Biological variation and quality control

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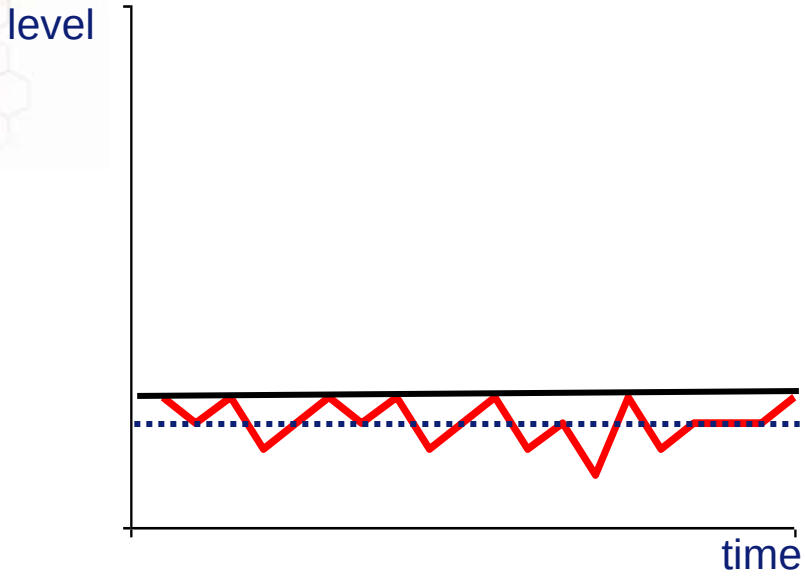
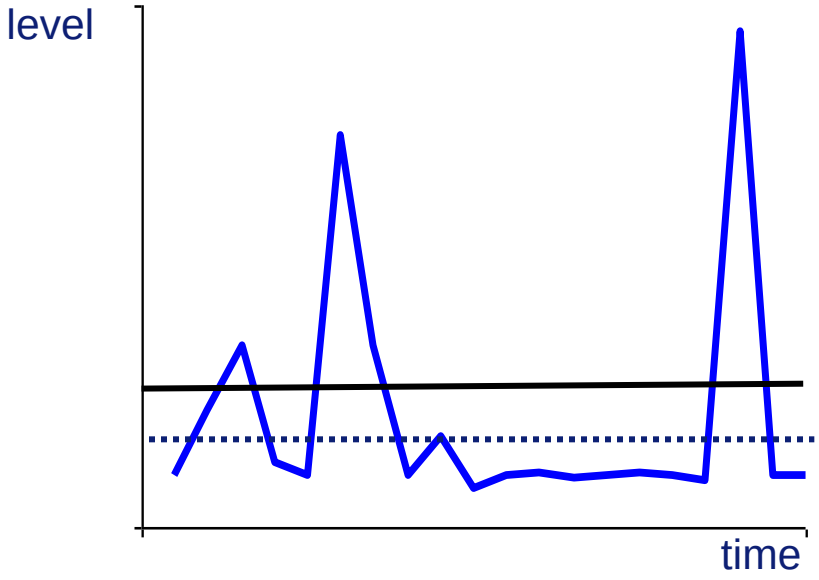
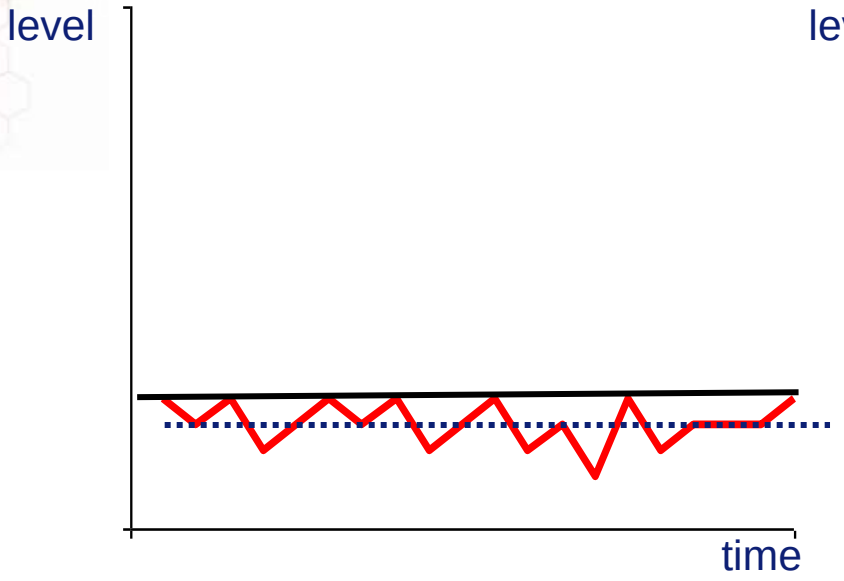
ECAT Participant meeting

