Biological Variation in haemostasis variables

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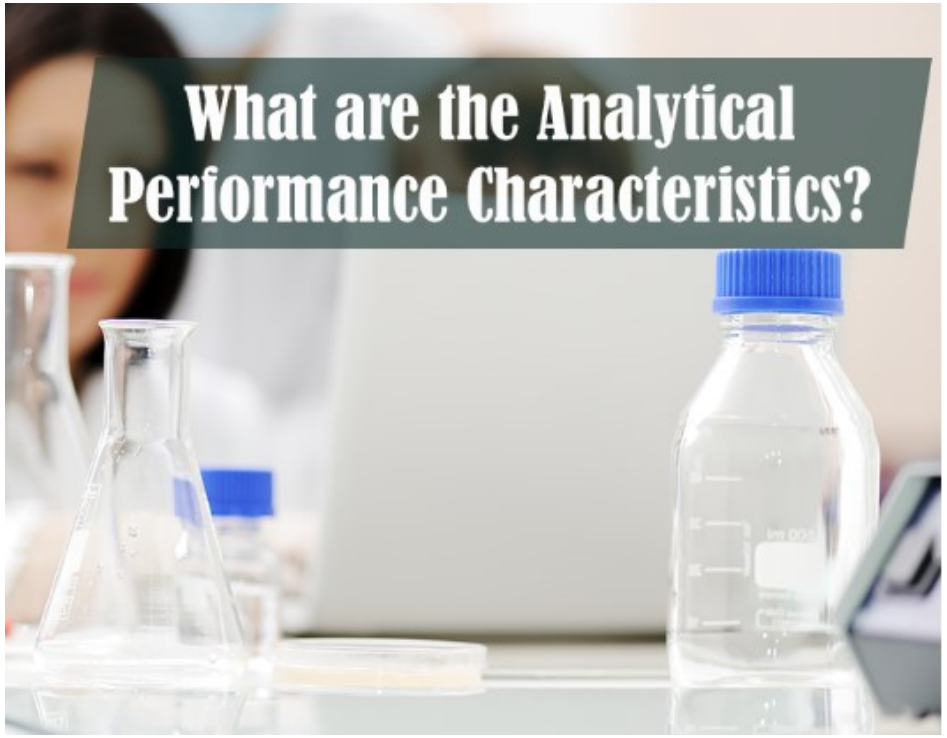
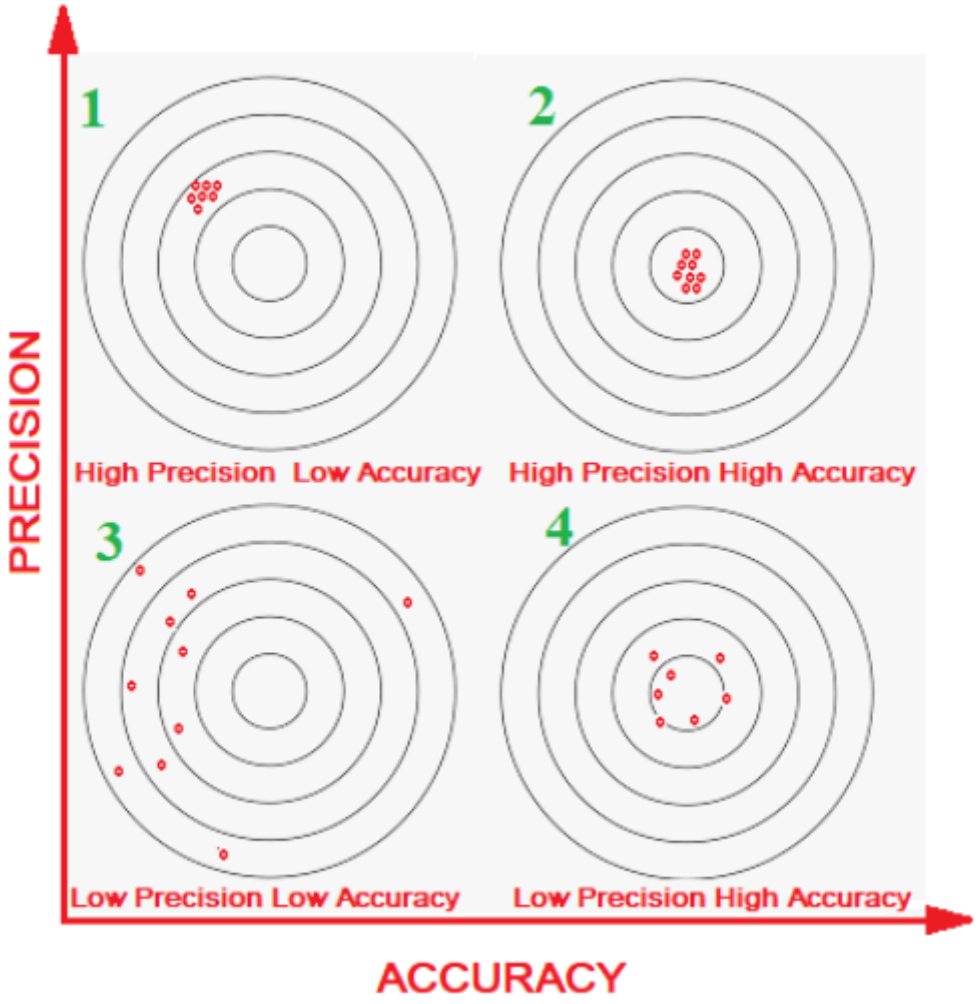
Outline presentation

