Assessing repeatability and reproducibility of dose-response experiments

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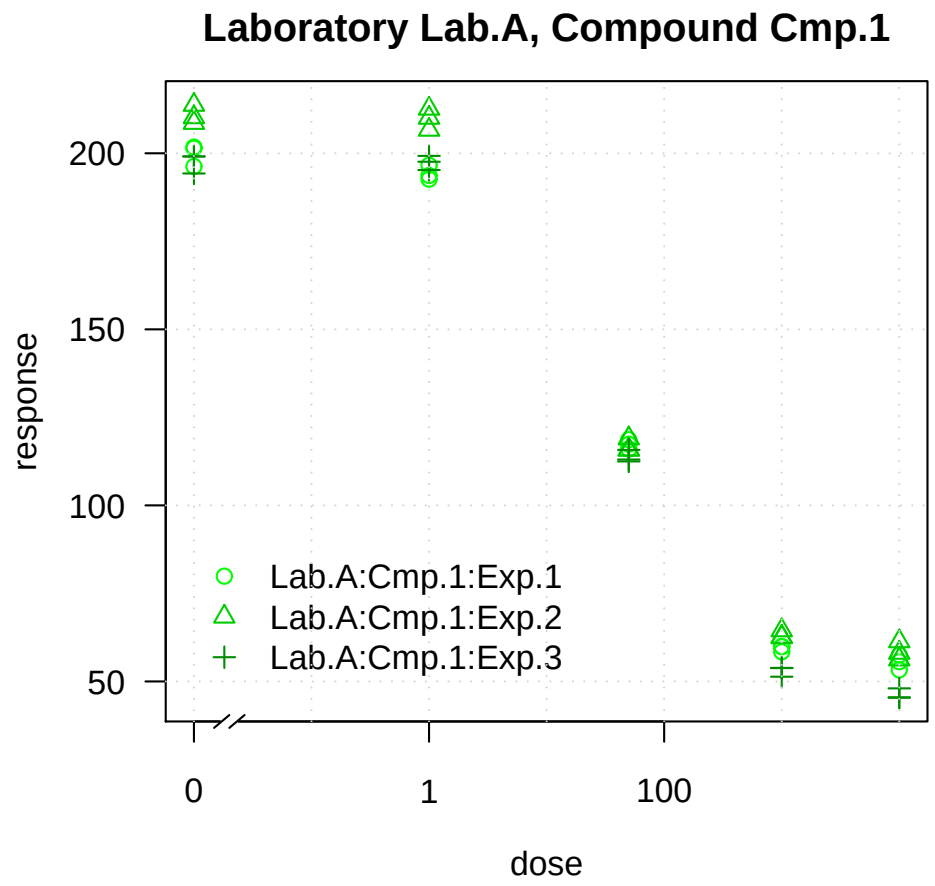
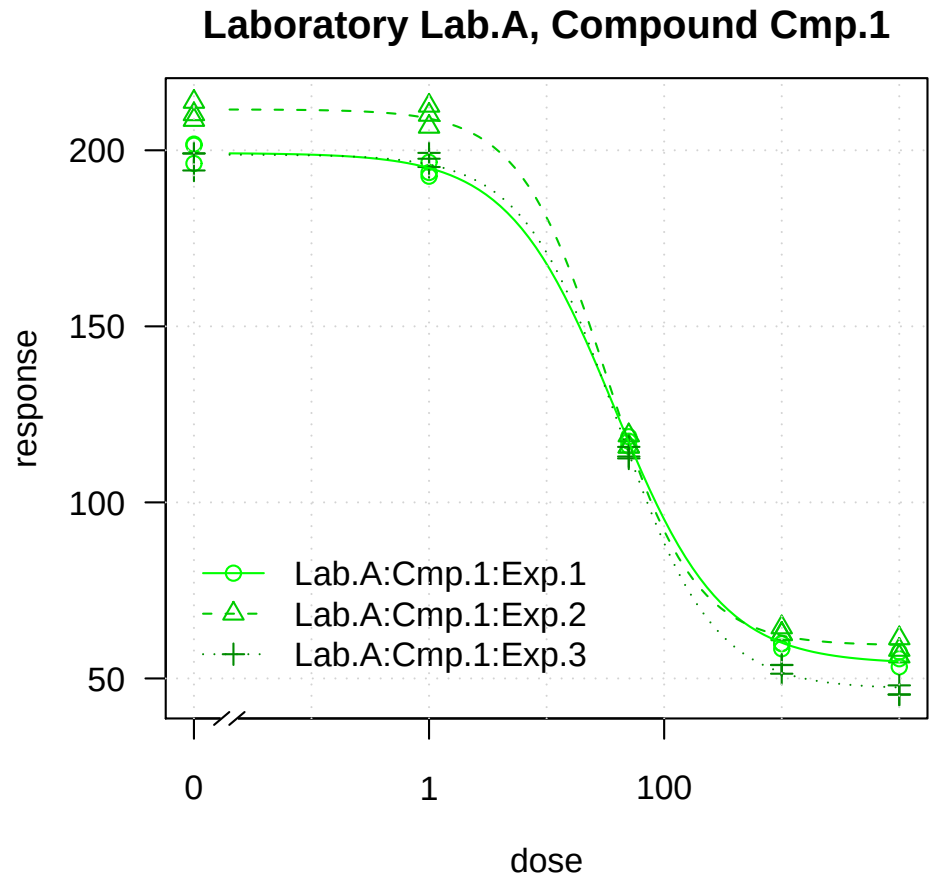
Dose response data



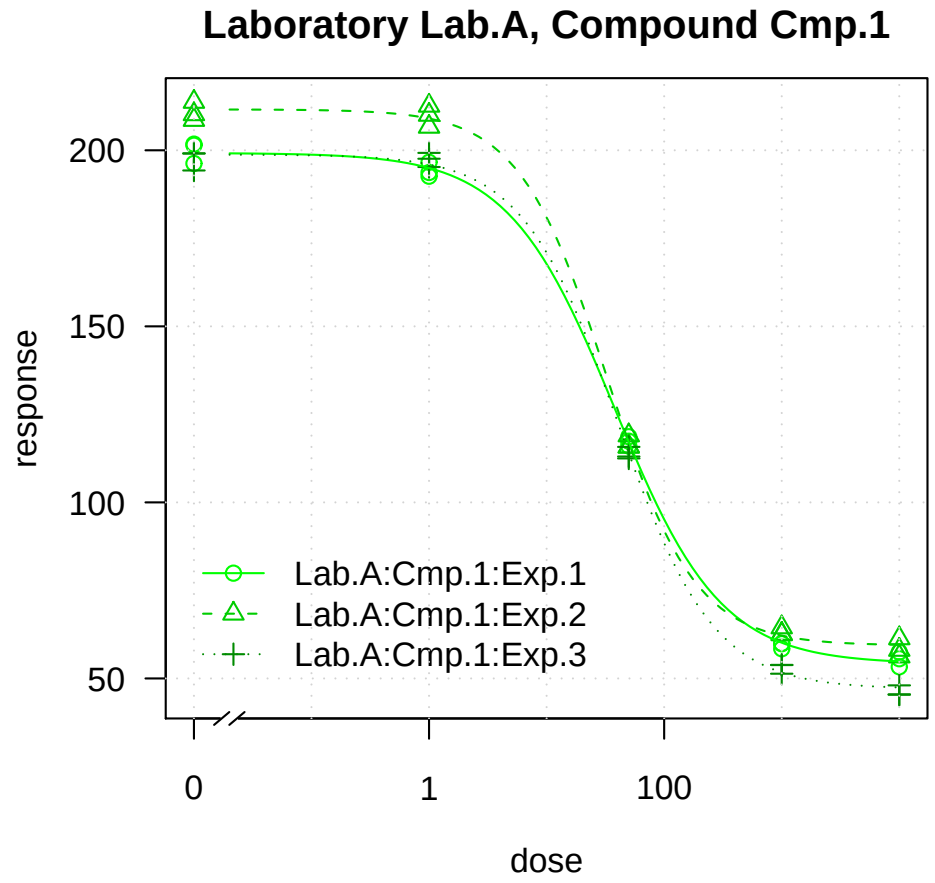
- Relationship between dose of a substance and biological response
- Quantitative toxicology: ED_{50} values as predictors for toxicity

