CORESTA Joint Study Groups Meeting
Smoke Science / Product Technology
2011 - Graz, Austria

Effect of the number of laboratories and replicates on the precision of the measurement methods

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Introduction

Would an increase in the number of replicates reduce the repeatability?

Would an increase in the number of laboratories have an impact on repeatability and reproducibility?

Is it possible to compensate a low number of laboratories by a high number of replicates?

The objective of this presentation is to give answers to these questions in order to clarify the confusion existing about the influence of these two parameters

Outline

1. Indicator of method performances

   r&R, collaborative study

2. Process to simulate dataset

   based on CM6 product and NFDPM parameter

3. Impact of the number of replicates and laboratories

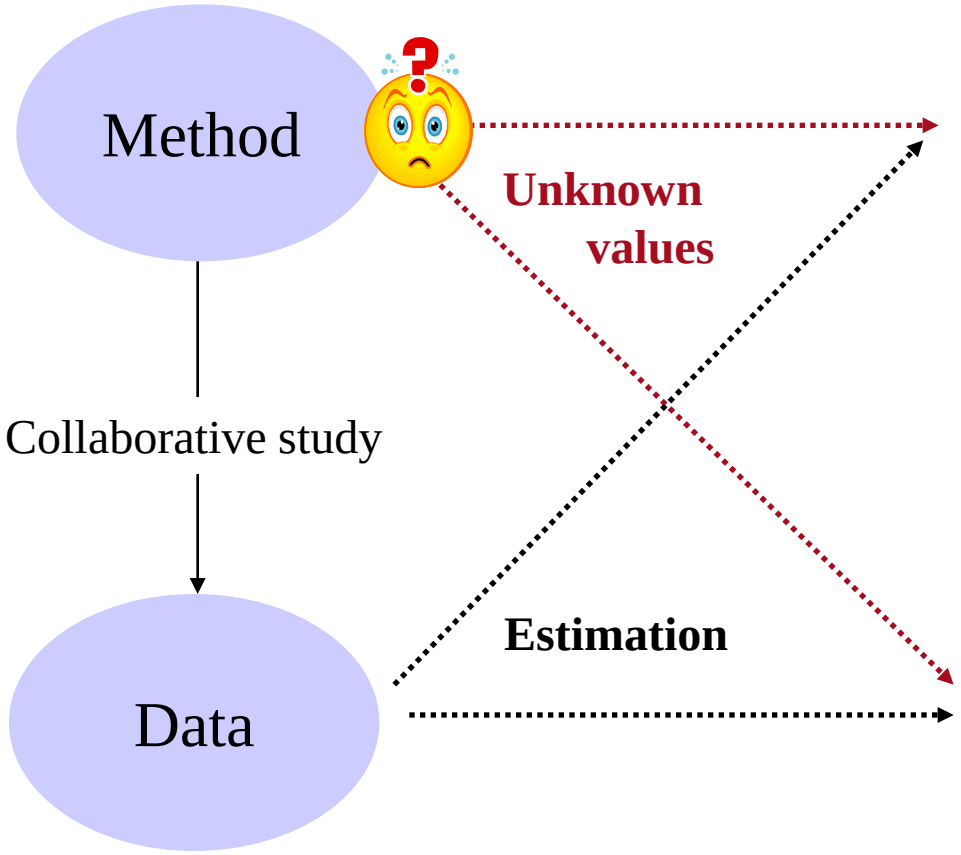
   on repeatability

   on Reproducibility

4. Generalization for any products & parameters

5. Conclusion

Method performance study



Number of replicates ***n***

Number of laboratories ***p***

Performance indicators

repeatability

Independent test results are obtained with the same method on identical test items in

the same laboratory by

the same operator using

the same equipment

within short intervals of time

Reproducibility

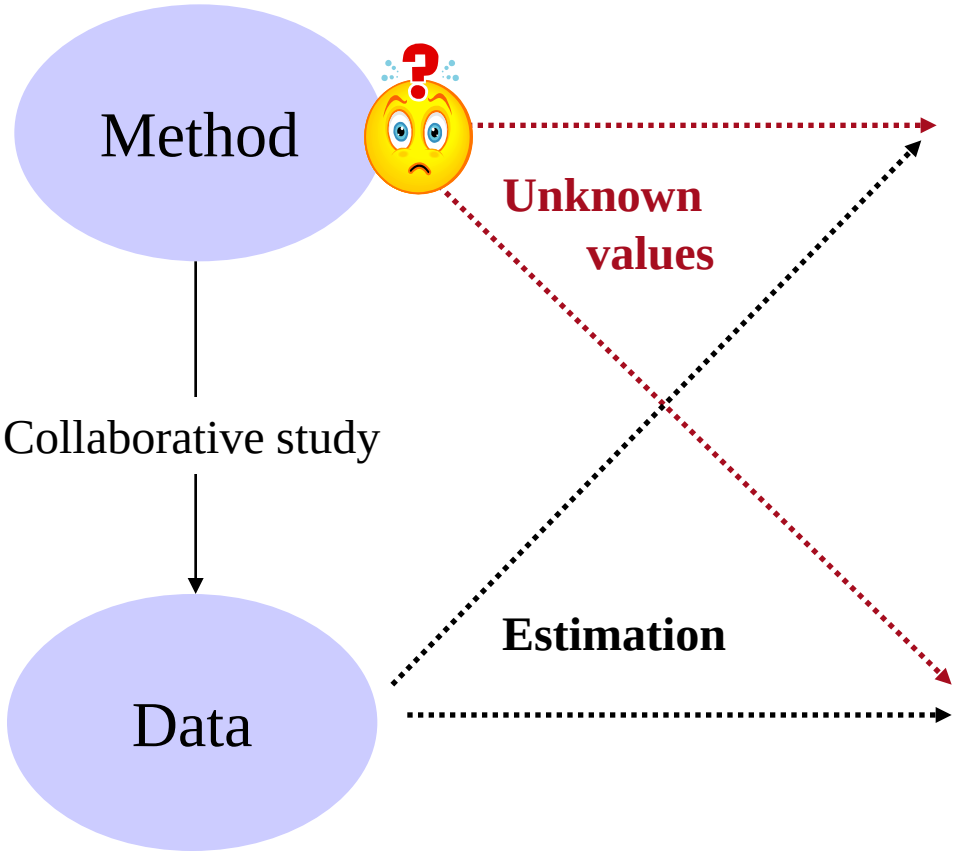
Independent test results are obtained with the same method on identical test items in

