Diagnostic challenges in current laboratory practice at Italian Hemophilia Centers: findings from a nationwide survey

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Availability of data and material: the complete list of 51 survey questions is provided in *Supplementary Material S1*. Furthermore, upon request, the de-identified and aggregated dataset is available to the reviewer for verification of the statistical analysis.

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ABSTRACT

Background: Laboratory diagnosis is essential for identifying inherited bleeding disorders and monitoring therapy. We report results of a national survey by the Italian Association of Hemophilia Centers (AICE) that assesses test and reagent availability relative to guidelines and identifies improvement areas in reference laboratories (RLs) affiliated with hemophilia centers.

Methods: Forty-three RLs participating in the ECAT External Quality Assessment completed an online questionnaire of 51 items across 11 sections.

Results: Of surveyed RLs, 46.5% are located within hemophilia centers, and 53.5% are external, most within the same hospital. Most employ dedicated hemostasis personnel and use analytical platforms from Werfen, Siemens, or Stago. Nearly all perform one-stage assays for FVIII and FIX activity; 86% also use chromogenic substrate assays (CSA). Availability of CSA for FIX and capacity for CSA-based inhibitor titration are limited. Significant heterogeneity exists in reagent selection and application, notably for activated partial thromboplastin time and FVIII/FIX assays, and in von Willebrand disease (VWD) diagnostic workflows. Turnaround times are generally longer, and reagent use is more variable in external RLs.

Conclusions: Practices across several RLs do not fully align with national and international recommendations for the diagnosis and monitoring of hemophilia and VWD. Regional procurement policies influence test availability. Findings support AICE's initiative to establish a working group to improve laboratory diagnostic standards and highlight the need for targeted training and collaboration among clinical, laboratory, and healthcare management stakeholders. These data provide a baseline for harmonizing practices, guiding procurement decisions, and prioritizing education, research, and quality improvement initiatives nationally.

Key words: inherited bleeding disorders; hemostasis laboratory; hemophilia diagnostics; von Willebrand disease diagnostics.

Introduction

Hemostasis is a dynamic biological system involving distinct players (cellular and plasma components) and complex processes. Its laboratory analysis is often challenging. Laboratory hemostasis exhibits several distinctive characteristics: the need to perform multiple tests for diagnosis, the variability of results owing to preanalytical variables, the persistent lack of standardization and harmonization of certain assays and diagnostic algorithms.[1] These analytical challenges are particularly critical in the laboratory diagnosis and therapeutic monitoring of subjects with inherited bleeding diseases.[2]

Inherited bleeding disorders, such as hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency), and Von Willebrand Disease (VWD) (the most common inherited bleeding disorder), require highly specialized and accurate laboratory support. These conditions significantly impact the lives of affected individuals, not only through acute bleeding episodes but also by affecting their overall quality of life (QoL) due to chronic joint disease, treatment burden, and psychological stress.[3]

The laboratory is a cornerstone of diagnosis, accurate classification (e.g., VWD subtyping), and therapeutic monitoring of inherited bleeding diseases,[4,5] particularly with the introduction of new non-factor replacement therapies such as emicizumab, that make laboratory's role even more critical for validating treatment efficacy and detecting potential complications.[2] Furthermore, the emergence of acute conditions such as acquired hemophilia A, a rare life-threatening bleeding disorder, needs immediate availability of reliable and rapid coagulation assays for prompt diagnosis and management.[6] Recognizing the need to enhance the quality of diagnostic testing and therapy monitoring nationwide, the Italian Association of Hemophilia Centers (AICE, Associazione Italiana Centri Emofilia) established a dedicated working group in 2016 whose first major action was establishing a collaboration with the ECAT Foundation (Voorschoten, The Netherlands), ensuring that all affiliated AICE laboratories participate (four times per year) in a dedicated ECAT External Quality Control Program, economically supported by AICE. While external quality assessment (EQA) is vital, it provides only a snapshot of analytical performance but doesn't reveal operational context, resource allocation, specific methodologies (e.g., mixing studies, VWD subtyping) and the capacity for complex assays (e.g., Factor XIII, Emicizumab) used in daily practice.

