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How to determine acceptability limits based on biological variation (BV) in External Quality Assessment Schemes (EQAS): lymphocyte subsets as a case study

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Outline

- ❑Components of variation in laboratory measurements
- ❑Desirable quality specifications based on BV
- ❑How to estimate BV
- ❑Application to lymphocyte subsets
- ❑Conclusion

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Components of Variation in Laboratory Measurements

- Pre-analytical variation ($CV_P \approx 0$)
- Analytical variation (CV_A)
- Within-subject biological variation (CV_I)
- Between-subject biological variation (CV_G)

$$CV_T \approx \sqrt{CV_B^2 + CV_A^2}$$

$where \;\; CV_B = (CV_I^2 + CV_G^2)^{1/2}$

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Desirable quality specifications in EQAS based on BV

Harris (1979)

- $CV_{A,max} = 0.5\ CV_I$

Gowans et al (1988)

- $B_{max} = 0.275\ CV_B \;\; and \;\; CV_{A,max} = 0.597\ CV_B$

Wytze P.Oosterhuis and Sverre Sandberg (2015)

- $B_{max} = 0.275\ (CV_B^2 + CV_{A0}^2)^{1/2}\ and$
  $CV_{A,max} = 0.597\ ((1.96/1.68)^2(CV_B^2 + CV_{A0}^2) - CV_B^2)^{1/2}$

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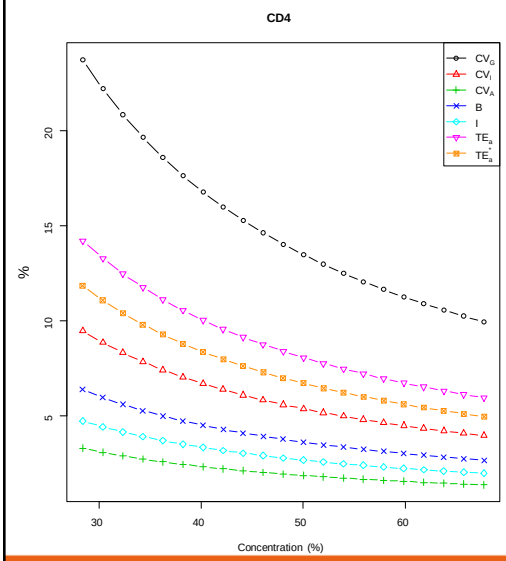
How to estimate the components of BV

- Collect blood samples from healthy volunteers
- Store the sample for analysis in optimal conditions
- Analyse samples in duplicate, simultaneously under conditions minimising analytical variation
- Remove outliers
- Determine the analytical variation, within-subject and between-subject variation by fitting linear mixed-effects models (GLMM) to data

BV for lymphocyte subsets

- 29 healthy donors (15 females and 14 males)
- Samples were collected at monthly intervals during 1-year period.
- Particular attention was paid to minimize pre-analytical sources of variation
- Samples were immediately analyzed after collection using the same methodology.
- Percentages and absolute numbers were determined for T (CD3+), B (CD19+) and NK cells, and for the CD4+ and CD8+ T cell subsets
- Analytical, within-subject and between-subject BV were derived using GLMM.

CD4 (%)



CONC	CV_G	CV_I	CV_A	B(%)	I(%)	TE_a
35.63	18.92	7.55	2.64	5.09	3.78	11.33
41	16.44	6.56	2.29	4.43	3.28	9.84
47.35	14.24	5.68	1.98	3.83	2.84	8.52
52.7	12.79	5.11	1.78	3.44	2.55	7.66
56.6	11.91	4.76	1.66	3.21	2.38	7.13

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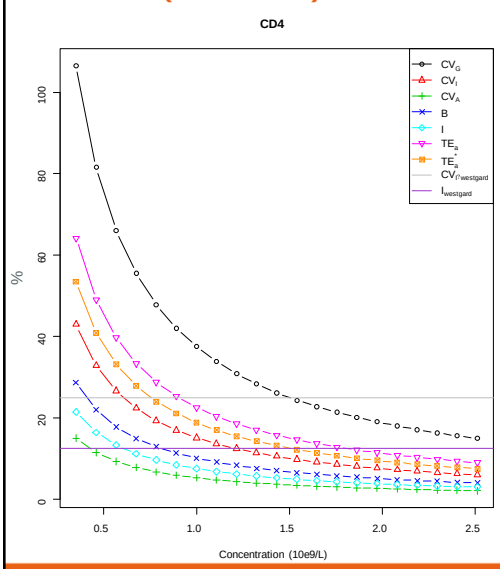
CD4 (10e9/L)