different laboratories with

different operators using

different equipment

Method performance study



Performance indicators

repeatability

$$s_r^2 = \frac{\sum_{i=1}^{p}(n_i - 1)s_i^2}{\sum_{i=1}^{p}(n_i - 1)}$$

Reproducibility

$$s_R^2 = \frac{\left[\frac{1}{p-1}\sum_{i=1}^{p} n_i(\bar{y}_i - \bar{\bar{y}})^2\right] - s_r^2}{\frac{1}{p-1}\left[\sum_{i=1}^{p} n_i - \frac{\sum_{i=1}^{p} n_i^2}{\sum_{i=1}^{p} n_i}\right]} + s_r^2$$

Recommendations from different standards

Number of Laboratories

- **DIN 38402** and **ISO 5725** standard recommend a minimum of 8-15 laboratories.
- **CIPAC (Collaborative International Pesticides Analytical Council Limited)** and **IUPAC (International Union of Pure and Applied Chemistry)** specify an absolute minimum number of five laboratories
- **AOAC (Association of Official Analytical Chemists)** recommend a minimum of 8

Number of Replicates

- **ISO 5725** standard does not specify the number of replicates.
- **CIPAC** requires a duplication of the experiments
- **DIN 38402** prescribes to perform at least two parallel determinations, but recommends a larger number (e.g. four). additionally this norm requires that the product of the number of laboratories and the number of replicates has to be equal to or larger than 24.

Comment

- **Youden and Steiner (AOAC)** suggest a larger number of laboratories instead of a larger number of replicates.