To achieve these objectives, the AICE Working Group developed and conducted a comprehensive national survey to rigorously map current practices, resource allocation, technical platforms, and quality control programs across laboratories affiliated with Italian hemophilia centers, thereby identifying potential variations and gaps relative to national and international guidelines.[5,7] The survey is the first comprehensive national effort to systematically describe the reality of specialized hemostasis laboratories in Italy.

This study aimed to provide a comprehensive, cross-sectional mapping of diagnostic and methodological practices across the AICE Reference Laboratories (Rls). Using a structured survey instrument, primary objectives were: i) to document the current state of staffing, analytical platforms, and assay availability for hemophilia and VWD; ii) to assess adherence to key quality control (QC) procedures and turnaround times (TAT); and iii) to identify significant differences in practices and performance between laboratories located Internal (iRLs) *vs* External (eRLs) to the Hemophilia Treatment Centers.

Materials and Methods

Survey instrument design and validation

The survey was distributed as an online questionnaire, comprising both open- and closed-ended questions, designed to map diagnostic and management protocols for bleeding disorders across Italian AICE reference laboratories. A total of 51 questions were divided into 11 detailed sections: i) staff and activities organization; ii) analytical platforms of laboratory and methods characteristics; iii) activated partial thromboplastin time (aPTT): analytical platform, reagents, mixing studies; iv) FVIII assays; v) FIX assays; vi) FVIII and FIX inhibitor titration; vii) FXIII measurement; viii) Emicizumab concentration measurement; ix) Screening of platelet function defects; x) VWD diagnosis; xi) external and internal quality control programs.

The questionnaire was developed by the AICE Working Group on "Quality of Laboratories" to ensure its content validity and relevance to current clinical practice. A pilot test (pre-test) was conducted on five non-participating laboratories to identify and resolve ambiguities in question wording or survey flow before the primary distribution. Given the predominantly descriptive nature of the survey, which focused on current practices and protocols rather than psychometric scales or latent constructs, Cronbach's Alpha or Factor Analysis were deemed inapplicable for instrument validation.

Dissemination, online tool, and CHERRIES compliance

Identification and recruitment: The target population comprised specialized laboratories identified by Hemophilia Centers of the AICE as their RLs. This process identified 43 RLs eligible for participation, establishing the sample frame.

Dissemination and repository: The referent for each eligible RL received a formal email from AICE in June 2023 that included the study's scope and a unique link to the survey. Answers were collected between June and July 2023. The raw data were stored on a password-protected institutional server, accessible only by the principal investigators.

CHERRIES compliance: The online survey design adhered to the relevant criteria of the CHERRIES (CHEcklist for Reporting Results of Internet E-Surveys) guidelines, thereby ensuring high methodological rigor. This included clear indication of mandatory fields, internal logic controls, and the ability to track completion rates.

Ethical oversight and data preservation: The study surveyed professional laboratory practices and did not involve patient data or clinical interventions, so a formal review by the Institutional Ethics Committee of the Liguria Region was not required. All collected data, including laboratory and respondent identification (required due to the non-anonymous nature of the survey), were handled in strict accordance with GDPR and institutional data confidentiality protocols. Informed consent for participation in the non-anonymous survey and data processing was explicitly

secured on the first page of the online questionnaire. The complete list of 51 survey questions is provided in *Supplementary Material S1*. Furthermore, upon request, the de-identified and aggregated dataset is available to the reviewer for verification of the statistical analysis.

Statistical analysis

The statistical analysis was conducted in two phases to provide both descriptive mapping and inferential insights. Findings were initially reported as relative frequencies (percentages) along with the total number of participants, providing a comprehensive national overview of methodologies. To assess simple associations between categorical variables (e.g., laboratory size and method used), Fisher's Exact test was performed for contingency tables. This choice was made due to the small sample sizes (iRLs n=20 and eRLs n=23), which would result in low expected cell counts, rendering the standard Chi-squared test (even with Yates' continuity correction) unreliable.

Results

Participation in the survey was complete, with all 43 RLs taking part. Of these, 46.5% are located within the Hemophilia Centre (referred to as iRLs), while the remaining 53.5% are situated outside the Hemophilia Centre (eRLs). Specifically, 21 of the eRLs are at the same hospital as the Hemophilia Centre, and 2 are at a different hospital. The nearly equal number of eRLs and iRLs at the AICE centers prompted us to examine the differences in the information provided by these two laboratory groups.