ED_{50} estimation



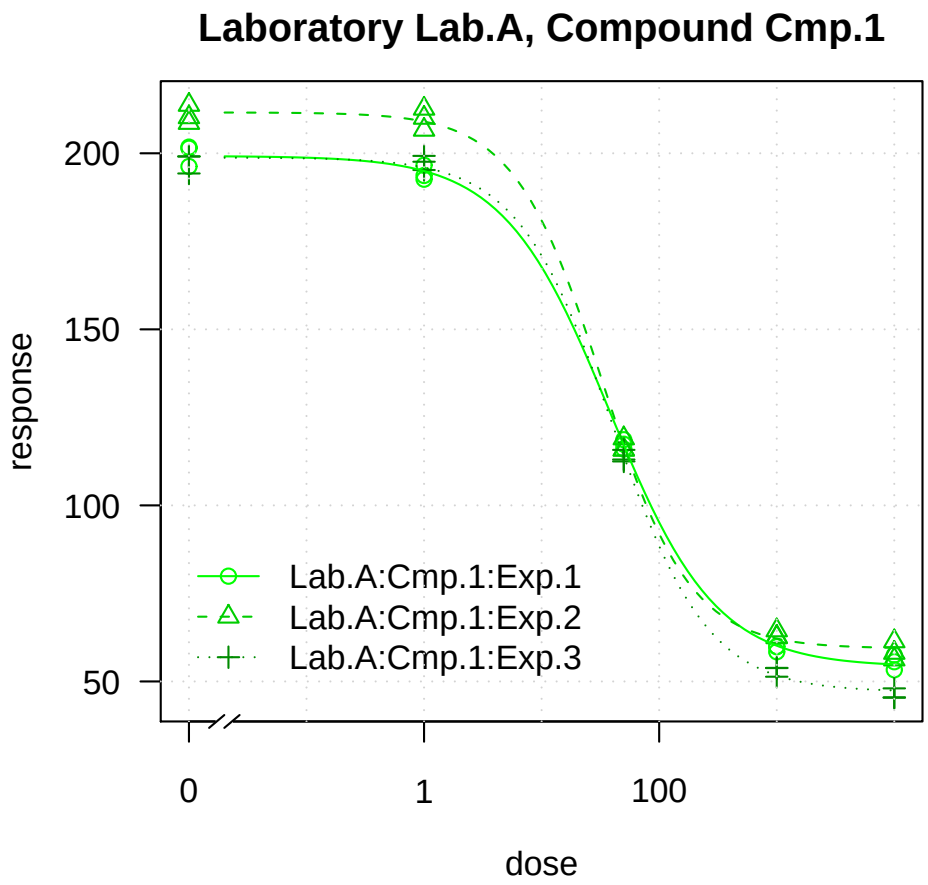
- Fit model $f(x) = c + \dfrac{d - c}{1 + \exp(b(\log(x) - e))}$
- Parameter $e \equiv \log(ED_{50})$

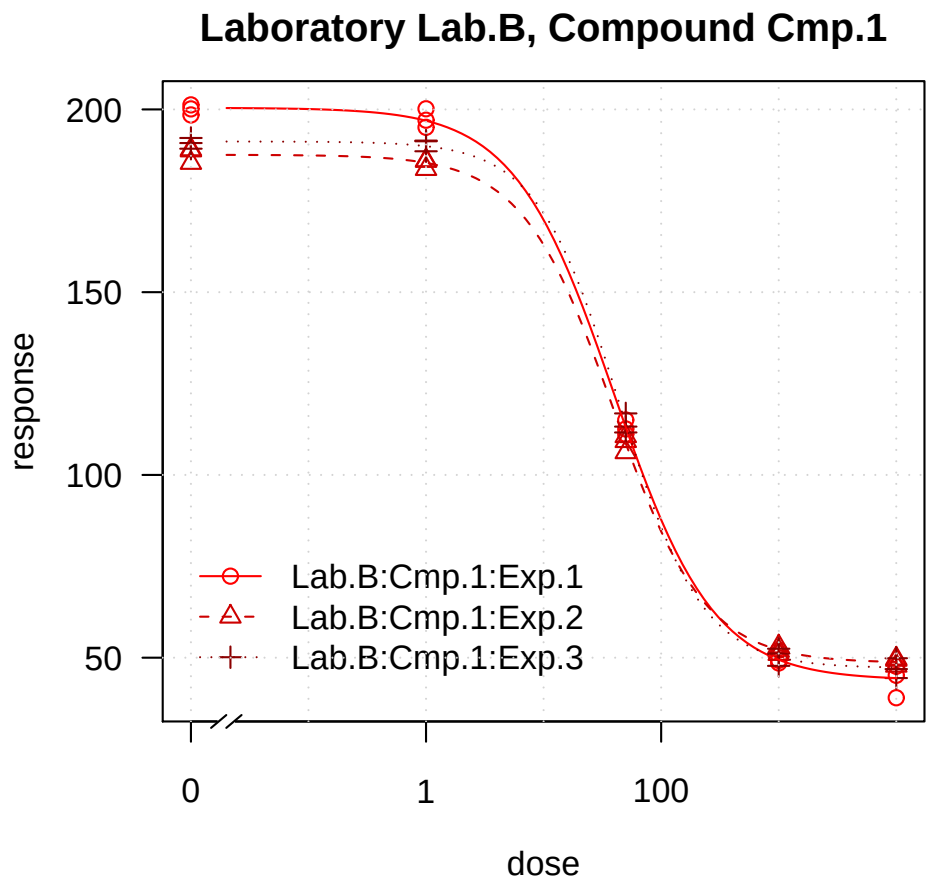
ED_{50} estimates



	Estimate	Lower	Upper
Lab.A:Cmp.1:Exp.1	37.97	34.29	42.05
Lab.A:Cmp.1:Exp.2	32.68	29.01	36.81
Lab.A:Cmp.1:Exp.3	40.14	36.93	43.62

Different laboratories





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Lab.A:Cmp.1:Exp.1	37.97	34.29	42.05
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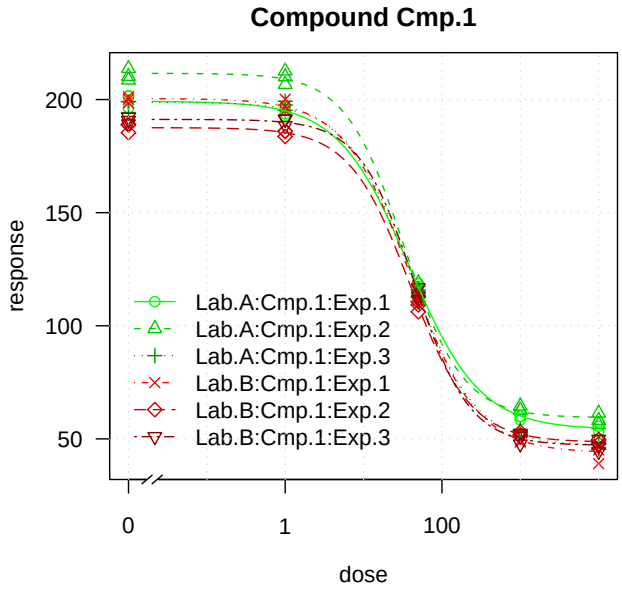
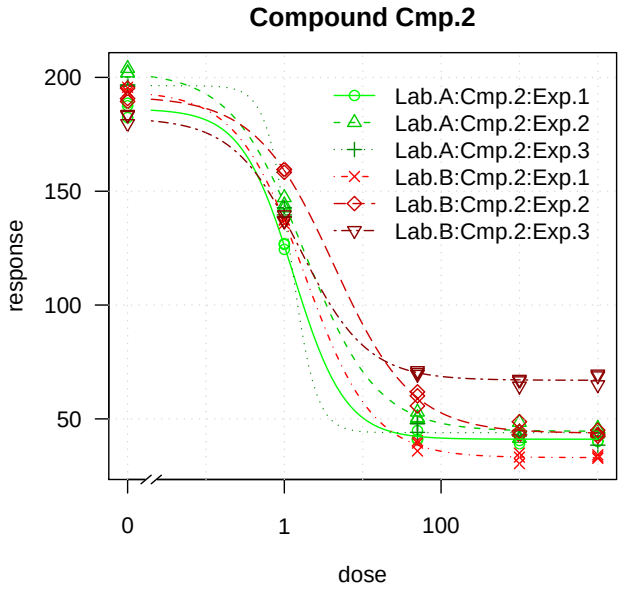
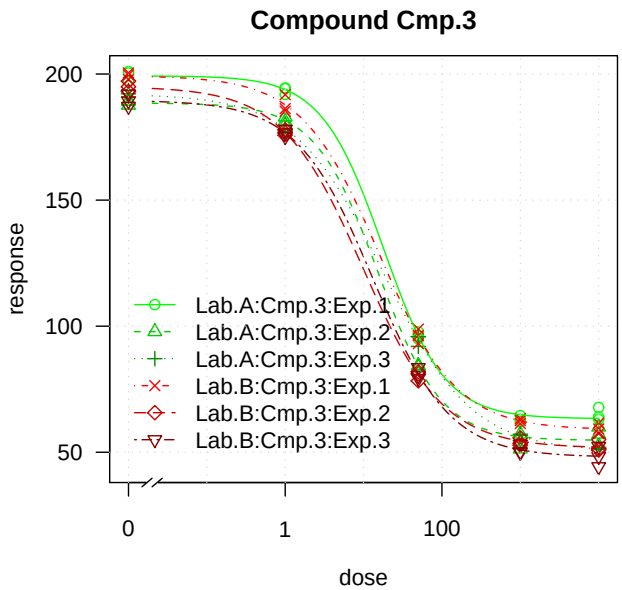
Lab.A:Cmp.1:Exp.3	40.14	36.93	43.62