- Introduction
- Aim
- Setup study
- Results
- Conclusion

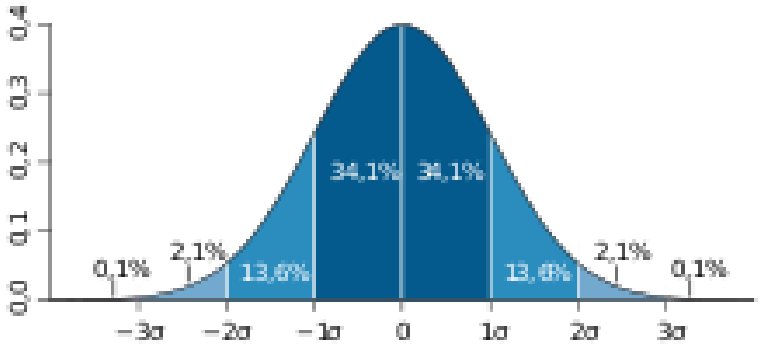


- **Correctness of measurements is defined by analytical performance specifications**

Precision and Accuracy



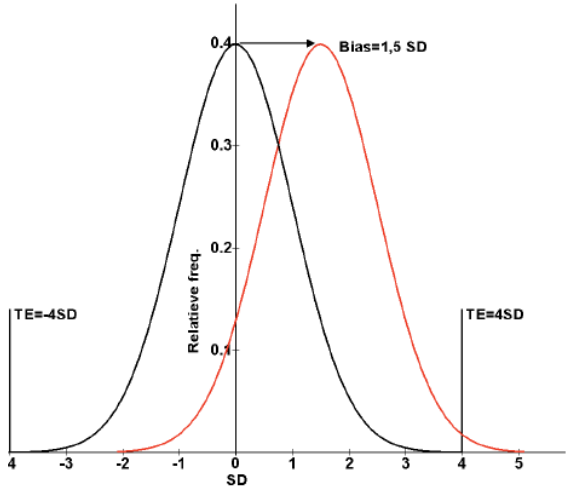
Correct test result



PRECISION

ACCURACY

Result



Every test result is subject to systematic (bias) and random errors (imprecision)

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

Sigma Score

$$\text{sigma} = \frac{(TE_A\text{-}bias_A)}{CV_A}$$

- **The higher the sigma score, the better the analytical performance of the test**

Sigma scores based on CV_A of internal QC data and applying the minimum, median and maximum BV estimates

		Sigma		
	CV_A	Min.	Median	Max.
PT	1.6	1.9	2.2	4.3
APTT	1.9	1.7	2.5	4.3
Fibrinogen	2.7	3.6	5.4	8.3
AT	5.6	0.3	0.8	1.4
CVa: Analytical variation of an internal QC sample. Yellow cells represent a sigma score above 3.				

- **The application of the six-sigma method to BV-derived APS is hampered by the large variation in published BV data**

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-analysis

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):
    - 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]

The Biological Variation Data Critical Appraisal Checklist

Table 1. BIVAC with criteria for achieving A, B, C, and D scores for the different quality items and their rationale. *(Continued from page 503)*