Staff and activities organization

Dedicated hemostasis staff are reported in 81% of RLs, with a median of four operators. Nine laboratories operate 24 h a day, while 12 have on-call staff available on weekends, holidays, and nighttime. An additional five laboratories reported having both arrangements, while 39% did not answer this question. For both iRLs and eRLs, emergency services are equally accessible, covering half of all RLs. Approximately 80% of RLs employ dedicated staff, with no significant differences observed between iRLs and eRLs (Table 1).

Turnaround times in routine and emergency/urgency settings

In routine testing, 86% of RLs report PT, aPTT, fibrinogen, and D-dimer results within 4 h. For single coagulation factor measurements, 62% of RLs deliver results within 24 h. Additionally, 66% of them complete FVIII and FIX inhibitor assessments within 48 h. In emergency and urgent scenarios, 61% deliver PT, aPTT, fibrinogen, and D-dimer results within 1 h, 62% complete single coagulation factor measurements within 8 h, 51% complete FVIII and FIX inhibitor assessments within 8 h. Under routine conditions, TAT comparisons show that iRLs report faster results than eRLs, whereas in an emergency, both groups perform similarly (4 h); iRLs are faster overall. Fewer iRLs exceed 8 h, compared to eRLs, even if this difference was not statistically significant (p=ns) (Table 2).

Analytical platform, calibrations, and reference plasmas

Eighty-one percent of RLs use a single analytical platform, while 19% use two different platforms. Fifty-three percent of RLs exclusively use coagulometers and reagents from Werfen (Bedford, MA, USA), making Werfen the dominant platform. Nineteen percent exclusively use Siemens (Siemens Healthcare, Erlangen, Munchen, Germany), 9% use Stago (Diagnostica Stago, Asnières-sur-Seine, France). Furthermore, 11% of RLs use platforms from both Werfen and Siemens, and 7% use platforms from both Werfen and Stago.

Regarding calibration, 93% of RLs plan their analytical system calibration methods; only 20% perform a calibration curve for factor assays before each test session, whereas 80% calibrate only at reagent batch change. Regarding reference plasma, 74% use commercial plasma, 40% use homemade Pooled Normal Plasma (PNP), and 53% of the latter calibrate their pool against an international standard.

Table 1. Staff and operational coverage across AICE reference laboratories (RLs).

	All RLs (n=43)	iRLs (n=20)	eRLs (n=23)	p-value (χ^2)
Dedicated hemostasis staff	35 (81%)	16 (80%)	19 (83%)	1.000
Median operators per lab (IQR)	4 [3-5]	4 [3-5]	4 [3-6]	N/A
24h/day operations	9 (21%)	4 (20%)	5 (22%)	1.000
On-call staff	12 (28%)	6 (30%)	6 (26%)	1.000
Both 24 and& on-call	5 (12%)	3 (15%)	2 (9%)	0.650

Table 2. Turnaround time in routine/emergency. Data are number (%).

	All (n=43)	iRLs (n=20)	eRLs (n=23)	p-value (Fisher's)
Routine factor assays >48h	8 (17%)	0 (0%)	8 (35%)	0.004
Routine inhibitor assays >48h	14 (32%)	3 (15%)	11 (48%)	0.027
Emergency factor assays <8h	22 (51%)	20 (100%)	12 (53%)	0.0003
Emergency inhibitor assays >8h	20 (46%)	8 (42%)	12 (53%)	0.544

aPTT: reagents, analytical platform, mixing studies

Ellagic acid activators predominate, being used by 35 (81%) of laboratories for aPTT and up to 33 (77%) for FVIII. Silica and Kaolin activators were used less frequently (less than 30%). Fewer RLs (7 iRLs and 5 eRLs) measure Emicizumab plasmatic concentration using a modified FVIII OSA, 9 labs employ Ellagic Acid, 2 use Silica, and 1 use Kaolin as activator (Table 3). A deeper analysis showed that 72% of RLs use a single aPTT reagent, 23% use two, and 5% use three (Table 4): 25% of eRLs use different reagents for aPTT testing than for factor assays, whereas only 5-10% of iRLs do the same. In the aPTT mixing procedure, nearly all RLs (98%) perform immediate testing without incubation; 93% of these repeat testing after 2 h at 37°C.

