# A Mixed Model for the Analysis of Enzyme Linked Immunosorbent Assay

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# Enzyme Linked Immunosorbent Assay (ELISA)

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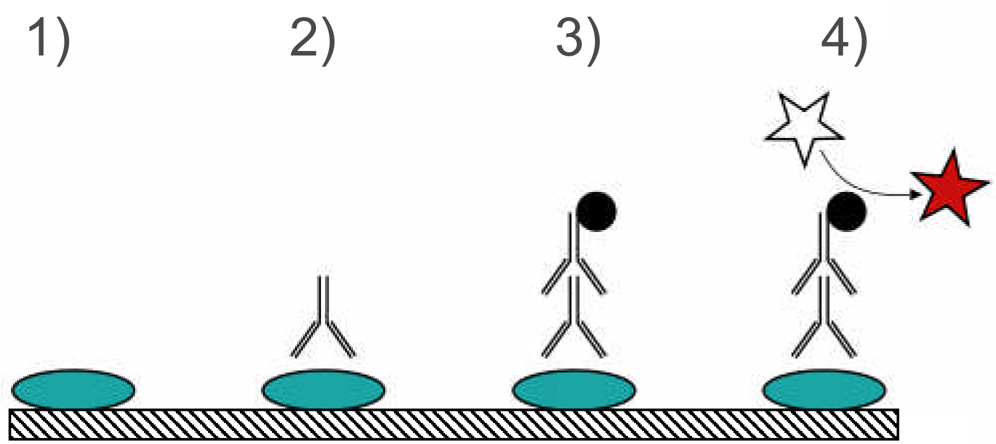


Steps:
(1) Plate is coated with a protein for capture.
(2) Serum containing a mixture of antibodies is added. Antibodies specific for the capture protein will be bound.
(3) A secondary antibody linked to an enzyme is added. It binds specifically to the first antibody.
(4) Substrate is added, and is converted by enzyme to detectable form.

# Cutoff analysis

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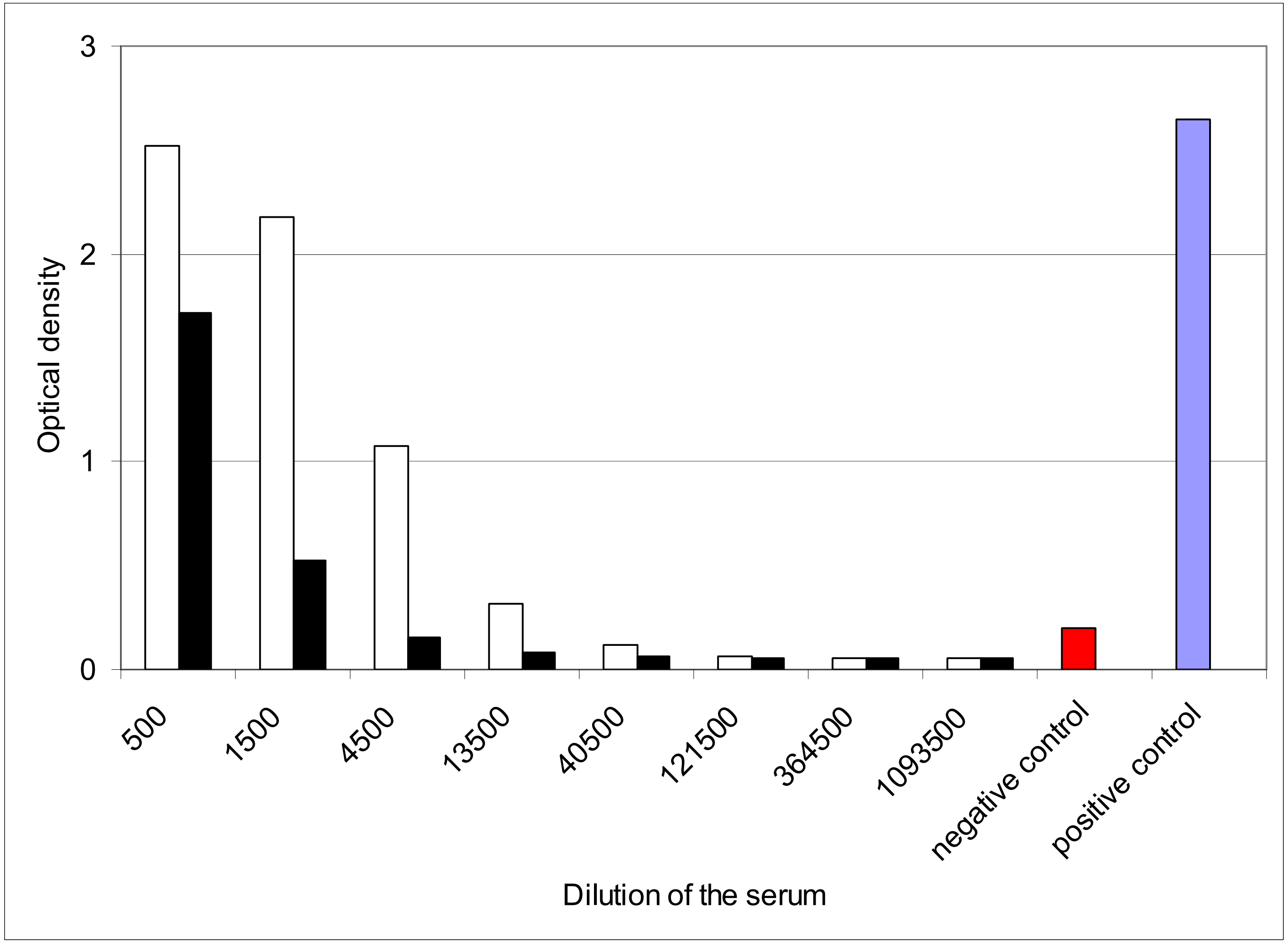

# Cutoff analysis
# Explanation

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## Why does it work?

Continuous inverse exists in measurement range

$$OD_c \cong f\left(\frac{1}{d_{j=c}} s_{ini}\right)$$

$$\Rightarrow \underbrace{f^{-1}(OD_c)}_{\text{constant}} \cong \frac{1}{d_{j=c}} s_{ini}$$

$$\Rightarrow \qquad s_{ini} \sim d_{j=c}$$

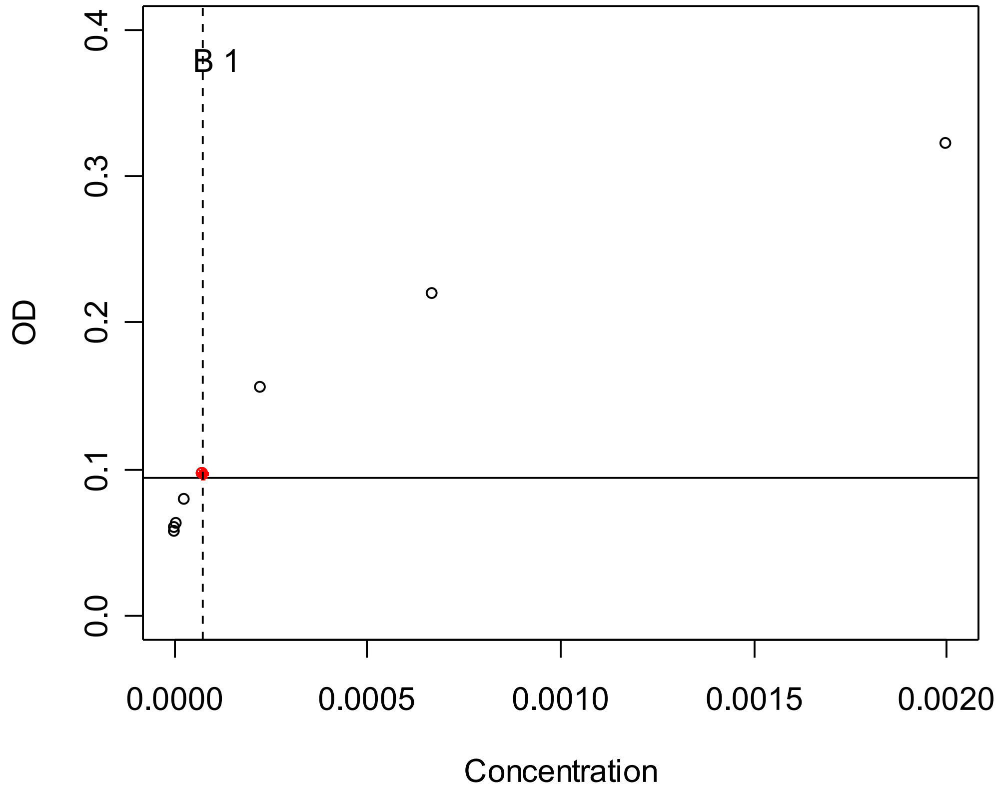
# Cutoff analysis
# Resume

- Cutoff analysis is simple and robust
- No calibration necessary
- Producing ordered categorical data
- Wasting information
- Treatment effects in arbitrary units

Statistical analysis:

- Stage 1: Each subject has its own curve characteristic: Value above the cutoff
- Stage 2: Characteristics is analyzed by conventional statistical techniques (e.g. Wilcoxon-test).

