

Survey on current practice in thrombophilia testing: from phenotype to genotype. Communication from the SSC of the ISTH

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Abstract

Diagnosing inherited thrombophilia as the cause of venous thromboembolism is important for patient management. Deficiencies in antithrombin, protein C, and protein S are usually diagnosed by plasma-based assays. Genetic testing can confirm the congenital nature of these deficiencies. Factor V Leiden can be detected using activated protein C resistance assays, followed by or replaced by molecular confirmation, whereas prothrombin G20210A can only be detected genetically. During the last decade, molecular techniques have evolved from single-gene sequencing to multigene sequencing panels. To understand current thrombophilia testing practices, a questionnaire was designed focusing on thrombophilia testing in coagulation laboratories and how genetic testing is placed in the diagnostic workflow. All International Society on Thrombosis and Haemostasis members and participants in external quality control schemes on thrombophilia testing were invited to complete the survey. Eighty-two unique responses were received. This international survey showed that laboratories perform plasma-based thrombophilia testing, but 42% restrict it to requests from thrombosis/hemostasis specialists, patients without anticoagulant treatment, or those with a strong personal or familial history of venous thrombosis. However, phenotypic

Manuscript handled by: Jerrold Levy

Final decision: Jerrold Levy, 20 October 2025

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testing is not always performed according to published guidelines. More specifically, the transference of reference intervals from manufacturers or literature is often suboptimal. For results interpretation, anticoagulant use and acquired causes were considered the most. Genetic testing is not systematically included in the diagnostic work-up algorithms and is mostly restricted to single-variant testing. Multigene panel testing is only performed by a minority of laboratories. Our results highlight the necessity for recommendations on how and when to perform this kind of testing.

KEYWORDS

activated protein C resistance, antithrombin, clinical laboratory testing, protein C, protein S, thrombophilia

1 | INTRODUCTION

Patients with deficiencies of physiological anticoagulants such as antithrombin, protein C, and protein S are usually identified by functional or antigenic assays performed on plasma. The congenital nature of these deficiencies can be confirmed using molecular analysis of the respective genes. Factor V (FV) Leiden and prothrombin G20210A (PTG20210A) are gain-of-function variants that result in a higher risk of venous thrombosis than in noncarriers and are also commonly included in screening protocols. FV Leiden can initially be detected with phenotypical activated protein C (APC) resistance assays and is generally confirmed with a molecular analysis; in some cases, only a molecular analysis is undertaken. On the other hand, PTG20210A can only be detected with a molecular analysis. Although considered rare, non-FV Leiden *F5* variants conferring APC resistance are not routinely sought, even in APC resistance-positive/FV Leiden -negative cases [1]. New genetic causes of thrombophilia, such as antithrombin resistance and FVIII and FIX Padua, more recently described in private families, have not been evaluated in large cohorts and their frequency among patients with thrombosis is still unknown [2–4].

During the last decade, molecular techniques have evolved from single-gene Sanger sequencing to high-throughput multigene sequencing panels [5]. This has led to the discovery of new genetic variants with, in many cases, uncertain pathogenic significance (named variants of uncertain significance or VUS). This multigene sequencing approach has also led to the identification of variants in other genes, in addition to those tested using plasma-based thrombophilia assays [6]. Two recent genetic studies for thrombophilia have used a 31-gene panel in 194 venous thromboembolism (VTE) patients, finding a 63% diagnostic rate (including FV Leiden and PTG20210A) and a 28-gene panel in 133 VTE patients, detecting variants in 35% of the patients (excluding FV Leiden and PTG20210A) [7,8]. The decreased cost of multigene sequencing, the increased number of pathogenic variants that are not detected by current plasma-based functional methods [3,9,10], and the effort of experts curating the variants in genes included in these panels have raised the discussion about potential changes in thrombophilia testing. It can indeed be debated whether genetic tests should be

used as a first-line screening and complemented by functional tests, at least for some selected patients.

To gain insight into the current practice of thrombophilia testing in clinical coagulation laboratories worldwide, we designed and distributed a survey focusing on phenotypic (eg, type of assays, source of reference ranges, interpretation of results) and genetic (eg, single-gene or multigene approach) testing for inherited thrombophilia. This survey was designed to also provide insight related to whether laboratories adhere to the recommendations for laboratory testing for thrombophilia previously published by the Scientific and Standardization Committee (SSC) subcommittee on Physiological Anticoagulants and Thrombophilia [11–14]. Laboratory testing for acquired thrombophilia, including lupus anticoagulant and antiphospholipid antibodies, was outside the scope of this study.

2 | METHODS

2.1 | Survey questionnaire

A questionnaire (see [Supplementary Material](#)) addressing phenotypic and genotypic testing strategies for inherited thrombophilia testing was formulated by consensus by the authors and hosted on the International Society on Thrombosis and Haemostasis (ISTH) website using REDCap. The language of the survey was English. The questionnaire was advertised and distributed to all ISTH members by email (ISTH Fast Five), social media (LinkedIn pages of ISTH and African Society on Laboratory Medicine), and advertised during the SSC sessions of the ISTH 2023 and 2024 congresses. Additionally, participating laboratories of external quality control schemes for thrombophilia from ECAT (External quality Control of diagnostic Assays and Tests), NASCOLA (North American Specialized Coagulation Laboratory Association), UK-NEQAS (United Kingdom National External Quality Assessment Service), and RCPAQAP (Royal College of Pathologists of Australasia Quality Assurance Programs) were invited to complete the questionnaire, as they represent the target audience of the survey. The survey was open from June 2023 to July 2024.