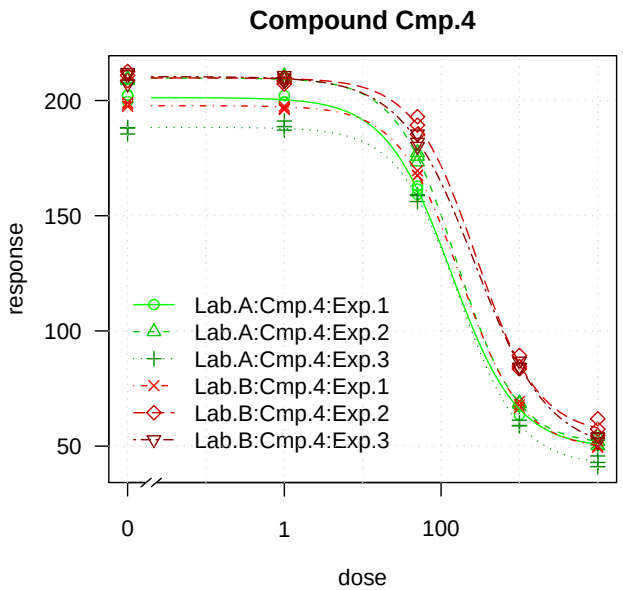
	Estimate	Lower	Upper
Lab.B:Cmp.1:Exp.1	39.77	36.68	43.12
Lab.B:Cmp.1:Exp.2	39.23	35.74	43.05
Lab.B:Cmp.1:Exp.3	44.43	41.44	47.63

Different labs and different compounds

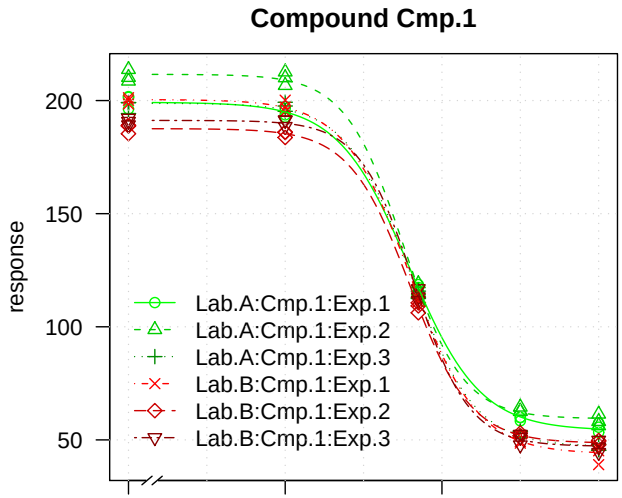


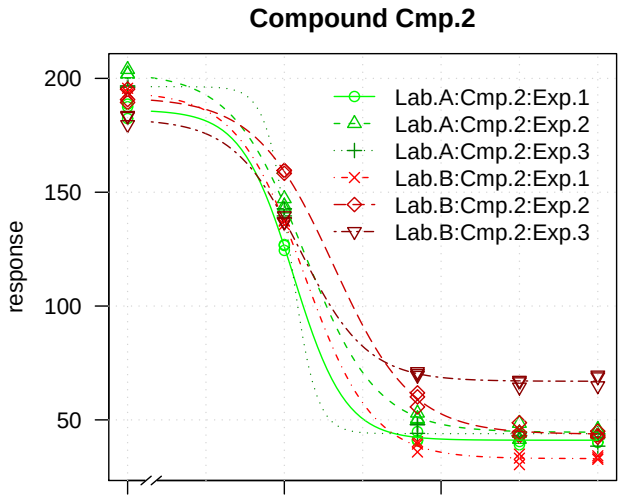




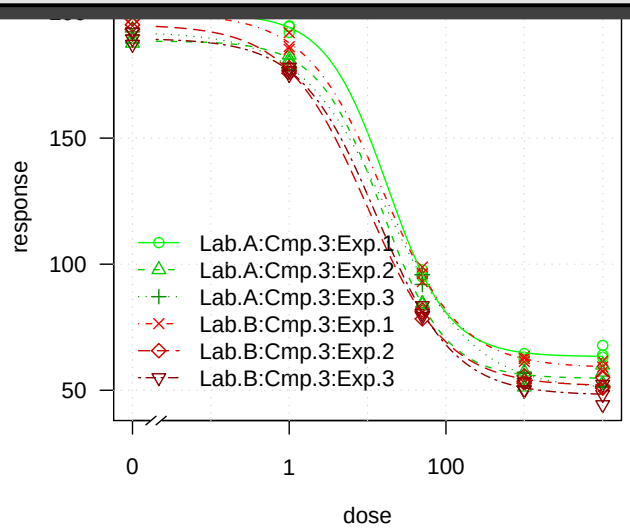


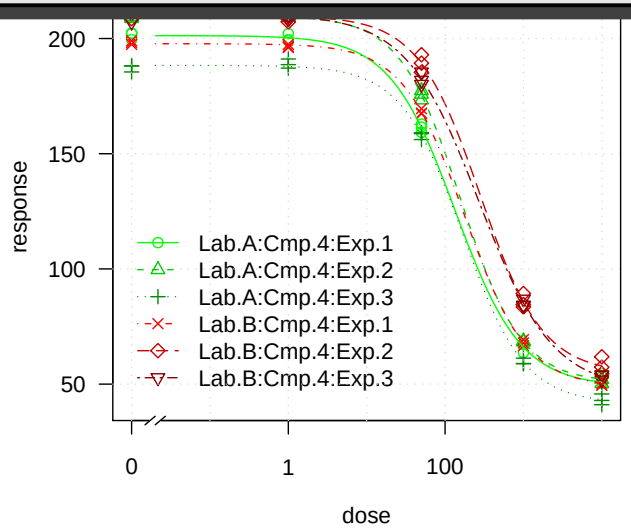
Different labs and different compounds



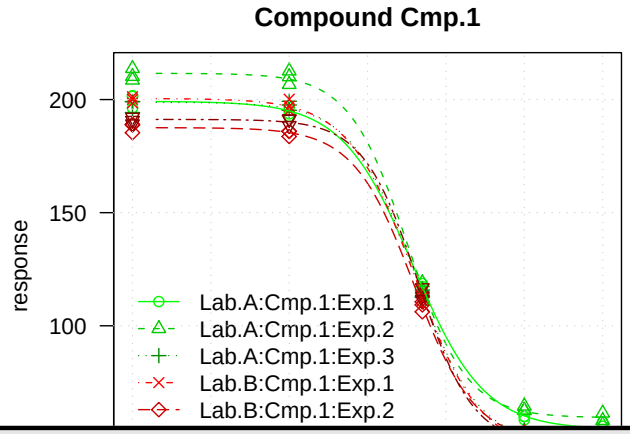


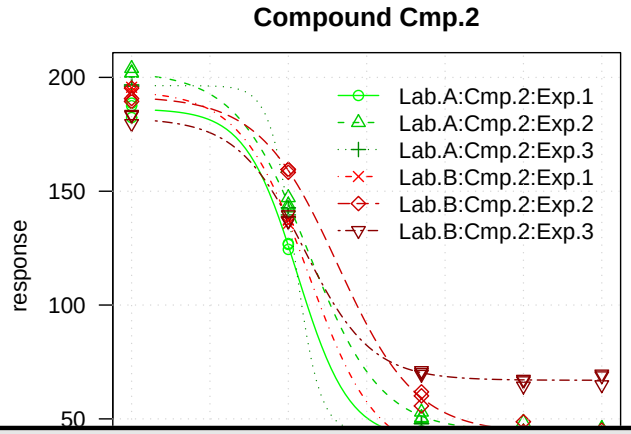
"What is the intra- and interlaboratory variability of the assay?"





Different labs and different compounds

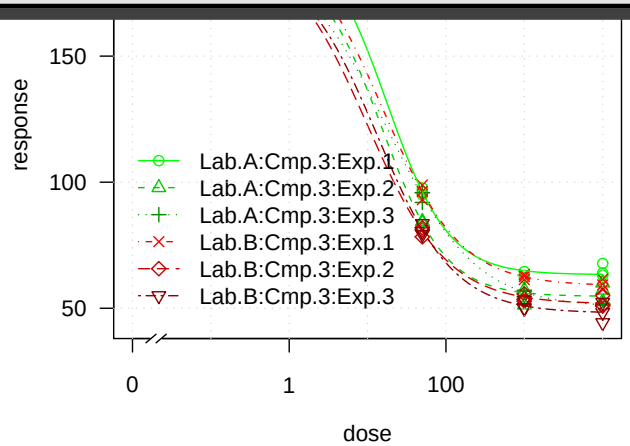


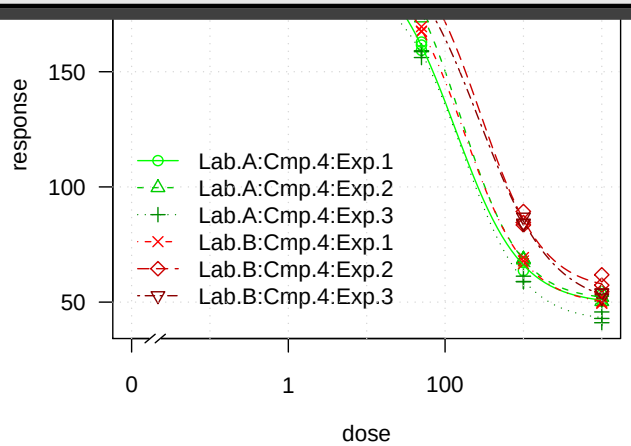


"What is the intra- and interlaboratory variability of the assay?"

basically means

"What is the intra- and interlaboratory variability of ED_{50} **values**?"