CONC	CV_G	CV_I	CV_A	B(%)	I(%)	TE_a
0.58	64.66	26.05	9.11	17.43	13.03	38.92
0.73	51.37	20.7	7.24	13.85	10.35	30.92
0.9	41.67	16.79	5.87	11.23	8.39	25.08
1.24	30.24	12.19	4.26	8.15	6.09	18.21
1.58	23.74	9.56	3.34	6.4	4.78	14.29

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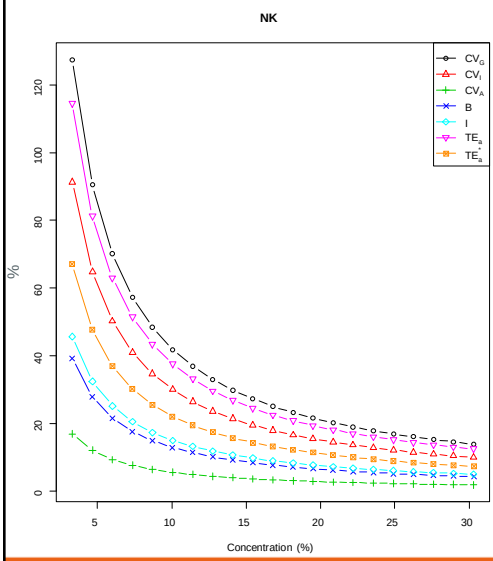
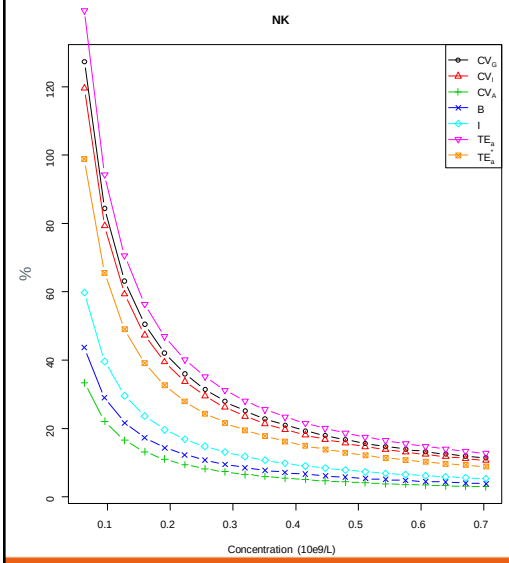
NK (%)



CONC	CV_G	CV_I	CV_A	B(%)	I(%)	TE_a
7.43	56.58	40.52	7.52	17.4	20.26	50.83
10	42.04	30.11	5.58	12.93	15.05	37.77
12.75	32.97	23.61	4.38	10.14	11.81	29.62
16.5	25.48	18.25	3.38	7.83	9.12	22.89
22.27	18.88	13.52	2.51	5.8	6.76	16.96

NK (10e9/L)

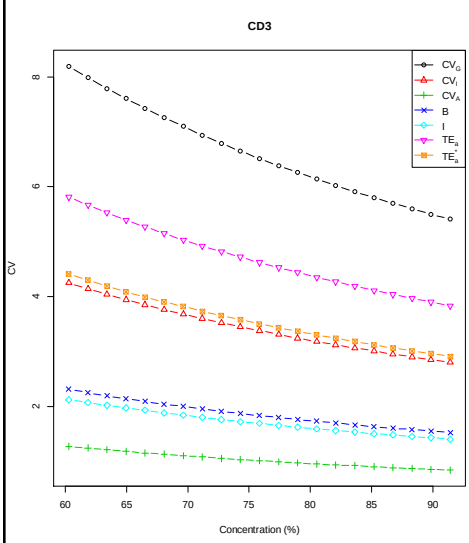


CONC	CV_G	CV_I	CV_A	B(%)	I(%)	TE_a
0.15	53.51	50.21	14.03	18.34	25.11	59.77
0.21	38.22	35.87	10.02	13.1	17.93	42.69
0.26	30.87	28.97	8.09	10.58	14.48	34.48
0.33	24.32	22.82	6.38	8.34	11.41	27.17
0.43	18.66	17.52	4.89	6.4	8.76	20.85