2.2 | Data analysis

Data were exported from the REDCap platform into an Excel (Microsoft) spreadsheet and were analyzed using basic descriptive statistics. When more than one submission from the same institution was received, these were checked for agreement and duplicates were removed.

3 | RESULTS

3.1 | General information

Eighty-six completed questionnaires were received between June 2023 and July 2024. After removal of blanks and duplicates, 82 unique responses were available for data analysis. The majority of responses were from laboratories located in Europe (55%), followed by North America and Australia/New Zealand (each 13%). Laboratories located in Asia, South America, the Middle East, and Africa accounted for 6%, 6%, 3%, and 1% of the respondents, respectively. Three percent of the respondents did not specify their location. The locations of the responding sites can be found in [Supplementary Figures S1 and S2](#). Half of the respondents (50%) worked in university hospital laboratories and 39% in non-university hospital laboratories. The remaining respondents worked in research or private laboratories (6%), in a hemostasis center (1%), in both a university hospital laboratory and research institute (1%), or did not specify their place of work (3%). Most laboratories (34%) undertake 100 to 500 thrombophilia tests annually, 21% conduct 500 to 1000 tests, 26% perform more than 1000 tests, and 15% perform less than 100 tests per year. The remaining respondents did not know the number of tests performed annually (3%) or did not specify (1%).

Four of the respondents performed neither phenotypic nor genotypic testing. These records were excluded from further statistical analysis.

3.2 | Plasma-based thrombophilia testing

3.2.1 | Restrictions for testing

From the 74 (90%) laboratories that performed phenotypic thrombophilia testing, 42% had restrictions in place for such testing. [Table 1](#) shows the main reasons to reject thrombophilia testing and the percentage of laboratories following these recommendations (laboratories were given the option to select more than one reason). The main reason for rejecting thrombophilia test requests was active anticoagulant treatment (42%), absence of early-onset or strong family history of VTE (26%), or request made by an MD who is not a specialist in thrombosis and hemostasis (23%), or other reasons (49%) such as the presence of an acute thrombotic event, adherence with published guidelines, recurrent miscarriage, or for reimbursement reasons. Laboratories located in Europe and North America tended to perform thrombophilia testing irrespective of the

TABLE 1 Reasons for restricting thrombophilia testing.

Reason	n (%)
Use of anticoagulant treatment	13 (43)
Absence of early onset or strong family history of VTE	8 (26)
Requests not made by MDs specialized in thrombosis/hemostasis	7 (23)
Other	15 (48)

MD, medical doctor; VTE, venous thromboembolism.

indication or condition of the patient, since only 39% and 22% of these labs, respectively, indicated to have restrictions in place for testing.

3.2.2 | Thrombophilia assays

All laboratories performing plasma-based thrombophilia testing indicated testing for antithrombin, protein C, and protein S, whereas only 55% also measured APC resistance. The remaining laboratories directly performed FV Leiden genotyping instead of performing the APC resistance assay. There were no regional differences in the choice to perform APC resistance or FV Leiden genotyping. Only 46% of the laboratories performing APC resistance confirmed their positive results by FV Leiden genotyping, irrespective of the test used (ie, with or without addition of FV-deficient plasma).

Laboratories were asked about the type of assay(s) they performed for each of the investigated thrombophilia parameters and were given the option to indicate more than one test per parameter. The distribution of the responses is depicted in [Figure 1](#). The most commonly used assays were anti-Xa activity for antithrombin deficiency (53%), chromogenic activity for protein C deficiency (78%), free protein S antigen for protein S deficiency (80%), and modified APC resistance assay (54%). For antithrombin, protein C, and protein S, the majority of laboratories performed only 1 assay per parameter (from 63% for protein S to 73% for protein C), as shown in [Figure 2](#). Only a minority of laboratories performed a combination of (one or more) activity and antigen assays for each thrombophilia parameter.

Nine of the laboratories performing phenotypic testing for inherited thrombophilia indicated they performed other tests besides antithrombin, protein C, protein S, and APC resistance, but only 6 of them specified which tests. These tests were FVIII ($n = 1$), lupus anticoagulant ($n = 2$), homocysteine ($n = 1$), thrombin generation ($n = 1$), plasminogen ($n = 1$), and plasminogen activator inhibitor 1 mutation analysis ($n = 2$).

