66th Tobacco Science Research Conference
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COMPARISON OF ANALYTICAL DATA PROVIDED BY DIFFERENT LABORATORIES

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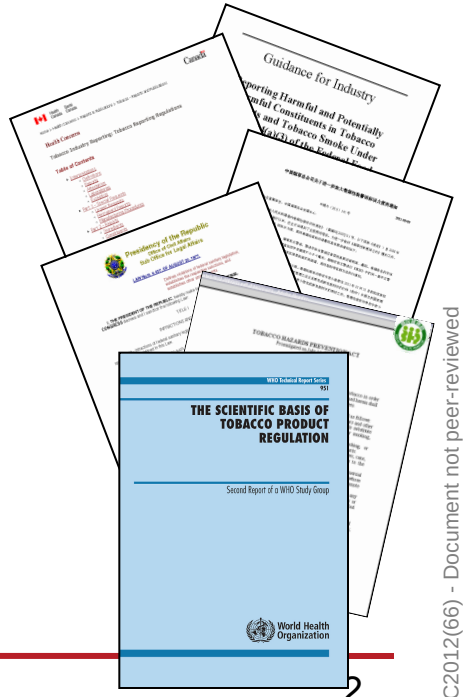
Context

- Manufacturers are increasingly being asked to report data on their products
- The objective of these requests is to use data for
  - Public communication
  - Product comparison
  - Introduction of smoke yield ceilings

- **FDA -** Testing of HPHCs
- **Health Canada -** Testing of 44 smoke emissions
- **Other Regulatory Authorities e.g. in Taiwan, Brazil, China, etc -**
- **WHO / TobReg** – Proposed ceilings on 9 smoke constituents

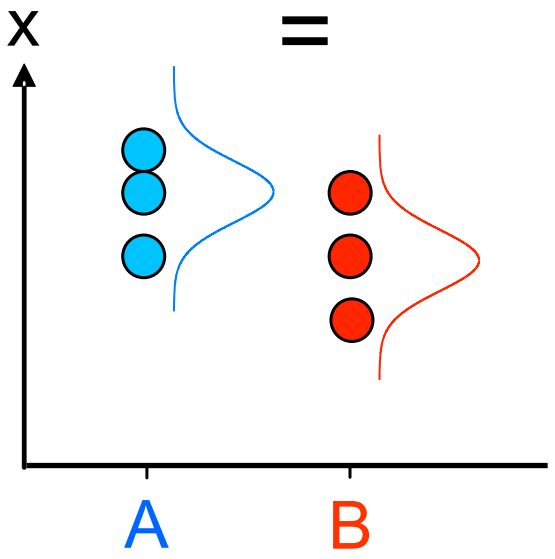


Issue

- Reporting data means
  - Different laboratories
    - Materials and apparatus
    - Operators
    - Laboratory supervision and quality system
  - At different times
  - With potentially different analytical methods

How to compare two sets of data?

Product comparison A and B; Parameter X



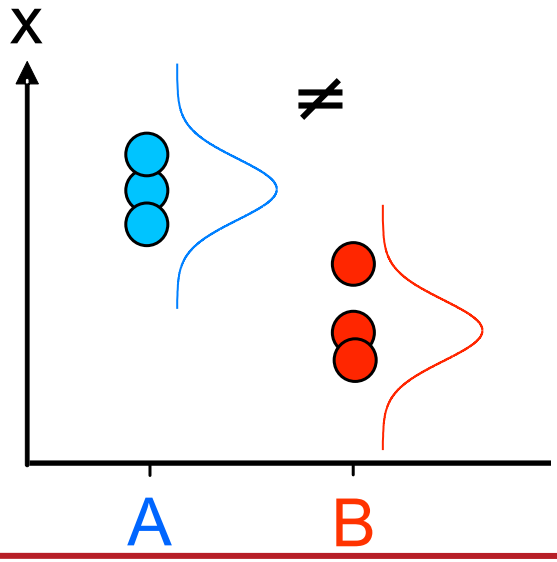
Comparison of means by Student's t-test

Hypothesis: $H : \overline{A} = \overline{B}$

Calculate: $t_{exp}\ (\overline{A}, S_A^2, \overline{B}, S_B^2)$ $\quad df = (n_A - n_B - 2)$

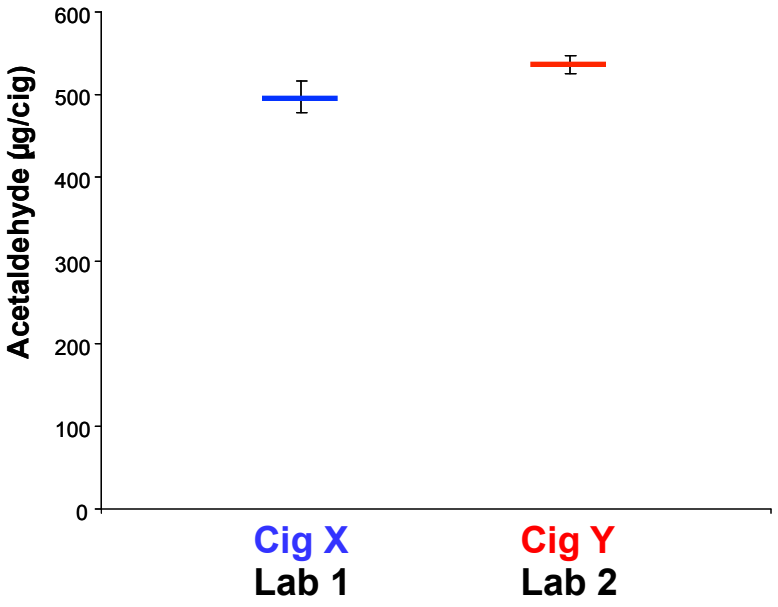
$t_{exp} < t_{th} \Rightarrow$ H accepted

Different days
or
Different labs



Acetaldehyde yields of 2 cigarettes from 2 labs

Cig X : Lab 1
Cig Y : Lab 2



5 replicates for each lab

Mean comparison

Using the Student's t-test the means for the two cigarettes are significantly different at $\alpha = 0.05$.

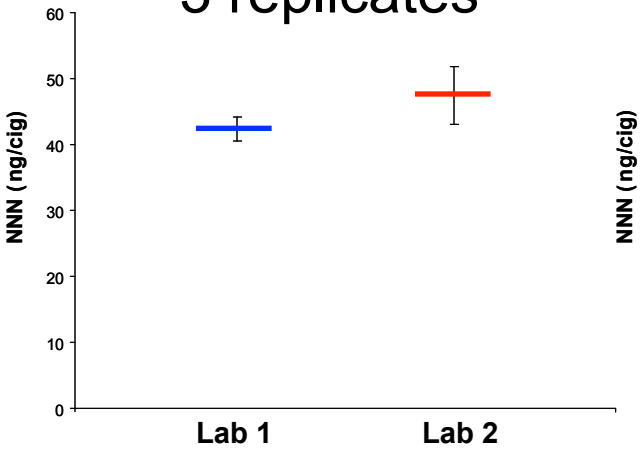
Cig X ≠ Cig Y

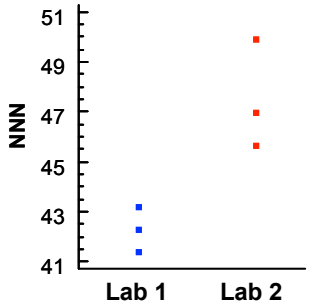
Cig X: 3R4F
Cig Y: 3R4F

*CORESTA Recommended Method 74 (2011)