Conclusion and discussion

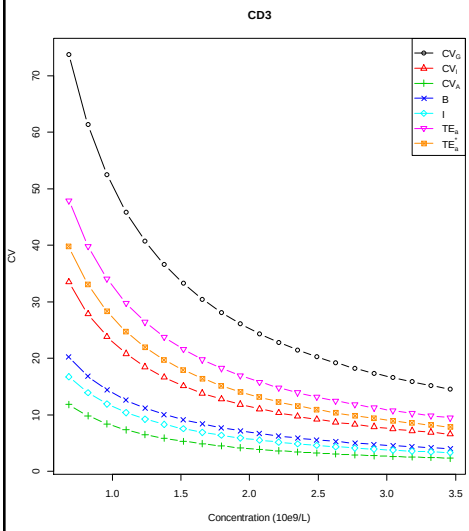
- The approach of limited data collection combined with mixed linear models is a workable alternative to compiling information from different sources.
- It offers a general solution for analytical quality setting and evaluation criteria for other EQAS
- Data collected could be used to derive reference intervals

CD3 (%)



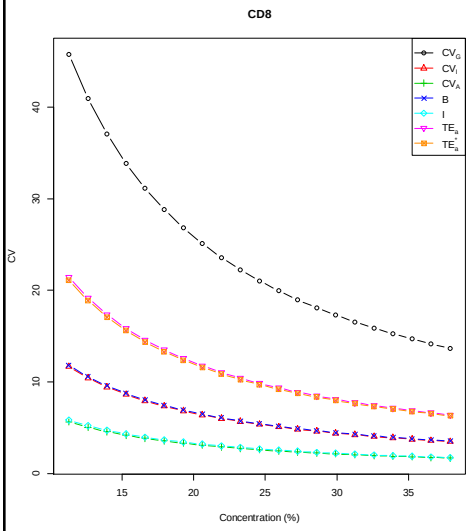
CONC	CV_G	CV_I	CV_A	B(%)	I(%)	TE_a	TE^*_a
67.2	7.35	3.81	1.14	2.07	1.91	5.21	3.95
70.7	6.99	3.62	1.09	1.97	1.81	4.96	3.76
75.1	6.58	3.41	1.02	1.85	1.71	4.67	3.54
78.8	6.27	3.25	0.97	1.77	1.63	4.45	3.37
82.3	6	3.11	0.93	1.69	1.56	4.26	3.23

CD3 (10e9/L)



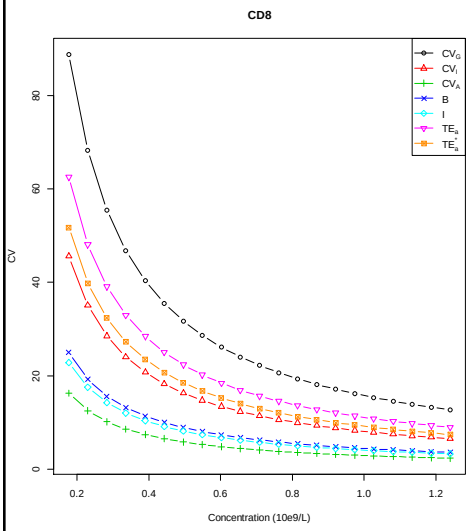
CONC	CV_G	CV_I	CV_A	B(%)	I(%)	TE_a	TE^*_a
0.98	51.5	23.38	8.25	14.14	11.69	33.43	27.76
1.2	42.06	19.1	6.74	11.55	9.55	27.3	22.67
1.52	33.2	15.08	5.32	9.12	7.54	21.55	17.9
1.9	26.56	12.06	4.26	7.29	6.03	17.24	14.32
2.35	21.48	9.75	3.44	5.9	4.88	13.94	11.58

CD8 (%)