# Cutoff analysis Revisited




Measurements of optical density (OD) over concentration of mouse B1,
concentration is presented in arbitrary units: $\frac{1}{54675}, \frac{1}{18225}, \frac{1}{6075}, \frac{1}{2025}, \frac{1}{675}, \frac{1}{225}, \frac{1}{75}, \frac{1}{25}$

# Michaelis-Menten model
# generalized Michaelis-Menten model

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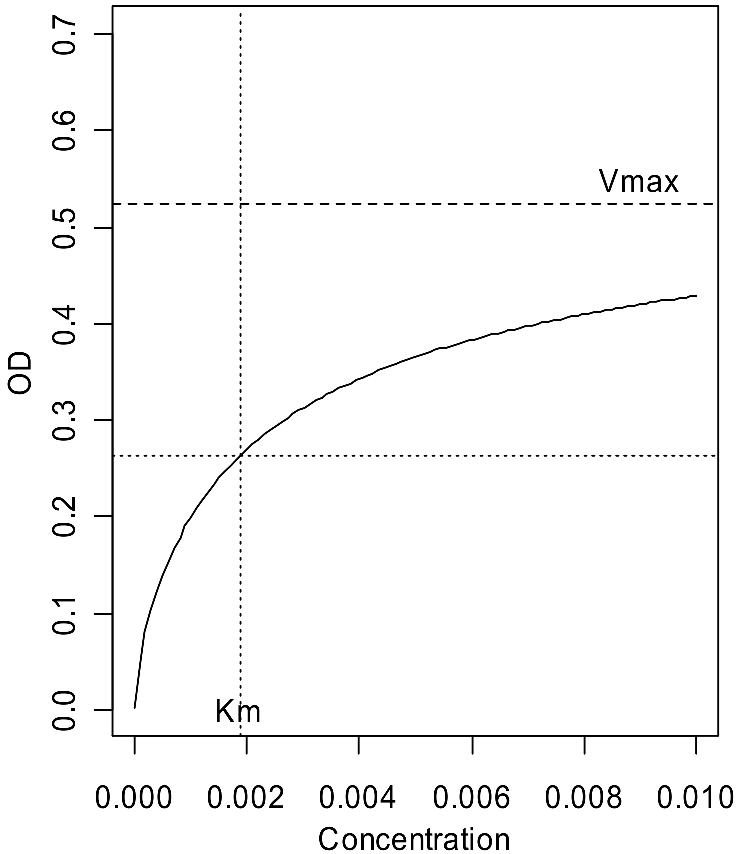
Leonor Michaelis 1875-1949
Maud Menten 1879-1960

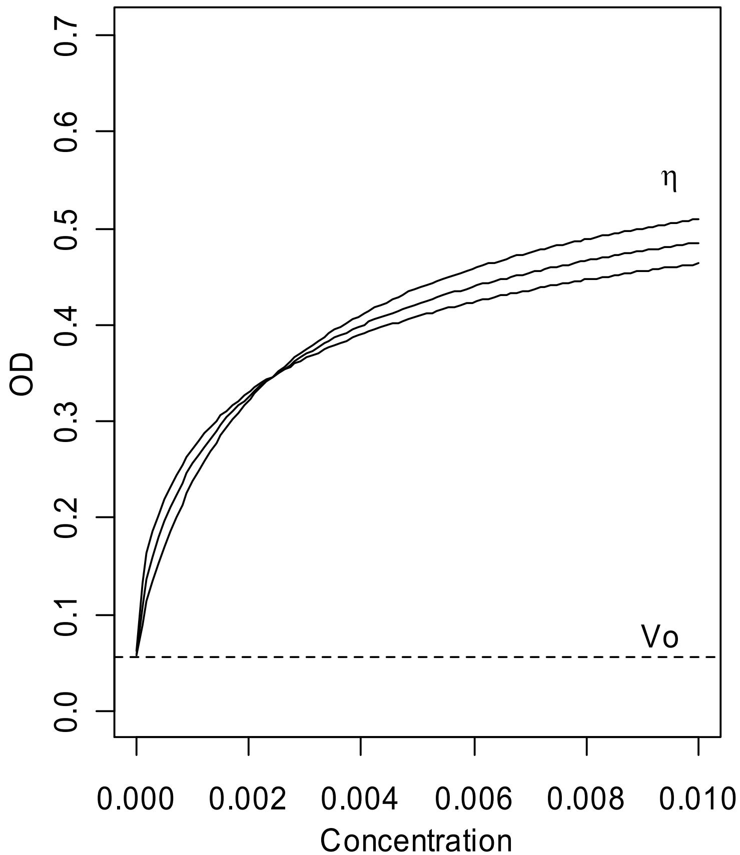
$$\mu = \frac{V_{\max} \cdot s}{K_m + s}$$

$$\mu = V_0 + \frac{V_{\max}^{'} \cdot s^{\eta}}{K_m + s^{\eta}} \;,\; V_{\max}^{'} = V_{\max} - V_0$$

Michaelis-Menten model




generalized Michaelis-Menten model



# Nonlinear calibration
# Calibrating step

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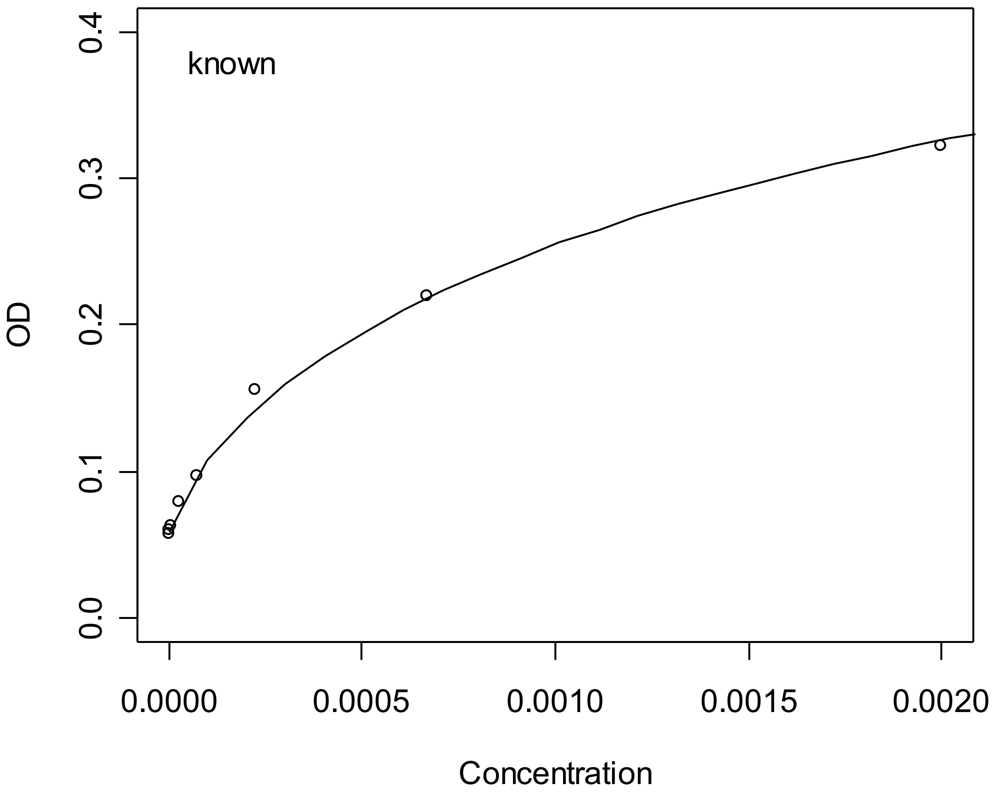
$$\mu = V_0 + \frac{V'_{\max} \cdot \left(\frac{1}{d_j} s^0_{ini}\right)^\eta}{K_m + \left(\frac{1}{d_j} s^0_{ini}\right)^\eta}$$