NNN of Ky-1R5F from 2 labs

3 replicates

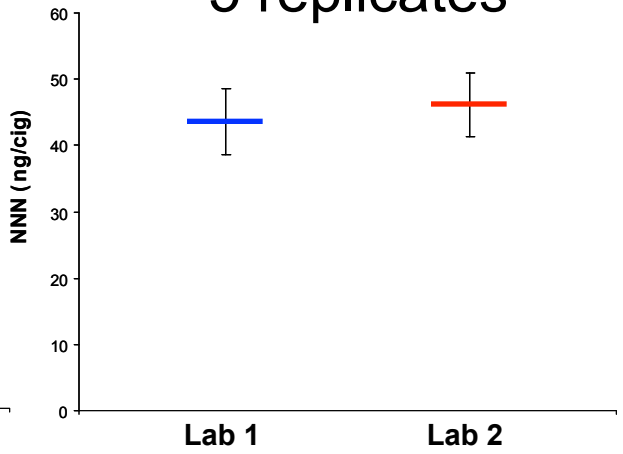


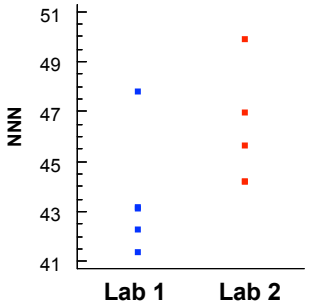


P = 0.0190

Lab 1 ≠ Lab 2

5 replicates

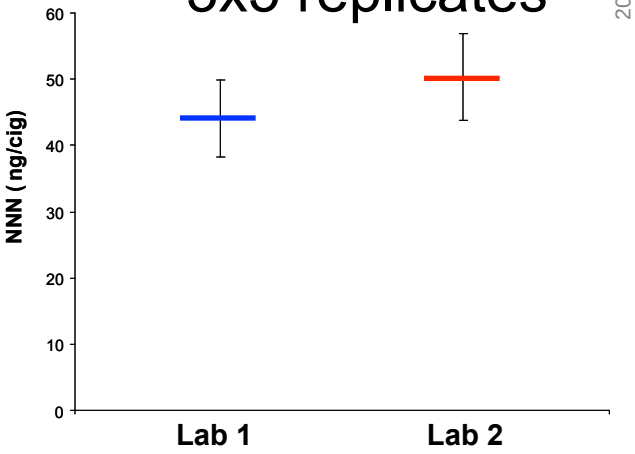


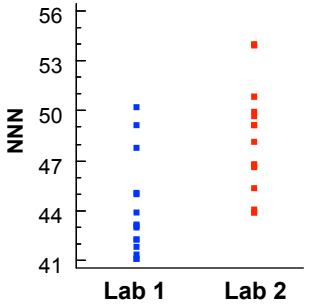


P = 0.1262

Lab 1 = Lab 2

3x5 replicates





P = 0.0002

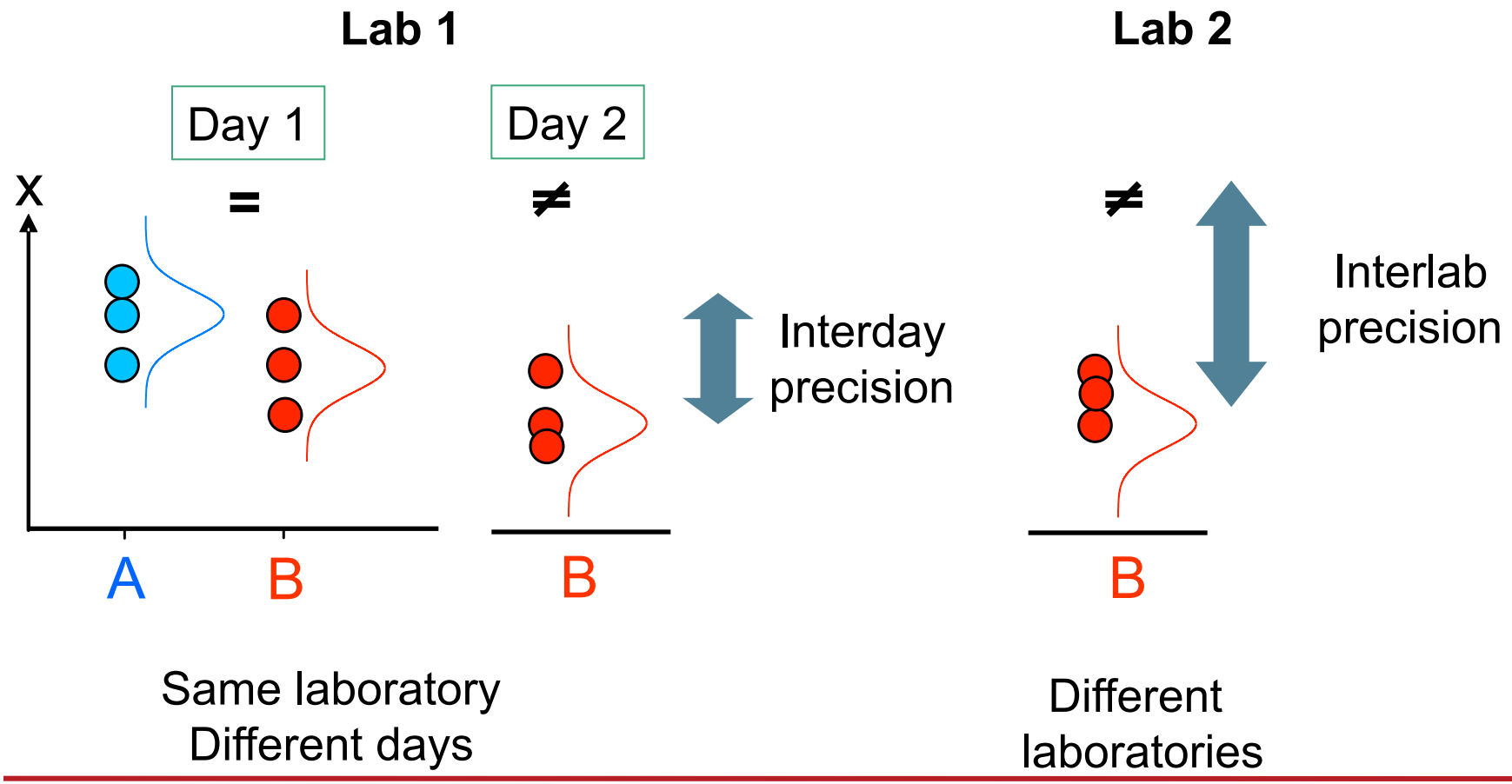
Lab 1 ≠ Lab 2

t-test is not appropriate to compare data coming from different labs

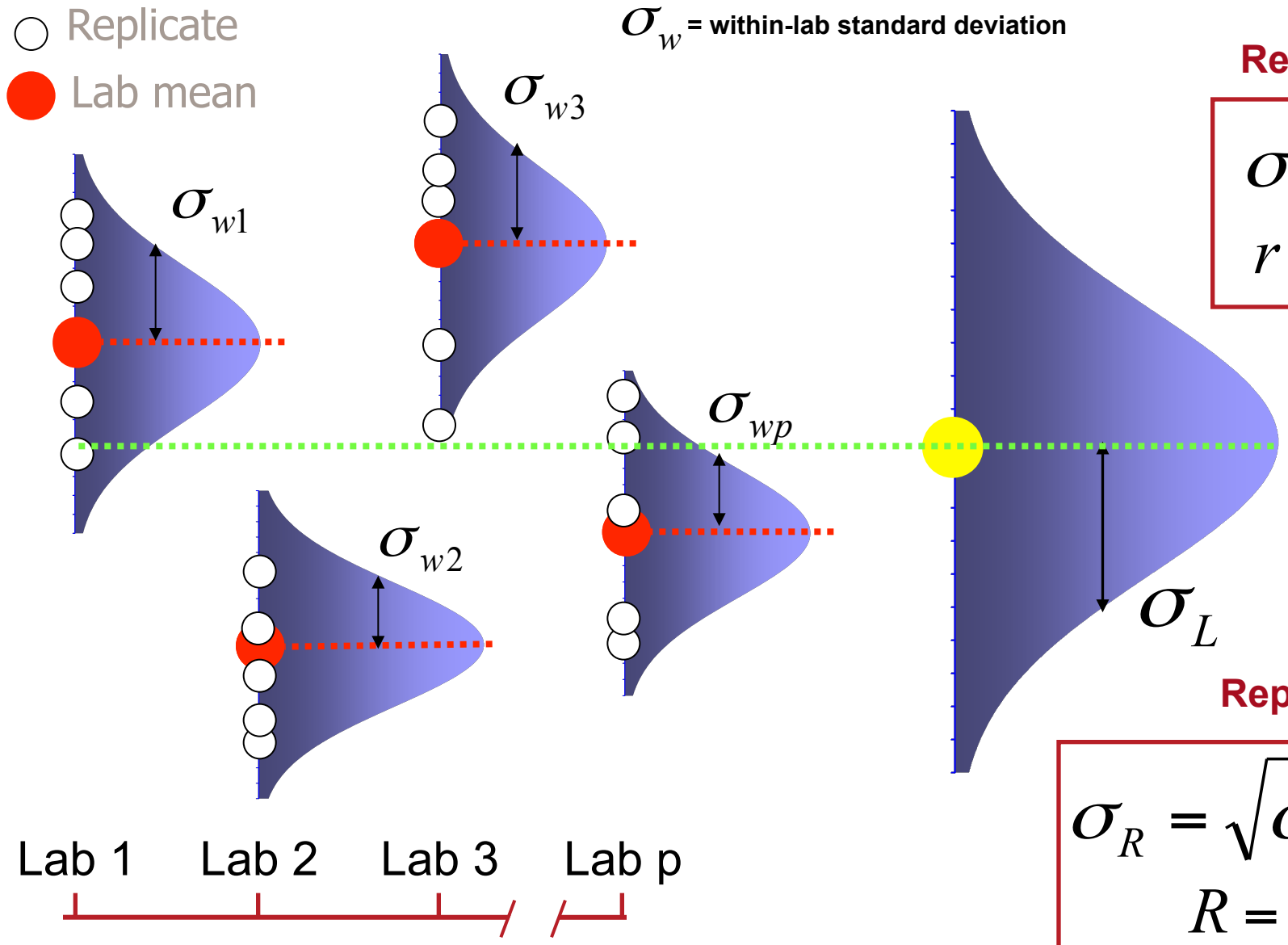
**CORESTA 2006 joint experiment data*

Product comparison A and B; Parameter X

it is important to take into account all the factors which affect variation



Intra- and Inter-laboratory variability



Repeatability

$$\sigma_r = \overline{\sigma_{wi}}$$

$$r = 2.8\sigma_r$$

Reproducibility

$$\sigma_R = \sqrt{\sigma_r^2 + \sigma_L^2}$$

$$R = 2.8\sigma_{R8}$$

Intra- and Inter-laboratories variability

Repeatability conditions stipulate that the test results are obtained by the same method on identical test items in the same laboratory by the same operator using the same apparatus within short periods of time.

Intermediate precision conditions allow for the varying of factors such as operators and apparatus over longer periods of time within a single laboratory

Reproducibility conditions stipulate that the same method is conducted on identical test items in different laboratories, which necessarily involves different operators and apparatus.

	Repeatability Conditions	Intermediate Precision Conditions	Reproducibility Conditions
Laboratory	**Same**	**Same**	**Different**
Operator	**Same**	**Different**	**Different**
Apparatus	**Same**	**Different**	**Different**
Time between Tests	**Short[a]**	**Multiple Days**	**Not Specified**