No agreement between the different recommendations and not clear information

Data simulation and evaluation

Unknow « true » parameters

CM6 (EUCS 2010)
m = 13.97
Sr = 0.202
SR = 0.479
SL = 0.454
SB = 0.444

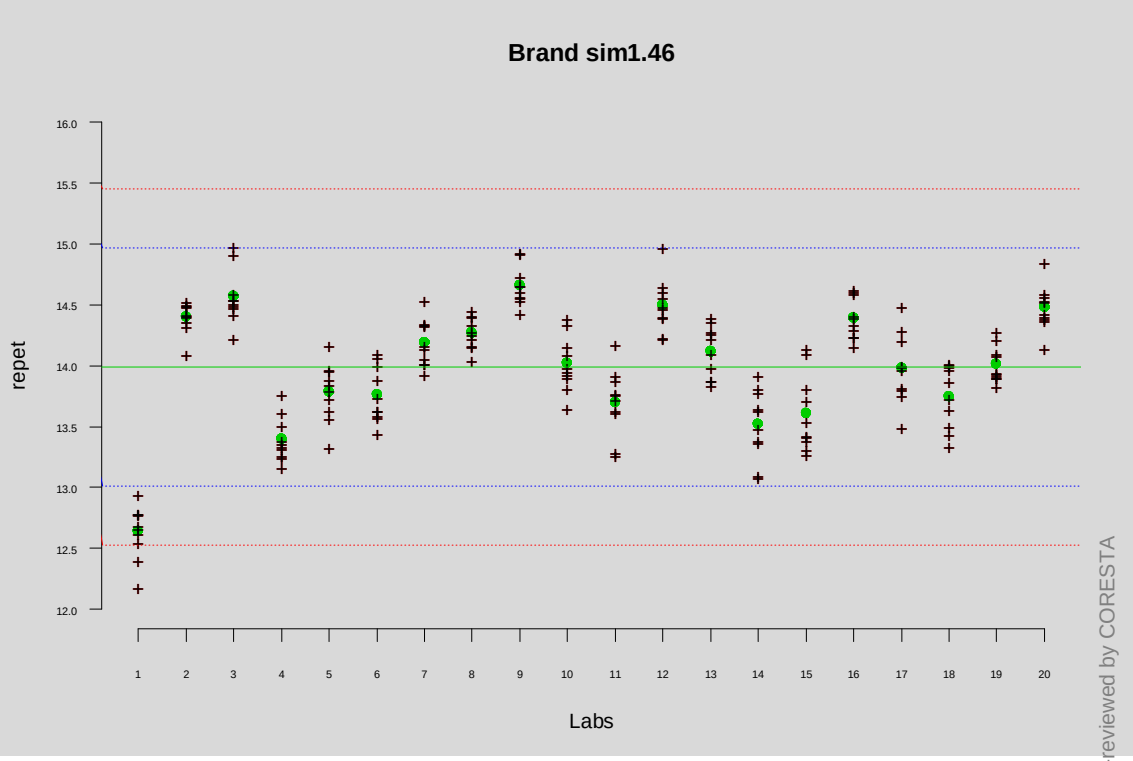
Simulation

Comparisons

Estimations
ISO 5725

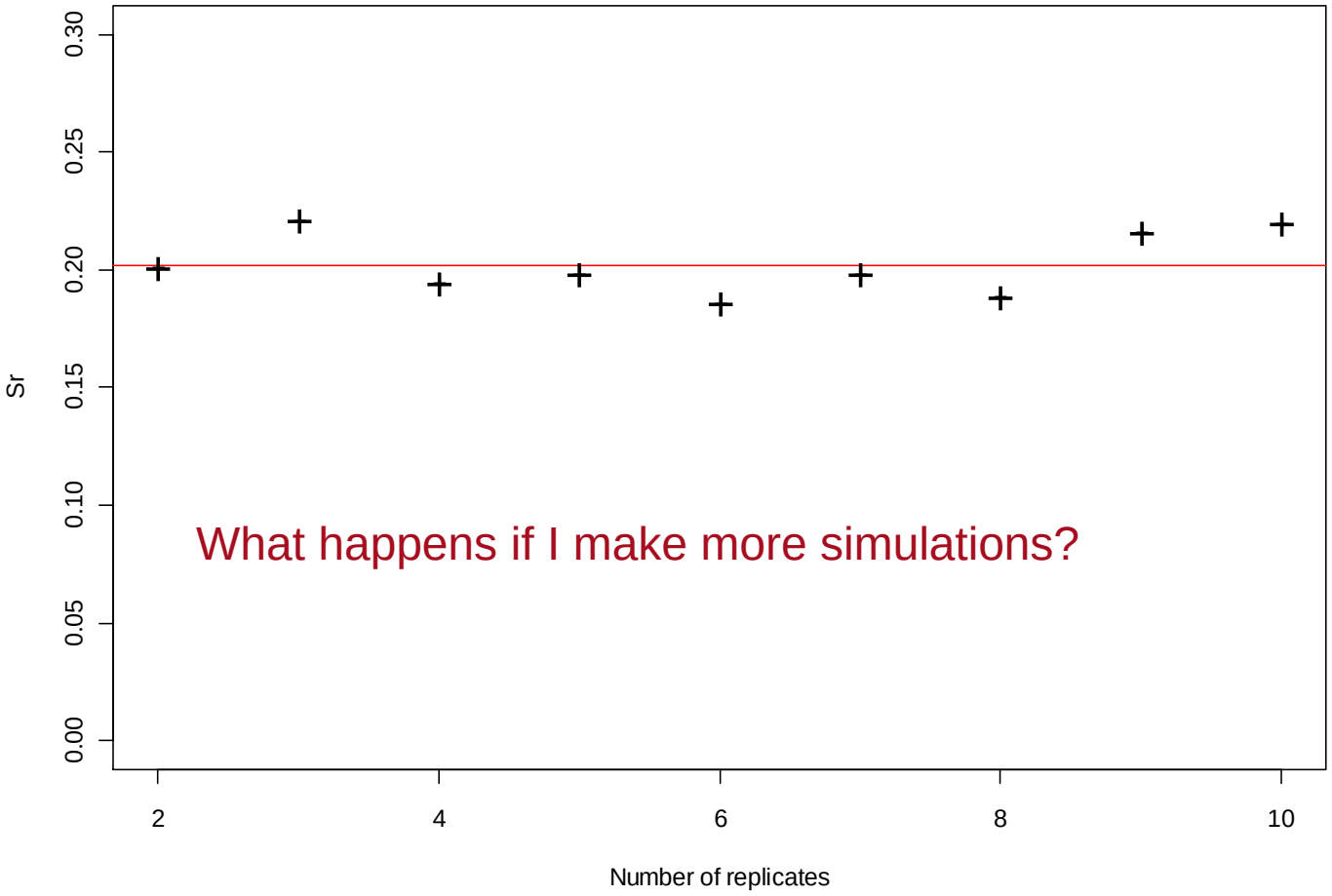
CM6 (EUCS 2010)
m = 13.99
Sr = 0.221
SR = 0.531
SL = 0.483
SB = 0.488

Simulated data



What happens if I consider
two replicates?
three replicates?
…

Simulations: impact of *n* on Sr



« True » Sr value

CM6 (EUCS 2010)
m = 13.97
Sr = 0.202
n = 2 to 10
p = 20

First observation: Increase in the number of replicates, doesn't decrease the repeatability

Second observation: Results with only two replicates can be closer to the true value than with 10 replicates

