Comparison of Thrombophilia Assay Results for the International Society on Thrombosis and Haemostasis Scientific and Standardization Committee Plasma Standard from Different External Quality Assessment Providers—for the External Quality Assurance in Thrombosis and Haemostasis Group

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Abstract

External quality assessment (EQA) is used to evaluate laboratory performance in tests of hemostasis; however, some esoteric tests are performed by too few centers in any one EQA program to allow valid statistical assessment. To explore the feasibility of pooling data from several EQA providers, an exercise was carried out by the External Quality Assurance in Thrombosis and Haemostasis group, using the International Society on Thrombosis and Haemostasis Scientific and Standardization Committee (SSC) plasma standard for thrombophilia screening assays. Six EQA providers took part in this exercise, distributing the SSC plasma standard as a "blinded" sample to participants for thrombophilia tests between November 2020 and December 2021. Data were collected by each provider, anonymized, and pooled for analysis. Results were analyzed as overall results from each EQA provider, and by kit/method-specific comparisons of data from all providers pooled together. For each parameter, median

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results and range were determined. Over 1,250 sets of data were returned in the six EQA programs. The overall medians (all data pooled) were <4% of the assigned values for each parameter with the exception of protein C activity by clot-based assay. Method-related differences in median results were observed for free protein S antigen and protein S activity—a pattern seen across data from the different EQA providers. Antithrombin antigen results reported in mg/dL provided an example where small numbers of results for a single EQA provider may be supplemented by pooling data from multiple providers with good agreement seen among results reported by the different EQA providers. This study demonstrated that a multicenter EQA provider collaboration can be carried out and demonstrated benefit for assays with smaller number of participants. In addition, results showed good agreement with the assigned values of the SSC plasma standard. Further exercises for tests performed by only small numbers of laboratories can be planned.

Laboratory testing plays a crucial role in the diagnosis and management of many hemostatic disorders. Part of the quality assurance process required to deliver accurate and precise laboratory test results is external quality assessment (EQA) or proficiency testing (PT) whereby identical aliquots of an unknown sample are distributed to multiple laboratories to perform the same test. Results are compared among participating centers, allowing the EQA provider to assess the deviation of each laboratory's result from the target value, typically the median of all peer-method results, giving the laboratory insight into the accuracy of their measurement.[1]

Any discrepancies in test results from an individual laboratory or with a specific method can be identified. This process is effective and statistically valid when the number of centers performing the test is large, as is the case for most routine hemostasis screening tests and the more commonly performed specialized assays. However, for more esoteric assays, the number of centers performing the test within an EQA program may be very small, preventing any meaningful between-center comparison of results.

The EQATH (External Quality Assurance in Thrombosis and Haemostasis) group, formed in 2005, was established to support collaboration among worldwide providers of EQA within the field of hemostasis and has previously investigated scoring systems and provided guidance on establishing hemostasis-based EQA programs.[2,3] A project was devised by this group to pool and compare data from multiple EQA programs for "esoteric" analytes, i.e., those performed by fewer than 10 centers in any one EQA program. In this way, it may be possible to combine data from different programs and carry out meaningful statistical analysis to assign performance for these assays.

To test the principle of collecting, anonymizing, pooling, and reporting data from several different EQA programs prior to distribution of an esoteric test sample, the EQATH executive committee decided to carry out a multicenter exercise using the International Society on Thrombosis and Haemostasis Scientific and Standardization Committee (SSC) plasma standard lot #5 in a thrombophilia screening exercise.

The SSC plasma standard, prepared in large volumes ($\sim$100,000 $\times$ 1 mL vials), is calibrated against World Health Organization standards for a range of hemostatic factors.[4] The standard is prepared primarily for use by diagnostic manufacturers to aid in assigning values to coagulation calibrators. However, the standard has also been made available to EQA providers for PT purposes, where it may be used to troubleshoot apparent calibration issues for individual centers, and has also been supplied as an EQA sample in routine exercises.[5] Following agreement with NIBSC (the National Institute of Biological Standards and Controls), custodians of the SSC plasma standard, EQATH group members were invited to take part in a study using this plasma for a combined EQA exercise for thrombophilia assays.