$$known : \frac{1}{d_j} s^0_{ini}$$

$$fit : V_0, V'_{\max}, K_m, \eta$$



# Nonlinear calibration
# Predicting step

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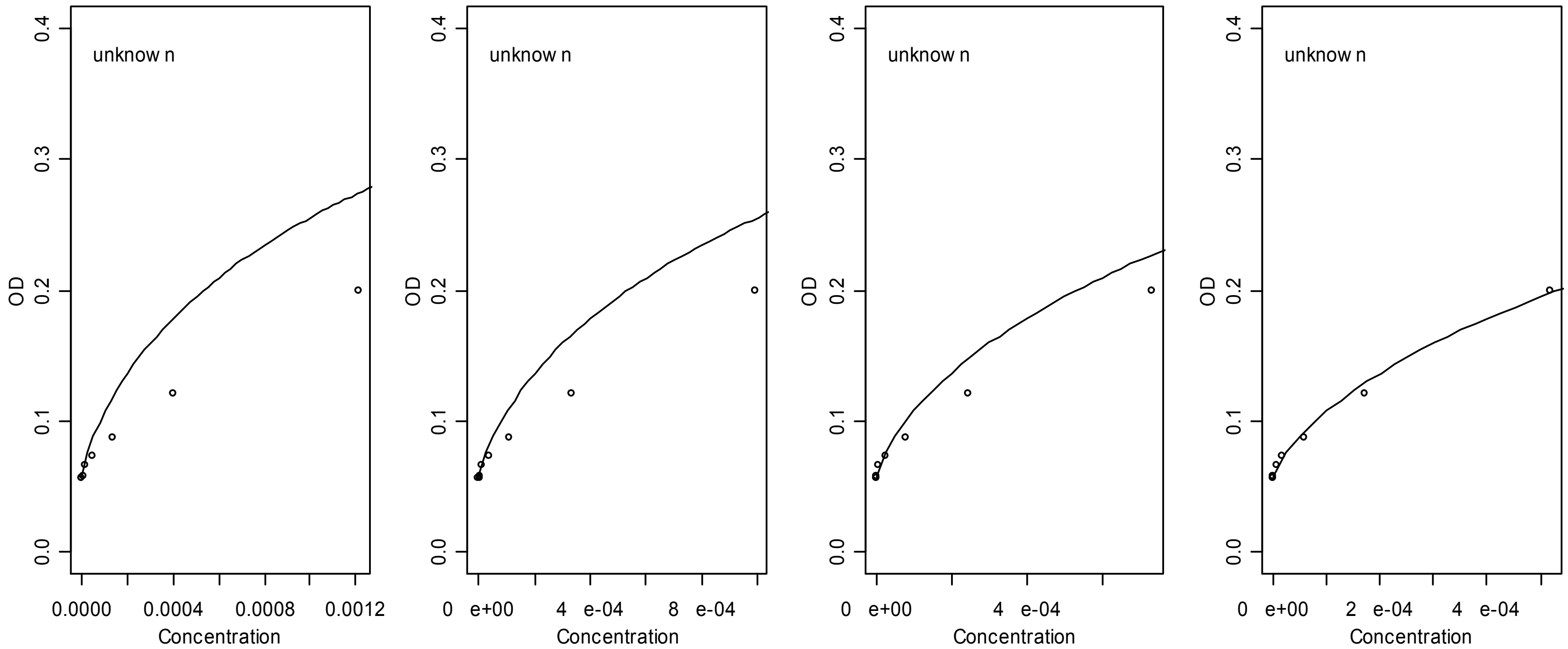


$$V_0, V'_{\max}, K_m, \eta \text{ known} \Rightarrow \mu = V_0 + \frac{V'_{\max} \cdot \left(\frac{1}{d_j} \alpha \cdot s^0_{ini}\right)^\eta}{K_m + \left(\frac{1}{d_j} \alpha \cdot s^0_{ini}\right)^\eta} \Rightarrow s^u_{ini} = \alpha \cdot s^0_{ini}$$

# Nonlinear calibration
# Resume

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- Using all measurements
- Curve characteristics $S^0_{ini}$ is continuous
- Treatment effects in physical units
- Calibration sample necessary
- Needs nonlinear fitting software

Statistical analysis:

- Stage 1: Each subject has its curve characteristic: $s^u_{ini}$
- Stage 2: Characteristics is analyzed by conventional statistical techniques (e.g. ANOVA).

- The parameters: $V_0, V'_{max}, K_m, \eta$ are method characteristics, they can be determined by the laws of thermodynamics. They can be considered as **fixed effects**!
- Biological variability is described in $\alpha$ ,$\alpha$ will be considered as **random effect**!

# Nonlinear mixed effect model

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Mean model

$$\mu_{ij} = V_0 + \frac{V'_{\max} \cdot s_{ij}^{\eta}}{K_m + s_{ij}^{\eta}} \quad \text{and} \quad s_{ij} = \tfrac{1}{d_j} \alpha_i \alpha_1^{trt_i} \overline{s^{init}}$$

$$\mu_{ij} = V_0 + \frac{V'_{\max}}{1 + \left[ e^{(\beta_0 + b_i + \beta_1 \cdot trt_i)} \cdot \frac{1}{d_j} \right]^{-\eta}}$$

$$with \quad e^{\beta_0} = e^{\frac{1}{-\eta}\log(K_m)} \overline{s^{init}}, \qquad e^{b_i} = \alpha_i, \qquad e^{\beta_1 trt_i} = \alpha_1^{trt_i}$$

Error model

| | |
|---|---|
| Autocorrelation | $AR(1)$ |
| Heteroscedasticity | $Var(\varepsilon_{ij}) = (\mu_{ij})^{2\delta} \cdot \sigma^2$ |
| Random effect | $b_i \sim N(0, \sigma_b^2)$ |
| Probability model | $normal \ or \ \log - normal$ |

# Nestlé animal trial (Mouse)

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**n/group = 5, primary: IgE, secondary IgG1**

B) Water (negative control)
E) Bacteria-DNA
F) Bacteria-DNAse
G) Calf-thymus-DNA (negative control)

| Contrasts | Expectation |
|---|---|
| E-B | negative |
| F-B | negative |
| G-B | +/- zero |
| F-E | ? |

# Nonlinear mixed effect model
# IgG1: Model selection, parameter estimates

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## Normal vs. log-normal

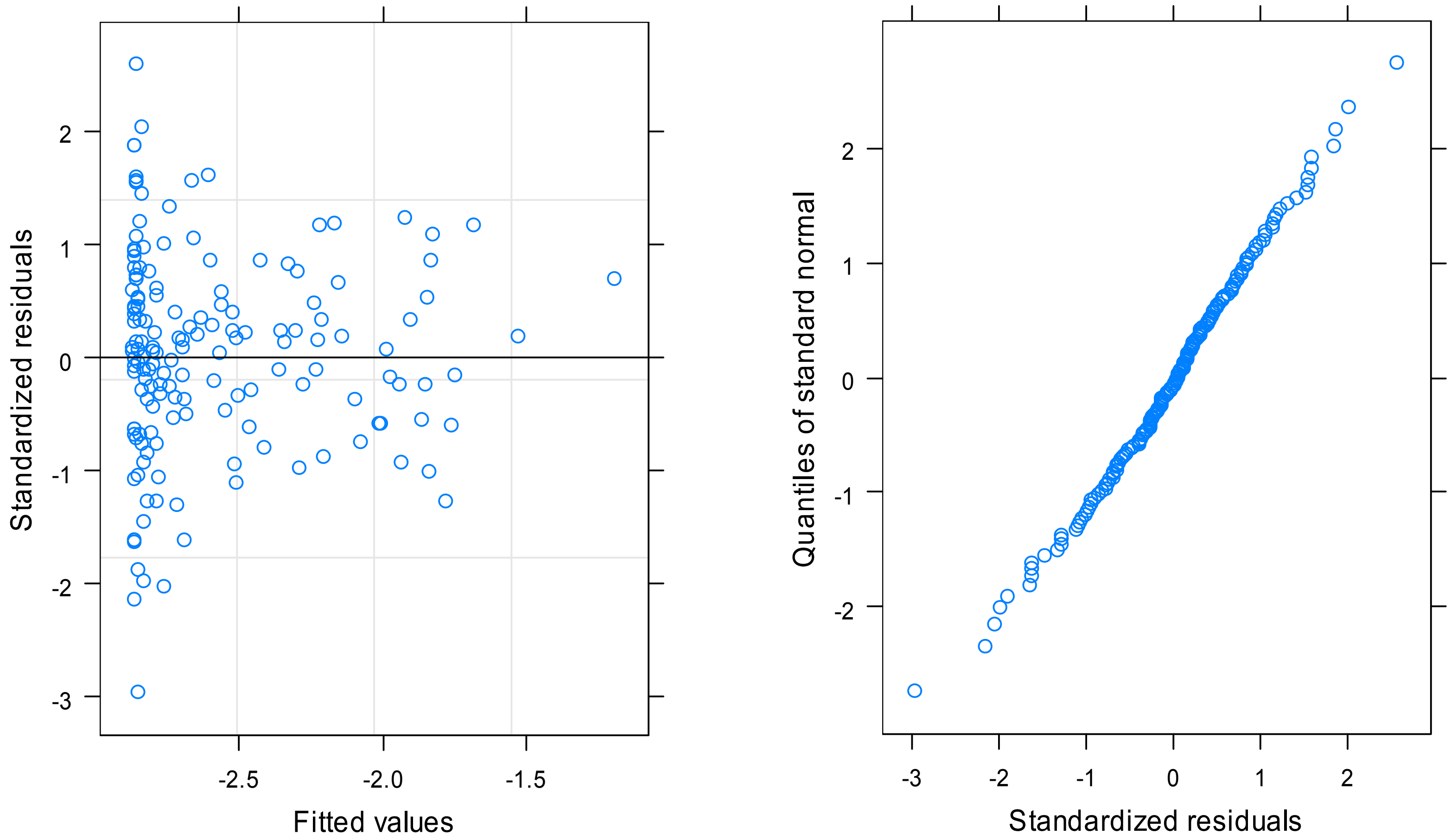
AIC(normal) = -1323.99
AIC(log-normal) = -1324.23

