Biological Variation in haemostasis variables

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Three models to set analytical performance specification

1. **Based on effect of analytical performance on clinical outcomes**
2. **Based on components of biological variation of the variable**
3. **Based on state-of-the-art: relates to the highest level of technically available performance of an assay**

➢ **Biological variation model is most widely used**

Reference: 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine Sandberg et al., CCLM (2015) 53: 883-5

Definitions

Within-subject biological variation (CV_I)

Random fluctuation around a homeostatic set point in an individual

Between-subject biological variation (CV_G)

Difference between the homeostatic set points of different individuals

The equations for desirable performance goals based on the biological variation

$$CV_A < 0.5\ CV_I$$

$$B_A < 0.25\ (CV_I^2 + CV_G^2)^{½}$$

$$TE_A < 0.25\ (CV_I^2 + CV_G^2)^{½} + 1.65\ (0.5\ CV_I)$$

Historical online BV database last updated in 2014

	Analyte	Number of papers	Biological Variation		Desirable specification		
			CV_I	CVg	I(%)	B(%)	TE(%)
P-	Antithrombin III	4	5.2	15.3	2.6	4.0	8.3
S-	D-Dimer (MoM)	1	23.3	26.5	11.65	8.82	28.04
P-	Factor V coagulation	1	3.6	---	1.8	---	---
P-	Factor VII coagulation	2	6.8	19.4	3.4	5.1	10.7
P-	Factor VIII coagulation	2	4.8	19.1	2.4	4.9	8.9
P-	Factor X coagulation	1	5.9	---	3.0	---	---
P-	Fibrinogen	5	10.7	15.8	5.4	4.8	13.6

Reference: https://www.westgard.com/biodatabase1.htm

Biological variation (BV) data haemostasis variables

	CV_I			CV_G		
	minimum	median	maximum	minimum	median	maximum
PT	2.3	2.6	5.8	4.0	4.9	6.8
APTT	1.7	3.3	6.8	7.1	7.8	8.9
Fibrinogen	6.8	11.5	18.6	14.7	16.4	20.2
AT	1.1	3.1	5.7	2.6	7.8	10.4

Median and range (minimum and maximum value) based on the results of the combined studies.
CV_I: within-subject coefficient of variation, CV_G: between-subject coefficient of variation

➢ **Large heterogeneity in BV data due to heterogeneity in study setup**

Reference: Hollestelle et al., CCLM (2019) 57: 1235-41

Aim

To generate up-to-date within-subject (CV_I) and between-subject (CV_G) BV data for coagulation and fibrinolytic variables by systematically appraising published BV data and subsequently combine the data in meta-analyses

Setup study

- Literature search including BV variables in healthy adults, study period at least 1 week and three or more samples collected per person
- Appraising publications by the Biological Variation Data Critical Appraisal Checklist (BIVAC):

  Reference: Aarsand et al., CC (2018) 64: 501-14

  - 4 independent assessors reviewed the papers
  - 14 quality items graded A to D
- D-graded studies were excluded from the meta-analysis, data was considered not fit for use
- Meta-analysis was performed with the BIVAC grades given weights [A=4, B=2, C=1]

Results

- 26 papers were included, representing BV data for 35 variables
- Most publication on BV were found for Fibrinogen (17), Antithrombin (9) and APTT (8).
- Majority of studies were graded as a C => 74%
- 20% were graded as a A, which is the highest score achievable