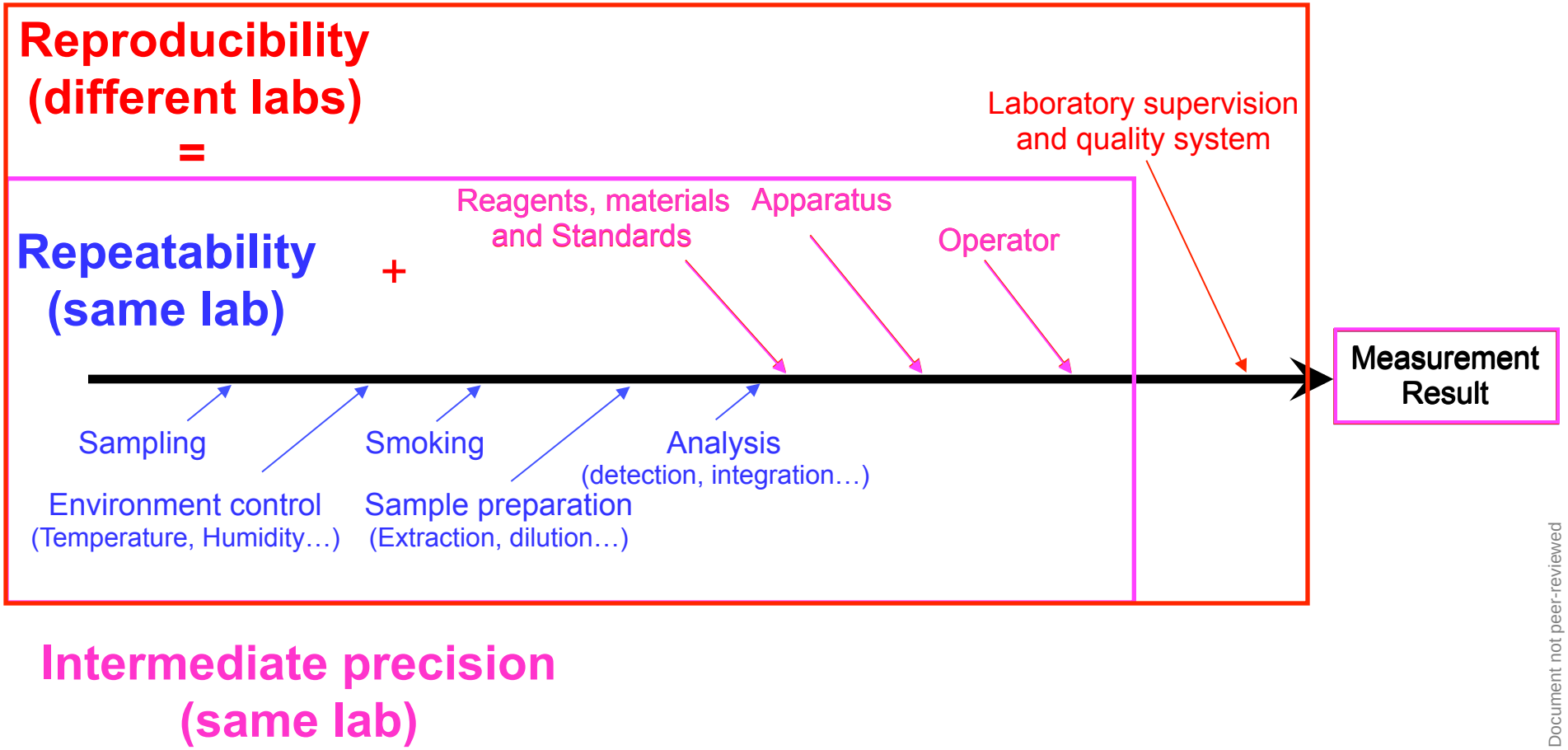
red: required

blue: optionnal

[a] *Standard test method dependent, typically does not exceed one day*

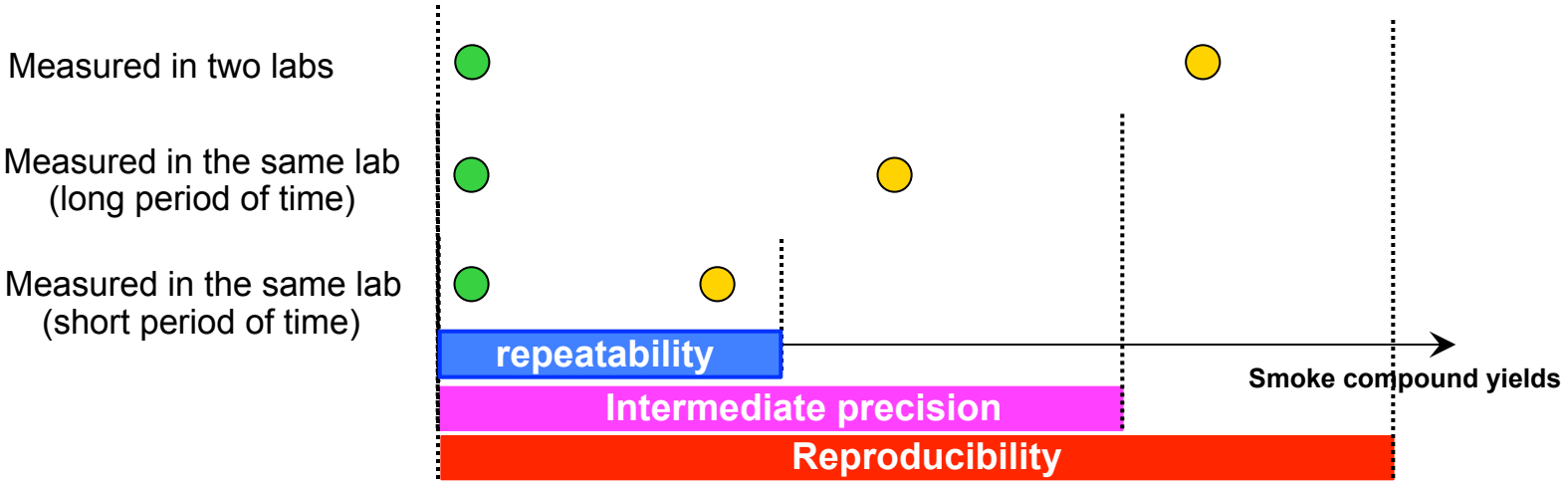
Intra- and Inter-laboratory variability

Factors Influencing Outcomes Include:



Intermediate precision (same lab)

Product comparison A and B; Parameter X



$$CD_r = 2.8\sigma_r \sqrt{\frac{1}{2n_A} + \frac{1}{2n_B}}$$

Critical Difference

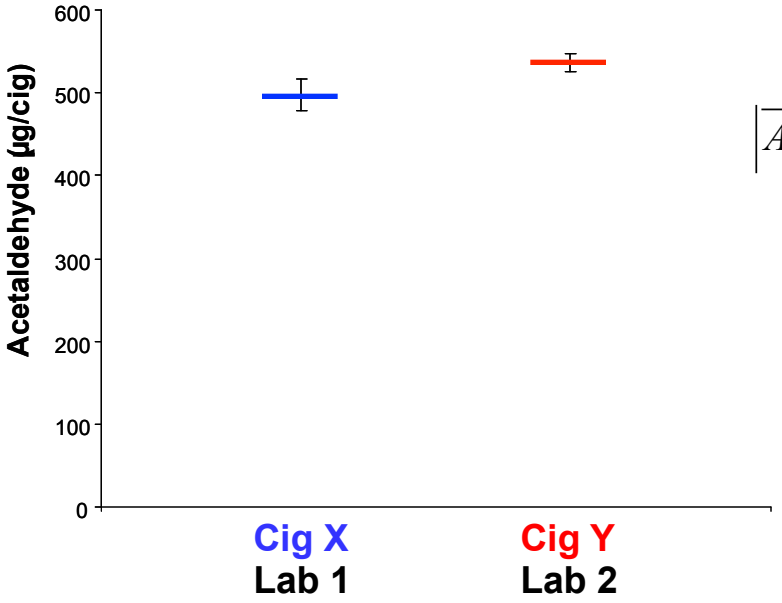
To determine whether or not data originating in the same lab or in two laboratories are in agreement, calculate the absolute difference between the two results and compare to the critical difference (CD):

$$CD_{IP} = \sqrt{(2.8\sigma_{IP})^2 - (2.8\sigma_r)^2\left(1 - \frac{1}{2n_A} - \frac{1}{2n_B}\right)}$$

$$CD_R = \sqrt{(2.8\sigma_R)^2 - (2.8\sigma_r)^2\left(1 - \frac{1}{2n_A} - \frac{1}{2n_B}\right)}$$

Acetaldehyde yields of 2 cigarettes from 2 labs

Cig X : Lab 1
Cig Y : Lab 2



5 replicates for each lab

Cig X: 3R4F
Cig Y: 3R4F

comparison

$\left|\overline{Acet}_{cigX} - \overline{Acet}_{cigY}\right|$ and $CD = \sqrt{(2.8\sigma_R)^2 - (2.8\sigma_r)^2\left(1 - \frac{1}{2n_A} - \frac{1}{2n_B}\right)}$

$$CD = \sqrt{R^2 - \frac{4}{5}r^2}$$

$\left|\overline{Acet}_{cigX} - \overline{Acet}_{cigY}\right| = 39.2\ \mu g / cig$ < CD=137.5 µg/cig

There is no significant difference between the two products

**CORESTA Recommended Method 74 (2011)*

Intra-lab comparison:

Cig A: US blended style cigarette (tar: **10** mg/cig ISO) – French market

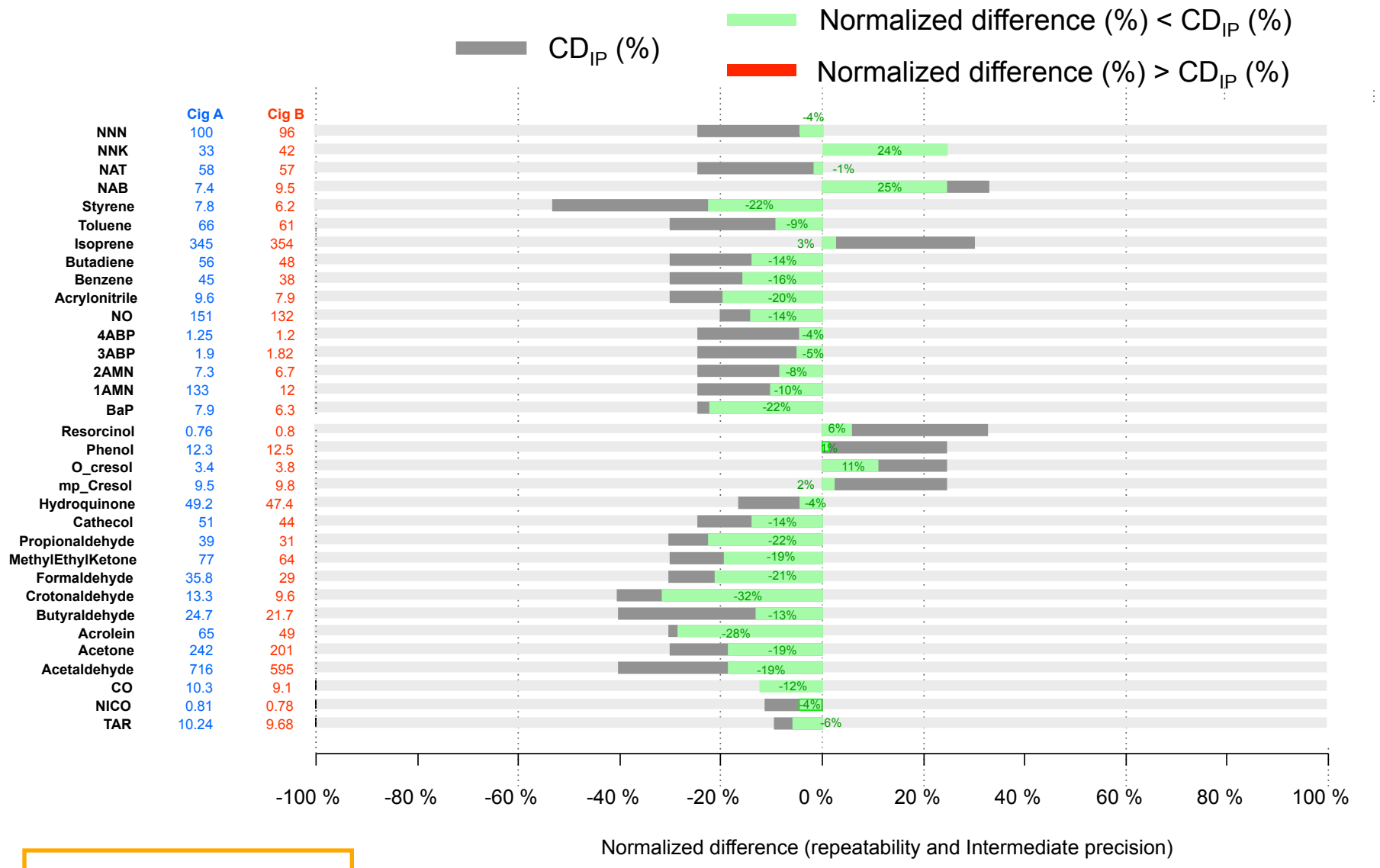
Cig B: US blended style cigarette (tar: **10** mg/cig ISO) – French market

Same laboratory =

- ✓ CD based on Repeatability and intermediate precision
  - •Long period of time
- ✓ Validated methods

Yields comparison of 33 smoke compounds generated under ISO smoking regime

Intra-lab comparison: 2 cigarettes



Cig A = Cig B

Intra-lab comparison:

Cig A: US blended style cigarette (tar: **10** mg/cig ISO) – French market

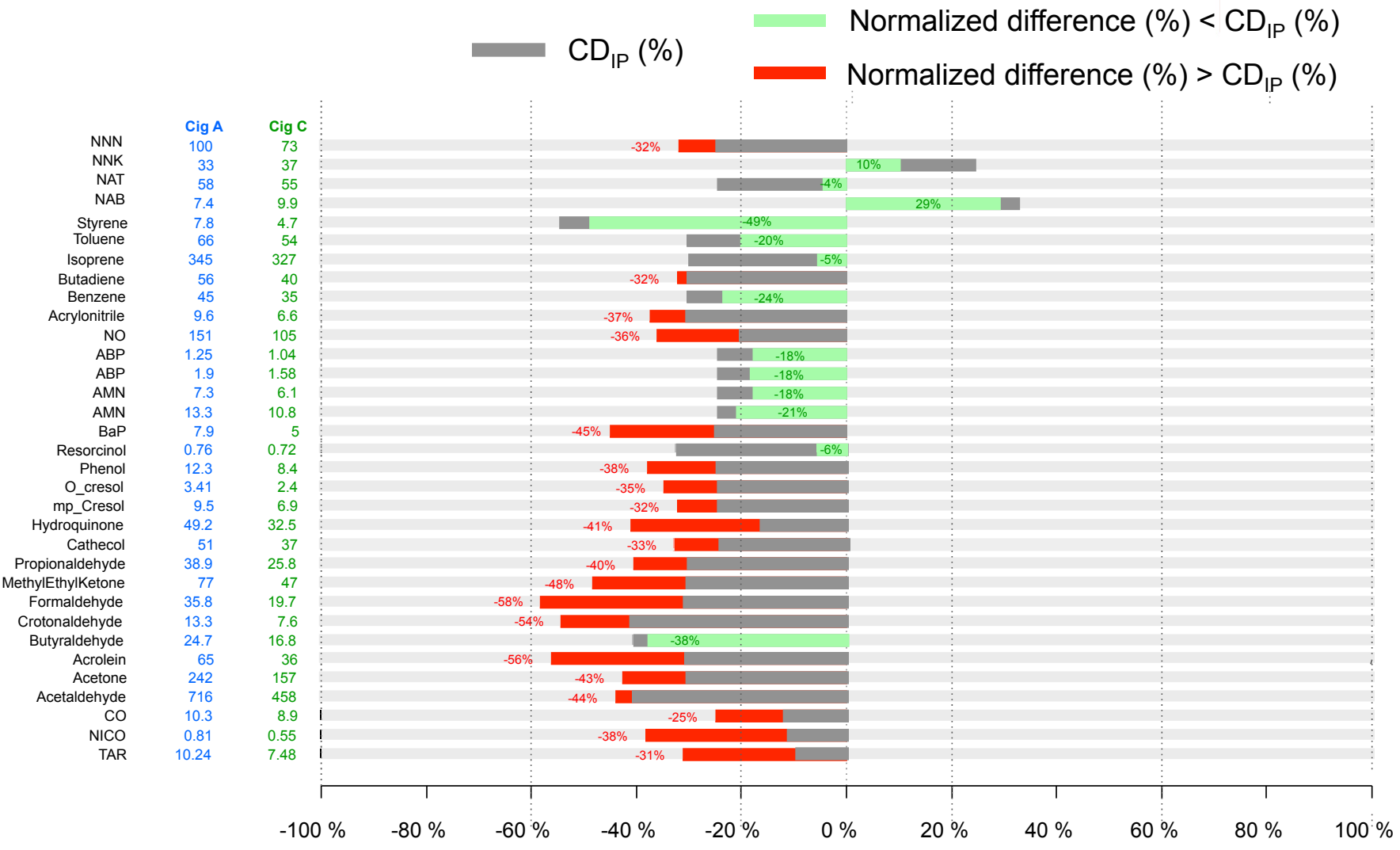
Cig C: US blended style cigarette (tar: **8** mg/cig ISO) – French market