3.2.3 | Reference ranges

For the most commonly used assays, the majority of laboratories (range 49%–58%) used reference ranges provided by the manufacturer ([Table 2](#)). Per good laboratory practice, when using reference

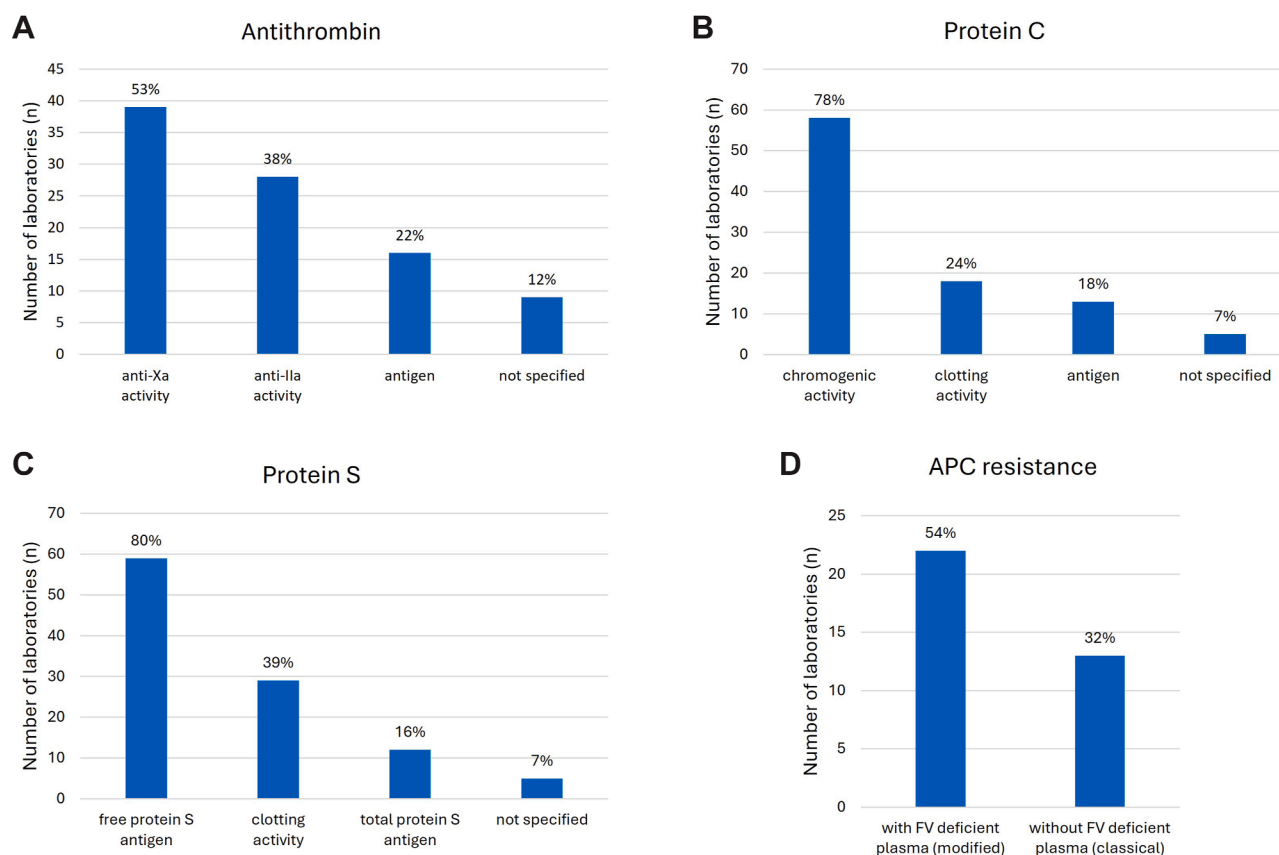


FIGURE 1 Assays performed for antithrombin (A), protein C (B), protein S (C), and APC resistance (D). The y-axis indicates the number of laboratories, and the percentage of responding laboratories following each restriction is provided on top of the bars. APC, activated protein C; FV, factor V.

ranges provided by the manufacturer of the assay kit, or when transferring reference ranges from the literature, these should be re-evaluated in-house. However, only 40% to 53% of the laboratories used reference ranges from the literature or manufacturers, depending on the specific thrombophilia test (Table 2). Moreover, the number of samples used to re-evaluate this reference range was often insufficient to validate the range; in some cases, only 5 samples were used.

Reference ranges were established in-house by 22% to 33% of the laboratories, depending on the parameter, and the number of individual samples used to establish this reference range varied between 15 and 250. For the less frequently used assays, the sources of reference ranges and whether they were checked in-house can be found in the [Supplementary Tables](#). Respondents were given the option to report the lower limit of the reference range used in their laboratory for the different assays they perform, and the corresponding data are presented in Figure 3.

For antithrombin anti-Xa activity (Figure 3A), the reported lower limit of the reference range varied from 0.70 to 0.87 IU/mL, with the majority of laboratories (55%) using 0.80 IU/mL. A comparable pattern was seen for antithrombin anti-IIa activity assays, ranging

from 0.70 to 0.86 IU/mL, with 0.80 IU/mL used by 73% of laboratories.

For protein C (Figure 3B), the widest variability for the lower limit of the reference range was observed for the chromogenic activity assay, ranging from 0.50 to 0.81 IU/mL. However, most laboratories (70%) used 0.70 IU/mL as the lower limit of their reference range.

Fifty-eight percent of the laboratories used sex-specific reference ranges for protein S. The greatest variability in the lower limit of the reference range was observed for the free protein S antigen assay (ranging from 0.49 to 0.70 IU/mL), even when sex-specific reference ranges were used (from 0.48 to 0.65 IU/mL for women and from 0.57 to 0.75 IU/mL for men) (Figure 3C, D).