Methods

EQA providers in the EQATH group were approached, and six providers agreed to take part in this exercise (College of American Pathologists [CAP], United States; External quality Control of diagnostic Assays and Tests (ECAT) Foundation, The Netherlands, in collaboration with the North American Specialized Coagulation Laboratory Association, United States; INSTAND, Germany; National External Quality Assessment Scheme (NEQAS) United Kingdom; Association for the Promotion of Quality Control in Medical Biology (ProBioQual), France; Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP), Australia. Agreement for the project was obtained from NIBSC, and EQA providers purchased vials of this plasma directly from NIBSC. All EQA providers distributed the sample as a "blinded" test sample to their participants with a request to perform thrombophilia screening tests (protein C, protein S, and antithrombin assays); exercises were carried out during the period November 2020 to December 2021. Participants were asked to perform their standard assay method(s) for each of the thrombophilia screening tests, and to return their results along with methodology details. Data were collected by each EQA provider, anonymized, and then forwarded to NEQAS for pooling and data analysis.

For each test, results were analyzed as overall results from each EQA provider, and by kit/method-specific comparisons of data from all providers pooled together. For each parameter, median results and range were determined for all provider data and for each kit/method; coefficients of variation (CVs) for analysis by provider were determined when five or more results were provided, and for analysis by kit or method when 10 or more results were received. Median data were compared using a Mann–Whitney U test. Outliers were calculated for each EQA provider separately, and for the overall pooled data, defined as any result >3 SD (standard deviation) from the median value. For those EQA providers where interpretations were routinely collected, this information was also recorded.

Results

Over 1,250 sets of data were returned from participants in the six EQA programs. ►**Table 1** details the overall median result for all centers for each test, along with number of centers returning results in this exercise, and for comparison shows the relative assigned value for the SSC plasma standard (lot #5), and the number of centers that took part in the original calibration process to assign the value.[6] The overall medians were within 4% of the assigned values for each parameter with the exception of protein C activity by clot-based assay.

For protein C antigen, results were returned by 101 centers, the majority of these from two EQA providers. The overall median for all data was 93 IU/dL against the assigned value of 89 IU/dL (►**Supplementary Table S1** and ►**Supplementary Fig. S1**).

For protein C activity, data were analyzed separately dependent on methodology, chromogenic, or clot-based assays. For chromogenic protein C assays, close agreement was seen across all EQA providers, with medians differing by 2.1% or less (►**Supplementary Table S2** and ►**Supplementary Fig. S2**). For the most widely used methods, when combined for all EQA providers, medians differed by 2.5% or less.

For clot-based protein C activity assays, there was good agreement across the values obtained by different EQA providers (maximum difference 3%) and also for results obtained with different assay kits (►**Table 2** and ►**Fig. 1**). However, the overall median for clot-based protein C activity assays was statistically higher than for chromogenic-based assays (105 vs. 98 IU/dL, $p < 0.0001$) and higher than the assigned value for the SSC plasma standard (97 IU/dL).

Total protein S antigen results were comparable across the EQA providers, with only two methods used by more than 10 centers (►**Supplementary Table S3** and ►**Supplementary Fig. S3**). The overall median for all data was 93 IU/dL against the assigned value of 96 IU/dL. For free protein S antigen, there were statistically significant differences between results for the two most widely used methods (Siemens Innovance, $n = 252$, median = 91.1 IU/dL; Werfen, $n = 352$, median = 99.0 IU/dL, $p < 0.0001$). These between-method differences were present within the data from EQA providers, for example for ECAT (Siemens Innovance, $n = 96$, median = 91.0 IU/dL; Werfen, $n = 105$, median = 99.0 IU/dL) and for NEQAS (Siemens Innovance, $n = 60$, median = 91.6 IU/dL; Werfen, $n = 152$, median = 98.0 IU/dL) (►**Supplementary Table S4** and ►**Supplementary Fig. S4**). However, the overall medians for each provider were comparable, and in close agreement (<5.2%) with the assigned value.