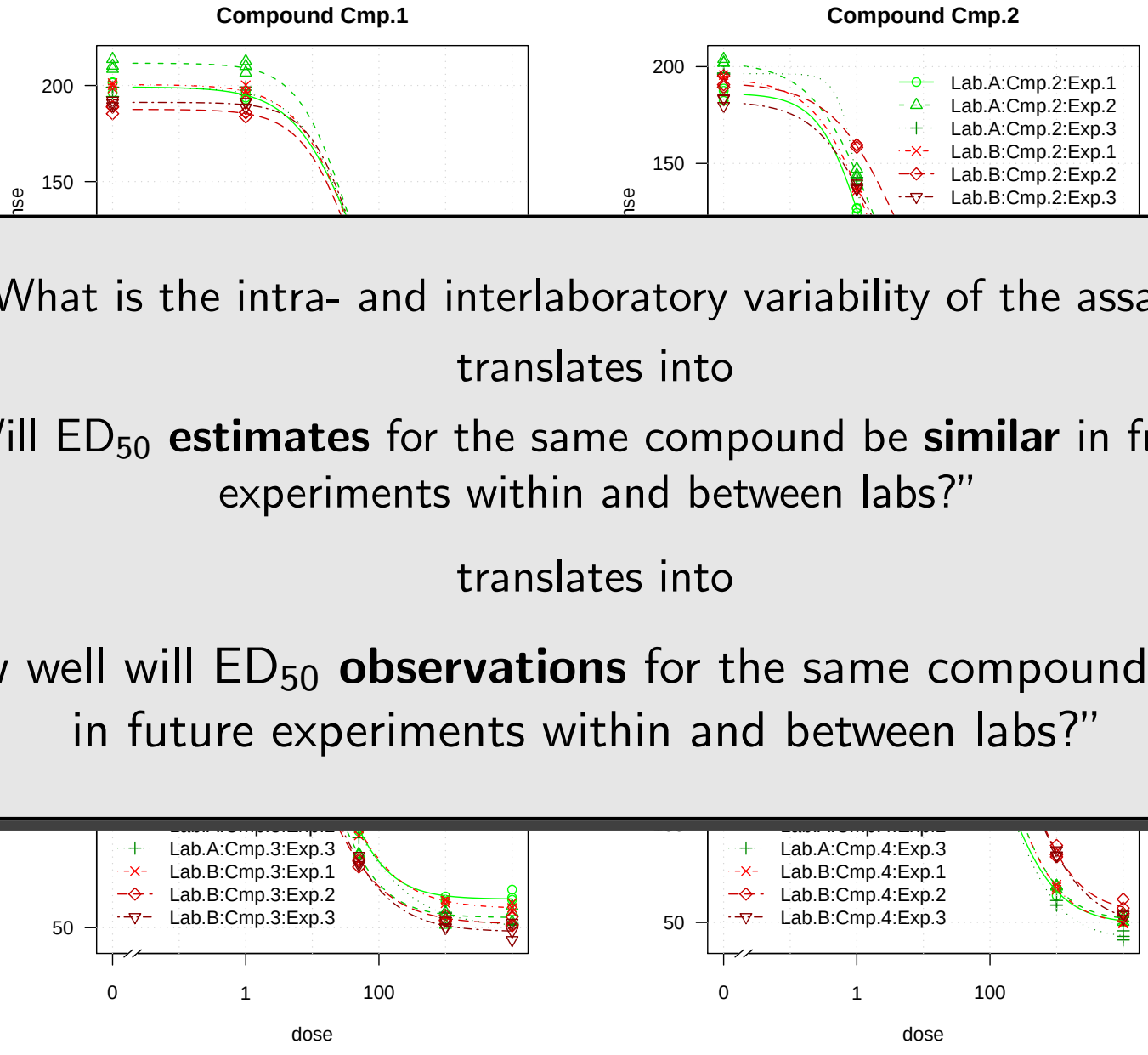




ED_{50} as parameter

- F-testing: Should we model with different ED_{50} parameters?
- Compound i, laboratory j, experimental run k
- e_{ijk} the ED_{50} parameter for a given experimental run
- Model M_b: ED_{50} parameter for each compound, $e_{ijk} = e_{ij'k'}$
- Model M_w: ED_{50} parameter for each cmp:lab, $e_{ijk} = e_{ijk'}$
- Model M_f: Different ED_{50} parameters for each run
- Reject M_b vs $M_w \stackrel{?}{\Rightarrow}$ Poor ED_{50} (inter-lab) reproducibility
- Reject M_w vs $M_f \stackrel{?}{\Rightarrow}$ Poor ED_{50} (intra-lab) repeatability
- Are we really interested in ED_{50} parameters?

ED_{50} as observation



"What is the intra- and interlaboratory variability of the assay?"

translates into

"Will ED_{50} **estimates** for the same compound be **similar** in future experiments within and between labs?"

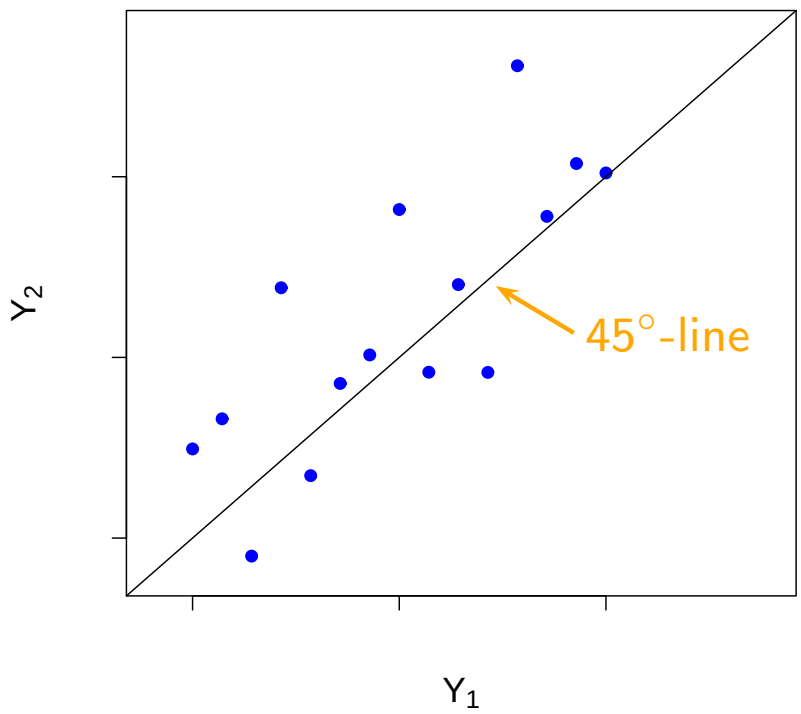
translates into

"How well will ED_{50} **observations** for the same compound **agree** in future experiments within and between labs?"