For APC resistance, 33% of the laboratories performing the classic APC resistance assay reported their cutoff value for positivity. Five of the laboratories reported using a standard ratio (ie, activated partial thromboplastin time with addition of exogenous APC divided by the baseline activated partial thromboplastin time, with values ranging from 1.80 to 2.14). Approximately half of the laboratories (46%) that performed the modified APC resistance assay reported their cutoff value. Seven laboratories employed a standard ratio

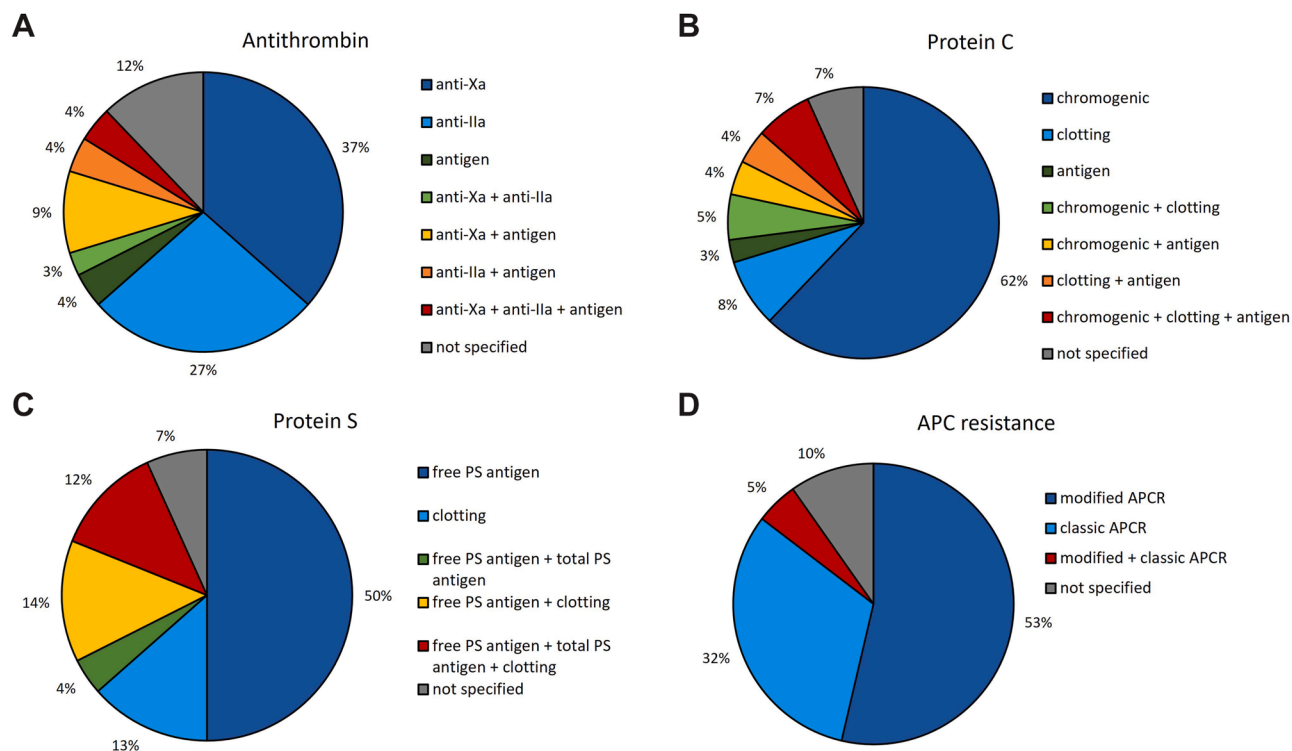


FIGURE 2 Assay panel used for evaluation of antithrombin (A), protein C (B), protein S (C), and APC resistance (D). APCR, activated protein C resistance; PS, protein S.

(range, 2.0-3.0), and 4 laboratories employed a normalized ratio (range, 0.72-0.90).

3.2.4 | Results interpretation

Participants were asked which variables they consider when interpreting thrombophilia test results. The presence of acquired causes and the use of anticoagulants were considered the most (Figure 4). Only 5% of the laboratories indicated taking patient ethnicity into account when interpreting test results. Most laboratories interpreted results according to the age of the patient (ie, adult vs pediatric), ranging from 60% of the laboratories for antithrombin to 76% of the laboratories for protein S.

3.3 | Genetic thrombophilia testing

Fifty-six percent of the responding laboratories indicated they performed genetic testing in the diagnostic workup of thrombophilia, while 32% outsource their requests for genetic testing to other institutions, which were mostly clinical hospital laboratories. Patient inclusion criteria were surveyed for the laboratories performing or outsourcing genetic testing. Only 22% of laboratories provided genetic testing secondary to prior abnormal thrombophilia screening,

whereas 38% of laboratories offered genetic testing irrespective of plasma-based testing results. Two laboratories specifically mentioned a strong family history of thrombosis as an indication for genetic testing. The survey showed wide heterogeneity in the type of genetic test that was used (Figure 5). Most laboratories (54%) still only performed single variant testing whereas 26% offered multigene panel testing for thrombophilia. Laboratories performing multigene panel testing were mainly based in Europe and North America (75%).