For protein S activity, there was close agreement among the medians determined for different EQA providers, but statistically significant method-related differences were observed among the Siemens kit ($n = 88$, median = 74.4 IU/dL) and both Werfen ($n = 130$, median = 80.0 IU/dL, $p < 0.0001$) and Stago ($n = 202$, median = 78.0 IU/dL, $p < 0.0001$) (►**Table 3** and ►**Fig. 2**). The median for all EQA provider data combined (78.0 IU/dL) was identical to the assigned value for this plasma standard.

Antithrombin antigen is reported in IU/dL by 74/92 centers across the EQA programs (80%). Median results for the two EQA providers with the largest number of results were within 1 IU/dL of each other (►**Table 4** and ►**Fig. 3**). There

Table 1 Summary of data from the exercise

Assay	*n*	Median (IU/dL)	Assigned value (IU/dL)	Centers in value assignment (*n*)
Protein C antigen	101	93.0	89.0	11
Protein C activity (chromogenic)	913	98.0	97.0[a]	31
Protein C activity (clot-based)	174	105.0	97.0[a]	31
Protein S total antigen	96	93.0	96.0	10
Protein S free antigen	827	95.6	98.0	16
Protein S activity	447	78.0	78.0	18
Antithrombin antigen (IU/dL)	74	97.3	94.0	13
Antithrombin antigen (mg/dL)	18	25.1 mg/dL	–	–
Antithrombin activity (IIa–based)	567	94.0	95.0[b]	26
Antithrombin activity (Xa–based)	701	97.0	95.0[b]	26

[a]Single value assigned for all PC activity methods.
[b]Single value assigned for all AT activity methods.

Table 2 Protein C activity analysis by EQA provider and by method—clot-based assays

Provider	*n*	Median, IU/dL	CV %	Min, IU/dL	Max, IU/dL
CAP	27	110.0	11.7	59.0	130.0
ECAT	73	104.0	8.6	88.0	134.0
INSTAND	15	101.0	11.5	92.0	131.0
NEQAS	10	105.0	11.8	82.0	122.2
ProBioQual	49	104.0	8.4	80.0	123.0
RCPAQAP	–	–		–	–
Kit/method (all programs)	***n***	**Median, IU/dL**	**CV %**	**Min, IU/dL**	**Max, IU/dL**
Precision Biologic	1	103.0	–	–	–
Siemens	33	103.0	9.1	88.0	125.0
Stago	98	107.0	9.6	59.0	131.0
Werfen	33	101.0	10.3	86.0	134.0
Overall	*174*	*105.0*	*9.6*	*59.0*	*134.0*

Abbreviations: CAP, College of American Pathologists; CV, coefficient of variation; ECAT, External quality Control of diagnostic Assays and Test; EQA, external quality assessment; Min, minimum; Max, maximum; NEQAS, National External Quality Assessment Scheme.

was also good agreement among the overall median for all EQA providers and the assigned value for the SSC plasma standard (97.3 vs. 94 IU/dL). Just 18 centers (20%) across all EQA providers report antithrombin antigen in mg/dL; however, good agreement was seen in the median results obtained from the three different EQA providers, and among methods (►**Table 4** and ►**Fig 3**). The SSC plasma standard is only assigned a value in IU, so no comparison was possible with results reported in mg/dL.

For antithrombin activity, data were analyzed separately dependent on the substrate employed for the assay (IIa or Xa). Thrombin (IIa)-based methods were used by 45% of centers, and Xa-based assays were used by 55% of centers. For IIa-based assays, there was very good agreement among medians for each EQA provider (<3 IU/dL difference), and only a 2 IU/dL difference in median results for the two most widely used kits. For Xa-based assays, identical medians were recorded for five of the six EQA providers, and only a 3 IU/dL difference in median results for the two most widely used kits (►**Supplementary Table S5**; ►**Supplementary Figs. S5** and **S6**). Only one value is assigned for the SSC plasma standard for antithrombin activity, 95.0 IU/dL, irrespective of substrate method used. This result fell close to and between the overall median for IIa-based methods (94 IU/dL) and Xa-based methods (97 IU/dL), despite the statistically significant difference between the median for the two methods ($p = 0.0003$).