FVIII, FIX, and inhibitor assays

All the RLs perform a one-stage assay (OSA) for FVIII activity measurement, while 98% perform OSA for FIX activity. The Chromogenic Substrate Assay (CSA) for FVIII activity is performed by 86% of RLs, while for FIX, it is performed by 44%. For OSA, the majority of RLs use FVIII-deficient plasma containing VWF, whereas all RLs exclusively use CSA bovine protein-containing reagents. FVIII inhibitor testing is performed by 42/43 laboratories, with one laboratory requiring that the assay be sent to another AICE RL. The Nijmegen-modified Bethesda assay (64%) is the most widely used method. For residual activity measurement, 55% of RL use CSA. FIX inhibitor testing is performed by 86%, also favoring the Nijmegen-modified Bethesda assay (60%). Heat inactivation is used by most RL to eliminate interference from circulating FVIII and to enhance measurement of low-titer inhibitors. Seventy-nine percent routinely apply heat inactivation before testing both FVIII and FIX inhibitor (Table 5). Additionally, 53% of labs freeze CSA reagents to enhance stability. No significant differences were observed between internal and external RLs regarding the adoption of CSA for FVIII (95 *vs* 78%) and FIX (40 *vs* 48%), nor in inhibitor titration practices. Both laboratory types consistently used the Nijmegen-modified Bethesda method and showed similar rates of FIX inhibitor detection (85% *vs* 74%) and of heat inactivation. While CSA reagent freezing and FVIII inhibitor titration were more frequent in iRLs, these trends did not reach statistical significance. When FVIII inhibitors are present alongside Emicizumab, FVIII residual activity is assessed using the CSA by 86% of RLs (95% of iRLs and 78% of eRLs).

FXIII measurement

Testing for FXIII is performed by 39 laboratories, with antigenic assays used more frequently than functional methods (63 *vs* 37%). Only 9% perform both assay types. FXIII testing is available in 95% of iRLs, compared with 87% of eRLs (p=ns).

Panel of tests for VWD diagnosis

All RLs measure VWF antigen (VWF:Ag), with 91% using a single assay. For platelet-dependent VWF activity (PD-VWF:Act), 84% use the ristocetin cofactor assay (VWF:RCo), and 19% the recombinant GPIbα binding (Table 6). To differentiate between type 2A and 2B VWD, 56% of RLs use ristocetin-induced platelet agglutination (RIPA), and only 14% perform genetic testing. Just 49% conduct the VWF-collagen binding assay (VWF:CB). A diagnostic panel, including FVIII measurement, is standard in 81% of RL. No significant differences were observed between iRL and eRL regarding the adoption of the different VWF assays, although RIPA showed a

Table 3. Type of activator utilized by reference laboratories according to a specific coagulation assay.

Activator	aPTT (n=43)	FVIII (n=43)	FIX (n=42)	Emicizumab (n=12)
Ellagic acid (n of labs)	35	33	31	9
Silica (n of labs)	12	7	6	2
Kaolin (n of labs)	9	8	8	1

Table 4. Distribution of one-stage reagents for coagulation assays. The table summarizes the number (n) and percentage (%) of reference laboratories by specific one-stage reagents used for aPTT, factor VIII (FVIII), factor IX (FIX) activity measurements, and emicizumab concentration analysis.