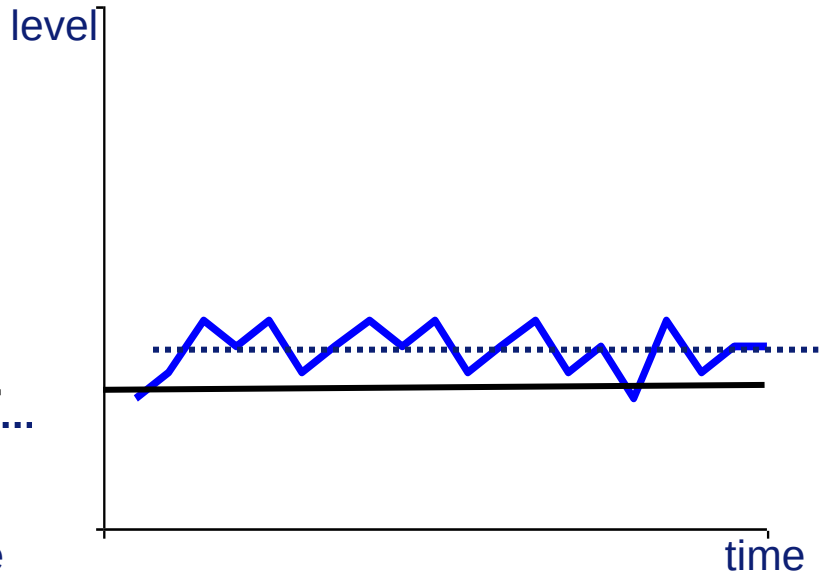
Variation in variable A



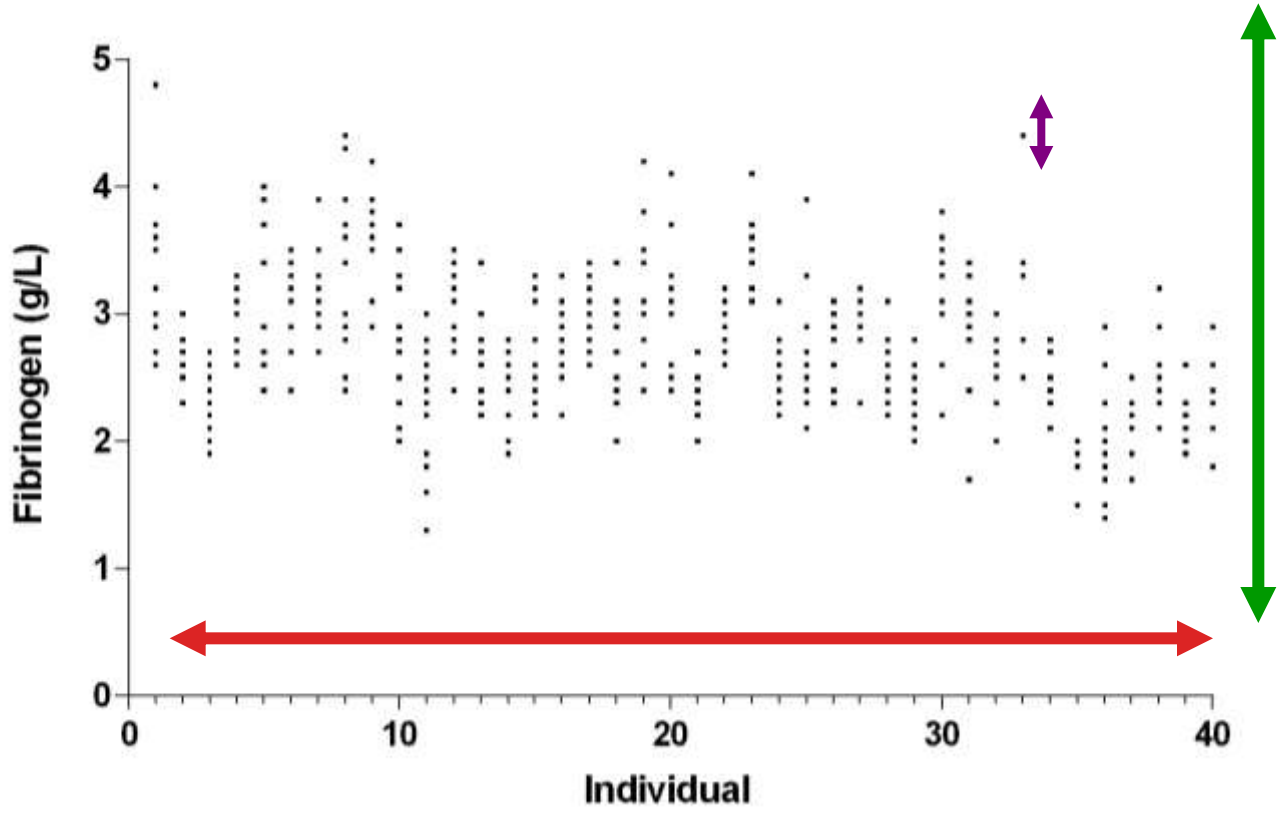


Variation in variable B





Total variation = **inter-individual variation +**
intra-individual variation +
analytical variation



Sources of analytical variation

- **Pre-analytical variation**:
  - **Subject-related**: fasting state, exercise / stress, posture

  - **Sample collection and handling**: type of sample, anticoagulant, tourniquet, transport time, centrifugation

- **Analytical variation**:
  - **Random**
  - **Systematic** (operators, reagents, apparatus, etc.)

Reducing analytical variation

- Limit pre-analytical variation
- Limit analytical variation
- Multiple measurements

What should the level of analytical variation be ?

Strategies to set quality specifications

- Assessment of the effect of analytical performance on **specific** clinical decision-making.
- Assessment of the effect of analytical performance on **general** clinical decision-making.
- Professional recommendations.
- Quality specifications laid down by PT or EQAS organisers.
- Published data on the state of the art.
- Biological variation

Introduction

Biological variation can help to determine Quality specifications of the assays:

- Inter-individual variation (between, CVg)
- Intra-individual variation (within, CVw, CVi)

- Monitoring
- Diagnosis

Components of variation

Total variation = inter-individual variation +
intra-individual variation +
analytical variation

$SD^2_{total} = SD^2_{between} + SD^2_{within} + SD^2_{analytical}$

How to express variation:

- Variance SD^2
- CV (SD / mean) * 100%

Quality specifications best based on biological variation

- Imprecision < 0.5 • CV_I
- Bias < 0.25 • $[CV_I^2 + CV_G^2]^{½}$
- Total error < 1.65 • 0.5 • CV_I + 0.25 • $[CV_I^2 + CV_G^2]^{½}$
- Other specifications based on biology include for reference methods and the allowable difference for two analysers in the same laboratory.