Overall, not only were median results obtained for each EQA provider data very similar, but both overall CVs and CVs for individual EQA providers were well below 10% for most assays (►**Tables 1–4** and ►**Supplementary Tables S1–S5**). The number of outliers for each assay (defined in this study as >3 SD from the median) was determined within individual EQA provider grouping and for all data combined. In some cases, there were the same number of outliers, when calculated against individual provider results compared to the overall group; in other cases, the overall pooled data created slightly more outliers, possibly as a consequence of a lower SD for the large pool of results (data not shown). Furthermore, 0.9% of results in total fell outside ± 3 SD from the median values. For antithrombin antigen reported in mg/dL, no results fell outside ± 3 SD when data were combined for all providers.

Several providers requested participants provided interpretations for their assays. There was good concurrence among the interpretations of the different providers, with the vast majority of centers (99%) reporting normal results for the SSC plasma standard, a pooled normal plasma.

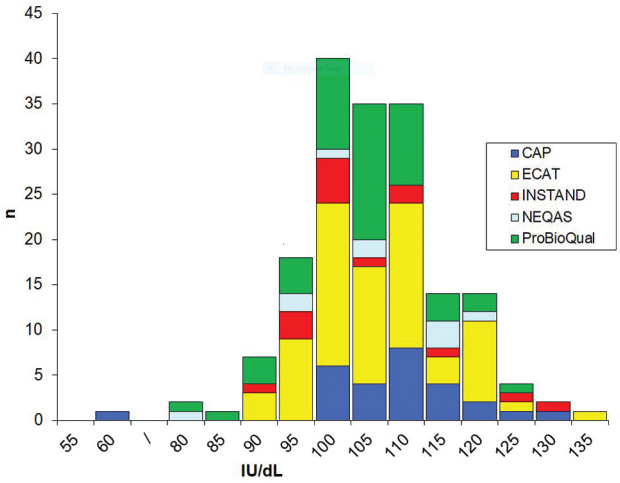

Fig. 1 Result distribution for protein C activity (clot-based assay) for each EQA provider. EQA, external quality assessment.

Discussion

EQA or PT is an important and powerful tool in the process of ensuring accurate and precise results for laboratory tests of hemostasis, and has been demonstrated to improve laboratory performance.[7–13] EQA is also a requirement for accreditation to international standards such as ISO15189.[14] However, in some cases it is not possible for a laboratory

Table 3 Protein S activity analysis by EQA provider and by method

Provider	*n*	Median, IU/dL	CV %	Min, IU/dL	Max, IU/dL
CAP	62	79.0	13.0	64.0	130.0
ECAT	161	78.0	11.5	47.8	110.0
INSTAND	87	78.0	14.5	53.0	129.0
NEQAS	51	80.5	10.0	59.0	101.0
ProBioQual	75	77.0	10.7	35.0	96.0
RCPAQAP	11	81.0	10.0	72.0	97.0
Kit/method (all programs)	***n***	**Median, IU/dL**	**CV %**	**Min, IU/dL**	**Max, IU/dL**
Gradipore	1	110.0	–	–	–
Helena	1	92.4	–	–	–
Hyphen Biomed	21	81.0	11.6	69.0	110.0
Precision Biologic	1	93.0	–	–	–
Siemens	88	74.4[a,b]	16.0	47.8	130.0
Stago	202	78.0[*]	10.3	35.0	99.0
Werfen	130	80.0[a]	10.5	59.0	129.0
Other/not stated	3	72.0	–	69.8	86.0
Overall	*447*	*78.0*	*12.1*	*35.0*	*130.0*

Abbreviations: CAP, College of American Pathologists; CV, coefficient of variation; ECAT, External quality Control of diagnostic Assays and Test; EQA, external quality assessment; Min, minimum; Max, maximum; NEQAS, National External Quality Assessment Scheme.
[a]Siemens vs. Werfen $p < 0.0001$.
[b]Siemens vs. Stago $p < 0.0001$.

to carry out EQA, for example, due to instability of material[15] or a lack of sufficient centers carrying out highly specialized or rarely performed tests for some disorders.

Three members of the EQATH group have previously been involved in collaborative exercises, where samples were distributed across the different programs, and data were pooled and analyzed resulting in stronger quantitative data.[16,17] EQATH has also carried out data analysis exercises across multiple different EQA providers[2] but had not previously carried out an exercise for multiple different assays using a single pool of results from all EQA providers to allow statistical analysis.