Reagent (manufacturer)	Activator type	Used for aPTT (n=43)	Used for FVIII (n=43)	Used for FIX (n=42)	Used for emicizumab (n=12)
HemosIL APTT-SP (IL/Werfen)	Silica	3 (6.9%)	0 (0.0%)	1 (2.4%)	1 (9.1%)
HemosIL SynthASil (IL/Werfen)	Ellagic acid	26 (60%)	27 (63%)	26 (62%)	8 (73%)
Syntafax (IL/Werfen)	Kaolin	2 (4.7%)	1 (2.3%)	1 (2.4%)	0 (0.0%)
Pathromtin SL (Siemens)	Kaolin	7 (16%)	7 (16%)	7 (16.6%)	1 (9.1%)
Actin (Siemens)	Silica	2 (4.7%)	1 (2.3%)	2 (4.8%)	0 (0.0%)
Actin FS (Siemens)	Ellagic acid	7 (16.3%)	5 (11.6%)	3 (7.1%)	0 (0.0%)
Actin FSL (Siemens)	Ellagic acid	2 (4.7%)	1 (2.3%)	0 (0.0%)	1 (9.1%)
CK Prest (Stago)	Silica	7 (16.3%)	6 (13.9%)	3 (7.1%)	1 (9.1%)
Total reference laboratories		43	43	42	12

numerical trend toward higher frequency in iRLs that did not reach statistical significance (p=0.081).

External and internal quality control programs

Ninety-one percent of RLs perform specific internal quality-control activities for all provided tests, and 93% participate in external quality-control programs (national or international) other than ECAT. Additional external quality control concerns FVIII and FIX assays in at least 93% of the RLs, and FVIII and FIX inhibitor titration in 81% and 72% of the RLs, respectively.

Discussion

This nationwide survey, encompassing all 43 laboratories supporting Italian Hemophilia Centers, provides a detailed, high-engagement analysis of diagnostic practices. Our findings confirm a shift toward laboratory centralization, with 53.5% of RLs now operating as eRLs. Operational differences between these two laboratory models are not extensively detailed in existing literature, making our comparative analysis a key strength of this study. For this aim, we compared TAT, the test portfolio, and key methodological issues.

Looking at TAT, overall, iRLs tend to deliver results faster than eRLs in both routine and emergency/urgency settings. While both groups perform similarly in some emergency scenarios (e.g., factor assays within 4 h), iRLs consistently show a more favorable TAT profile, particularly fewer delays beyond 48 h in routine conditions and beyond 8 h in emergency. Overall, only approximately 50% of RLs comply with the EU-HANET (European Hemophilia Network) guidelines published in 2014.[8] This is a relevant issue in the quality of care.

Analytical platforms are supplied by Werfen, Siemens, and Stago, with Werfen clearly dominant, likely due to regional procurement preferences. This variation impacts access to specialized diagnostics, as discussed below. Only a few RLs own two or three platforms; we believe this rare situation offers the greatest flexibility to meet clinicians' needs, especially for extended half-life coagulation factor concentrates that require multiple aPTT-based reagents. Most RL routinely calibrate their analytical systems: 40% use homemade pooled normal plasma as a ref-

Table 5. Diagnostic panel for hemophilia A and B and FXIII among all RLs, and according to iRL and eRLs.

	All (n=43)	iRLs (n=20)	eRLs (n=23)	*p*-value (Fisher's test)
One-stage clotting assay (OSA)				
FVIII OSA	43 (100%)	20 (100%)	23 (100%)	1.000
FIX OSA	42 (98%)	20 (100%)	22 (96%)	1.000
Chromogenic assays (CSA)				
FVIII CSA	37 (86%)	19 (95%)	18 (78%)	0.192
FIX CSA	19 (44%)	8 (40%)	11 (48%)	0.760
CSA reagents frozen	23 (53%)	15 (75%)	8 (35%)	0.014
CSA routinely available	21 (49%)	14 (70%)	7 (30%)	0.015
Inhibitor measurement				
FVIII OSA inhibitor titration	42 (98%)	20 (100%)	22 (96%)	1.000
FIX OSA inhibitor titration	37 (86%)	17 (85%)	20 (87%)	1.000
FVIII Bethesda OSA inhibitor titration	15 (36%)	7 (35%)	8 (35%)	1.000
FVIII Nijmegen modification OSA inhibitor titration	27 (64%)	13 (65%)	14 (61%)	1.000
FVIII CSA inhibitor titration	23 (55%)	11 (55%)	12 (52%)	1.000
FVIII Bethesda CSA inhibitor titration	5 (22%)	3 (15%)	2 (9%)	0.650
FVIII Nijmegen modification CSA inhibitor titration	18 (78%)	8 (40%)	10 (43%)	1.000
Heat inactivation	34 (79%)	17 (85%)	17 (74%)	0.467

Table 6. Von Willebrand factor assays.