Agreement statistics: Standard problem specification

- Two observers Y_1 and Y_2
- Each subject i measured once by each observer: Observations y_{i1}, y_{i2}
- Observers measure the same quantity
- No gold standard
- Agreement if Y_1 is "close to" Y_2

Standard problem	ED_{50} estimation
Observer	Laboratory
Subject	Compound
Observation	ED_{50} estimate

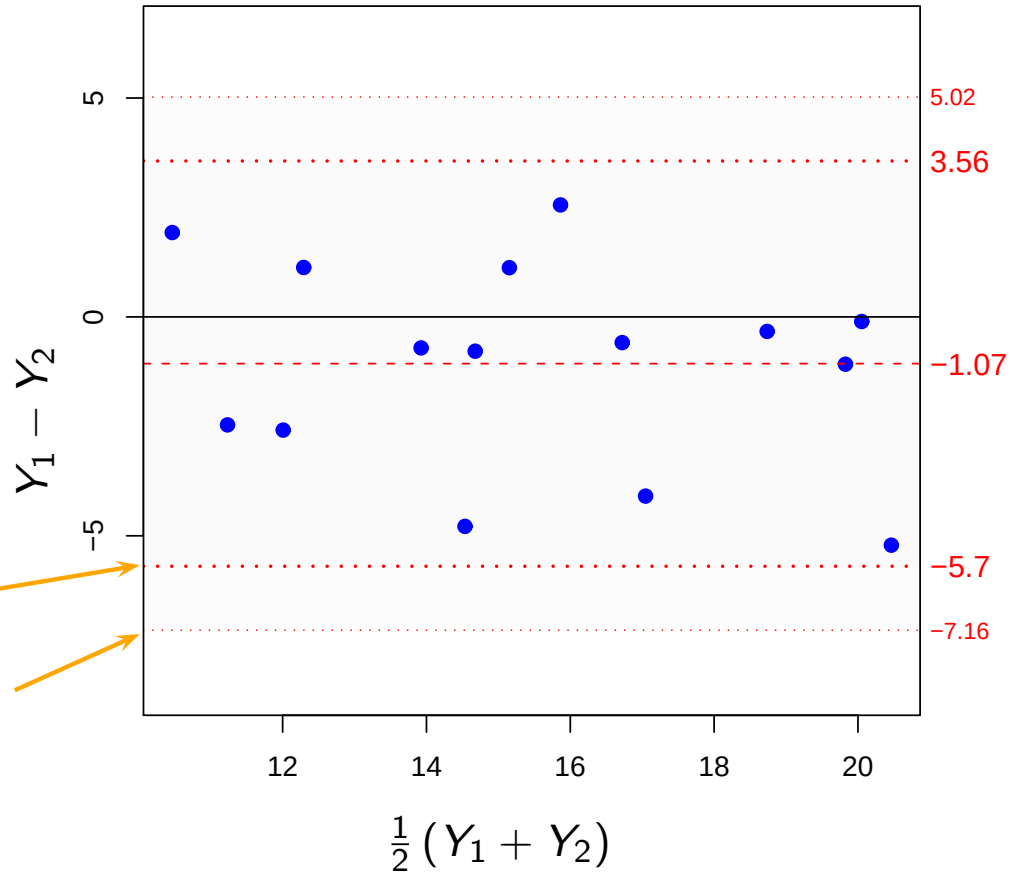


Limits of Agreement (LOA): Basic idea

$$\Delta := Y_1 - Y_2$$

$$\Delta \sim \mathcal{N}\left(\mu_\Delta, \sigma^2_\Delta\right)$$

$$\text{LOA}_{u,l} := \mu_\Delta \pm 1.96\sigma_\Delta$$



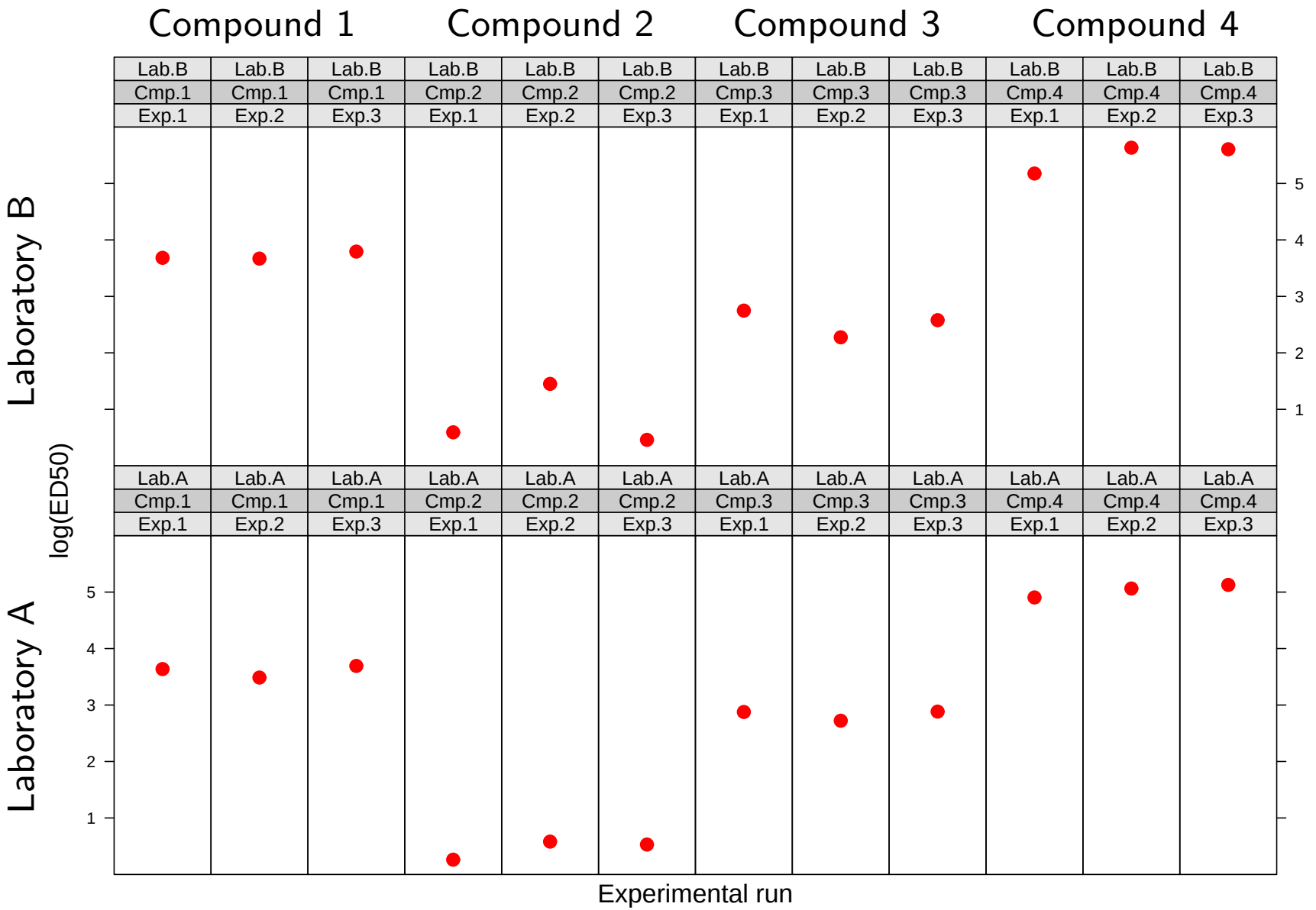
lower LOA

lower bound of CI of lower LOA

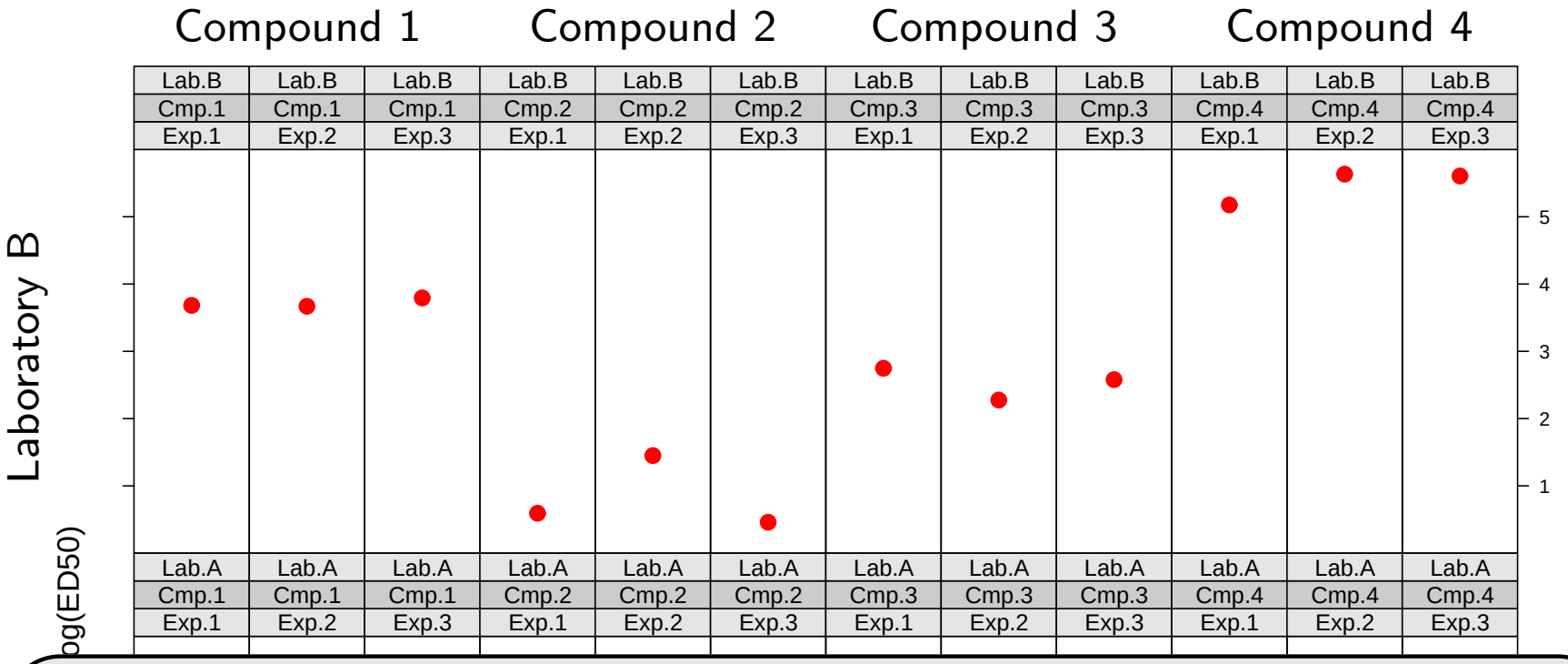
"Reference interval": LOA expected to contain the difference of observations for 95% of pairs of future observations