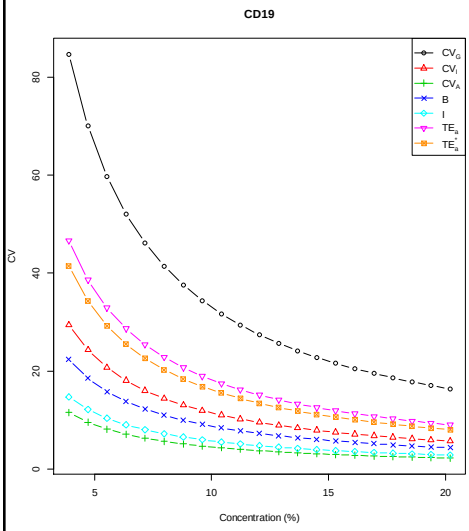
CONC	CV_G	CV_I	CV_A	B(%)	I(%)	TE_a	TE^*_a
15.63	33.09	8.43	4.07	8.54	4.21	15.49	15.25
20.4	25.35	6.46	3.12	6.54	3.23	11.87	11.69
24.2	21.37	5.44	2.63	5.51	2.72	10	9.85
27	19.16	4.88	2.36	4.94	2.44	8.97	8.83
29.9	17.3	4.41	2.13	4.46	2.2	8.1	7.97

CD8 (10e9/L)



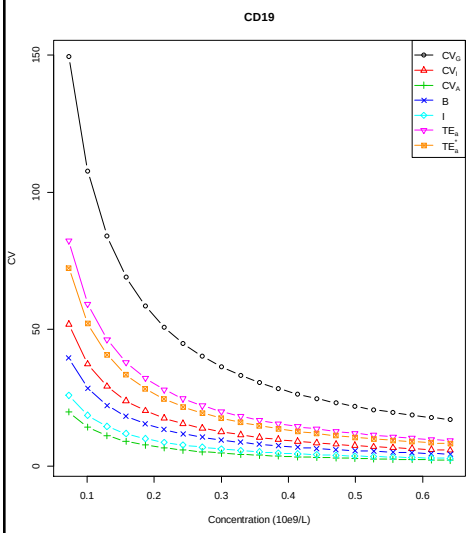
CONC	CV_G	CV_I	CV_A	B(%)	I(%)	TE_a	TE^*_a
0.29	54.23	27.83	9.91	15.24	13.91	38.2	31.6
0.37	42.51	21.81	7.77	11.94	10.91	29.94	24.77
0.48	32.77	16.81	5.99	9.21	8.41	23.08	19.09
0.61	25.78	13.23	4.71	7.24	6.62	18.16	15.02
0.73	21.54	11.06	3.94	6.05	5.53	15.17	12.55

CD19 (%)



CONC	CV_G	CV_I	CV_A	B(%)	I(%)	TE_a	TE^*_a
6.6	50.04	17.36	6.81	13.24	8.68	27.57	24.48
8	41.28	14.33	5.62	10.92	7.16	22.74	20.2
10.9	30.3	10.51	4.13	8.02	5.26	16.69	14.82
13.6	24.28	8.43	3.31	6.43	4.21	13.38	11.88
15.5	21.31	7.39	2.9	5.64	3.7	11.74	10.43

CD19 (10e9/L)



CONC	CV_G	CV_I	CV_A	B(%)	I(%)	TE_a	TE^*_a
0.12	90.96	31.52	12.09	24.07	15.76	50.07	44.02
0.15	72.77	25.22	9.67	19.25	12.61	40.06	35.22
0.21	51.98	18.01	6.91	13.75	9.01	28.61	25.15
0.29	37.64	13.04	5	9.96	6.52	20.72	18.22
0.42	25.99	9.01	3.46	6.88	4.5	14.31	12.58

Desirable quality specifications in EQAS based on BV

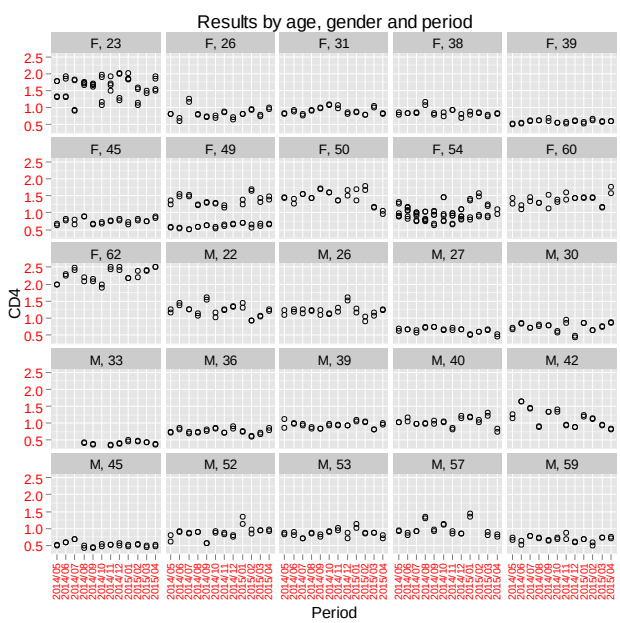
Westgard (1974)