Same laboratory =

- ✓ CD based on Repeatability and intermediate precision
  - •Long period of time
- ✓ Validated methods

Yields comparison of 33 smoke compounds generated under ISO smoking regime

Intra-lab comparison: 2 cigarettes



Cig A ≠ Cig C

Inter-lab comparison:

Cig A: US blended style cigarette (tar: **10** mg/cig ISO) – French market

- Laboratory 1
- Laboratory 2

Different laboratories =

- ✓ CD based on Repeatability and Reproducibility
- ✓ Standardized methods

NIC: ISO 10315 :2000
TAR: ISO 4387 :2000
CO: ISO 8454 :2007

B(a)P: ISO 22634:2008

Formaldehyde
Acetaldehyde
Acetone
Acrolein
Propionaldehyde
Crotonaldehyde
Methyl EthylKetone
Butyraldehyde

→ CRM 74 (2011)

Benzene
Toluene
1,3-butadiene
Isoprene
Acrylonitrile

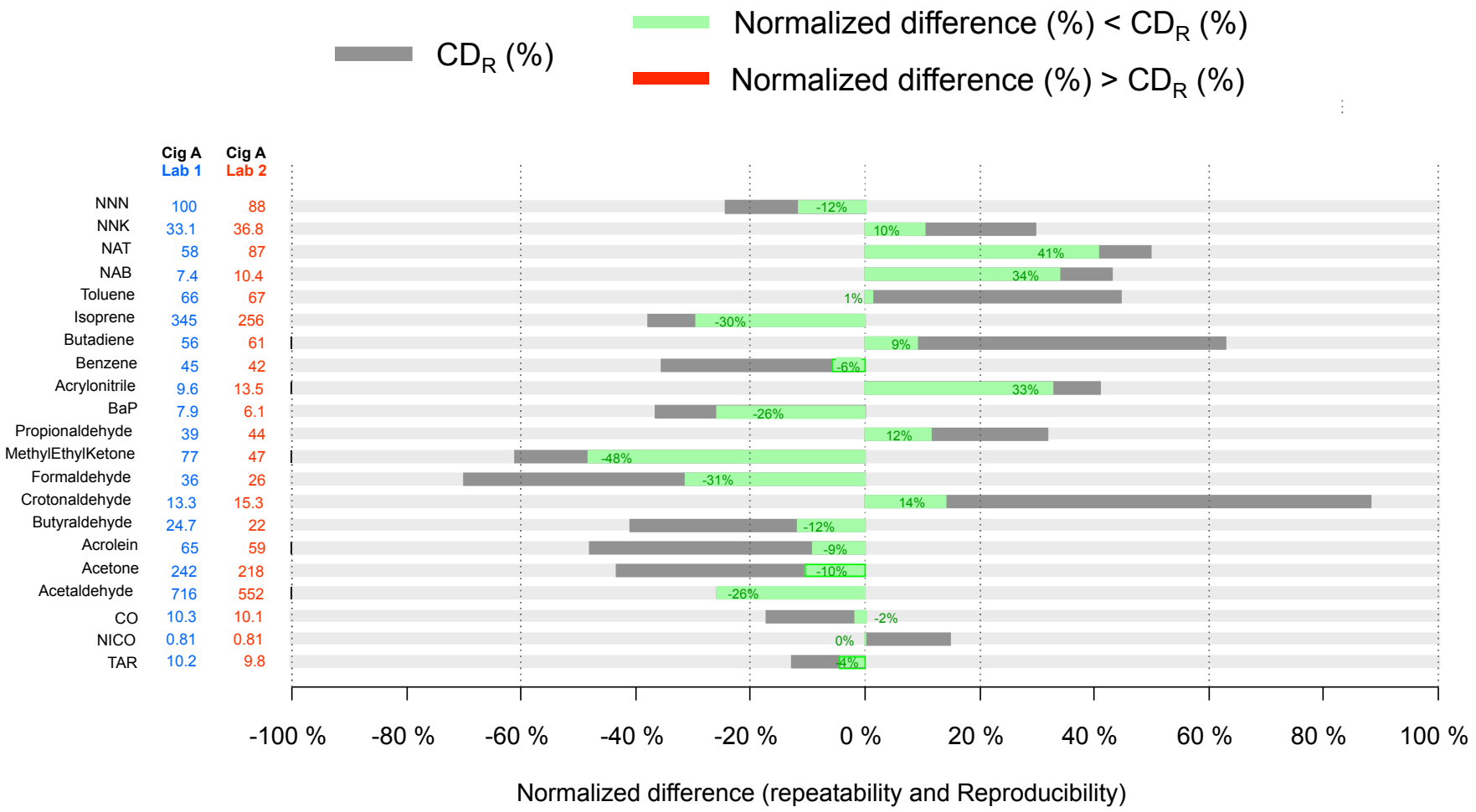
→ CRM 70 (2010)

NNN
NNK
NAT
NAB

→ CRM 75 (2012)

Yields comparison of 21 smoke compounds generated under ISO smoking regime

Inter-lab comparison: 2 cigarettes



Cig A (Lab 1) = Cig A (Lab 2)

Inter-lab comparison:

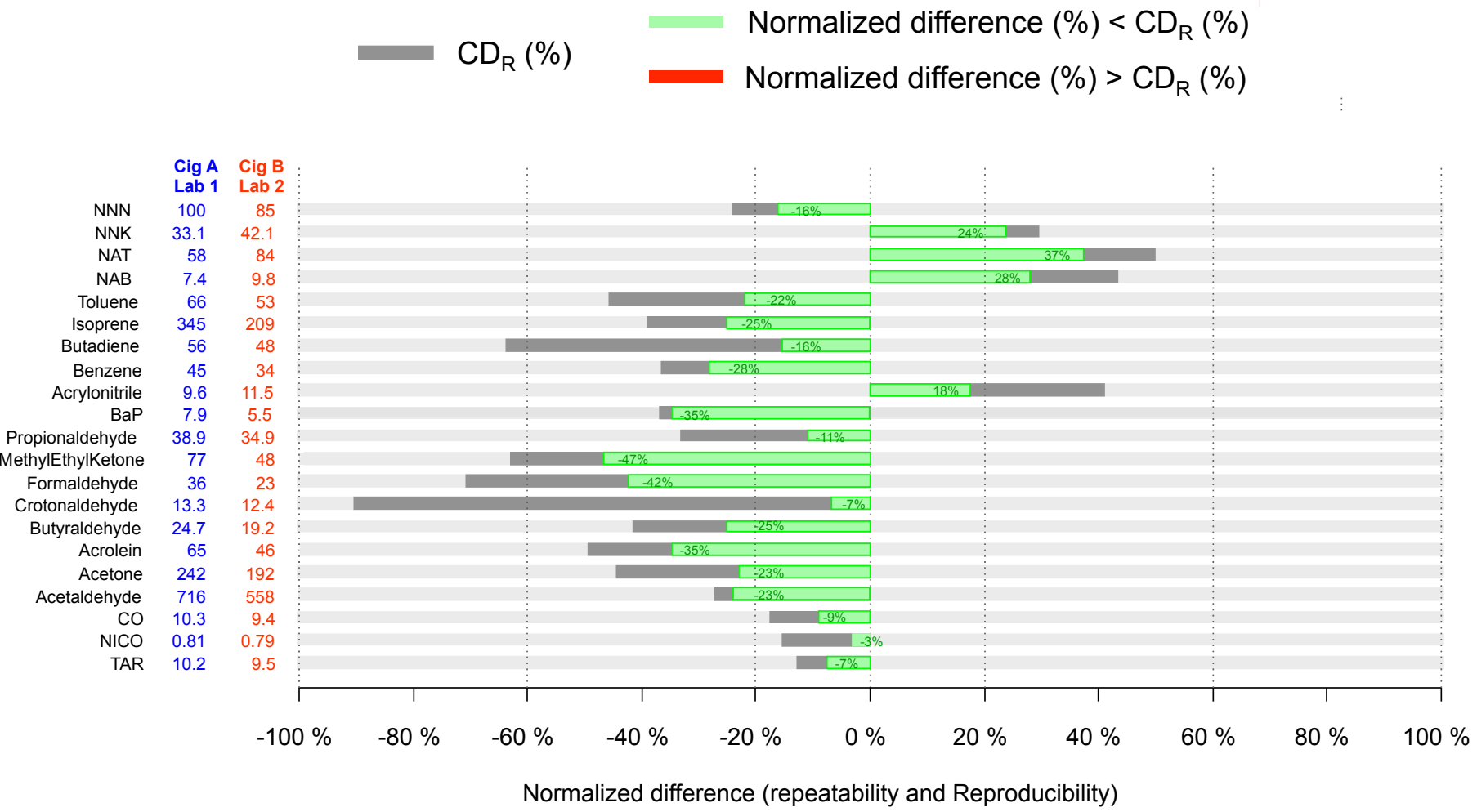
Cig A: US blended style cigarette (tar: **10** mg/cig ISO) – French market
- ☞ LAB 1