For laboratories that performed single-gene testing, the variant detection rate for genes encoding the natural anticoagulants, *PROC*, *PROS1*, and *SERPINC1*, was only known in a small proportion of laboratories (5%) and ranged between 20% and 100%. In the case of multigene panel testing, performed by 12 laboratories, 66% of the laboratories included genes based on the ISTH list of diagnostic-grade genes (TIER1, https://www.isth.org/page/GinTh_GeneLists) [15]. Only 17% of laboratories included additional genes, such as *MTHFR* and *PLAT*, and the remaining 17% did not know which genes are included in their panel. If multigene panel testing was performed, the diagnostic rate was known by only 58% of the laboratories and was widely variable, ranging from 1% to 10% to 40% to 50%. The detection of variants in more than one gene was only provided by 8 laboratories and ranged between 1% to 10% and 30% to 40%. Finally, the management of VUS was questioned. Only a few laboratories ($n = 13$) performed family studies and additional laboratory assays including recombinant protein expression studies and splicing assays

TABLE 2 Overview of the source of reference ranges for the most commonly used thrombophilia assays.

	AT anti-Xa activity (n = 39)	PC chromogenic activity (n = 58)	Free PS antigen (n = 59)	APC resistance with FV deficient plasma (n = 24)
Source of the used reference ranges, n (%)				
Manufacturer	20 (51%)	30 (52%)	29 (49%)	14 (58%)
Literature	6 (15%)	11 (19%)	12 (20%)	1 (4%)
In-house	10 (26%)	13 (22%)	16 (27%)	8 (33%)
Unknown	3 (8%)	4 (7%)	2 (3%)	1 (4%)
Literature or manufacturer reference range re-evaluated in-house				
Yes	10/25 (40%)	19/40 (48%)	18/38 (47%)	8/15 (53%)
No	15/25 (60%)	21/40 (52%)	20/38 (53%)	7/15 (47%)
Number of samples used to re-evaluate the reference range from manufacturer or literature, median (range)				
	40 (5-120)	30 (2-120)	22 (8-40)	40 (20-120)
Number of samples used to establish the in-house reference range, median (range)				
	40 (15-250)	40 (15-120)	50 (15-250)	35 (24-200)

APC, activated protein C; AT, antithrombin; FV, factor V; PC, protein C; PS, protein S.

to support variant reclassification for VUS. Nine of these laboratories were in Europe and the remainder in North America, Africa, and Australia.

Twelve percent of the responding laboratories indicated that the option for genetic testing was not offered.

4 | DISCUSSION

We collected information on phenotypic and genetic thrombophilia testing in specialized laboratories worldwide. Seventy-four laboratories performed phenotypic thrombophilia testing. Only 42% of these laboratories had restrictions in place for testing, which was mostly related to the use of anticoagulant drugs. It is well-known that the use of heparin, vitamin K antagonists, and direct oral anticoagulants can influence the results of thrombophilia testing, either leading to false-negative or false-positive results, depending on the type of anticoagulant and the type of assay used [16]. Restricting thrombophilia testing to requests made only by physicians specialized in thrombosis or hemostasis (23%) or patients with clear early onset VTE or strong family history of VTE (26%) avoids overtesting. There are several guidelines available on who, when, and what to test for inherited thrombophilia [17–19], and although not always in complete agreement, they all agree that testing in unselected patients is not warranted.

All laboratories performing phenotypic thrombophilia testing performed at least one assay for antithrombin, protein C, and protein S. Resistance for APC was performed by half of the laboratories, while the other half performed genetic testing for FV Leiden. Genotyping FV Leiden without measuring APC resistance implies that other *F5* variants that confer APC resistance could be missed [13,20]. This is particularly important in populations with low or null prevalence of FV

Leiden and a higher proportion of other *F5* variants causing APC resistance. Surprisingly, only half of the laboratories performing APC resistance confirmed their positive results with FV Leiden genotyping, which is encouraged by current recommendations [13]. As a result of not performing FV Leiden genotyping, laboratories cannot distinguish between inherited or acquired APC resistance when a classical APC resistance assay is used and heterozygous or homozygous FV Leiden status cannot be confirmed. The choice of test, ie, APC resistance versus Factor V Leiden genotyping, might be driven by reimbursement restrictions, but our survey did not inquire about that.

Published ISTH recommendations encourage the use of a chromogenic protein C, free protein S antigen, and a modified APC resistance assay as first-line assays, which is followed by the majority of the laboratories in this survey [12–14]. For antithrombin, the recommendation advises the use of an activity assay but does not favor FXa-based activity assays over FIIa-based assays [11]. Surprisingly, up to 4% of laboratories are performing only antigen assays for antithrombin and protein C, which indicates that only type I antithrombin and protein C deficiencies will be diagnosed and type II deficiencies will be missed. However, there is no single assay for antithrombin, protein C, or protein S able to detect every deficiency type and subtype, indicating that some defects will remain undiagnosed [1]. This, and the fact that certain pathogenic variants are not detected by functional methods, might be overcome by genetic testing [21,22].