More Simulations: impact of *n* on Sr

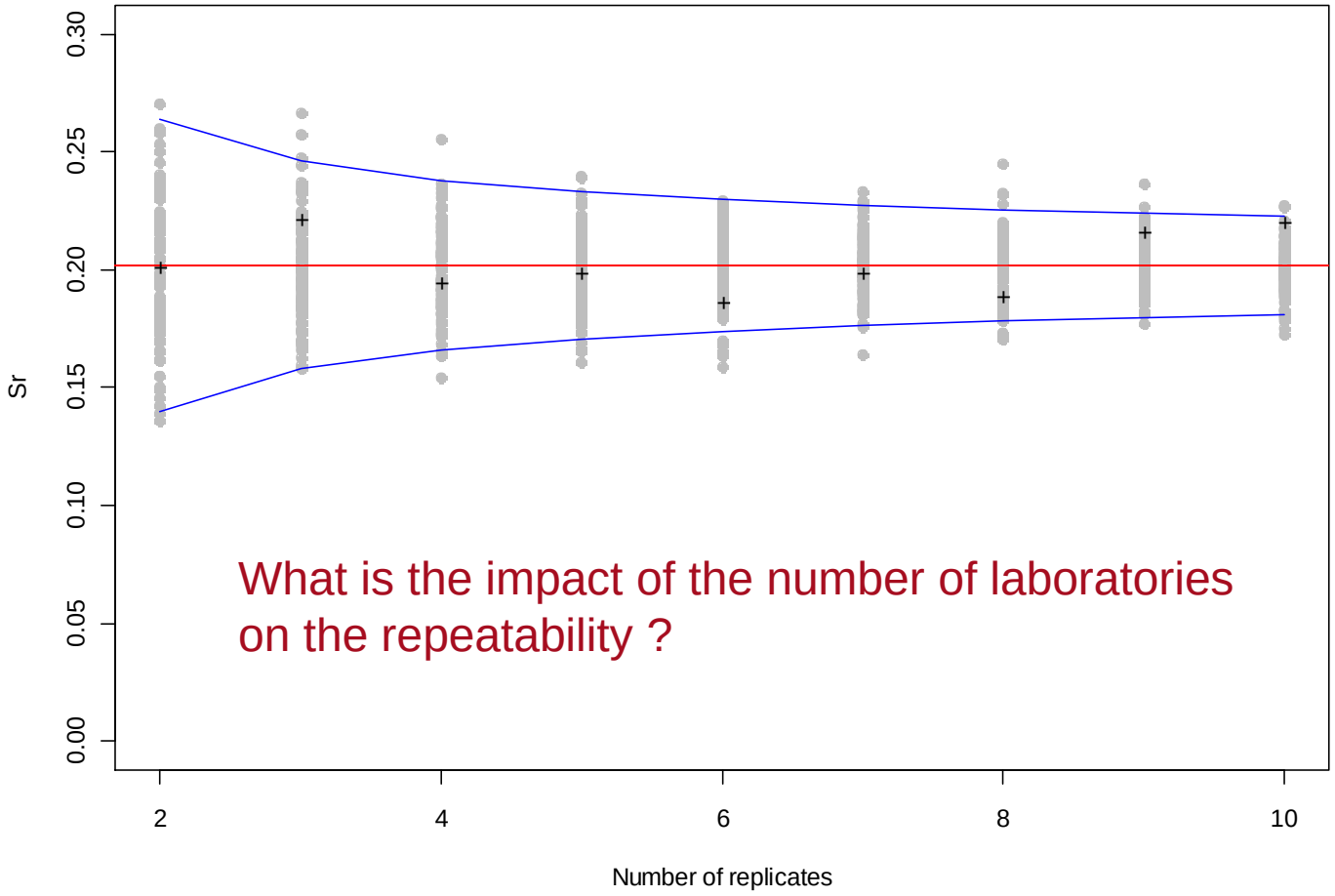
« True » Sr value

CM6 (EUCS 2010)
m = 13.97
Sr = 0.202
n = 2 to 10
p = 20

First observation: Increase in the number of replicates, doesn't decrease the repeatability
Second observation: Results with only two replicates can be closer to the true value than with 10 replicates
But if the number of replicates increase, the chance to be closer to the « true »value increases.

Increase in the number of replicates decreases the uncertainty of the repeatability estimation

Theory: uncertainty of Sr



« True » Sr value

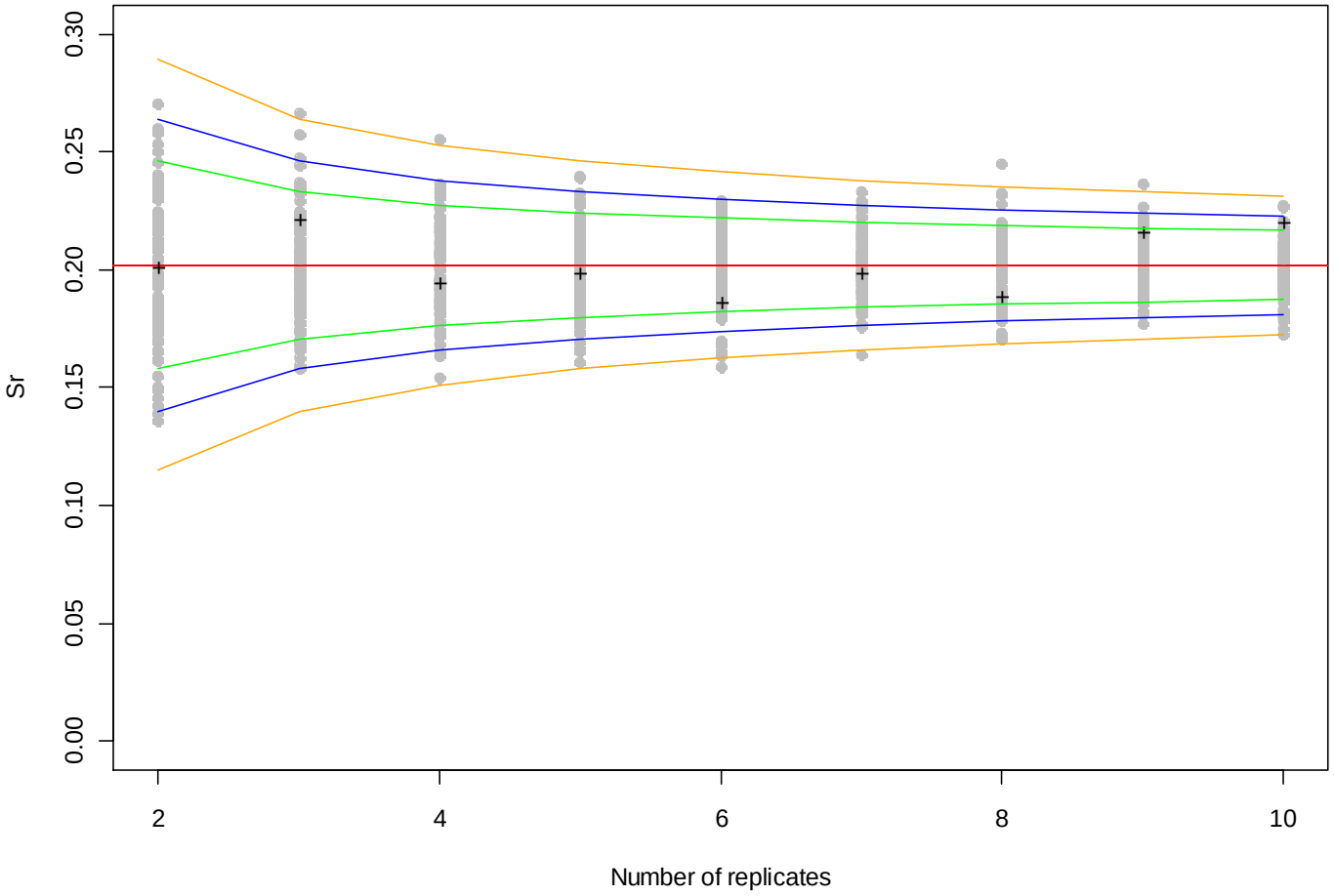
CM6 (EUCS 2010)
m = 13.97
Sr = 0.202
n = 2 to 10
p = 20

Theoretical interval of confidence (95%) of the standard deviation of repeatability

$$\sqrt{\frac{\chi^2_{0.025}}{p(\mathrm{n}-1)}}\times\sigma \le s \le \sqrt{\frac{\chi^2_{0.975}}{p(\mathrm{n}-1)}}\times\sigma$$