	All (n=43)	iRLs (n=20)	eRLs (n=23)	*p*-value (Fisher's test)
VWF antigen (VWF:Ag)	43 (100%)	20 (100%)	23 (100%)	-
VWF activity	38 (88%)	18 (90%)	20 (87%)	1.000
VWF ristocetin cofactor test	35 (81%)	16 (80%)	19 (83%)	0.827
VWF recombinant GPIb alpha binding	8 (19%)	2 (10%)	6 (26%)	0.172
RIPA (ristocetin-induced platelet agglutination)	24 (56%)	14 (70%)	10 (43%)	0.081
VWF:CB (collagen binding)	20 (47%)	9 (45%)	11 (48%)	0.853
VWF/FVIII binding assay	13 (30%)	6 (30%)	7 (30%)	1.000
VWF multimeric analysis	9 (21%)	4 (20%)	5 (22%)	1.000

erence, and 60% commercially available reference plasma, a useful approach in the event of shortages of in-house pooled normal plasma. Of particular concern, only 53% of RL use homemade plasma to calibrate their preparations against international reference standards. This procedure is necessary to ensure the accuracy, reproducibility, and comparability of results, mainly for FVIII activity or inhibitor measurement in patients with hemophilia.[9] The adoption of CSA enables the RLs to measure FVIII inhibitors in patients receiving Emicizumab.

Approximately 30% of RLs use different aPTT reagents; some RLs may use different reagents for screening tests *vs* factor assays; alternatively, some RLs (those equipped with multiple analytical platforms) may use one platform for routine tests and another one for urgent or specialized tests. Given the variable sensitivity of reagents to factor deficiencies and their different interactions with extended-half-life FVIII molecules,[2] such a choice could pose clinical challenges. This issue (primarily observed in eRLs) will be addressed in future AICE educational meetings.

Nearly all RLs perform aPTT mixing test, including a two-hour incubation; only two laboratories don't perform this step.

All laboratories perform OSA for FVIII; 56% use VWF-repleted FVIII-deficient plasma, 21% VWF-depleted or deficient plasma, 23% use both. These limitations could affect inhibitor titration.[10] Moreover, for FIX measurement, the limited availability of CSA test in some RL restricts the monitoring of at least one extended half-life FIX.[11]

Approximately 67% of laboratories freeze CSA reagents, a cost-effective practice that extends their shelf life while maintaining analytical performance. However, the survey did not assess whether this practice complies with manufacturer specifications for reagent stability, satisfies quality standards, and maintains optimal performance. The discrepancy between iRLs and eRLs in freezing CSA reagents may derive from iRLs conducting fewer tests or facing stricter budget constraints.

Significant variability exists in RLs inhibitor-detection capabilities: 98% perform FVIII inhibitor detection, but only 79% offer FIX inhibitor detection. Approximately two-thirds of RL utilize the Nijmegen-buffered procedure, which improves the analytical quality of the Bethesda method, enhancing sensitivity and precision.[12] Slightly more than half of the RL (53%) use CSA for FVIII inhibitor titration, which offers greater precision, sensitivity, and reliability; however, nearly half of the centers are unable to titrate inhibitors during Emicizumab therapy.

Analysis of FXIII assay methodologies reveals no location-based preference (iRLs/eRLs) between antigenic and functional testing; decisions are influenced by the availability of analytical platforms. Technical limitations of ammonia-release-based chromogenic FXIII assays in detecting low levels[13] underline the importance of procurement choices for patients' treatment: centers that lack sensitive FXIII assays are forced to maintain higher prophylactic factor levels (through higher concentrate doses or shorter infusion intervals), increasing drug use and overall treatment costs.