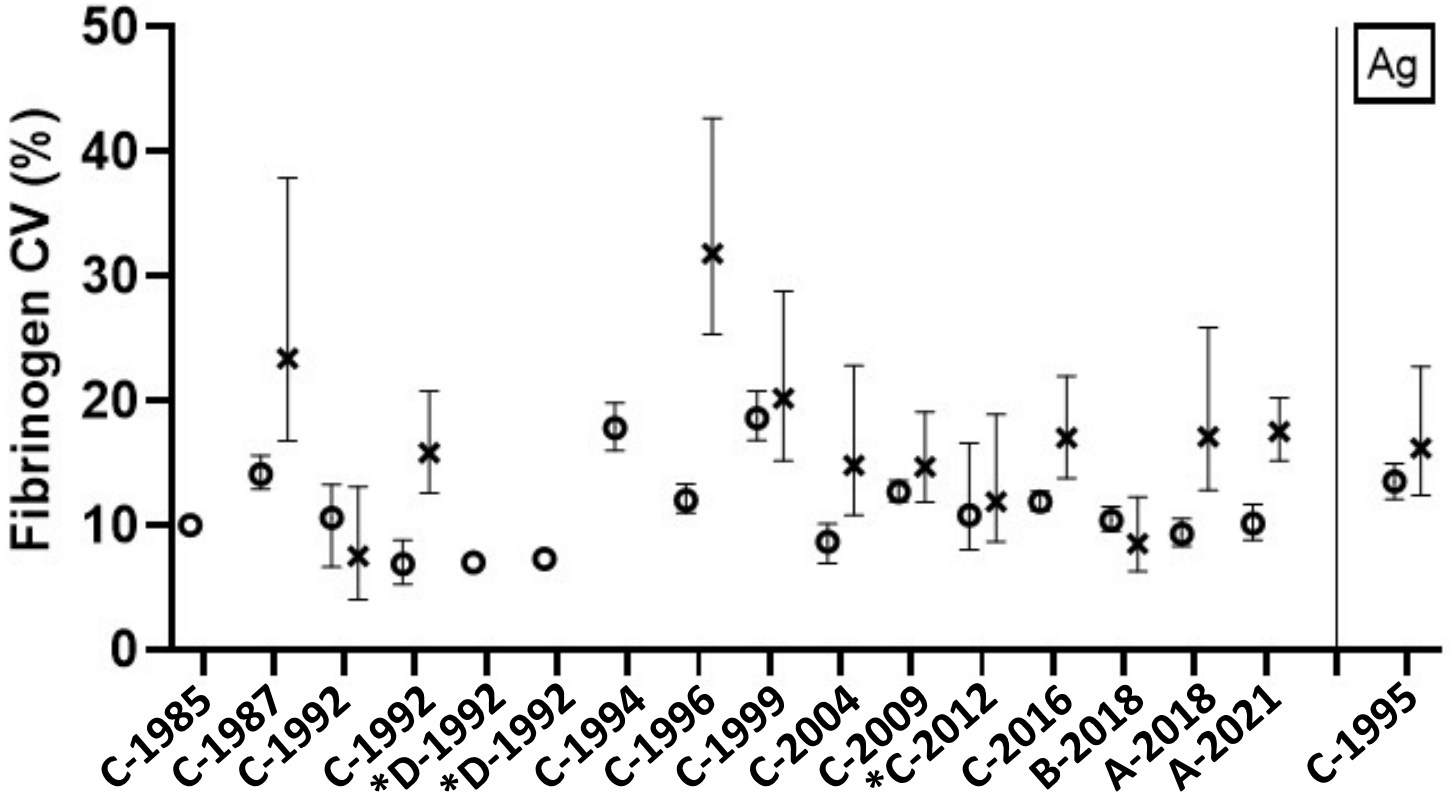
Reason grading C

- Mostly related to statistical issues; related to outlier analysis and testing for homogeneity