Effect of analytical performance in general terms

- Formulae using biological variation data look complex

BUT

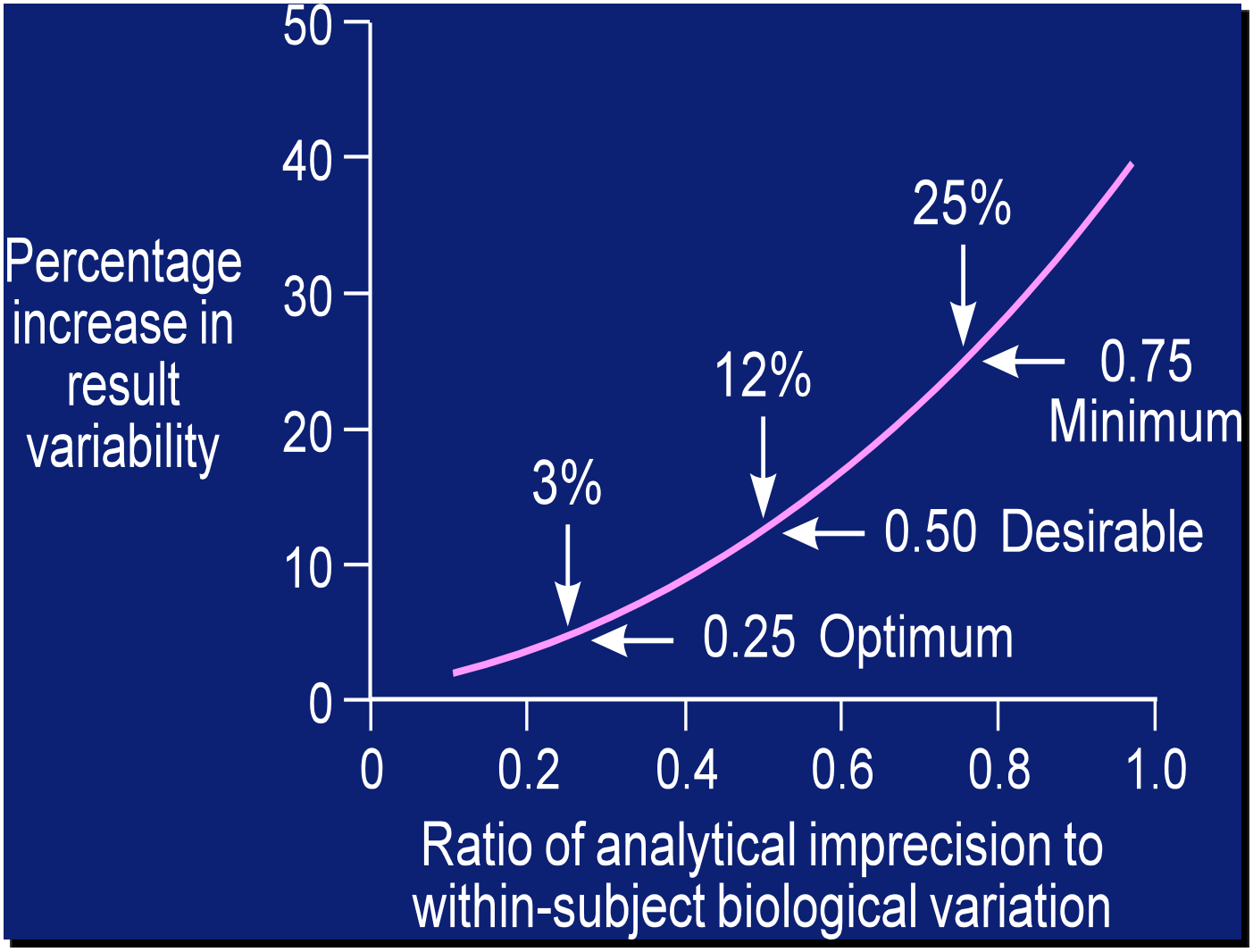
- directly related to use of tests clinically
- many different applications
- many data on biological variation
- widely supported by professionals

Analytical Quality Specifications (AQS)