When n increase, then $\chi^2_{0.975}/(n-1)$ decrease and the confidence interval decrease too

Simulations: impact of *n* & *p* on Sr



« True » Sr value

CM6 (EUCS 2010)
m = 13.97
Sr = 0.202
n = 2 to 10
p = **10**, **20** and **40**

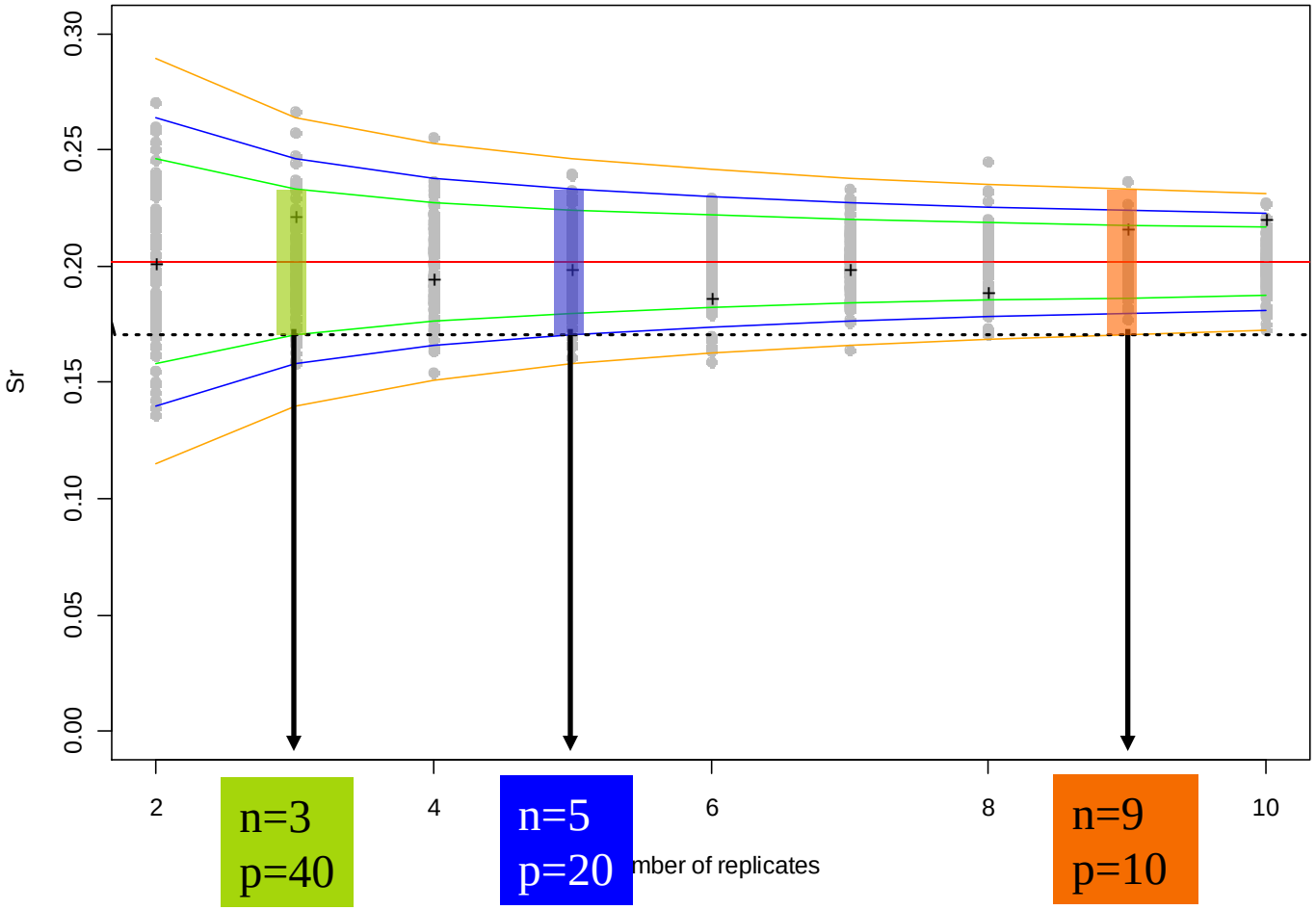
Theoretical interval of confidence of the standard deviation of repeatability

$$\sqrt{\frac{\chi^2_{0.025}}{p(n-1)}} \times \sigma \leq s \leq \sqrt{\frac{\chi^2_{0.975}}{p(n-1)}} \times \sigma$$

When n increase, then $\chi^2_{0.975}/(n-1)$ decrease and the confidence interval decrease too

When p increase, then $\chi^2_{0.975}/p$ decrease and the confidence interval decrease too