Cig B: US blended style cigarette (tar: **10** mg/cig ISO) – French market
- ☞ LAB 2

Different laboratories =
- ✓ CD based on Repeatability and Reproducibility
- ✓ Standardized methods

Yields comparison of 21 smoke compounds generated under ISO smoking regime

Inter-lab comparison: 2 cigarettes



Cig A (Lab 1) = Cig B (Lab 2)

Inter-lab comparison:

Cig A: US blended style cigarette (tar: **10** mg/cig ISO) – French market
☞ LAB 1

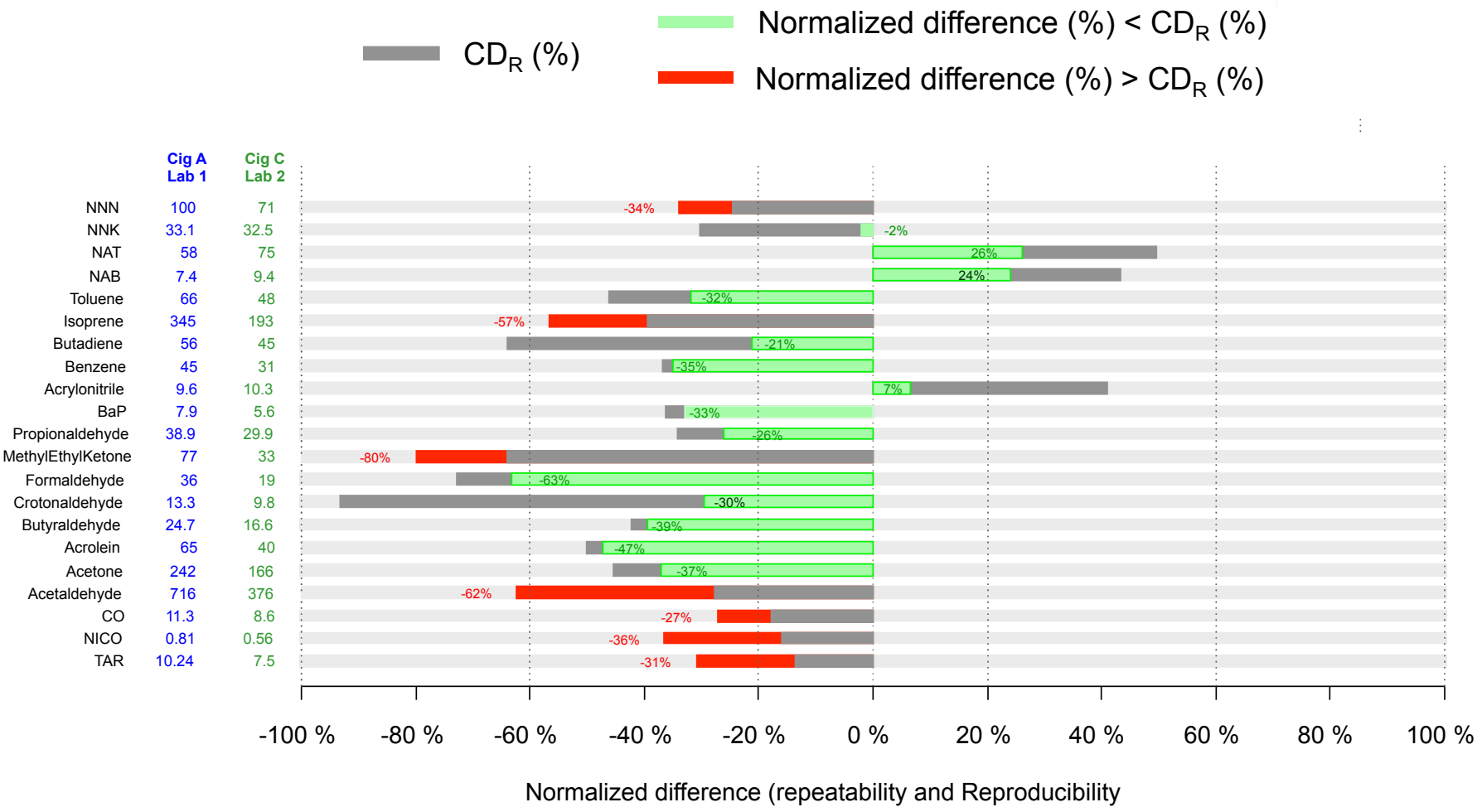
Cig C: US blended style cigarette (tar: **8** mg/cig ISO) – French market
☞ LAB 2

Different laboratories =

- ✓ CD based on Repeatability and Reproducibility
- ✓ Standardized methods

Yields comparison of 21 smoke compounds generated under ISO smoking regime

Inter-lab comparison: 2 cigarettes



Cig A (Lab 1) ≠ Cig C (Lab 2)