When considering reference ranges, most laboratories utilize reference ranges provided by the manufacturer. Transference of a manufacturer's reference interval is acceptable only if verified by each laboratory [23]. However, the results of our survey indicate that the appropriateness of the reference interval was evaluated in-house by half or less than half of the laboratories, depending on the parameter tested. Moreover, the number of samples used for this check was insufficient in some cases. Although recommendations

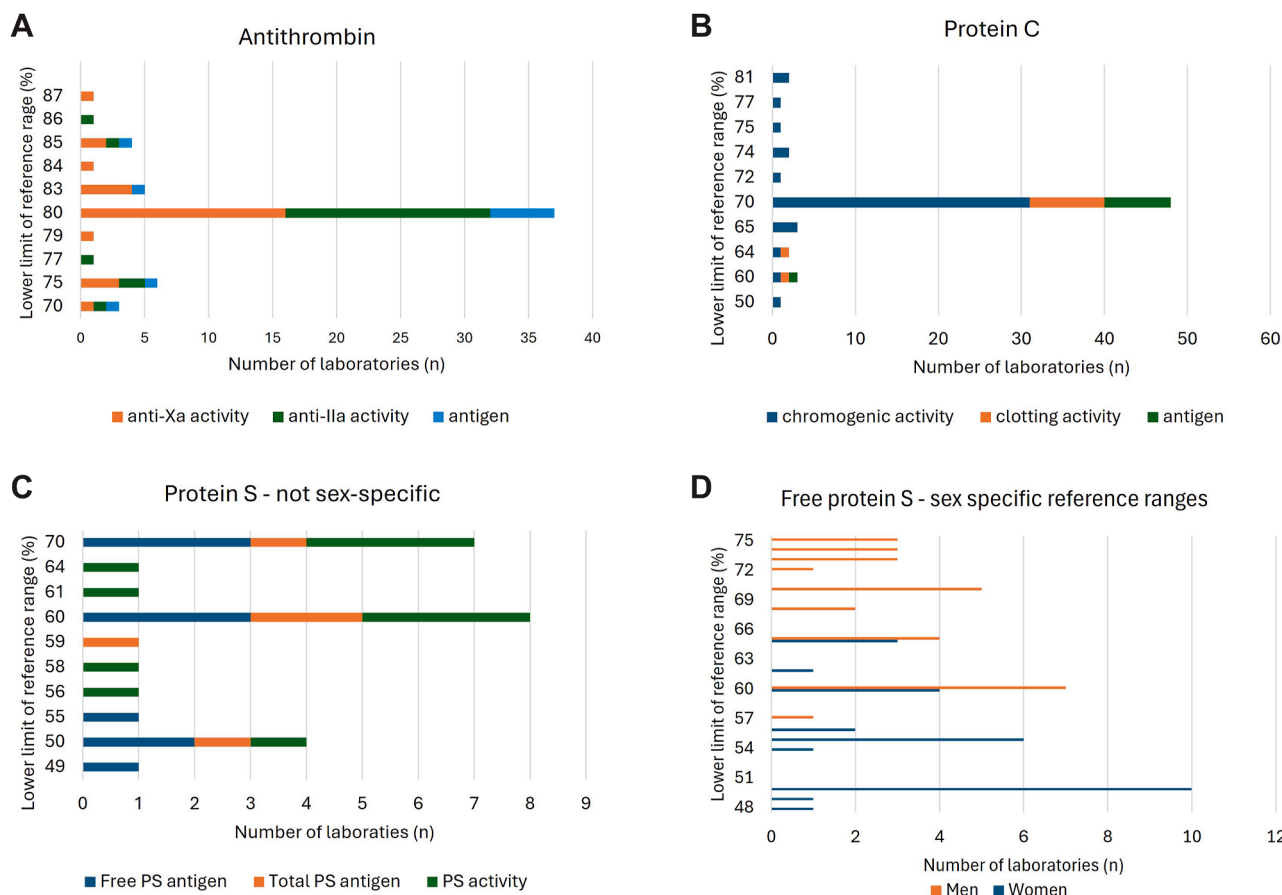


FIGURE 3 Lower limit of the reference ranges for the different assays for antithrombin (A), protein C (B), and protein S (C). Lower limit of reference range for free protein S antigen for males and females (D). PS, protein S.

advise testing of a minimum of 20 to 40 healthy donors, our results show that some laboratories use a much lower number of samples, and they might thus reach an inappropriate conclusion after their validation protocol. Reference ranges were established in-house by only up to one-third of laboratories, depending on the parameter. Establishing a reference interval can be undertaken with a minimum of 39 samples if the distribution of the results is Gaussian, although using more samples is ideal [24]. The Clinical and Laboratory Standards Institute guidelines recommend 120 samples from healthy individuals, and if distribution is non-Gaussian, 120 samples are the definite minimum. Outliers should be detected as described in the guidelines [23,24]. However, only a minority of laboratories fulfilled this requirement, probably due to the difficulty of obtaining such a large number of samples. This implies that the majority of laboratories that establish their reference range use a range that is not appropriately determined, which might ultimately lead to erroneous interpretation of the results of patient samples.

For protein S, sex-specific variations in healthy individuals are well known [25] and are accounted for in clinical practice in just over one-half of the laboratories. Despite the use of sex-specific reference ranges, protein S was the parameter for which the widest variability in the lower

reference was observed, emphasizing that laboratories still struggle with the verification and establishment of specific reference ranges.

An intriguing observation in our study was the reported cutoff values for the normalized APC resistance ratio. By definition, normalized ratios should be at or close to 1.0, which was not the case in this survey. This is a matter of concern and suggests that these laboratories are using inappropriate normal plasmas.

Most laboratories indicated taking the age of the patient into account when interpreting results of thrombophilia parameters. Indeed, the hemostatic system changes and matures throughout the time from fetal to adult life, and the use of age-specific reference ranges is recommended [26]. Moreover, pediatric reference ranges differ largely between reagent/analyzer combinations and are different from adult reference ranges [27–29].