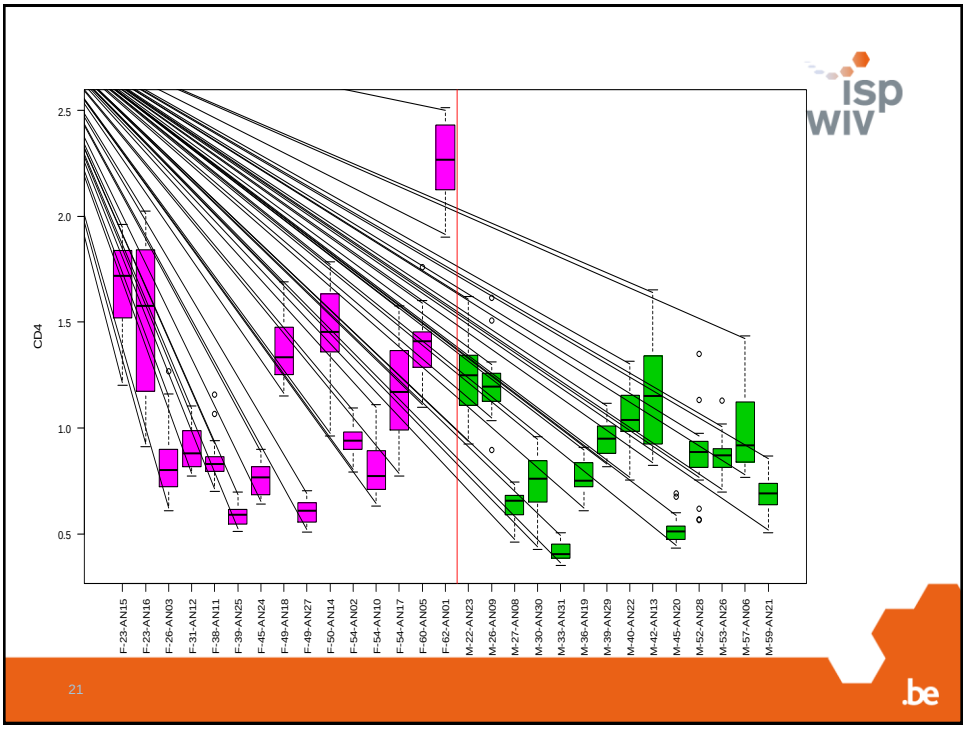
- $TE = B + 1.65\ CV_A$
- $TE_a = B_{max} + 1.65\ CV_{A,max}$
- $TE_a = 0.25\ CV_B + 1.65\ (0.5\ CV_I)$

How to estimate the components of BV

fit linear mixed-effects models to data

- $Y_{ik} = \mu + \alpha_i + \beta * G_i + \gamma_{ik} + \varepsilon_{ik}$
- $\gamma_{ik} \sim N(0, \sigma_k)$: The effect of the period k on the subject i
- $\alpha_i \sim N(0, \sigma_i)$: The individual random effect of subject i
-
- $\varepsilon_{ik} \sim N(0, \sigma_\varepsilon)$:: the residual error
- *gender* is "*dummy*" *variable* equal to 1 if the subject i is male, 0 otherwise.
- $Var(Y_{ik}) = \sigma_i^2 + \sigma_k^2 + \sigma_\varepsilon^2$




CD4
2.5
2.0
1.5
1.0
0.5
F-23-AN15
F-23-AN16
F-26-AN03
F-31-AN12
F-38-AN11
F-39-AN25
F-45-AN24
F-49-AN18
F-49-AN27
F-50-AN14
F-54-AN02
F-54-AN10
F-54-AN17
F-60-AN05
F-62-AN01
M-22-AN23
M-26-AN09
M-27-AN08
M-30-AN30
M-33-AN31
M-36-AN19
M-39-AN29
M-40-AN22
M-42-AN13
M-45-AN20
M-52-AN28
M-53-AN26
M-57-AN06
M-59-AN21
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