Simulations: impact of *n* & *p* on Sr



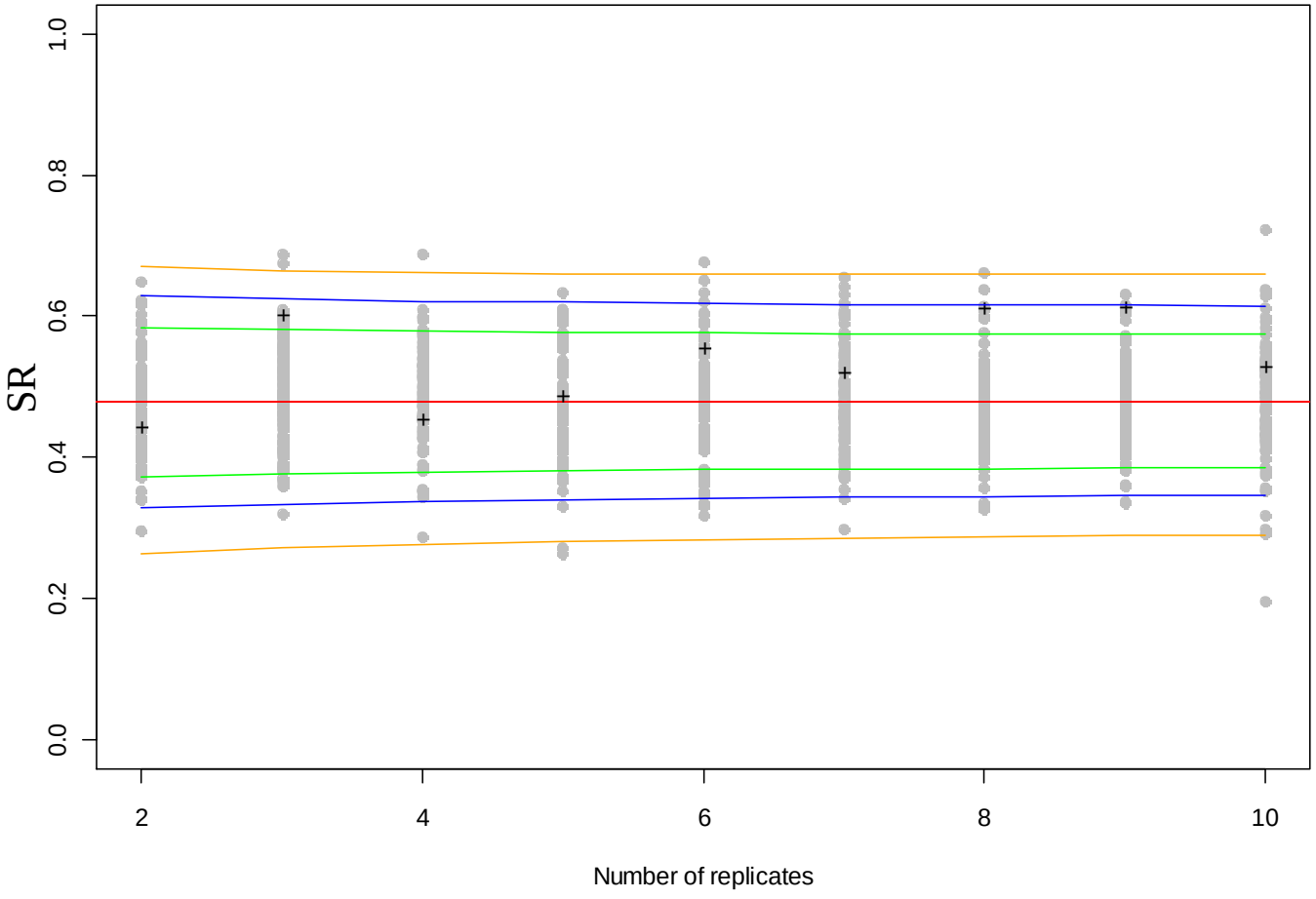
« True » Sr value

CM6 (EUCS 2010)
m = 13.97
Sr = 0.202
n = 2 to 10
p = **10**, **20** and **40**

$$p*(n-1) = 40*(3-1) = 20*(5-1) = 10*(9-1) = 80$$

The uncertainty of repeatability depends on the number of replicates *n* and the number of laboratories *p*

Theory: impact of *n* & *p* on SR



« *True* » S_R *value*

CM6 (EUCS 2010)
m = 13.97
SR= 0.479
n = 2 to 10
p = **10**, **20** and **40**

Theoretical interval of confidence of the standard deviation of repeatability

$$\ldots \leq s_R \leq \sqrt{\frac{\chi^2_{0.975;p-1}}{(p-1)}\left(\sigma_R{}^2 - \left(1-\frac{1}{n}\right)\sigma_r{}^2\right) + \left(1-\frac{1}{n}\right)\frac{\chi^2_{0.975;p(n-1)}}{p(n-1)} \times \sigma_r{}^2}$$

Here, the uncertainty of the reproducibility depends only on the number of laboratories

Intermediate conclusion

- Would an increase in the number of replicates reduce the repeatability?

No The repeatability depends only on the methodology and not the number of replicates.

But The number of replicates decreases the uncertainty of repeatability.

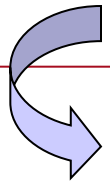
- Would an increase in the number of laboratories have an impact on repeatability and reproducibility?

No An increase in the number of laboratories has an impact on the uncertainties of repeatability and Reproducibility.

- Is it possible to compensate a low number of laboratories by a high number of replicates?

No The number of replicates have an impact on the uncertainty of repeatability but not on the uncertainty of reproducibility.

All these answers are right for the parameter NFDPM of the CM6 cigarette



Is it possible to generalize?

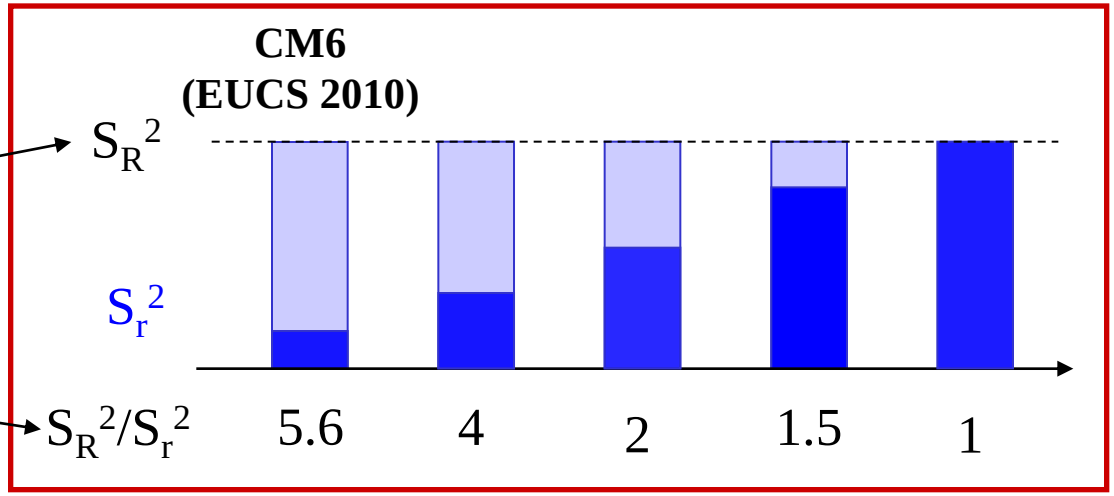
Generalization

We must consider other levels of repeatability and Reproducibility

Strategy:

- Fix S_R^2
- Then vary S_r^2 : $0<S_r^2<S_R^2$

To give different ratios



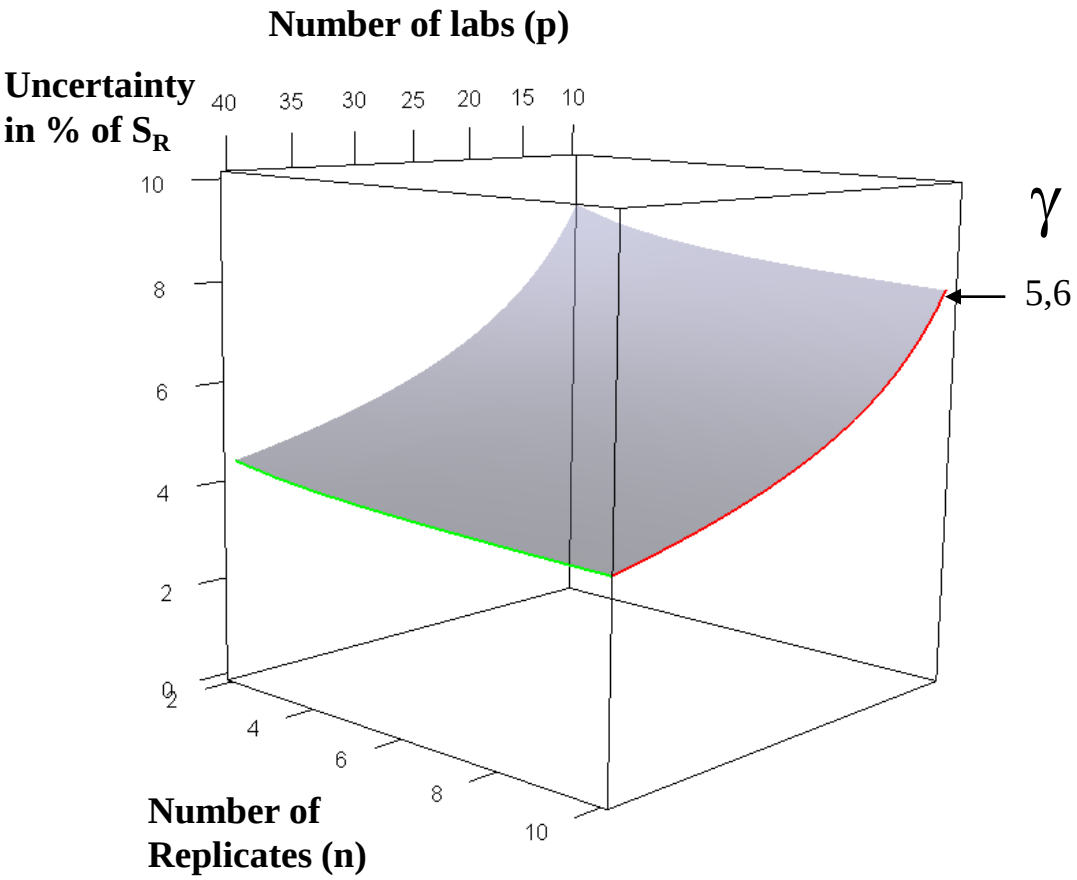
Formula:

$$\ldots \le s_R \le \sqrt{\frac{\chi^2_{0.975;p-1}}{(p-1)}\left(\sigma_R^{\,2}-\left(1-\frac{1}{n}\right)\sigma_r^{\,2}\right)+\left(1-\frac{1}{n}\right)\frac{\chi^2_{0.975;p(n-1)}}{p(\mathrm{n}-1)}\times\sigma_r^{\,2}}$$

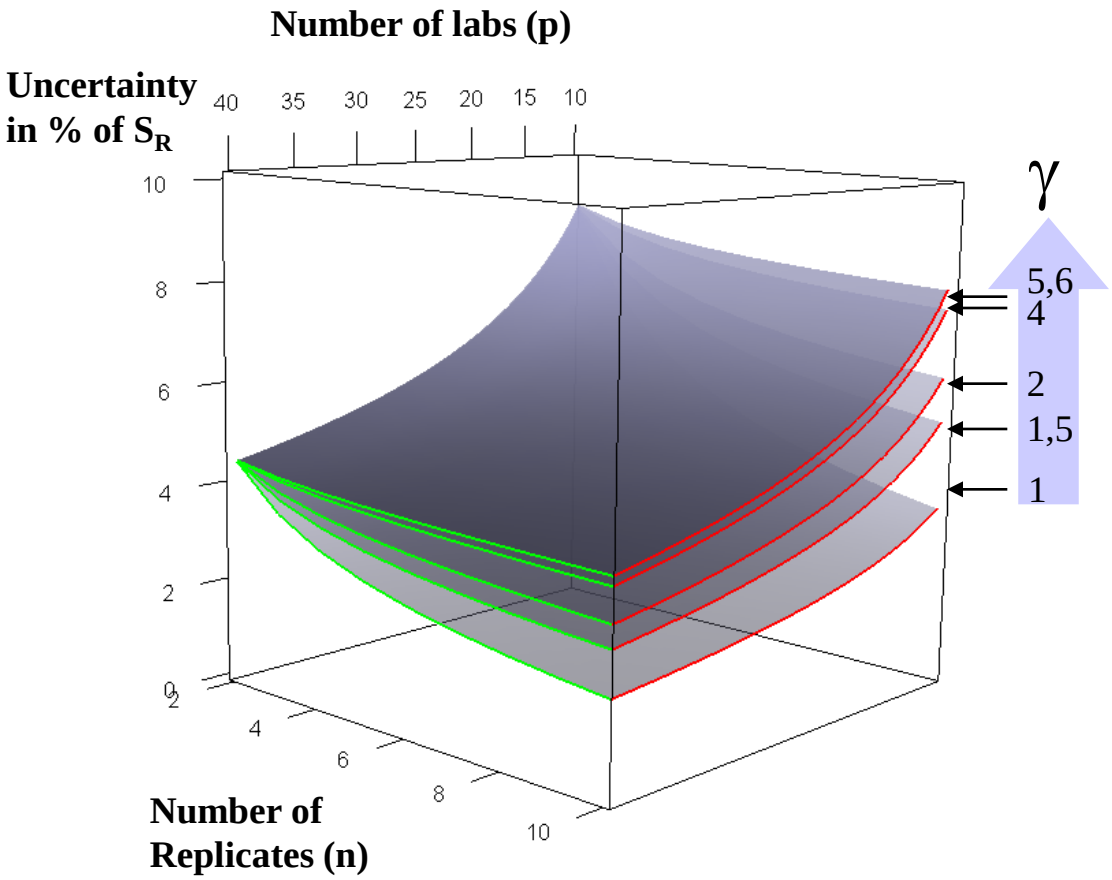
$$\gamma=\frac{\sigma_R^{\,2}}{\sigma_r^{\,2}}$$