## IgG1 analyzed by nonlinear mixed effect model, parameter estimates and 95% confidence intervals

| **Description** | **Parameter** | **Lower** | **Estimate** | **Upper** |
|---|---|---|---|---|
| background velocity | $V_0$ | 0.056 | 0.057 | 0.058 |
| maximum velocity | $V_{max}$ | 0.269 | 0.426 | 0.674 |
| power trans. substrate | $\eta$ | 0.685 | 0.749 | 0.818 |
| treatment effects | $\beta_0^B$ | 56.0 | 181.7 | 589.0 |
| | $\beta_1^E$ | 0.198 | 0.554 | 1.551 |
| | $\beta_1^F$ | 0.191 | 0.536 | 1.500 |
| | $\beta_1^G$ | 0.064 | 0.181 | 0.514 |
| random effect standard deviation | $\sigma_b$ | 0.555 | 0.815 | 1.198 |
| autocorrelation parameter | $\phi$ | 0.137 | 0.357 | 0.544 |
| power of the variance function | $\delta$ | -1.881 | -0.996 | -0.110 |
| residual standard deviation | $\sigma$ | 0.042 | 0.101 | 0.245 |

# Nonlinear mixed effect model IgG1: Quality of the fit





# Nonlinear mixed effect model
# IgG1: Prediction

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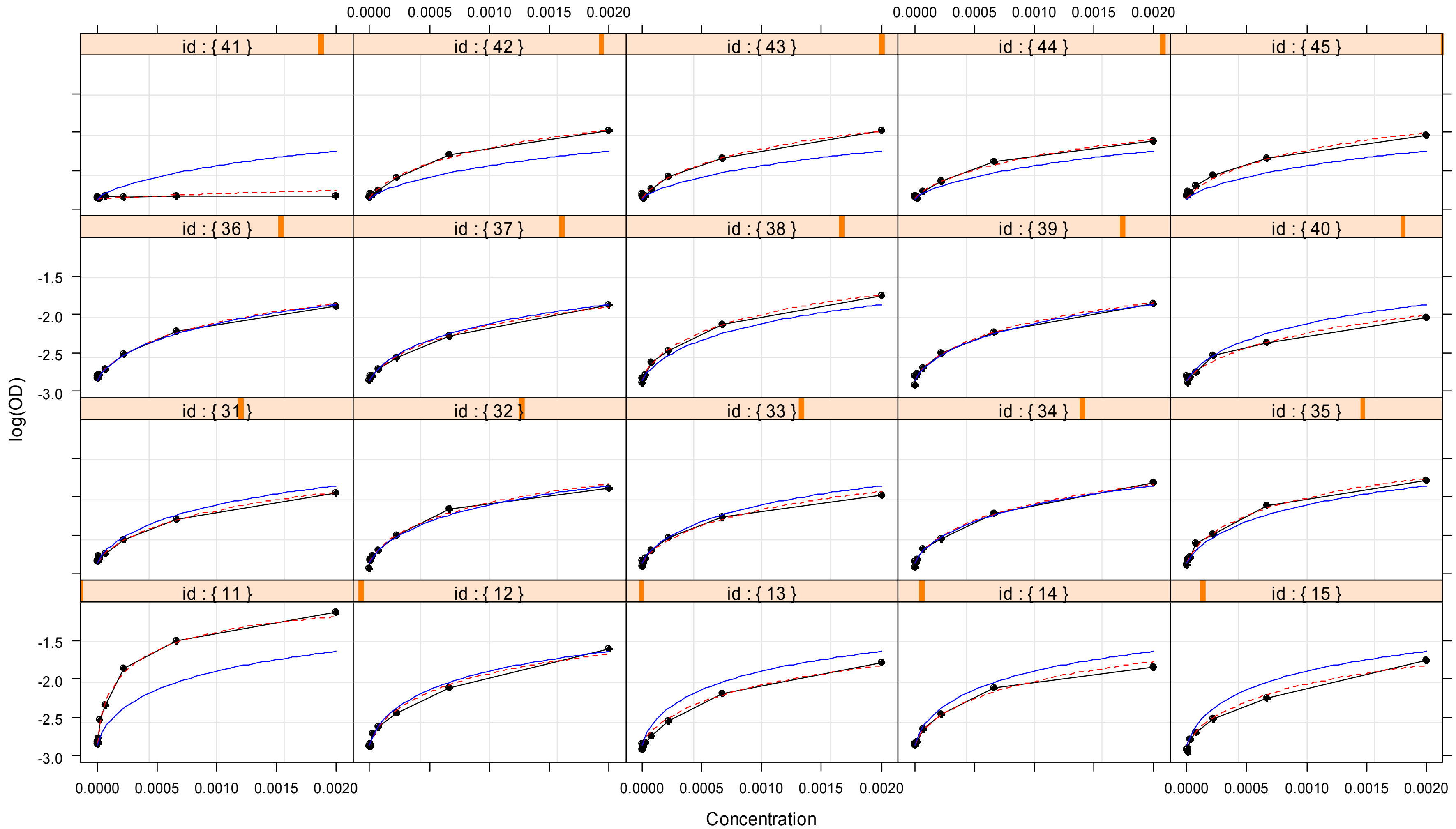
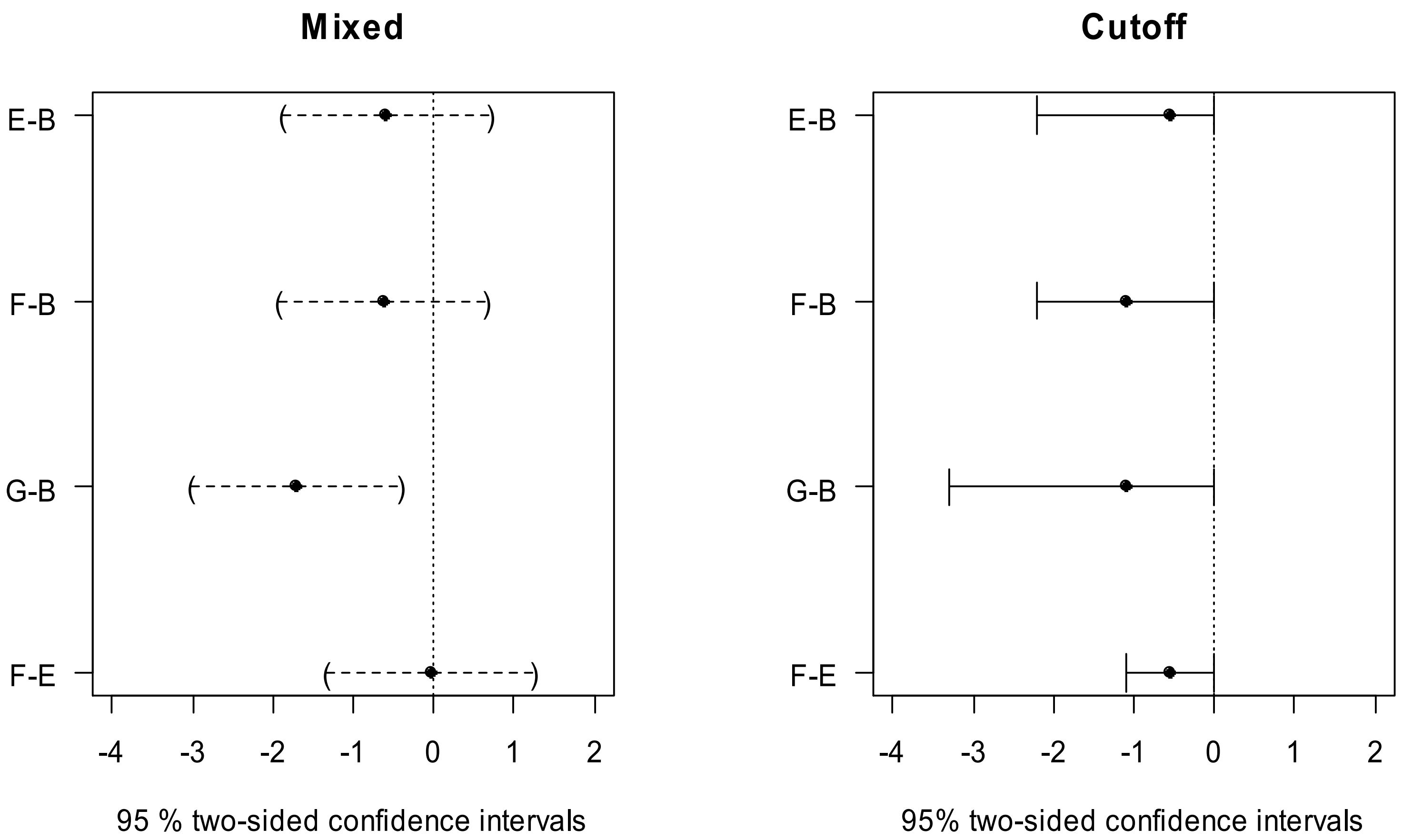