A VWD diagnosis requires combining several specific laboratory tests with a detailed evaluation of the patient's bleeding history using the Bleeding Assessment Tools (BAT).[7] All RLs test for VWF antigen, but not all can assess PD-VWF activity or perform the VWF:CB assay. Some lack the necessary platform, others choose not to use it. Some use RIPA or genetic testing to distinguish VWD types 2A and 2B. Only a few RLs perform multimer analysis. Consequently, diagnosis of VWD types cannot be performed in laboratories that perform only the VWF:Ag assay, because the PD-VWF:Act/VWF:Ag ratio cannot be calculated to distinguish at least type 1 (ratio >0.7) from type 2 (ratio <0.7) VWD. Moreover, approximately a quarter of RLs exclude the VWF:CB assay, which is essential for characterizing certain types of VWD. At last, 19% of laboratories did not include FVIII measurement in their diagnostic protocol for VWD. Current diagnostic strategies for VWD of most RL present three major limitations: i) limited diagnostic accuracy; ii) restricted therapeutic options; iii) inadequate monitoring (particularly evident in the widespread unavailability of VWF:CB). This diagnostic gap reflects local procurement policies that mainly prioritize cost containment over analytical completeness, ultimately impacting patient care.

These data suggest that RL practice doesn't meet requirements set by national and international guidelines on the diagnosis and management of hemophilia and VWD;[4,7,8,14] moreover, they do not adhere to the standard of care for hemophilia patients established by Italian legislation.[15]

The survey reveals that nearly all RLs implement comprehensive quality assurance measures: i) 100% perform internal quality control for all coagulation assays; ii) most labs participate in external quality assessment (EQA) programs beyond ECAT program; iii) focus areas include FVIII, FIX, and inhibitor testing; iv) approximately 50% of these programs are international, reflecting a strong commitment to maintaining diagnostic standards excellence.

Our survey shows significant limitations. First, it describes only the Italian context; however, we believe that some emerging issues (e.g., faster TAT of iRLs and their greater diagnostic potential) may be useful to readers in other countries. Secondly, reliance on self-reported data introduces a risk of social desirability bias, whereby laboratories may over-report compliance with ideal practices. Third, categorizing TAT into broad ranges (e.g., <4 h) limits the precision of our assessment. Finally, the absence of qualitative data (e.g., open-ended interviews) limits our ability to definitively explain the underlying causes of non-standard practices (e.g., cost pressures, regional governance).

Conclusions

Our survey conducted on 43 RLs affiliated with AICE confirms the presence of essential analytical capabilities for diagnosing and monitoring inherited bleeding disorders. Still, it highlights the persistence of significant critical challenges and marked methodological heterogeneity. Collected data provides solid evidence for healthcare policies and supports AICE initiative to establish a dedicated working group. It is essential to implement targeted training programs to standardize practices, particularly within eRLs, to optimize the national diagnostic network and overcome financial constraints.

Regarding methodological limitations, we can report the standardization of the FIX assay and the optimal selection of reagents for aPTT testing and FVIII/FIX activity assays. Regarding diagnostic gaps, we must consider the inhibitor detection and titration protocols, a comprehensive VWD diagnostic panel, and the availability of FXIII activity measurement. Our survey

did not show significant differences in diagnostic capabilities between iRLs and eRLs, but it indicated a trend toward shorter TATs for iRLs in both routine and emergency situations. The survey also highlighted substantial variability in reagent selection practices for aPTT and FVIII/IX assays, as well as the availability of RIPA testing in eRLs; these findings underscore the need for training programs for eRL staff. A subsequent analysis of EQA program data (started by AICE in 2017) comparing iRLs *vs* eRLs performance could yield valuable insights for quality improvement.

Our study highlights some critical issues: first, economically driven limitations; procurement decisions prioritize cost, and platform selection directly affects test availability, compromising accuracy of diagnosis and therapy monitoring. We also highlighted problems related to laboratory centralization: current laboratory workflows hinder specialized testing; centralization creates barriers to expert staff involvement, reduces diagnostic precision, and delays the delivery of results. These findings demonstrate how financial constraints in healthcare systems can compromise diagnostic quality and patient care standards. Moreover, our findings carry significant implications for healthcare policy, as they: i) Validate the clinical relevance of our nationwide survey data; ii) Provide evidence-based guidance for optimizing laboratory networks in hemophilia care; iii) Highlight opportunities for healthcare system planning to improve diagnostic efficiency. We hope this could serve as a starting point for a deeper analysis of these issues, involving healthcare and managerial figures as well.

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Online supplementary material:

Supplementary Material S1. List of 51 survey questions.