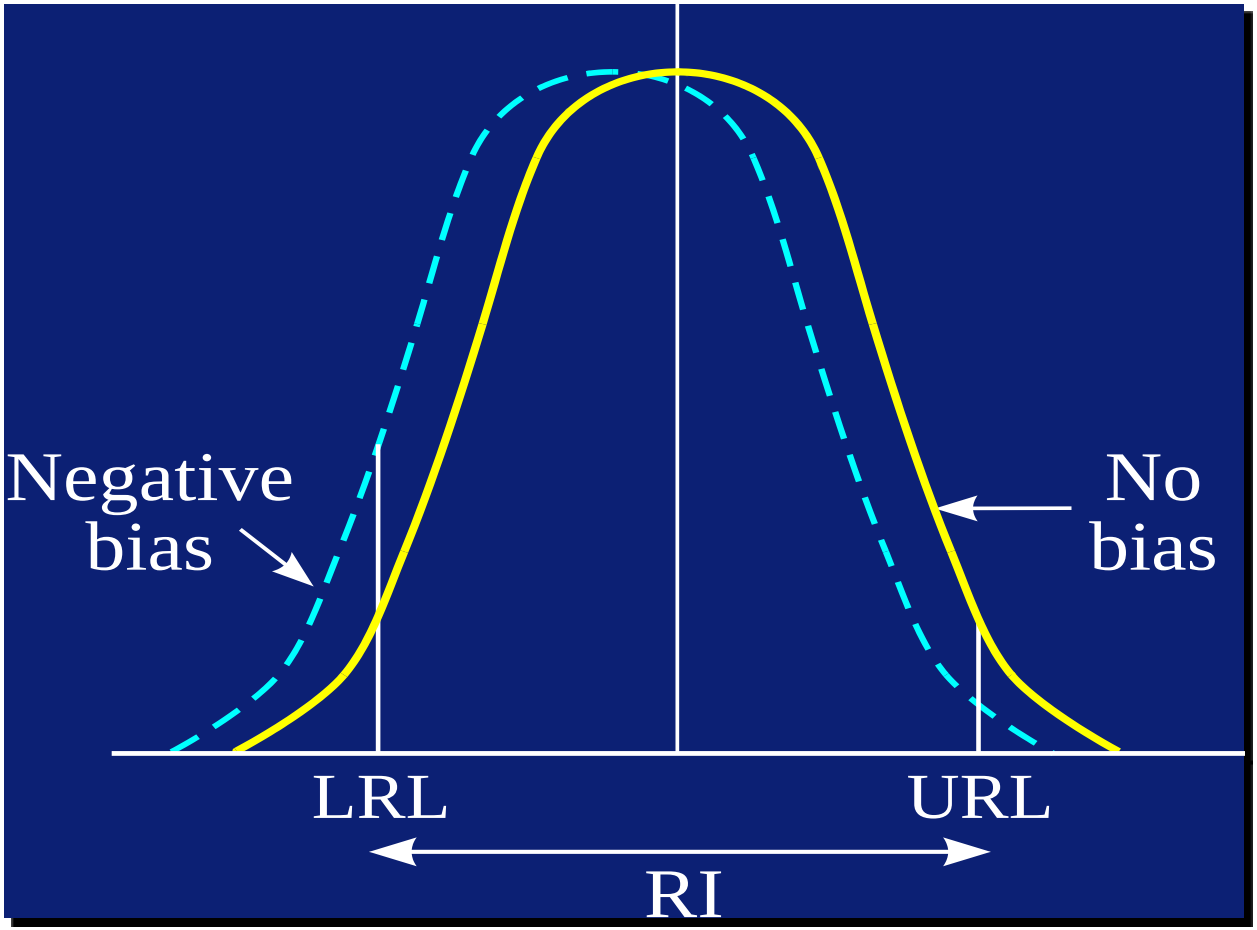
Performance goal	Imprecision (CV_A) (monitoring)	Imprecision (CV_A) (diagnostic testing)
Minimum quality	$CV_A < 0.75\ CV_W$	$CV_A < 0.87\ CV_T$
Desirable quality	$CV_A < 0.50\ CV_W$	$CV_A < 0.58\ CV_T$
Optimum quality	$CV_A < 0.25\ CV_W$	$CV_A < 0.29\ CV_T$