Ethnicity was only considered by a minority of laboratories when interpreting the results of thrombophilia testing. It is known that the prevalence of inherited thrombophilia is highly variable among populations [30,31]. Also, reference values may change according to population. A study performed in the UK found that healthy Black individuals have significantly lower protein C and protein S levels compared to the reference range derived from the White population, with a trend toward

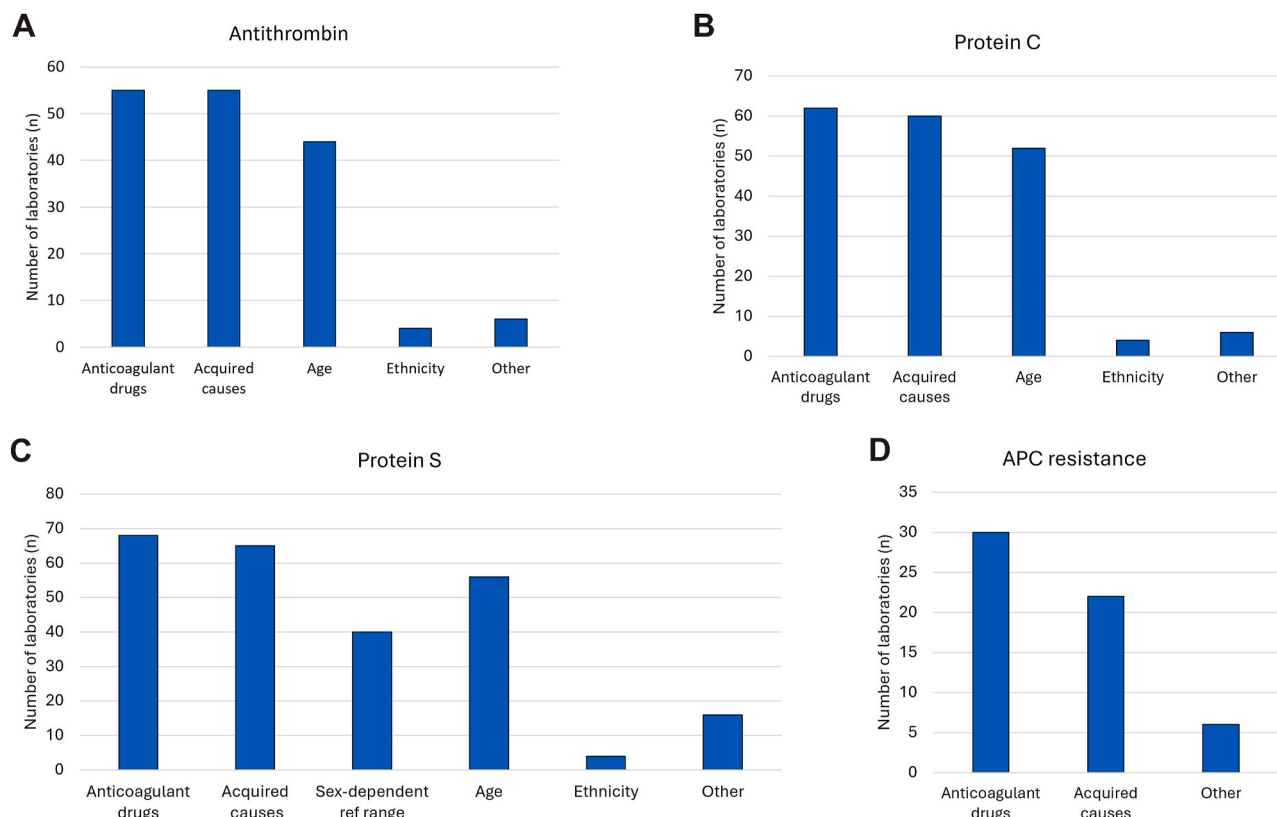


FIGURE 4 Suggested options for consideration of results interpretation for antithrombin (A), protein C (B), protein S (C), and APC resistance (D). APC, activated protein C.

lower antithrombin levels [32]. In addition, a study performed on healthy Nigerian blood donors found a significantly lower reference range for antithrombin activity than the range for Caucasians [33]. Therefore, laboratories are encouraged to consider preparing local, population-specific reference ranges, where possible.

Although new high-throughput sequencing approaches for thrombophilia testing have been developed during the last few years [5], and the cost of genetic testing has substantially declined, these survey results indicate that the conventional, phenotypic thrombophilia tests remain common practice in laboratories worldwide. Indeed, 90% of the respondents indicated they performed phenotypic thrombophilia testing. Although genetic testing for thrombophilia is also widely performed, it is mostly limited to single-variant testing (ie, FV Leiden and/or PTG20210A variants) in half of the laboratories. Genetic testing for thrombophilia diagnosis is provided by just over half of the laboratories, while most others outsource genetic testing. Although multigene panel testing recently became available in clinical diagnostics [5,6], only a minority of the surveyed laboratories perform multigene panel testing, and generally base it on the ISTH TIER1 gene list [15]. Depending on the laboratory, the indication to perform genetic testing is irrespective of the result of the phenotypic thrombophilia testing, meaning that genetic testing can also be initiated in cases with a normal plasma-based thrombophilia panel. This survey provided limited data about