# Nonlinear mixed effect model, cutoff IgG1: Treatment differences





# Nonlinear mixed effect model
# IgE: Model selection, parameter estimates

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## Normal vs. log-normal

AIC(normal) = -1048.54
AIC(log-normal) = -1073.40

## IgE analyzed by nonlinear mixed effect model, parameter estimates and 95% confidence intervals

| **Description** | **Parameter** | **Lower** | **Estimate** | **Upper** |
|---|---|---|---|---|
| background velocity | $V_0$ | 0.041 | 0.043 | 0.044 |
| maximum velocity | $V_{max}$ | 0.326 | 0.762 | 1.777 |
| power trans. substrate | $\eta$ | 0.579 | 0.646 | 0.721 |
| treatment effects | $\beta_0^B$ | 1.012 | 9.773 | 94.417 |
| | $\beta_1^E$ | 0.064 | 0.504 | 4.002 |
| | $\beta_1^F$ | 0.121 | 0.960 | 7.615 |
| | $\beta_1^G$ | 0.023 | 0.189 | 1.533 |
| random effect standard deviation | $\sigma_b$ | 1.137 | 1.628 | 2.333 |
| autocorrelation parameter | $\phi$ | 0.623 | 0.775 | 0.871 |
| power of the variance function | $\delta$ | -2.020 | -1.653 | -1.286 |
| residual standard deviation | $\sigma$ | 0.317 | 0.440 | 0.612 |

# Nonlinear mixed effect model
# IgE: Quality of the fit

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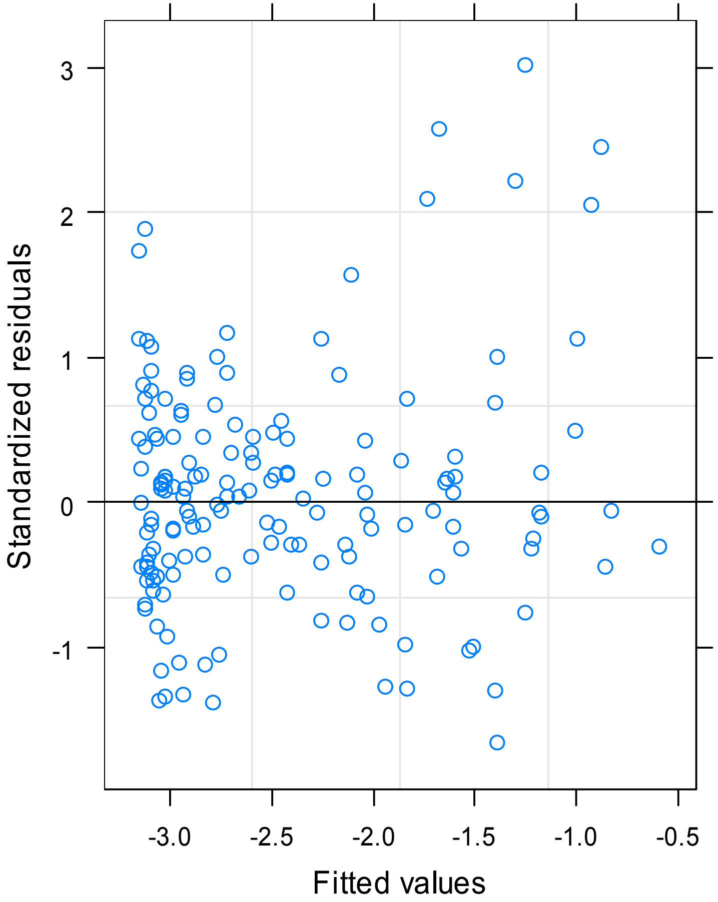