Fraser CG *et al.* Ann Clin Biochem 1997

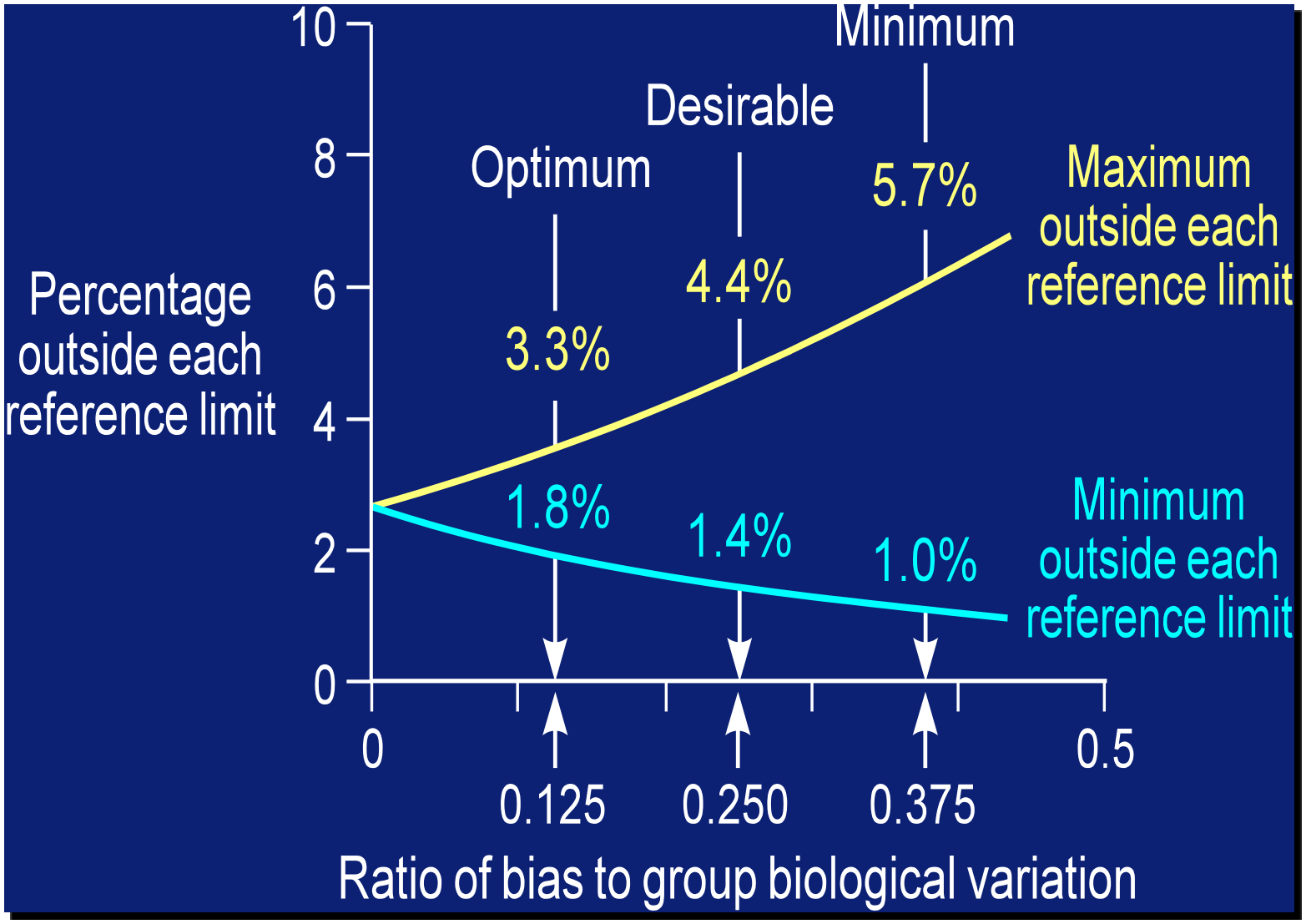
The effect of imprecision on test result variability



Effect of negative bias



Effect of bias on reference values



Generation of estimates of components of variation

- select a small number of reference individuals and apply exclusion criteria
- take samples at intervals - minimizing pre-analytical sources of variation [subject preparation and sample collection and handling]
- store as to ensure stability
- analyze in random duplicate in one batch
- look for outliers [complex]
- use random nested effects ANOVA to determine CV_A, CV_I, CV_G

Study design

- 40 healthy subjects living or working in Rotterdam region
- 15 blood samples were taken during 1-year from each participant (between January 2005 and December 2006)

- A total of 520 samples collected on 197 different days

Standardization

- Pre-analytical
  - Blood collection while sitting and resting
  - Right antecubital vein
  - Participants were allowed to have a light breakfast
  - Medical questionnaire (smoking, medication, common influenza, etc.)
- Analytical
  - From each participant all samples were assayed in 1 run
- Circadian variation
  - Samples were collected between 9 – 11 AM

Characteristics of the study population

Variable	Study population (n=40)
Age (years)	41 ± 15
Females	26 (65%)
BMI (kg/m^2)	22.6 ± 2.0
Smokers	7 (18%)
Oral contraceptives	9 (23%)

Contribution of biological to total variation after N repeated measurements

Variable	1	3
Fibrinogen	44%	21%
ln[CRP]	34%	15%
ETP	35%	15%
Prothrombin time	32%	13%
Antithrombin	18%	7%
Protein C clotting	23%	9%
Protein C chromogenic	13%	5%

Factors responsible for biological variation

- Age
- Gender
- Diet
- Smoking
- Drugs
- Time of blood sampling (diurnal, season)
- Particulate matter

Biological Variation

- How to define the group in which to determine biological variation ?