QI	Quality question	Quality scoring				
		A	B	C	D[a]	Rationale
3: Samples	Are the following documented? (*a*) Number of samples collected (*b*) Type of sample material used (*c*) Timing of sample collections (*d*) Length of study period	Yes	(*a*), (*c*), and (*d*) are documented. (*b*) Sample material; insufficient detail is given, but this is not of importance for the measurand in question.	(*a*), (*c*), and (*d*) are documented, but no information or insufficient detail on (*b*) sample material is given, with consequences for the measurand in question.	(*a*) Number of samples, (*c*) timing of sample collections, and/or (*d*) length of study period are not presented or deducible.	Necessary to characterize how and in what material the study has been performed and to evaluate if variation in timing gives different estimates.
4: Measurand	Are the measurand and the measurement procedure documented?	Yes A detailed method description is presented Or A reference to article where the method is described in detail is provided, Or An identifiable method has been applied and is described with sufficient detail.[b]	Insufficient detail on the method is given, but it is not of importance for the measurand in question.	Insufficient detail on the method is given or an outdated method has been used, which may be of importance for the measurand in question.	The method is obsolete and no longer valid, i.e., methods in use today estimate another measurand, or the method is not fit for the purpose of estimating biological variation in the chosen population.	Adequate description of the measurand and measurement procedure is necessary to ensure transferability of data.