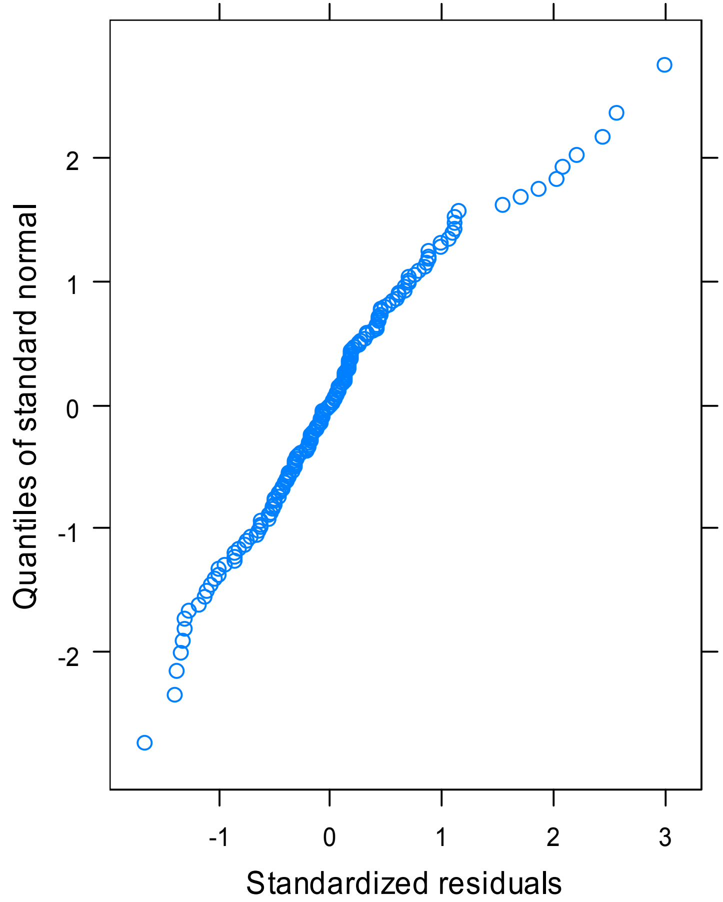

# Nonlinear mixed effect model
# IgE: Prediction

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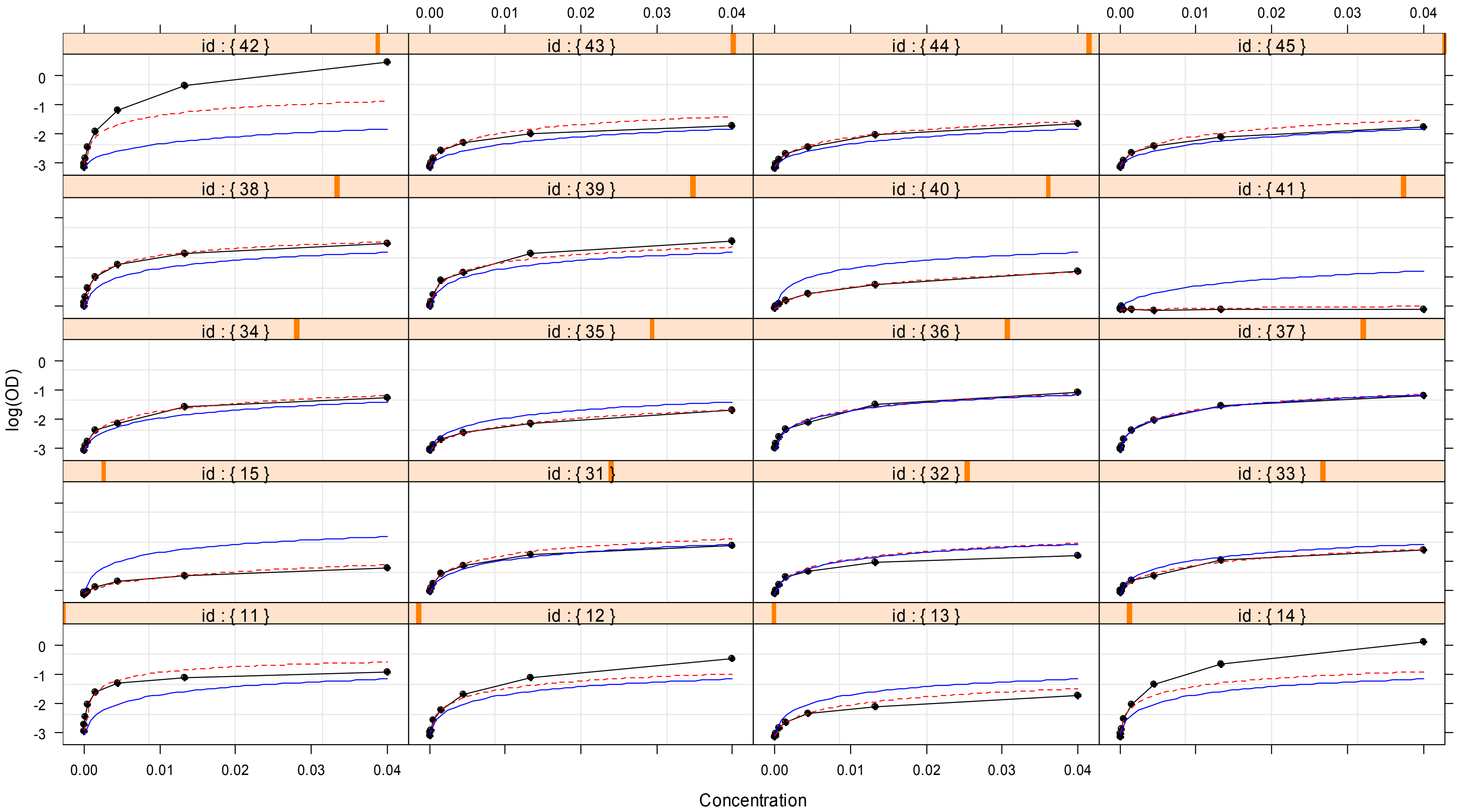
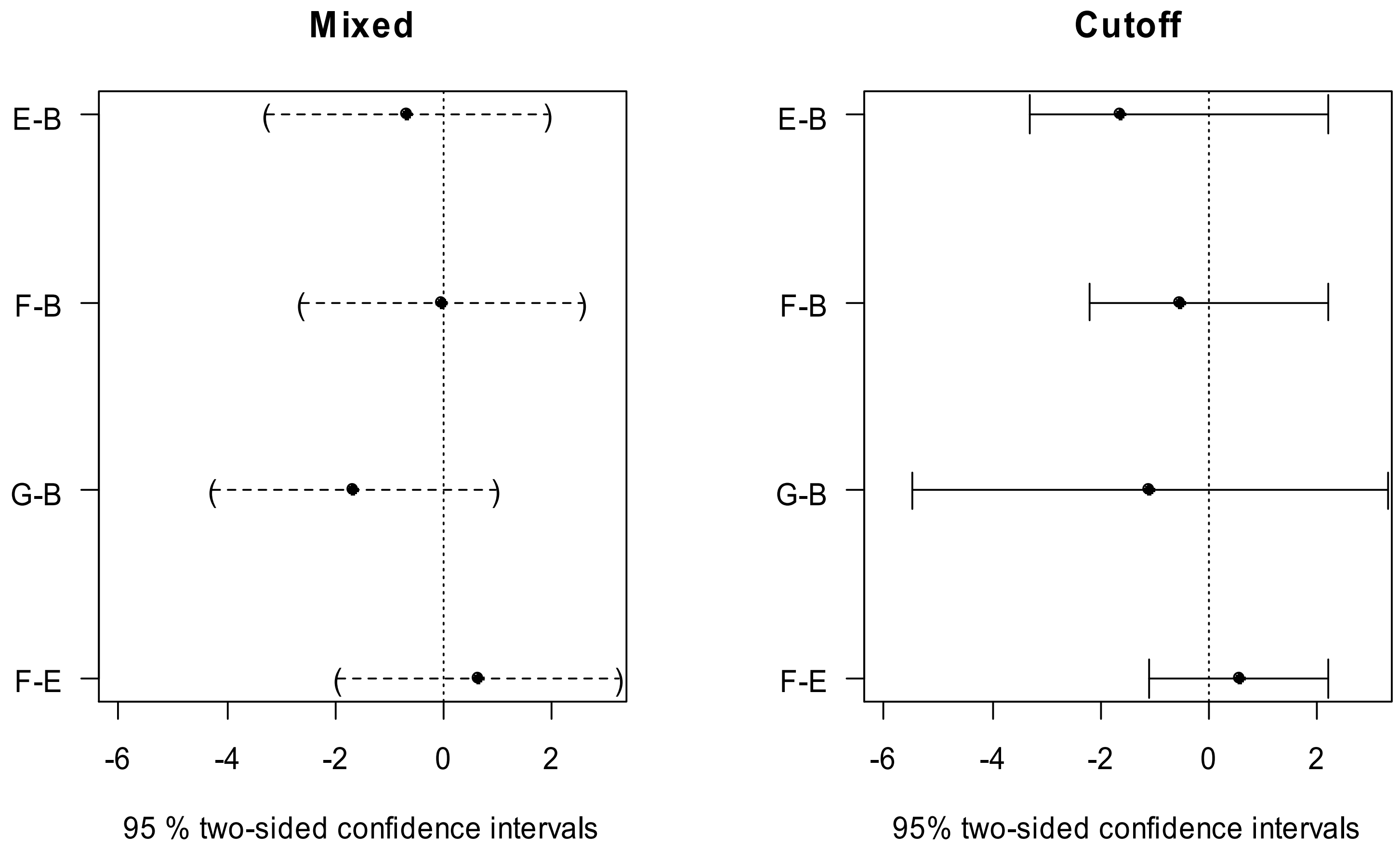


# Nonlinear mixed effect model, cutoff IgE: Treatment differences



# Nonlinear model, IgE: All parameters free

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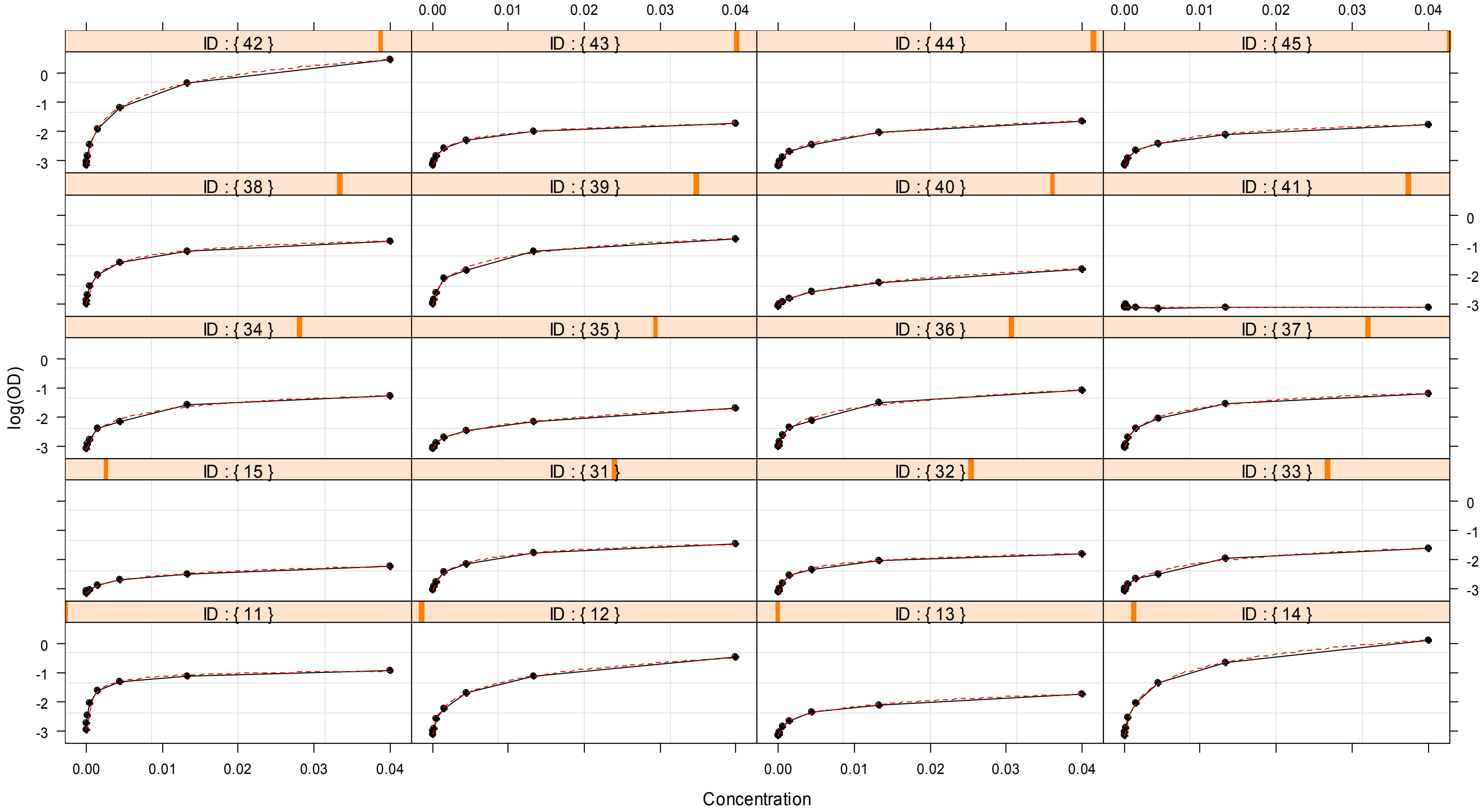