Reason grading D

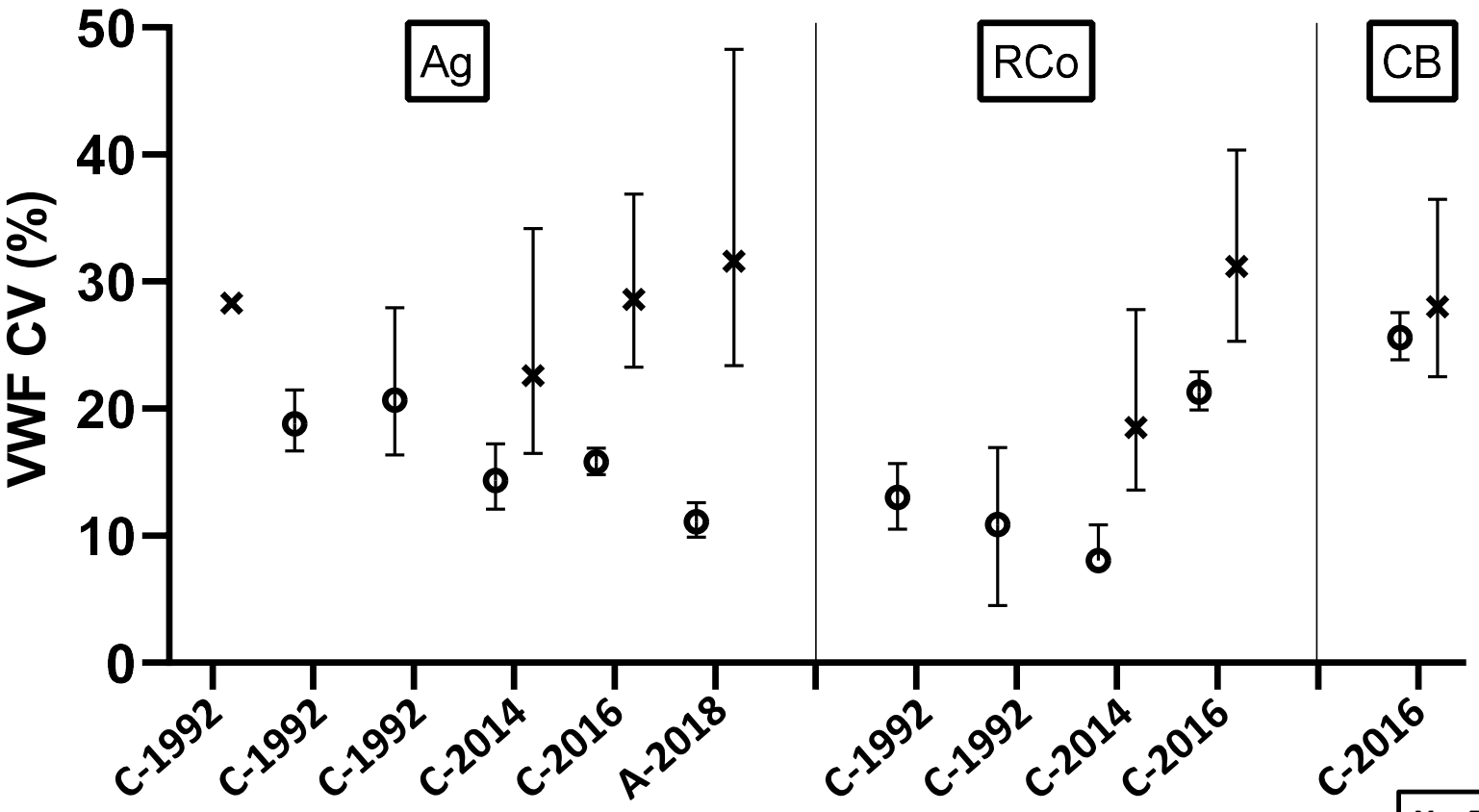
- Due to obsolete methods being applied

Fibrinogen



X Between subject CV_G
O Withing subject CV_I

VWF


VWF CV (%)
50
40
30
20
10
0
Ag
RCo
CB
C-1992
C-1992
C-1992
C-2014
C-2016
A-2018
C-1992
C-1992
C-2014
C-2016
C-2016
X Between subject CV_G
O Withing subject CV_I

Meta-analysis derived within-subject (CV_I) and between-subject (CV_G) estimates with 95% CIs of coagulation and fibrinolytic variables

	CV_I	CV_G
APTT	2.8 (1.7-6.8)	7.2 (4.9-8.9)
APCR ratio	1.5 (1.3-6.7)	4.5 (3.8-5.4)
ADAMTS13 Act	12.7 (9.7-15.8)	9.6 (5.6-16.5)
ADAMT13 Ag	9.8 (0.0-13.4)	6.3 (1.9-11.6)
Antithrombin Ag	7.2 (6.3-8.2)	5.0 (3.4-8.3)
Antithrombin Act	3.4 (1.1-7.0)	7.8 (2.6-25.2)
D-Dimer	25.2(17.4-56.4)	35.4 (26.5-89.5)
Factor II	5.8 (5.7-5.9)	9.7 (7.0-15.4)
Factor V	5.3 (3.6-6.6)	18.7 (14.1-27.5)
Factor VII	8.2 (6.9-14.2)	17.8 (16.7-19.4)
Factor VIII	8.7 (4.9-16.0)	22.5 (15.5-31.4)
Factor IX	6.9 (5.8-9.1)	16.3 (15.7-18.2)
Factor X	5.9 (4.6-8.5)	11.4 (8.2-18.2)
Factor XI	5.1 (4.2-6.3)	11.5 (8.5-17.5)
Factor XII	4.0 (3.0-5.1)	23.3 (17.6-34.5)
Fibrinogen Clauss	10.2 (9.3-11.9)	17.1 (8.5-17.3)
Fibringen Ag	13.5 (12.1-14.9)	16.2 (12.4-22.7)