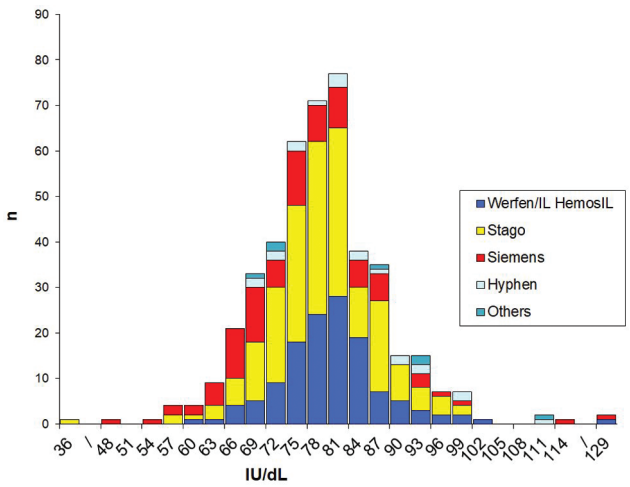

Fig. 2 Result distribution for protein S activity for the most widely used methods.

The SSC plasma standard used in this exercise has previously been used by individual EQA providers,[5,18] and it has been useful to provide post-assignment confirmation of values across a wider range of methods than usually included in the value assignment process. The plasma was readily available material for this large collaborative study. No issues were noted with the process of the study, and all EQA providers were able to complete the exercise within a period of 13 months.

Overall median results from all EQA providers were very close when compared to the assigned values for the SSC plasma standard for the majority of thrombophilia parameters studied. Thus, value assignment originally provided by a small number of centers (between 10 and 26) has now been supported by data from a much larger number of centers (between 96 and 913) using a large variety of methods. For antithrombin activity assays, despite a statistically significant difference in results for separately analyzed IIa-based and Xa-based methods ($p = 0.0003$), the small size of this difference (3 IU/dL) supports combination of IIa-based and Xa-based data in the value assignment process to provide a single assigned value for the SSC plasma standard. For protein C activity assays, a single value was again assigned to the SSC plasma standard, irrespective of the end-point principle of the assay (chromogenic or clot-based). Here, the assigned value and median for chromogenic assays in this study were in good agreement. However, the overall median for clot-based assays was significantly higher than for

Table 4 Antithrombin antigen analysis by EQA provider and by method—IU/dL and mg/dL

	Antithrombin (IU/dL)					Antithrombin (mg/dL)				
Provider	***n***	**Median, IU/dL**	**CV %**	**Min, IU/dL**	**Max, IU/dL**	***n***	**Median, mg/dL**	**CV %**	**Min, mg/dL**	**Max, mg/dL**
CAP	6	100.5	10.2	88.0	117.0	1	25.0	–	–	–
ECAT	48	97.0	7.3	75.0	110.0	13	24.2	13.7	17.6	27.4
INSTAND	1	82.0	–	82.0	82.0	–	–	–	–	–
NEQAS	19	98.0	8.9	68.0	112.0	4	25.1	–	20.0	30.1
ProBioQual	–	–	–	–	–	–	–	–	–	–
RCPAQAP	–	–	–	–	–	–	–	–	–	–
Kit/method (all programs)	***n***	**Median, IU/dL**	**CV %**	**Min, IU/dL**	**Max, IU/dL**	***n***	**Median, mg/dL**	**CV %**	**Min, mg/dL**	**Max, mg/dL**
Hyphen Biomed	21	96.0	5.2	85.0	104.6	–	–	–	–	–
Siemens (IEA)	1	68.0	–	–	–	–	–	–	–	–
Siemens (not stated)	1	82.0	–	–	–	–	–	–	–	–
Siemens (NorPartigen)	3	100.0	–	98.0	105.0	2	26.9	–	26.5	27.2
Siemens (Nephelometric)	–	–	–	–	–	10	24.2	14.9	17.6	24.2
Stago	43	98.7	7.9	75.0	117.0	1	30.1	–	–	–
Homemade (Siemens antibodies)	2	93.5	–	87.0	100.0	1	24.0	–	–	–
Others/not stated	3	98.0	–	94.0	105.0	4	25.1	–	20.0	25.1
Overall	*74*	*97.3*	*8.1*	*68.0*	*117.0*	*18*	*25.1*	*13.6*	*17.6*	*30.1*