Bland and Altman 1986, 1999

ED_{50} estimates as observations

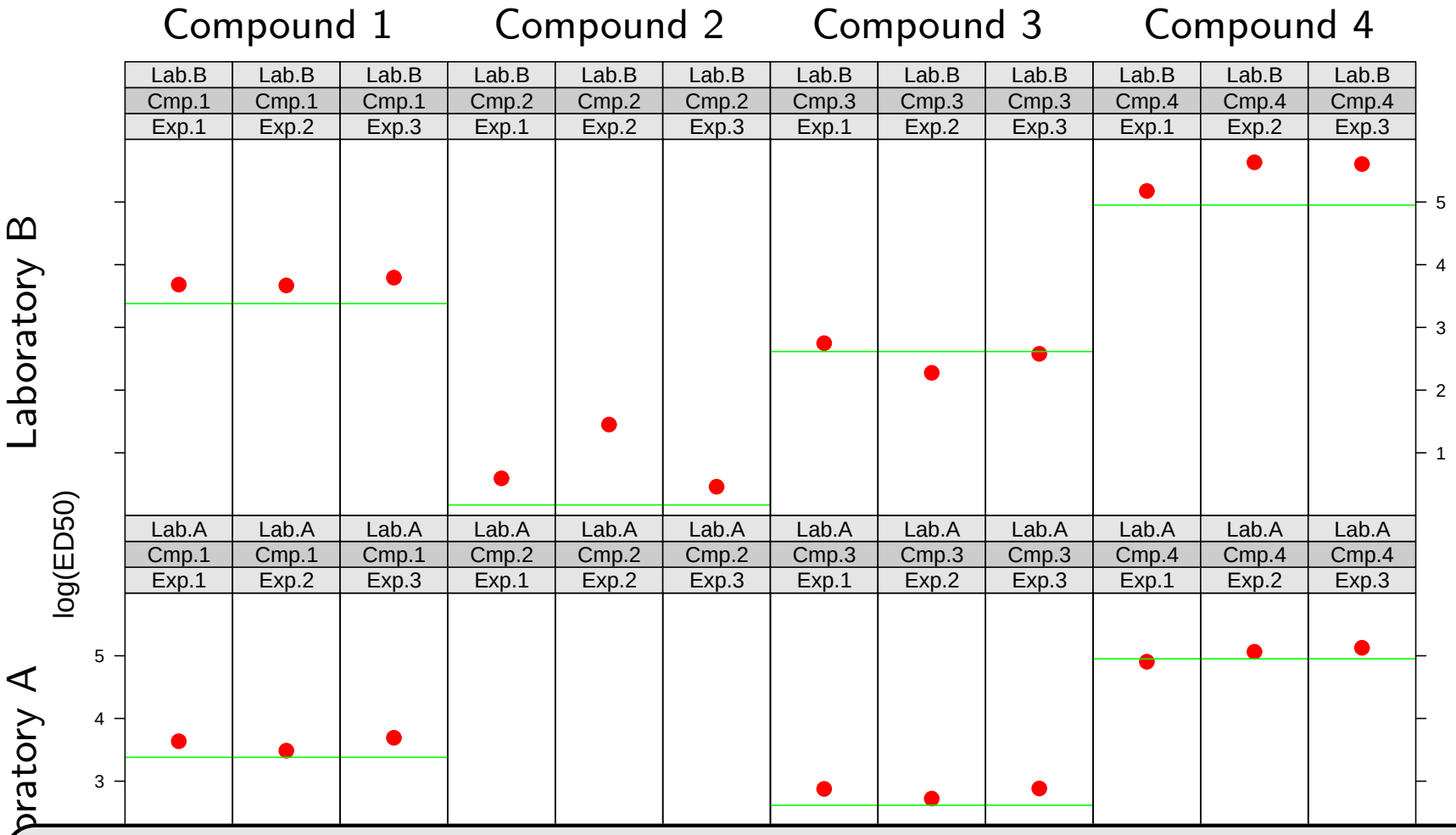


ED_{50} estimates as observations



- 2 random variables L_A and L_B
- Realization of L_X is a $\log(ED_{50})$ observation in laboratory X for a randomly chosen compound
- Idea: Use LOA to assess $\log(ED_{50})$ reproducibility between labs
- Difference of logs with convenient interpretation in terms of ratios
- Multiple ED_{50} observations for each compound in each lab