# Parameter estimates

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| ID | V0 | Vmax | eta | beta | sigma |
|---|---|---|---|---|---|
| 11 | -3.17 | -0.92 | -0.33 | 5.80 | 0.021 |
| 12 | -3.22 | 4.75 | -0.44 | -5.01 | 0.023 |
| 13 | -3.25 | -0.52 | -0.67 | 0.85 | 0.028 |
| 14 | -3.21 | 2.35 | -0.24 | 0.48 | 0.016 |
| 15 | -3.19 | -1.74 | -0.53 | 2.30 | 0.024 |
| 31 | -3.10 | -1.22 | -0.33 | 3.86 | 0.022 |
| 32 | -3.22 | -1.57 | -0.53 | 3.83 | 0.020 |
| 33 | -3.08 | -0.90 | -0.33 | 2.50 | 0.053 |
| 34 | -3.17 | -0.67 | -0.39 | 2.99 | 0.044 |
| 35 | -3.13 | 11.03 | -0.57 | -19.85 | 0.017 |
| 36 | -3.11 | 5.43 | -0.58 | -8.72 | 0.042 |
| 37 | -3.09 | -0.71 | -0.31 | 3.34 | 0.017 |
| 38 | -3.15 | -0.41 | -0.42 | 3.66 | 0.008 |
| 39 | -3.13 | 0.19 | -0.38 | 2.25 | 0.054 |
| 40 | -3.09 | 5.90 | -0.42 | -9.07 | 0.010 |
| 41 | -3.12 | -1.35 | 0.62 | -6.86 | 0.038 |
| 42 | -3.18 | 2.52 | -0.16 | 0.95 | 0.014 |
| 43 | -3.21 | -1.44 | -0.46 | 3.72 | 0.012 |
| 44 | -3.25 | 9.08 | -0.68 | -18.40 | 0.013 |
| 45 | -3.22 | -1.22 | -0.49 | 2.89 | 0.025 |

# Identifying stable subpopulation

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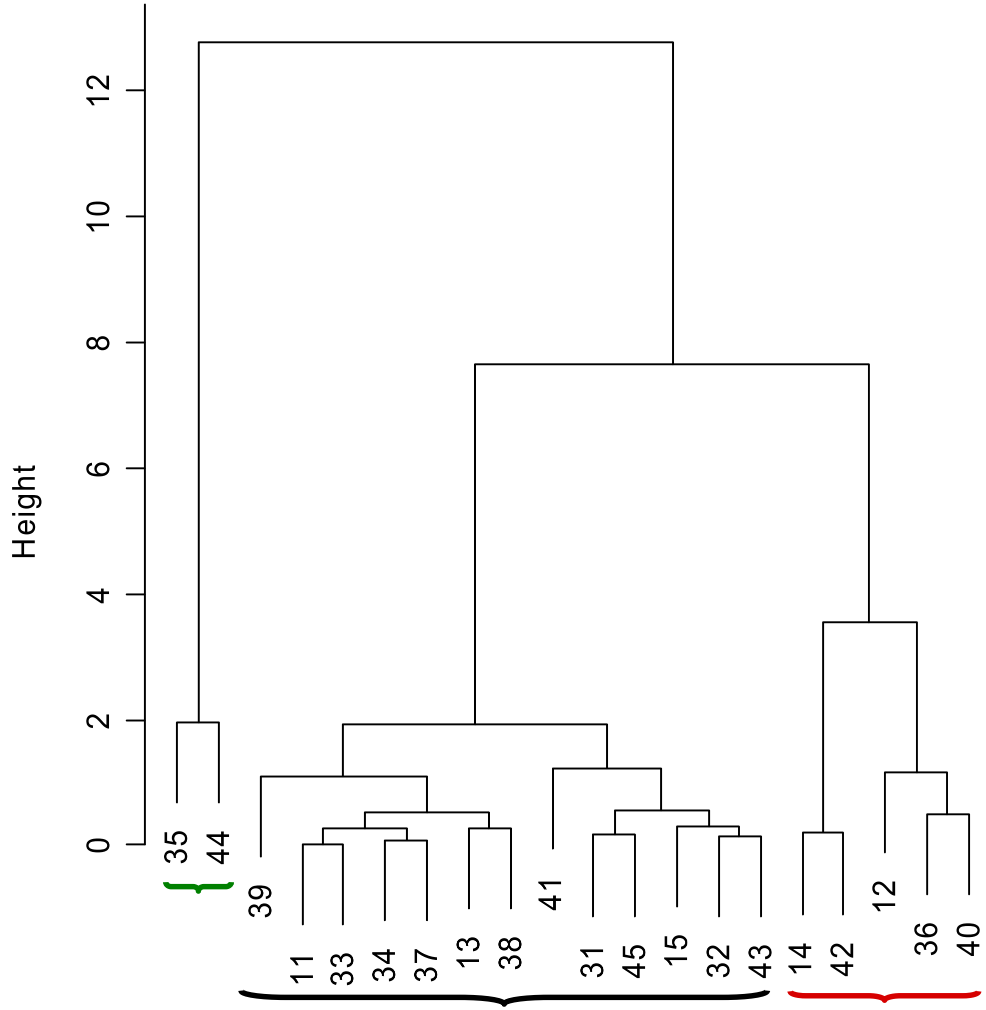
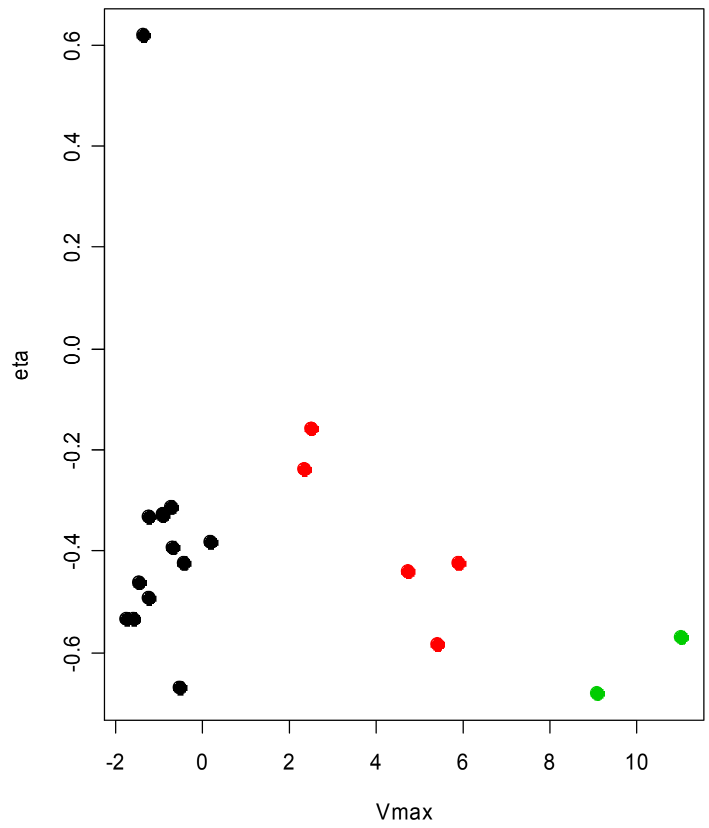
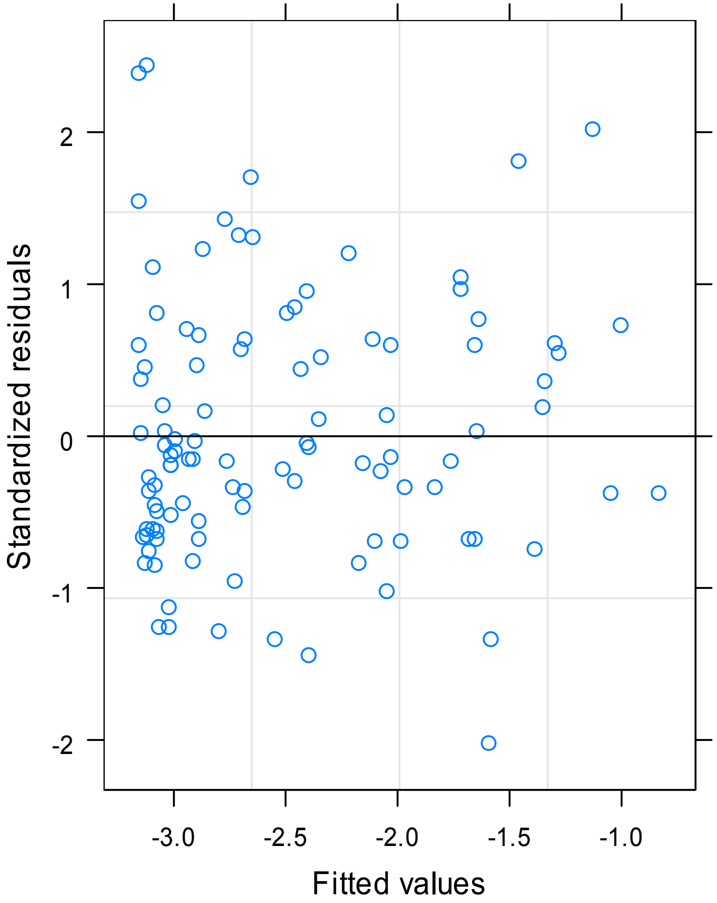
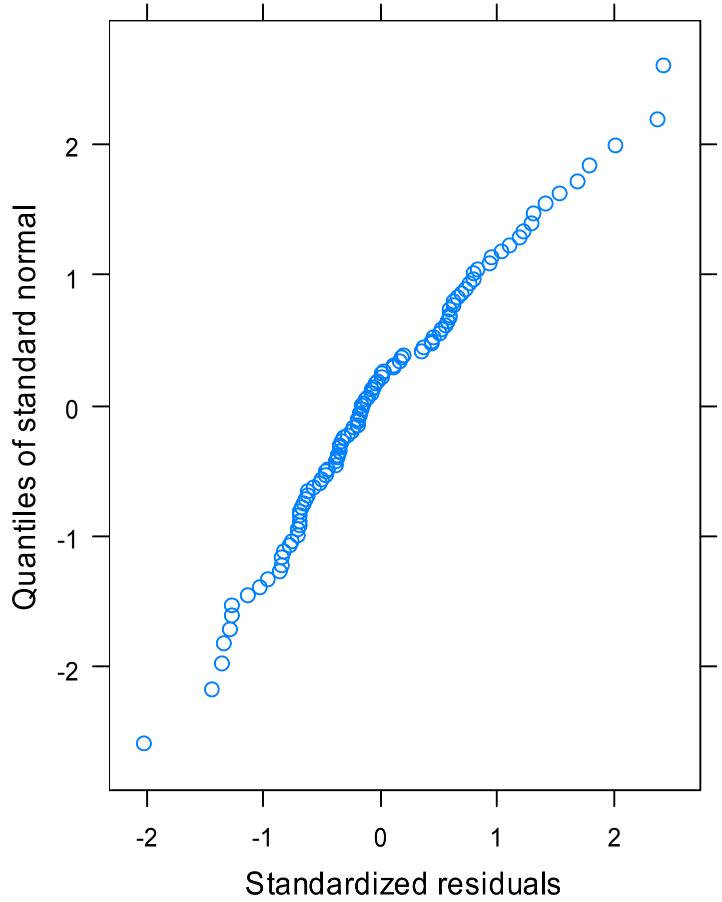