diagnostic rates for *PROC*, *PROS1*, or *SERPINC1* variants but showed widespread results. This is probably due to differences in genetic test requests and patient inclusion criteria. Similar findings were observed for the detection of variants in multiple genes in a single patient. This survey also showed widespread clinical practices of thrombophilia diagnosis based on genetic testing and only limited use of multigene panel testing in clinical settings. Practical guidelines that include genetic testing in the thrombophilia diagnostic workup are necessary, as are specific patient inclusion criteria for clinicians requesting genetic testing. However, since genetic testing has become more feasible, laboratories should be aware of complexities regarding variant classification and genetic interpretation. Additionally, a clear management of VUS is needed, as a high yield of VUS is expected in (anti)coagulation-related genes [5,6]. Reports as recently described by Verstraete et al. [7] and Suchon et al. [8] are of clinical value in evaluating genetic testing in thrombophilia work-up. Future studies should focus on the specific benefit of using multigene panel-based genetic testing based on the integration of clinical, familial, and laboratory data. The place of both genetics and laboratory thrombophilia testing should be addressed. Which patient populations should be submitted for thrombophilia testing, and more importantly, what is the best testing strategy, including genetic and phenotypic thrombophilia testing? The ISTH SSC Subcommittee on Physiological Anticoagulants and Thrombophilia is

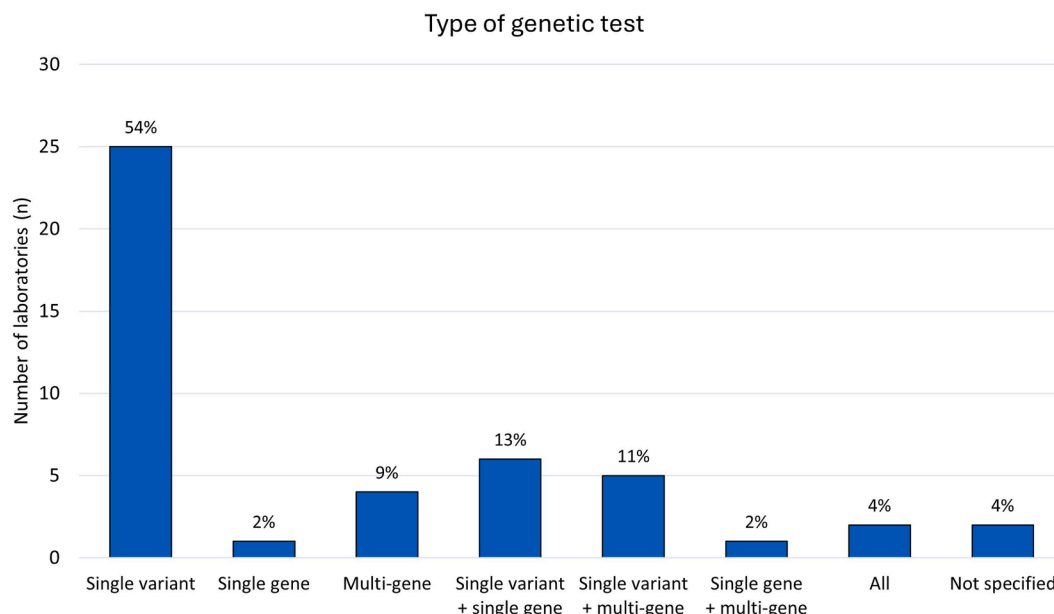


FIGURE 5 Type of genetic testing for inherited thrombophilia if performed by the participating laboratories.

currently working on a clinical and laboratory guidance paper on inherited thrombophilia testing to answer these questions.

An important limitation of this study is the uneven geographic representation of the respondents, which may introduce a potential bias in the results and limit the generalizability of the conclusions. More specifically, laboratories from Africa are largely underrepresented in our study.

5 | CONCLUSION

This survey provides a comprehensive overview of the current status of laboratory detection of inherited thrombophilia worldwide. Our results show that the current phenotypic thrombophilia testing strategy is often inconsistent and suboptimal compared to published guidelines. Genetic testing is not systematically included in diagnostic work-up algorithms and is mostly restricted to single-variant testing. Although multigene panel testing is being incorporated into clinical practice, our results emphasize the need for specific recommendations on how and when to perform this kind of testing to ensure standardization across laboratories and countries.

ACKNOWLEDGMENTS

This is a joint project from the ISTH SSC Subcommittees on Physiological Anticoagulants and Thrombophilia (PAT) and OMICs in Thrombosis and Haemostasis. We thank ECAT, NASCOLA, UK-NEQAS, and RCPAQAP for their assistance in the dissemination of the survey among their participants. The authors wish to thank all the respondents to the survey.

AUTHOR CONTRIBUTIONS

C.O. conceived the idea. C.O., G.W.M., J.C., R.M., and V.I. prepared the survey questions. C.O. analyzed the data. C.O. and C.V. wrote the first draft of the manuscript and prepared the figures. G.W.M., J.C., R.M., K.F., and V.I. critically reviewed the manuscript. All authors revised and accepted the manuscript, which was then approved by the Scientific and Standardization Committee subcommittees on Physiological Anticoagulants and Thrombophilia, and Genomics in Thrombosis and Haemostasis.

DECLARATION OF COMPETING INTERESTS

Gary W. Moore reports consultancy fees from Technoclone.

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SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at <https://doi.org/10.1016/j.jtha.2025.10.032>.