ED_{50} estimates as observations: Variance components

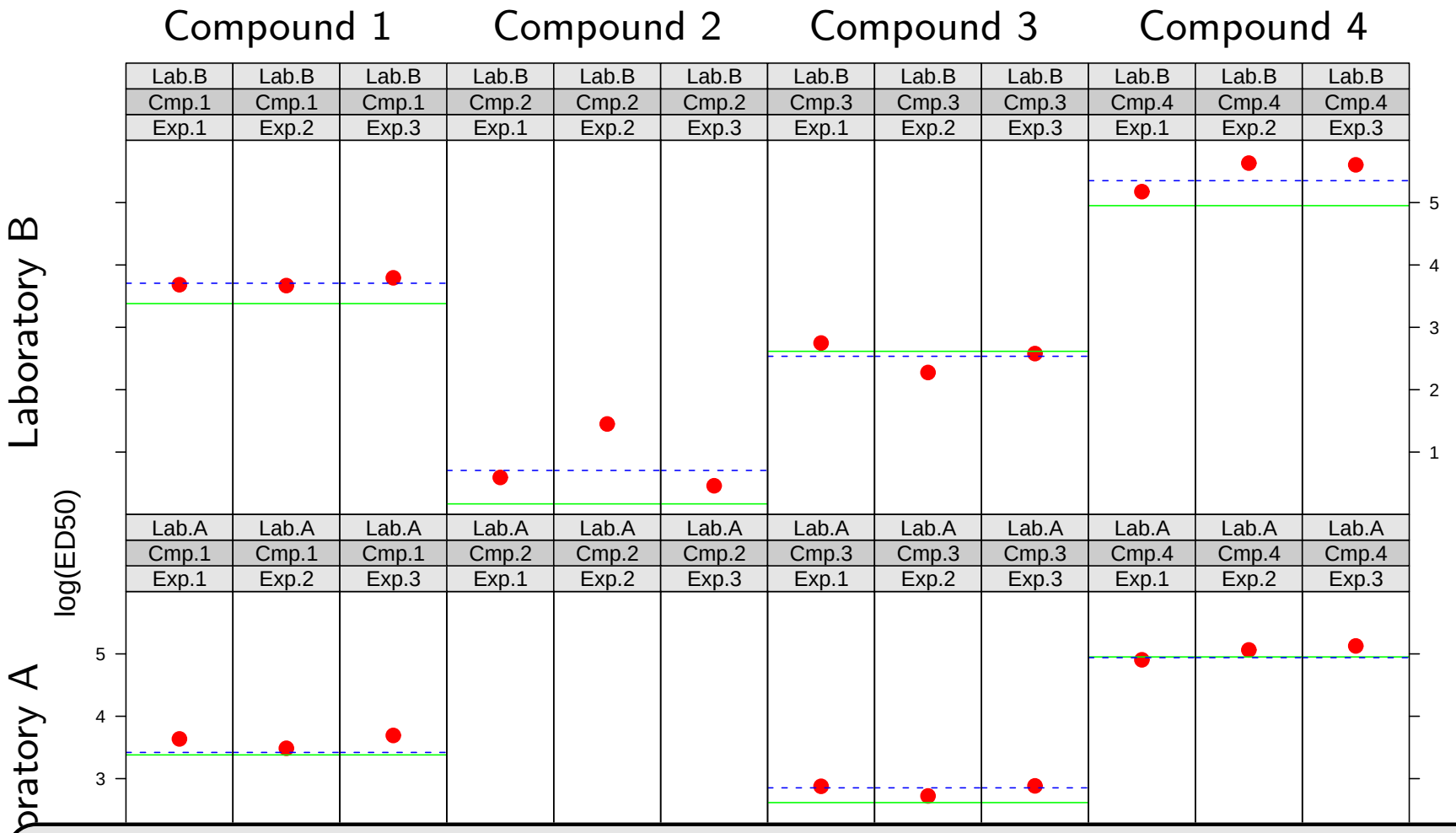


Variability of true $\log(ED_{50})$ value across compounds

$$\mathrm{Var}(L_A) = \sigma_t^2 + \ldots$$

$$\mathrm{Var}(L_B) = \sigma_t^2 + \ldots$$

ED_{50} estimates as observations: Variance components

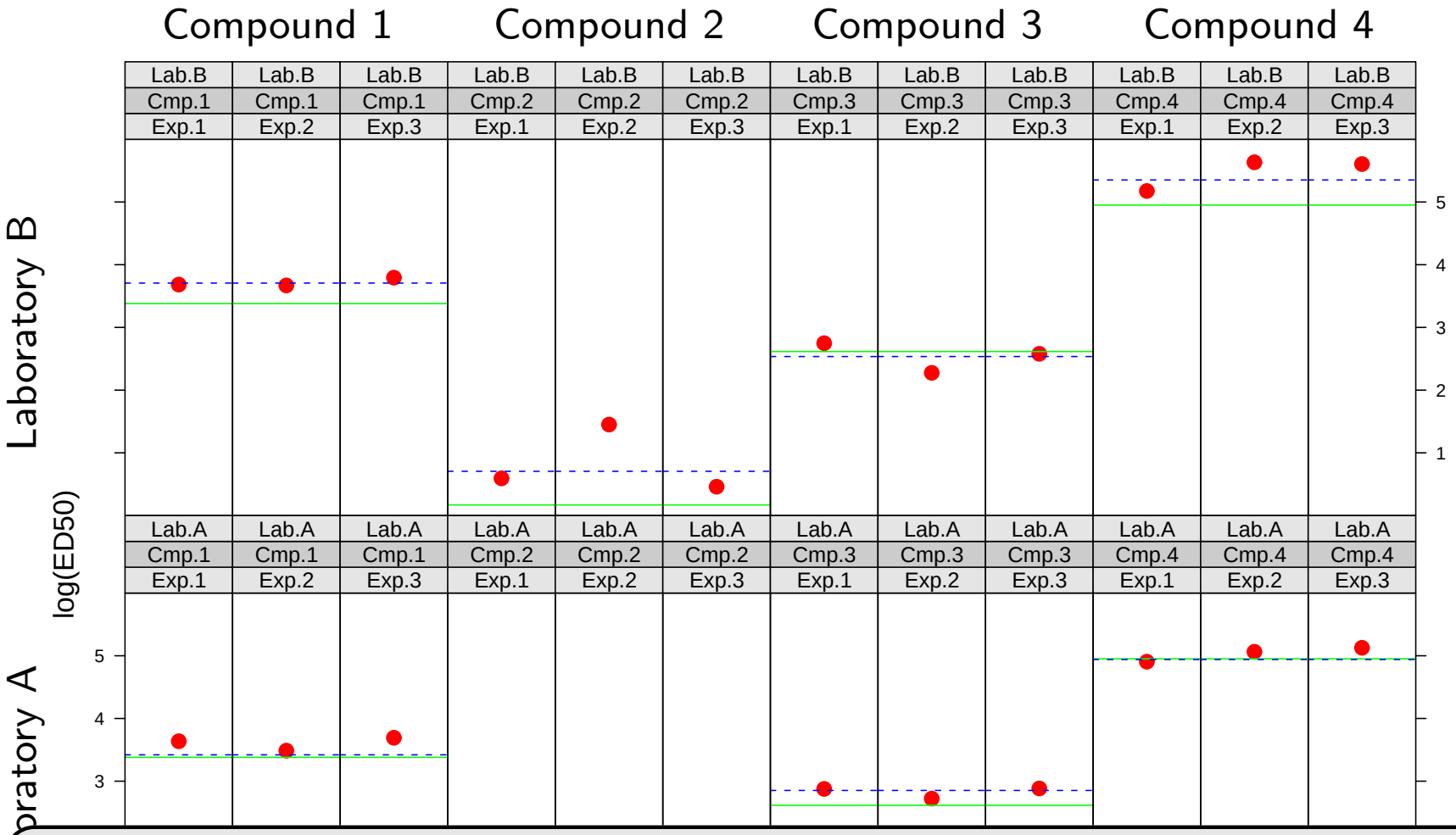


Variability of compound-laboratory interaction bias

$$\mathrm{Var}(L_A) = \sigma_t^2 + \sigma_{c_A}^2 + \ldots$$

$$\mathrm{Var}(L_B) = \sigma_t^2 + \sigma_{c_B}^2 + \ldots$$

ED_{50} estimates as observations: Variance components



Within-compound variance of observations in the same lab

$$\mathrm{Var}(L_A) = \sigma_t^2 + \sigma_{c_A}^2 + \sigma_{w_A}^2$$

$$\mathrm{Var}(L_B) = \sigma_t^2 + \sigma_{c_B}^2 + \sigma_{w_B}^2$$

LOA with multiple observations: Variance

$$\begin{aligned}
\mathrm{Var}\left(L_A\right) &= \sigma_t^2 + \sigma_{c_A}^2 + \sigma_{w_A}^2 \\
\mathrm{Var}\left(L_B\right) &= \sigma_t^2 + \sigma_{c_B}^2 + \sigma_{w_B}^2 \\
\mathrm{Var}(L_A - L_B) &= \sigma_{c_A}^2 + \sigma_{c_B}^2 + \sigma_{w_A}^2 + \sigma_{w_B}^2
\end{aligned}$$

Mean of multiple observations

Number of observations per compound

$$\begin{aligned}
\mathrm{Var}\left(\bar{L}_A\right) &= \sigma_t^2 + \sigma_{c_A}^2 + \frac{\sigma_{w_A}^2}{n_A} \\
\mathrm{Var}\left(\bar{L}_B\right) &= \sigma_t^2 + \sigma_{c_B}^2 + \frac{\sigma_{w_B}^2}{n_B} \\
\mathrm{Var}\left(\bar{L}_A - \bar{L}_B\right) &= \sigma_{c_A}^2 + \sigma_{c_B}^2 + \frac{\sigma_{w_A}^2}{n_A} + \frac{\sigma_{w_B}^2}{n_B}
\end{aligned}$$

$$\mathrm{Var}(L_A - L_B) = \mathrm{Var}\left(\bar{L}_A - \bar{L}_B\right) + \left(1 - \frac{1}{n_A}\right)\sigma_{w_A}^2 + \left(1 - \frac{1}{n_B}\right)\sigma_{w_B}^2$$

Bland and Altman 1999

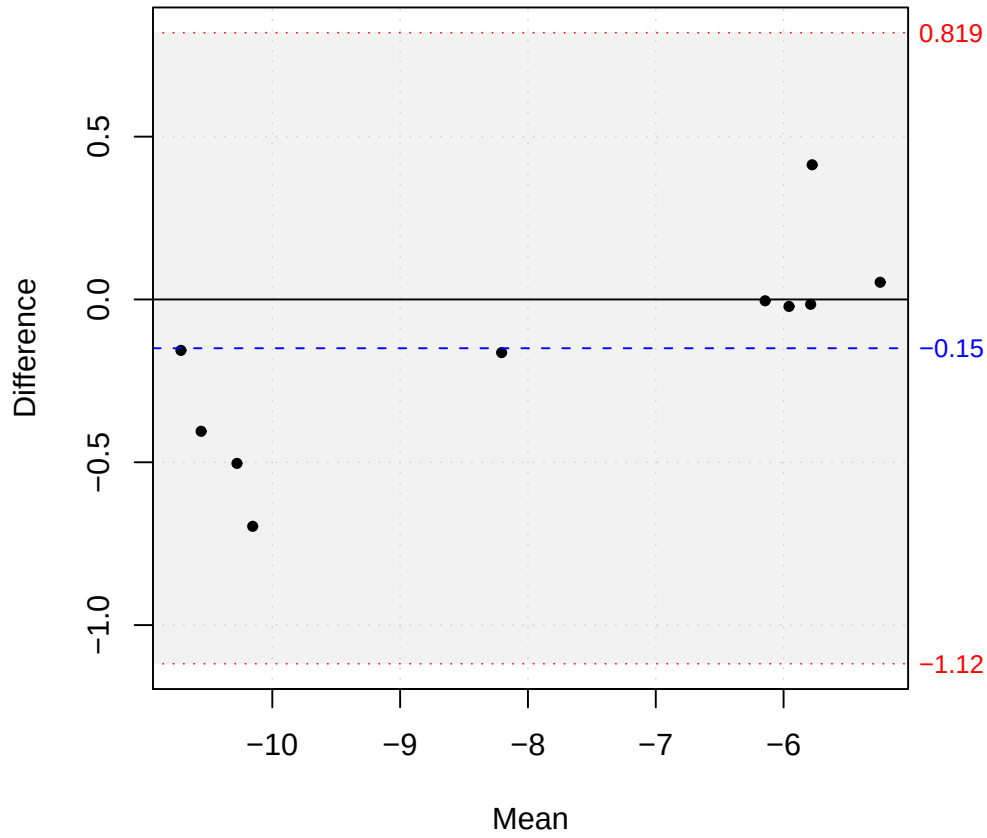
Real world data set

- Same assay with identical SOP in 2 laboratories
- Each lab analyzes the same 10 compounds
- 3 observations of ED_{50} in each lab for each compound

What are the limits expected to contain the ratio of observations for 95% of pairs of future ED_{50} observations in the two labs?