$$\ldots \le s_R \le \frac{\sigma_R}{\sqrt{\gamma}}\sqrt{\frac{\chi^2_{0.975;p-1}}{(p-1)}\gamma+\left(1-\frac{1}{n}\right)\left[\frac{\chi^2_{0.975;p(n-1)}}{p(\mathrm{n}-1)}-\frac{\chi^2_{0.975;p-1}}{(p-1)}\right]}$$

Uncertainty of SR in function of γ, *p* and *n*



Uncertainty of SR in function of γ, *p* and *n*

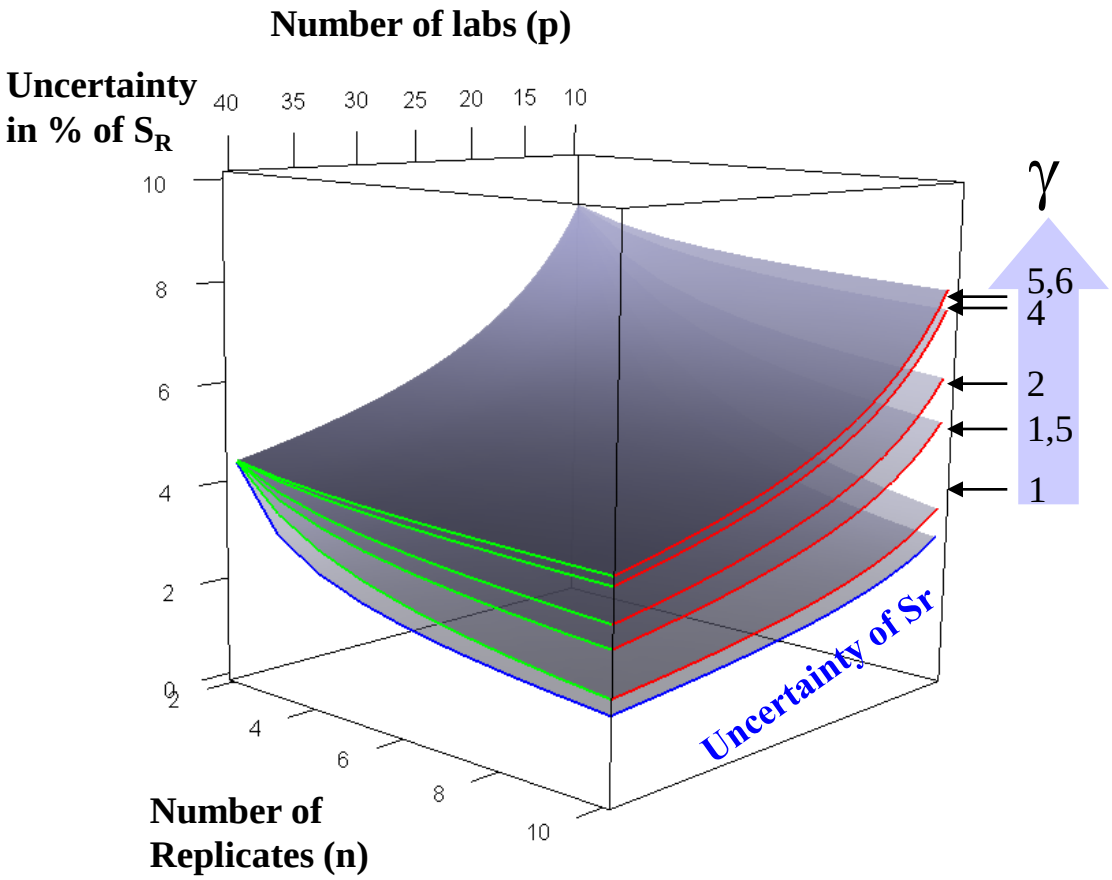


The number of replicates has small impact on the uncertainty

SR if γ is higher than 1,5

The number of laboratories has an impact on the uncertainty SR whatever γ

Uncertainty of SR in function of γ, *p* and *n*



The number of replicates has no impact on the uncertainty of SR if γ is higher than 1,5

The number of laboratories has an impact on the uncertainty of SR whatever γ

When γ is close to 1, the parameters p and n impact the uncertainty of SR to make it similar to the uncertainty of Sr.

Conclusion

- The number of replicates and the number of laboratories both impact the uncertainty of repeatability

- If the reproducibility is close to the repeatability (γ close to 1) then it is possible to change the combination of the number of replicates and the number of laboratories to achieve lower uncertainty of Reproducibility.

- However if the reproducibility is much higher than the repeatability (γ > 2) as is the case in collaborative studies on smoke components then an increase in the number of laboratories is the only way to achieve lower uncertainty on Reproducibility.

	EUCS (2008, 2009, 2010)				
Parameters	Nber of products	Nber of labs	yields	γ min	γ max
NFDPM	15	50 - 80	0.8 - 14	2.2	5.8
Nicotine	15	50 - 80	0.1 - 1.4	2.8	9.4
CO	15	50 - 80	1.1 - 14.6	2.3	6.0