# Nonlinear mixed effect model
# On "stable" subpopulation (quality)



# Nonlinear mixed effect model
# On “stable” subpopulation (prediction)

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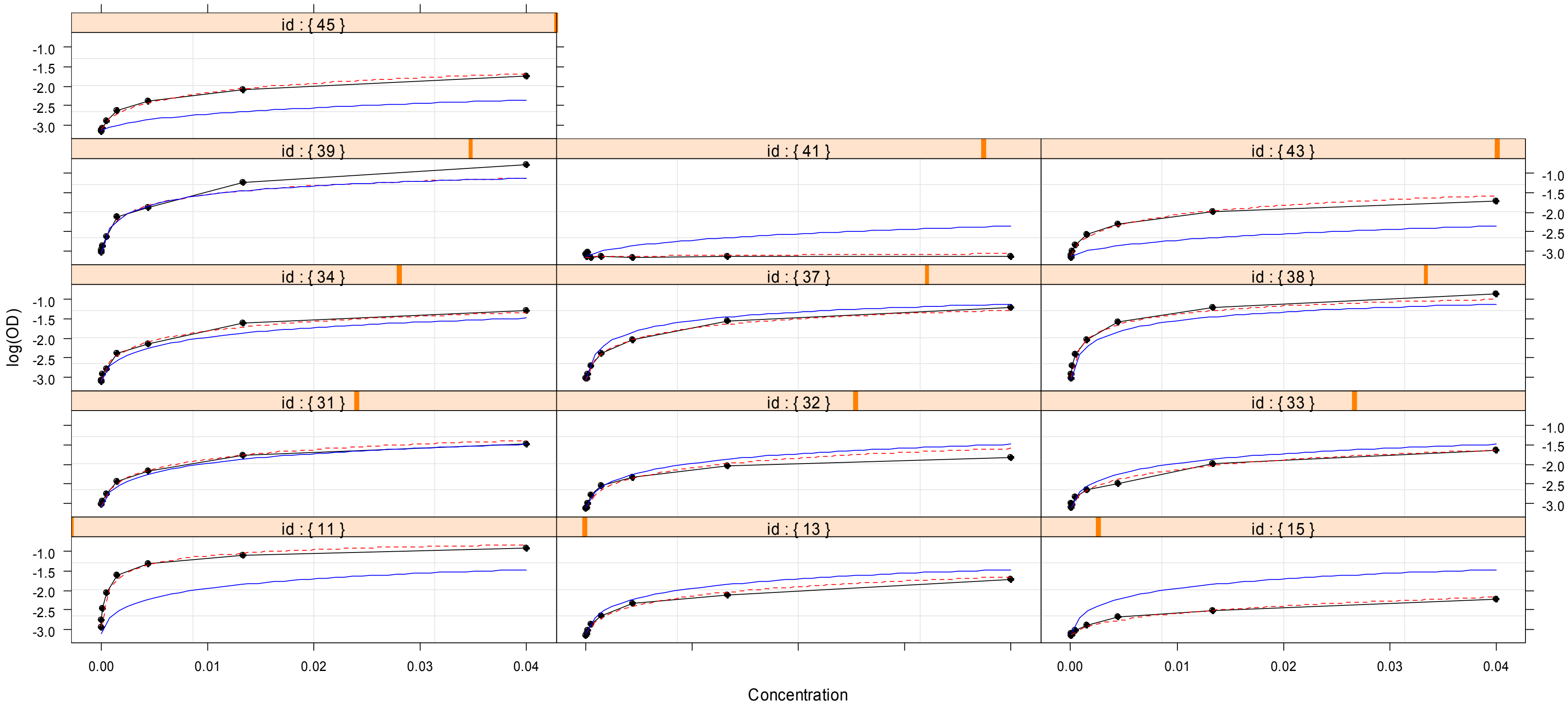

# Nonlinear mixed effect model
# Resume

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- Mechanistic model with clear interpretation of parameters
- Enables to identify cross reactions
- Enhance power of analysis by using all measurements
- No calibration necessary
- Treatment effects in arbitrary units
- Needs nonlinear fitting software

Statistical analysis:

- Stage 1 and Stage 2 are merged together.
  The calibration step is done “en passant”.