- Very defined
- Very broad
- Different definitions for different populations
- Different definitions for different diseases

Subgroup analyses and adjustments

- The results for the between- and within-subject variation only slightly changed when:
  - Outliers were not included
  - The analyses were performed for nonsmokers only
  - in men and women separately
  - after excluding periods of reported disease (common flu)
  - or for nonusers of contraceptives

- Adjustments for age and BMI did not affect significantly the within-subject (biological) variation.

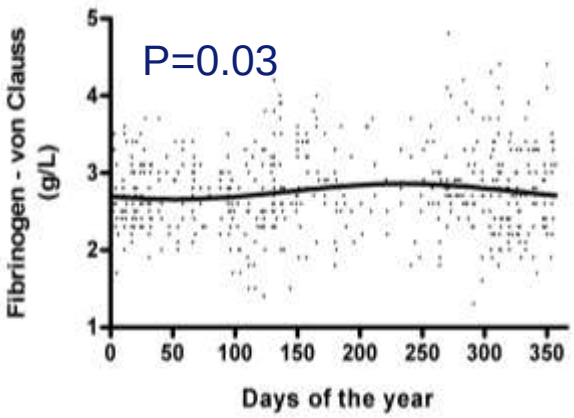
Seasonal variation

$$y = a + b*\sin(2\pi(t-1)/365) + c*\cos(2\pi(t-1)/365)$$

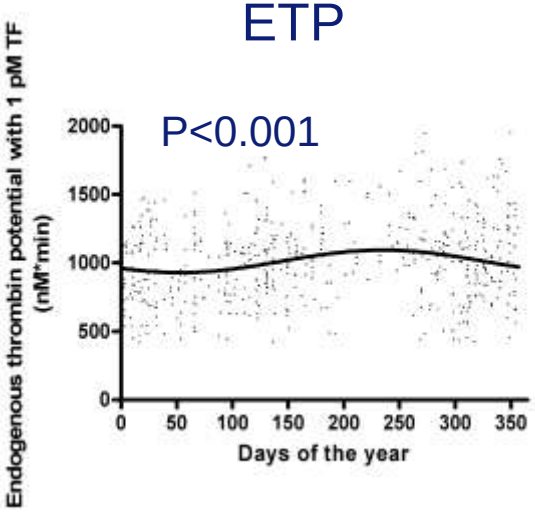
- *a*: annual mean
- *b* and *c*: components of seasonal variation
- *t*: day of the year