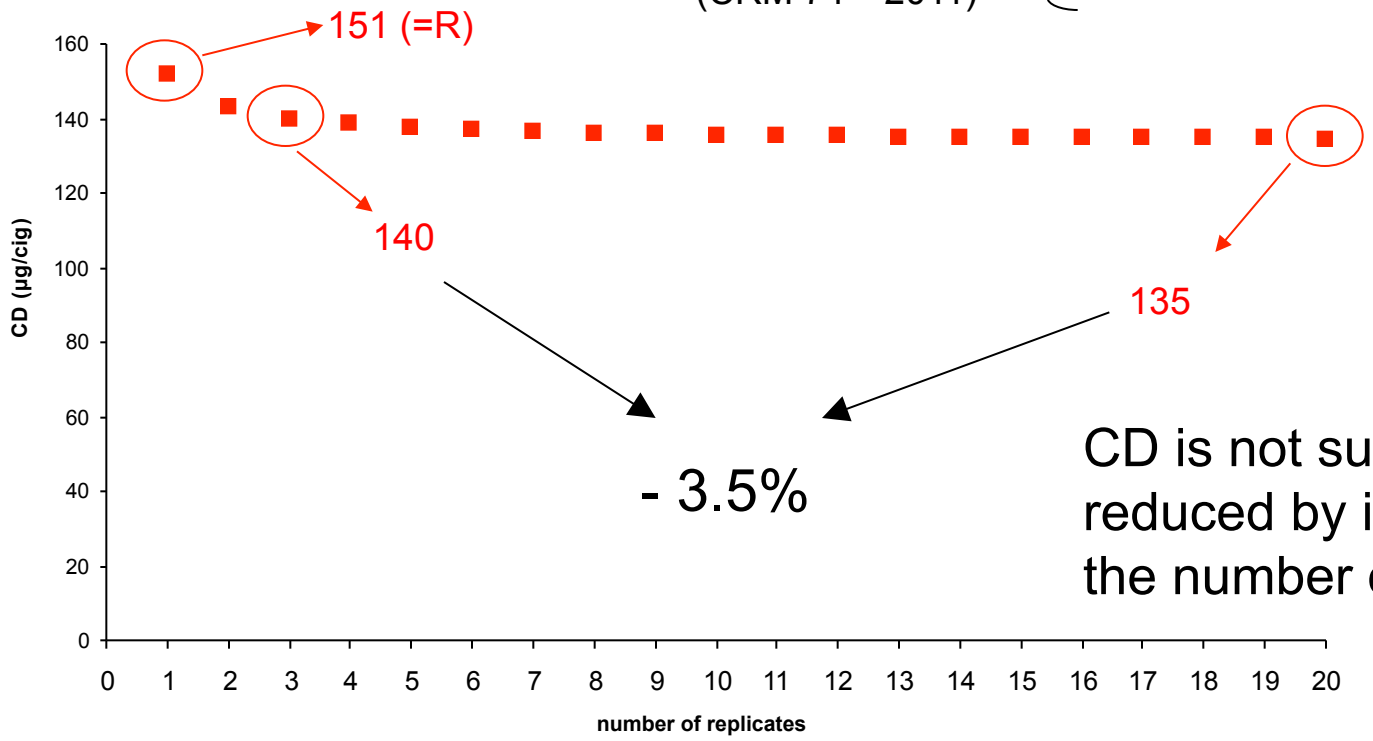
Impact of the number of replicates on CD

$$CD = \sqrt{R^2 - r^2\left(1 - \frac{1}{2n_A} - \frac{1}{2n_B}\right)} \xrightarrow{n_A = n_B} CD = \sqrt{R^2 - r^2\left(\frac{n-1}{n}\right)}$$

Acetaldehyde
Mean 517 µg/cig
(CRM 74 – 2011)

R= 151 µg/cig
r = 73 µg/cig

$\gamma_{R/r} = 2.1$



CD is not substantially reduced by increasing the number of replicates

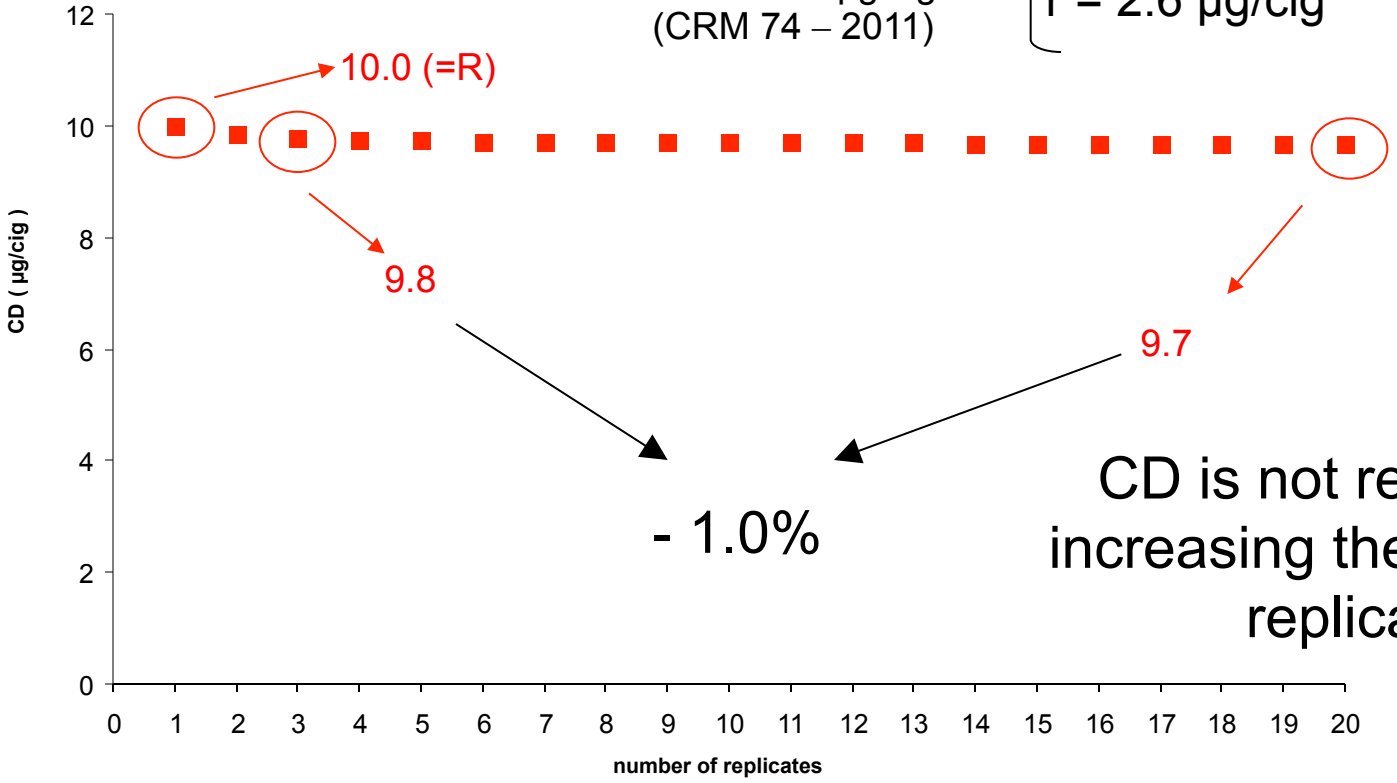
Impact of the number of replicates on CD

$$CD = \sqrt{R^2 - r^2\left(1 - \frac{1}{2n_A} - \frac{1}{2n_B}\right)} \xrightarrow{n_A = n_B} CD = \sqrt{R^2 - r^2\left(\frac{n-1}{n}\right)}$$

Crotonaldehyde
Mean 10.3 µg/cig
(CRM 74 – 2011)

R= 10.0 µg/cig
r = 2.6 µg/cig

$\gamma_{R/r} = 3.8$



CD is not reduced by increasing the number of replicates

Conclusions

- Using the t-test for product comparison can give wrong **conclusions***
- In the context of reporting data for regulatory purposes, **it is fundamental to consider the variability in its full definition (accuracy)** to avoid misleading information.
- Critical difference based on method reproducibility, as defined in the ISO 5725, is the best way to compare data from different laboratories
- Number of replicates does not have an impact on the critical difference

*Poster 11: **Limitation of standard deviation to express variability**
Teillet B, Verron T, Cahours X, Colard S, Purkis S.

COMPARISON OF ANALYTICAL DATA PROVIDED BY DIFFERENT LABORATORIES

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THANK YOU !