	CV_I	CV_G
Plasmin Inhibitor	5.8 (4.8-5.8)	7.1 (5.2-10.8)
Plasminogen	5.7 (4.2-7.7)	10.5 (7.8-15.8)
Protein C Act	5.5 (5.3-7.9)	16.9 (9.1-55.2)
Protein C Ag	2.2 (0.0-6.2)	13.3 (10.5-17.5)
Protein S Act	7.3 (7.1-8.1)	20.3 (18.8-23.8)
Protein S Total	6.7 (2.9-7.3)	13.3 (8.9-63.4)
Protein S Free	4.2 (4.0-8.7)	16.9 (16.2-25.0)
Prothrombin time	2.6 (2.4-5.8)	5.1 (2.8-5.7)
TAT	19.0 (11.0-26.0)	33.3 (20.0-60.5)
Thrombomodulin	11.4 (9.1-13.2)	16.5 (12.1-25.1)
t-PA Ag	13.3 (11.0-30.9)	38.1 (23.9-191.1)
t-PA Act	32.0 (27.6-37.4)	NA
PAI-1 Ag	48.6 (35.6-55.0)	59.8 (26.0-90.0)
PAI-1 Act	34.9 (30.3-49.0)	90.2 (62.0-181.8)
VWF:RCo	17.0 (8.1-21.3)	24.6 (18.5-31.2)
VWF:Ag	12.7 (11.1-19.4)	29.9 (22.6-31.6)
VWF:CB	25.6 (23.9-27.5)	28.0 (22.6-36.3)

Yellow marked variables were present in the historical online BV database last updated in 2014

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Red marked variables represent the highest estimates

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Reasons for high BV estimates PAI-1

- PAI-1 is an acute phase reactant
- Influence should be minimized since only healthy adults were included

- PAI-1 levels are influenced by a strong diurnal variation
- Since most samples were taken early in the morning this should be minimized too

➡ High BV estimates mean wider APS

Green marked variable represent the lowest estimates

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Fibringen Ag	13.5 (12.1-14.9)	16.2 (12.4-22.7)

➡ **Low BV estimates values means strict APS**

Protein C Ag	2.2 (0.0-6.2)	13.3 (10.5-17.5)
Protein S Act	7.3 (7.1-8.1)	20.3 (18.8-23.8)
Protein S Total	6.7 (2.9-7.3)	13.3 (8.9-63.4)
Protein S Free	4.2 (4.0-8.7)	16.9 (16.2-25.0)
Prothrombin time	2.6 (2.4-5.8)	5.1 (2.8-5.7)
TAT	19.0 (11.0-26.0)	33.3 (20.0-60.5)
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Prothrombin Time, INR: In healthy adults versus patients

Healthy adults	Patients using anticoagulation treatment (data derived from three studies)
• CV_I = 2.5% (2.3-3.0)	• CV_I = 9.0 to 23.8%
• CV_G = 4.6% (2.9-6.8)	• CV_G = 12.8 to 18.5%

- ➢ Much higher estimates observed in patients compared to healthy adults
- ➢ APS criteria based on patients is less strict compared to healthy adults
- ➢ These results show the need for more BV studies in clinical patients to adapt quality criteria for specific clinical questions

Reference: Solvik et al., Scand J Clin Lab Invest (2014) 74:470-6
Lassen et al., Clin Chem (1995) 41:444-7
van den Besselaar et al., Thromb Res (2012) 130:535-7

Conclusion

- Systematic review and updated estimates of CV_I and CV_G (with 95% confidence interval) for an expanding number of haemostasis variables

- These estimates can form the basis for APS for haemostasis tests used in the diagnostic work-up in bleeding- and thrombosis events and for risk estimation

- Further expansion of high-quality BV studies is necessary to gain more knowledge of BV estimates for different population groups and states of health

Haemostasis BV estimates will be included in the new database

https://biologicalvariation.eu/

EUROPEAN FEDERATION OF CLINICAL CHEMISTRY AND LABORATORY MEDICINE

EFLM Biological Variation Database

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