Seasonal variation

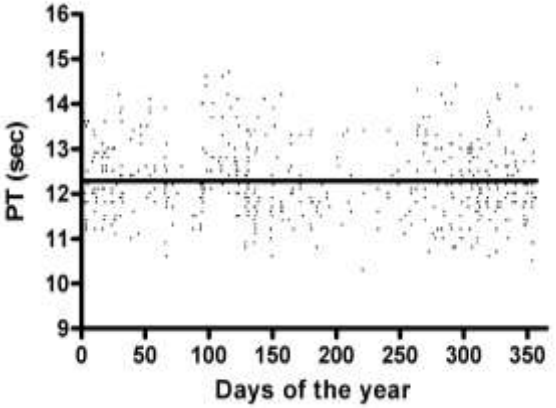
Fibrinogen



ETP

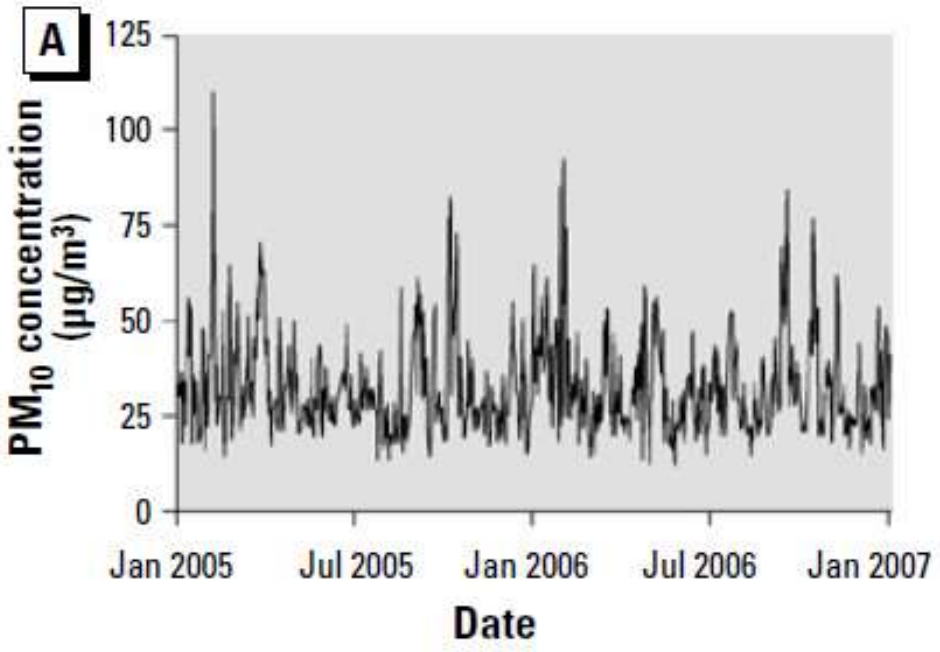


PT



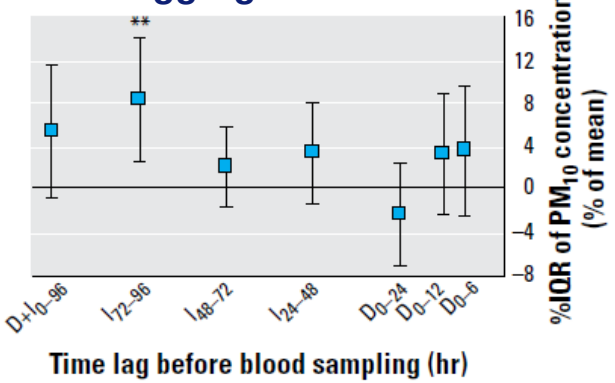
Rudez 2009

Particulate matter

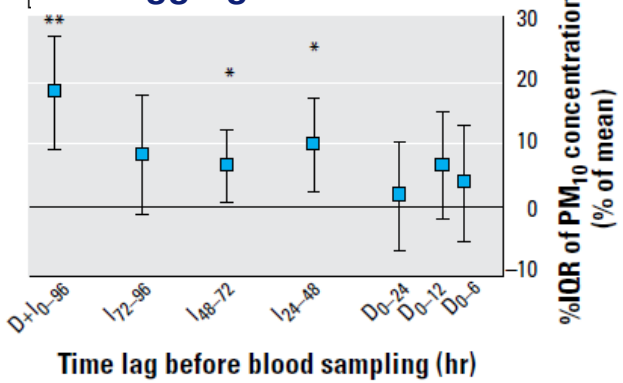


Particulate matter

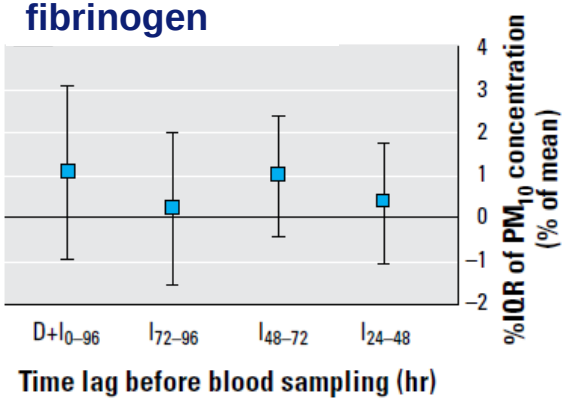
max aggregation



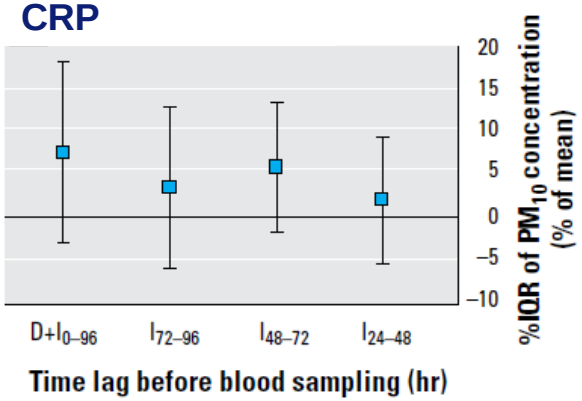
late aggregation



fibrinogen



CRP



Conclusions

- Within versus between-subject variation is different for different variables
- Analytical variation should be as low as possible
- For most hemostasis tests: analytical variation follows the desired level, for some even levels for optimum quality
- Biological variation is determined by many factors

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