- Analysis of $\log_{10}(ED_{50})$ values
- LOA of difference easily transformed into LOA of ED_{50} ratio

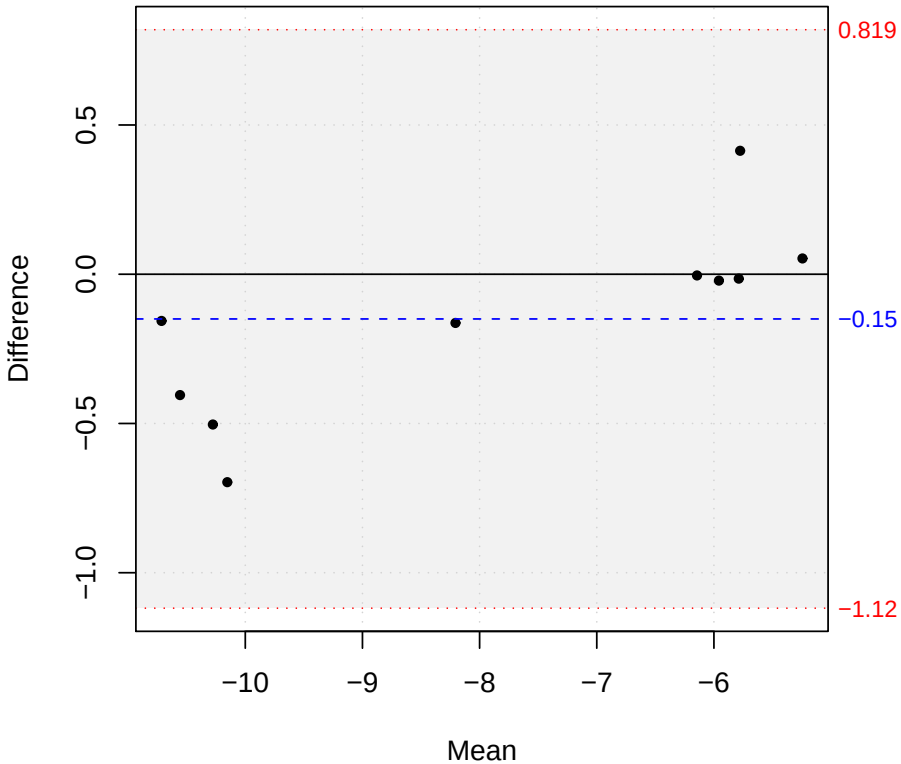
Real world data set



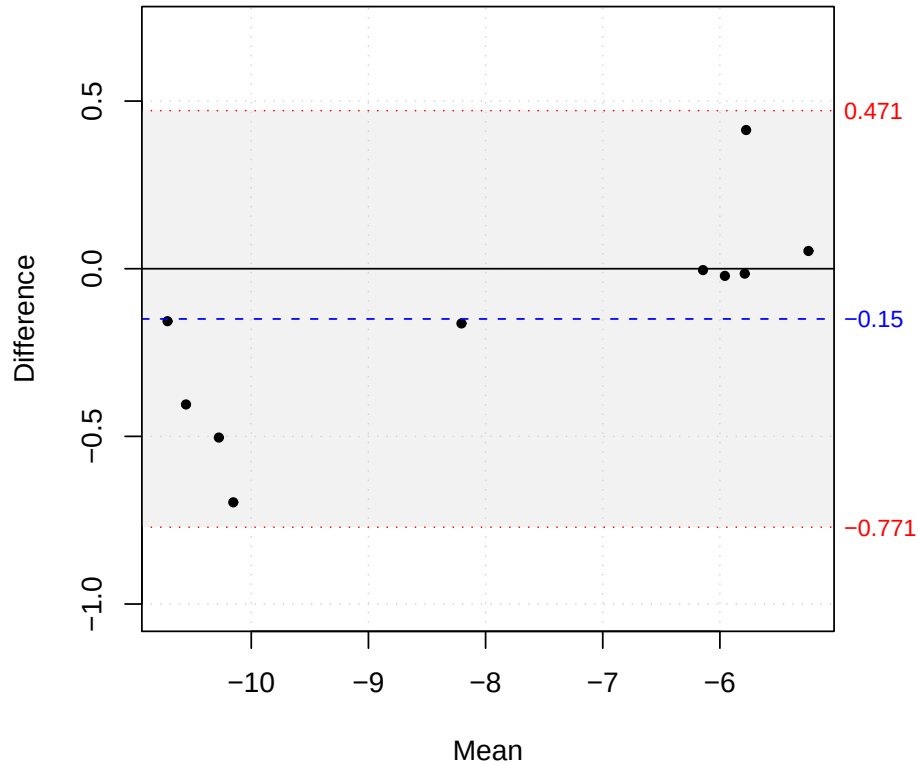
- LOA for pair of one observation from each lab (random variable $L_A - L_B$)
- Observations expected to differ by a factor between 0.08 and 6.59
- Plotted points are averages of 3 observations

Real world data set

- LOA for single observation in each lab ($L_A - L_B$)



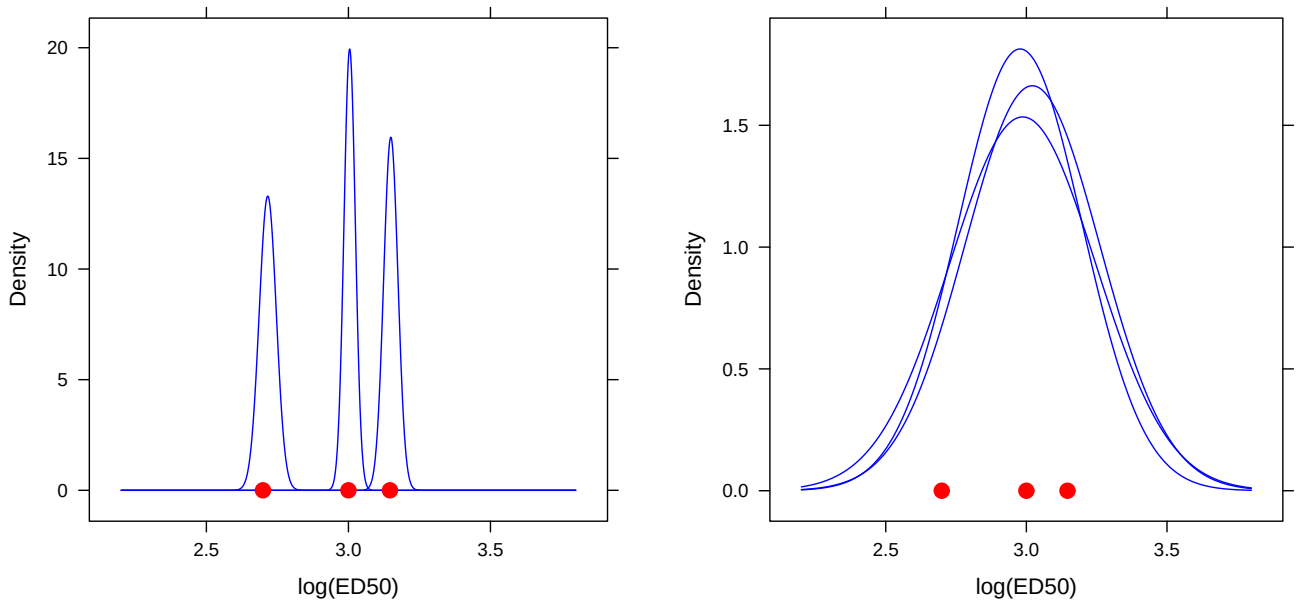
- LOA for average of three observations in each lab ($\bar{L}_A - \bar{L}_B$)



- Observations expected to differ by a factor between 0.08 and 6.59
- Averages expected to differ by a factor between 0.17 and 2.96

Perspectives

- Application of other agreement indices, e.g.
  - Total deviation index $\Pr\left(|Y_1 - Y_2| < \mathrm{TDI}_\pi\right) = \pi$
  - Coverage probability $\mathrm{CP}_\delta := \Pr\left(|Y_1 - Y_2| < \delta\right)$
- ED_{50} standard error and agreement



Summary

ED_{50} values in dose-response assay as predictors for toxicity

"What is the intra- and interlaboratory variability of the assay?"

translates into

"How well will ED_{50} **observations** for the same compound **agree** in future experiments within and between labs?"

Use agreement statistics to analyze differences of $\log(ED_{50})$ observations: Limits of agreement, coverage probability, total deviation index, ...

Standard problem	ED_{50} estimation
Observer	Laboratory
Subject	Compound
Observation	ED_{50} estimate