemostasis and this often leads to abnormalities of the bleeding time and the number of platelets (54-56). APTT is commonly used for monitoring UFH therapy. Historically, the therapeutic range for the treatment of venous thrombosis had been empirically fixed at APTT values corresponding from 1.5 to 2.5-fold the baseline value (57). Subsequently, it was realized that this APTT prolongation does not accommodate the wide variety of commercially available APTT reagents; it has also been shown that the indiscriminate application of this therapeutic range, regardless of the reagent used, could lead to the patient being under- or over-anticoagulated, when poorly or overly responsive APTT reagents are used for dose adjustment to UFH, effectively preventing a correct heparin therapy (58-70).

Attempts have been made to translate the concept of INR for APTT as well, by providing an international APTT standard against which to calibrate commercial APTT reagents [66]. However, the calibration model was excessively complex to apply in practice and was abandoned also because UFH was subsequently and gradually replaced by LMWHs, since these drugs do not usually require strict laboratory control for dose adjustment. However, there are still clinical conditions in which UFH is prescribed, so clinical laboratories measuring APTT and physicians prescribing UFH need to be aware of these pitfalls: in other words, therapeutic ranges need to be validated for the APTT reagent in use in each individual laboratory. This can be achieved by two methods: titration with protamine sulfate (58) or assay of anti-FXa activity (60,71-73); the latter method is certainly to be preferred due to the excellent analytical reproducibility of the test. The therapeutic range of UFH, when measured by the chromogenic method for measuring anti-Xa activity, is 0.3 to 0.7 UFH units/mL (74-75).

In the absence of a result expression system that harmonizes the measurements obtained with different reagents, it is recommended to use the reagent and the therapeutic range for which there is a consolidated clinical experience. When it is necessary to change the reagent, it is mandatory to determine the sensitivity of the new APTT reagent against UFH and to verify its therapeutic range as a function of the response to the dose of the heparin administered using the anti-Xa test in a concentration range between 0.3 and 0.7 units of heparin/mL.

APTT may be prolonged in the presence of LMWHs, vitamin K antagonists and DOACs (9,43,47-49,76). Finally, APTT is sensitive to the presence of circulating anticoagulants directed against single coagulation factors (for example anti-factor VIII antibodies) or against phospholipid binding proteins (LAC). It is of extreme clinical importance that laboratory diagnostics addresses, with the aid of the APTT mixture test (77-81), and identifies, with the help of specialized coagulation tests, the type of defect responsible for the alteration of APTT. Actually the factor deficit, the presence of anti-factor autoantibodies and LAC are associated with opposite clinical manifestations: hemorrhage in factor deficit and in the presence of anti-factor antibodies or thrombosis in the case of antibodies type LAC (43,49,80).

APTT is defined as the time, expressed in seconds, for an aliquot of plasma, made platelet-poor by appropriate centrifugation (9,82) to clot following the addition of a contact phase activator, of partial thromboplastin as a platelet substitute, plus calcium ions at 37°C (43,47-49). The partial thromboplastins (cephalins) used to perform the APTT are phospholipid extracts of animal tissue or plant origin or are synthetic products. Phospholipids act as platelet substitutes in the intrinsic pathway. The lipid composition of different APTT reagents, however, varies considerably. These discrepancies markedly affect responses to coagulation defects, heparin, and coagulation inhibitors (43-50).

Other components that can influence the coagulation response of the APTT, include the type of activator, the length of the plasma incubation time, and the composition of the buffers (43-50). Activators include particulate activators such as kaolin, celite, and micronized silica, while other activators, such as ellagic acid, are non-particulate. The amount of activator present in the different commercial reagents, and the length of the incubation time of the tests, show considerable variations. The tendency of most of the reagents on the market is to use less opaque activators to avoid interference in the reading of the formation of the clot in photo-optical type coagulometers. Further differences in the clotting times of the different APTT reagents can be ascribed to the

methodology used to determine clot formation: photo-optical or electromechanical/magneto-mechanical (44-50).

The combination of activator-phospholipids with other components such as the type of instrument and the incubation times provide, as a consequence significantly different APTT results (9,43-50).

Different units to report Activated Partial Thromboplastin Time results

Seconds

Absolutely NOT to be used as the times obtained are strongly affected by the different phospholipid composition of the cephalins, their preparation methods, the type of activator present in the reagents, the incubation times, the type of coagulometer used, the methods to reconstitute the reagents, the batch-to-batch variability.

Ratio

The reporting of the results as APTT Ratio (the coagulation time of the patient divided by the "normal value") as reported in the literature improves the comparability of APTT results between different laboratories and within the same laboratory, in particular for the adjustment of heparin dose in the monitoring of UFH therapy (16) and in the laboratory diagnosis of LAC, as suggested by recent ISTH guidelines (77). As shown above for PT (17,18), for APTT the ratio should be derived from the geometric mean of APTT of 20 (or more) apparently healthy subjects, obtained under the same experimental conditions as those in which examinations are performed in patients.

RECOMMENDATIONS FOR THE ACTIVATED PARTIAL THROMBOPLASTIN TIME LABORATORY REPORT

We recommend the use of only one unit of measurement:
-Ratio

TAKE HOME MESSAGES

-The use of multiple units to express the results of PT and APTT is strongly discouraged; double results (seconds and ratio for APTT) or even triple or quadruple (i.e. coagulation time, prothrombic activity, INR and ratio for PT) are strictly not recommended, as they are not educational, do not meet the requirements of proper reporting and can be misleading (83-90).
-The reference values of the PT-ratio and the APTT-ratio should be calculated locally, checked periodically (at least once a year) and recalculated in case of instrumental, reagent and batch changes.
-The INR results, due to the different therapeutic ranges relating to the patient specific thrombotic disease (venous or arterial, biological or mechanical heart valves), should always be accompanied by a note (for example "*The therapeutic range varies according to the disease for which the patient is treated with the anticoagulant drug*").
- The APTT should be reported only as a ratio, indicating the reference range, possibly calculated locally and with the same suggestions as above for the PT. Even if UFH therapy is nowadays much less used than in the past, it would be appropriate to indicate the suggested (and possibly locally calculated) therapeutic range for the APTT reagent in use in the laboratory, due to the great variability of response of commercial cephalins to the presence of heparin.
- PT and APTT must not be used to evaluate the anticoagulant activity of DOACs; for these drugs, the specific tests suggested by the reference Guidelines have to be utilized.

CONFLICT OF INTEREST

None

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