Abbreviations: AT, antithrombin; CAP, College of American Pathologists; CV, coefficient of variation; ECAT, External quality Control of diagnostic Assays and Test; EQA, external quality assessment; IEA, immunoelectrophoretic assay; Min, minimum; Max, maximum; NEQAS, National External Quality Assessment Scheme.

chromogenic methods ($p < 0.0001$), and did not demonstrate agreement with the assigned value, being 8 IU/dL higher. The calibration report from NIBSC for protein C activity did not identify any difference between clotting and chromogenic assay results, so the source of this discrepancy is unknown *(Craig Thelwell, personal communication)*. Lot #5 was the first lot of the SSC plasma standard to have method-specific values assigned for von Willebrand factor activity assays,[6] acknowledging different test principles of the different assays, and consideration should be given to separate values for chromogenic and clot-based protein C assays for future lots of this standard as well.

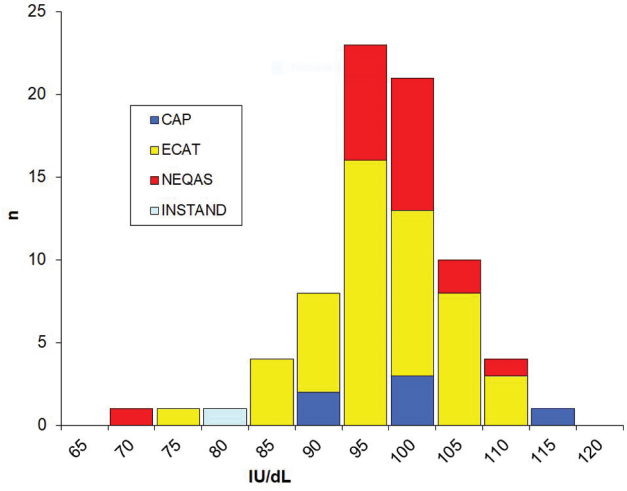

Fig. 3 Result distribution for antithrombin antigen for each EQA provider. EQA, external quality assessment.

These EQA results must be interpreted with caution as over-interpretation of EQA data in the absence of commutability studies may lead to incorrect conclusions.[19,20] Commutability studies comparing results for the lyophilized SSC plasma standard and clinical samples using different methods have not been carried out. However, the SSC plasma standard is widely used by manufacturers for value assignment of commercial controls, then used by all laboratories, and traceable to international plasma standards, which define the international unit for each clotting factor, e.g., factor VIII and factor IX.[21] It is also possible that the good agreement observed between methods for this pooled normal plasma standard may not show the same degree of agreement for different samples with reduced or abnormal levels of these factors.

The data for antithrombin antigen reported in mg/dL provide an example of where small numbers of results for

a single EQA provider may benefit by pooling data from multiple providers together. It was useful to see good agreement among results reported by the different EQA providers. It is also feasible to carry out performance analysis where numbers are sufficient to be statistically valid. It is interesting to note, using arbitrary limits of 3 SD, that there were no outlying results when all data were pooled and no outlying results for the set of data for the one provider with more than 10 participants.

For the other assays, when outlying results were determined either within the EQA provider group or against the overall data, very similar CVs and median values among the providers led to little or no difference in the number of results that were more than 3 SD from the median. Therefore, there were no advantages to pooling these large data sets when assessing performance.

Conclusion

This study successfully demonstrated that a multicenter EQA provider collaboration can be carried out, maintaining participant confidentiality whilst allowing pooling of data for survey analysis. Further exercises for tests performed by only small numbers of laboratories can now be planned.

A secondary benefit of this study is demonstration of good agreement between the values assigned to the SSC plasma standard in the calibration process and the medians obtained by the large number of centers in this study. Ongoing distribution of material in such EQA exercises is a useful monitor of assigned values for this and other or future plasma standards. It may be also feasible to use EQA provider data to assign target values for plasma standards where a calibration exercise using international standards has not been performed.

Conflict of Interest

None declared.

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