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

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

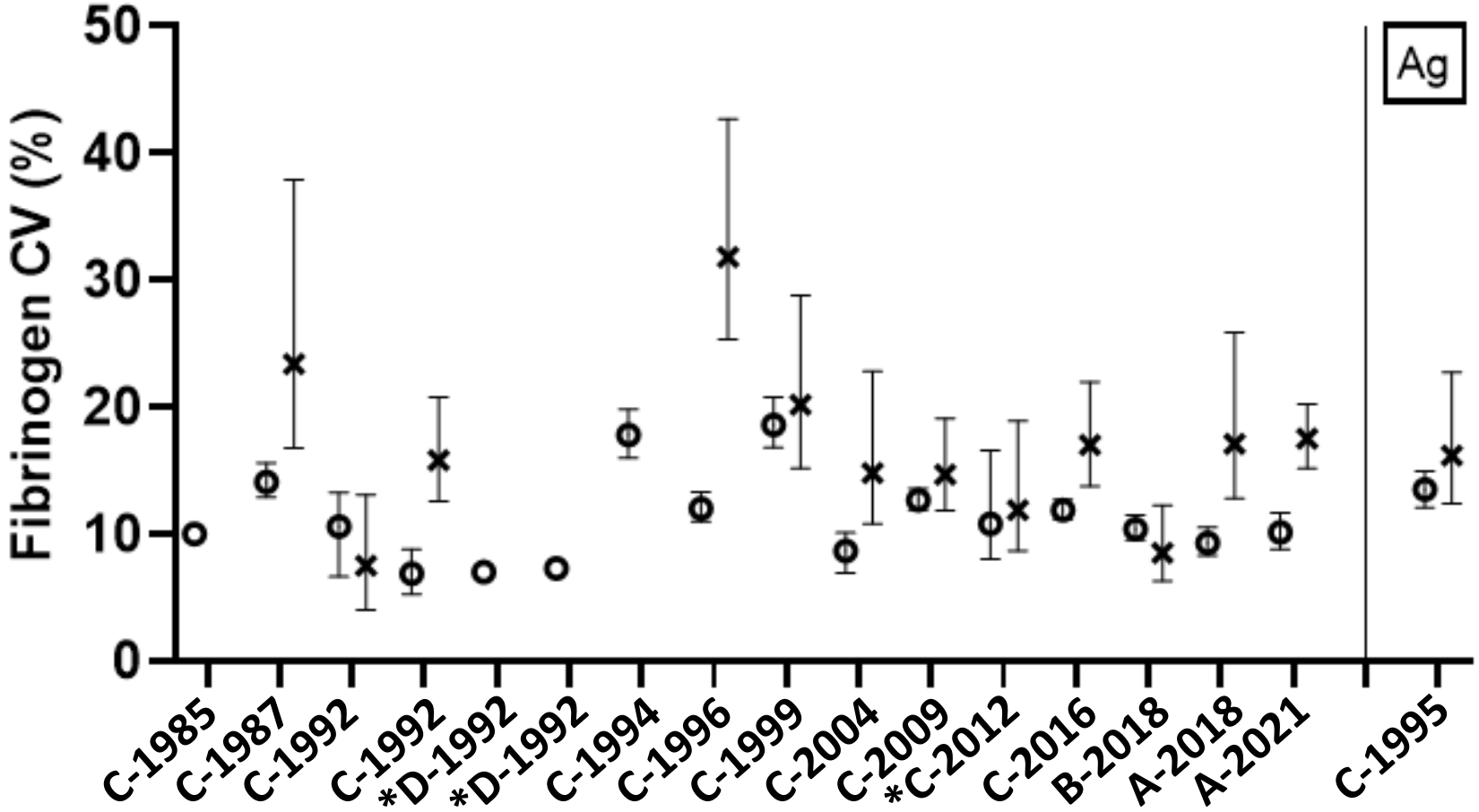
Reason grading C

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

Reason grading D

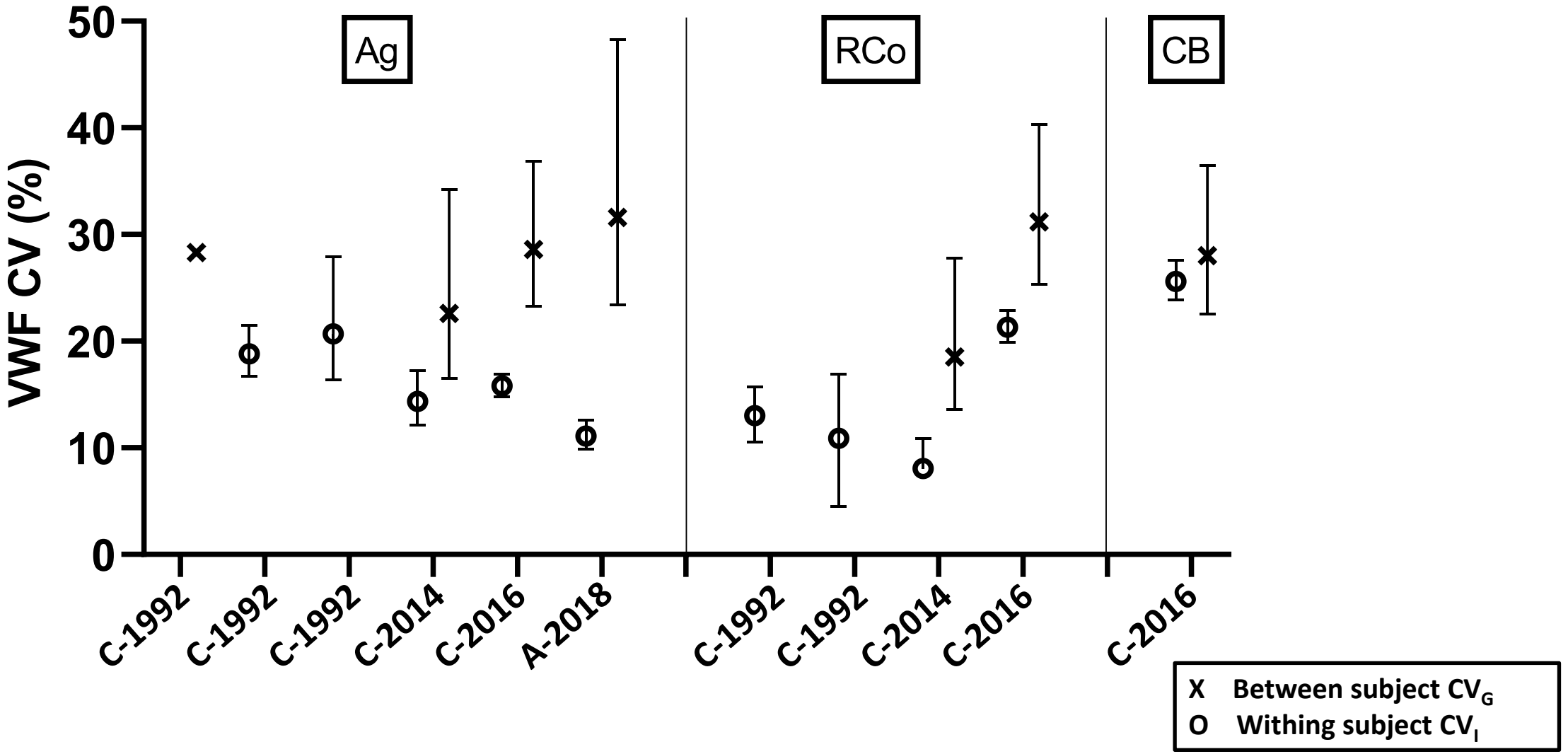
- Due to obsolete methods being applied

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

Comparison of data of different studies

- No trend observed in BV estimates of different grading
- No trend observed in BV estimates of different years
- Since robust statistics were used for the meta-analysis, no changes were observed after exclusion of the most extreme BV data

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 means wider APS

Green marked variable represent the lowest estimates

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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)
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)

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 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/

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