Physikalisch-Technische Bundesanstalt

Bayesian Concentration Estimation from ELISA Measurements

Katy Klauenberg, Bernd Ebert, Jan Voigt and Clemens Elster

WGs Data Analysis & Measurement Uncertainty / Tissue Optics & Molecular Imaging

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- Introduction to ELISA
- Design of an International Comparability Study of ELISAs
- Experiment and Model Setup
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- Discussion

Introduction to ELISA

Immunoassay:

- A biochemical test
- Measures even very small amounts of substance
- Uses the highly specific binding between an antibody and its antigen

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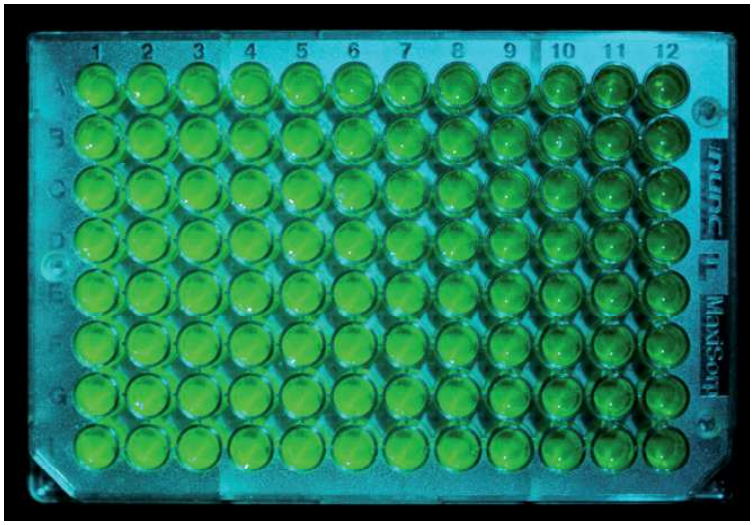

Source: [Voigt et al., 2008, Fig. 1]

Enzyme-Linked ImmunoSorbent Assay: (e.g. [Wild, 2005])

- Detects antibodies or antigens by labelling antibodies with an enzyme to generate a detectable signal (e.g. fluorescence)
- Focus on sandwich ELISAs: detect antigens by ‘sandwiching’ them between 2 antibodies (one is bound to a microplate and the other linked to an enzyme)
- protocol involves a high number of steps (with unknown uncertainty budgets)

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An international comparability study to determine the sources of uncertainty associated with a non-competitive sandwich fluorescent ELISA

James E. Noble[1,*], Lili Wang[2,*], Eleonora Cerasoli[1], Alex E. Knight[1], Robert A. Porter[1], Elaine Gray[3], Chris Howe[4], Elisabeth Hannes[5], Philippe Corbisier[5], Jing Wang[6], Liqing Wu[6], Ilaria Altieri[7], Marina Patriarca[7], Angelika Hoffman[8], Ute Resch-Genger[8], Bernd Ebert[9], Jan Voigt[9], Yasushi Shigeri[10], Maxim S. Vonsky[11], Leonid A. Konopelko[12], Adolfas K. Gaigalas[2] and Marc J.A. Bailey[1]

[1] Analytical Science Group, National Physical Laboratory, Teddington, UK
[2] Biochemical Science Division, National Institute of Standards and Technology, Gaithersburg, MD, USA
[3] Biotherapeutics, National Institute for Biological Standards and Control, Potters Bar, UK

estimation of a protein cytokine concentration using a fluorescent ELISA.
Methods: The intercomparison study method was based on a non-competitive sandwich immunoassay with an enhancement step to generate a fluorescent readout. The intercomparison was performed in two phases, with the uncertainty of the instrument determined separately from that of the assay. The 11 laboratories participating in the study represented national metrology institutes or nominated expert laboratories.
Results: Participants were asked to determine an undisclosed concentration of interferon using a supplied standard. The mean participant estimate and experimental standard deviation of the mean was 3.54 ± 0.22 mg/L, with the spread of data ranging

Experiment and Model Setup

X Unknown Concentration

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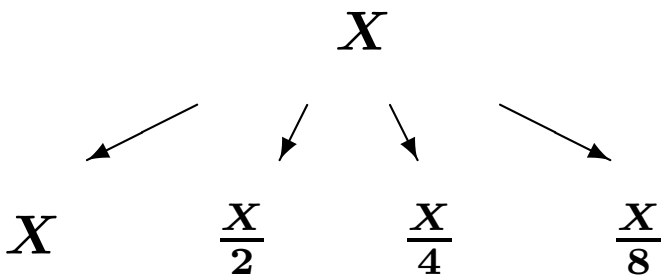

Unknown Concentration

Dilutions

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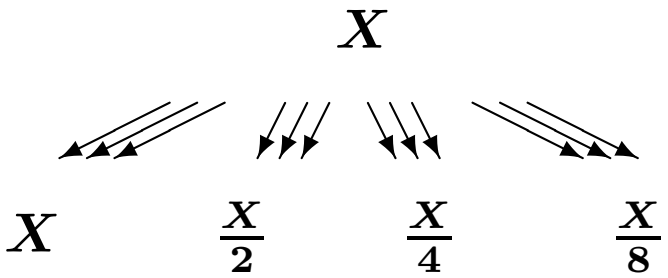

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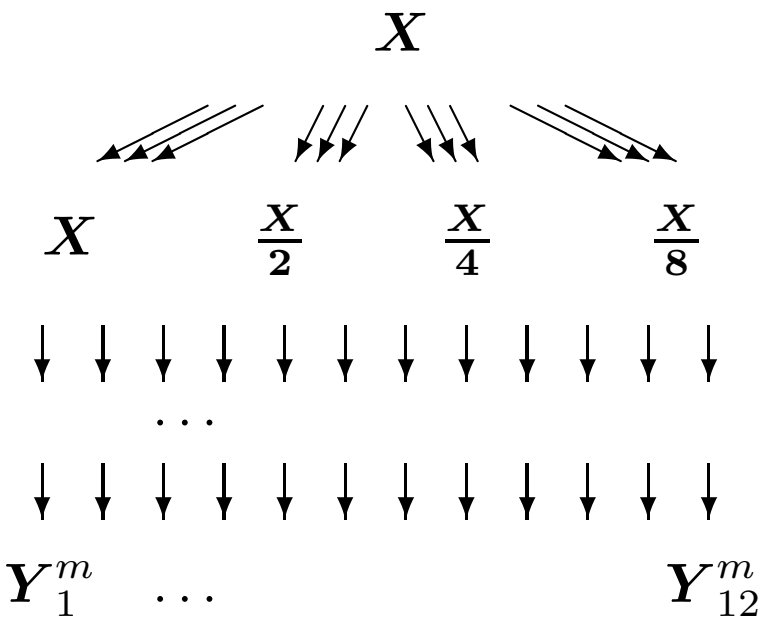

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Dilutions

Chemistry

Fluor. intensity

Experiment and Model Setup

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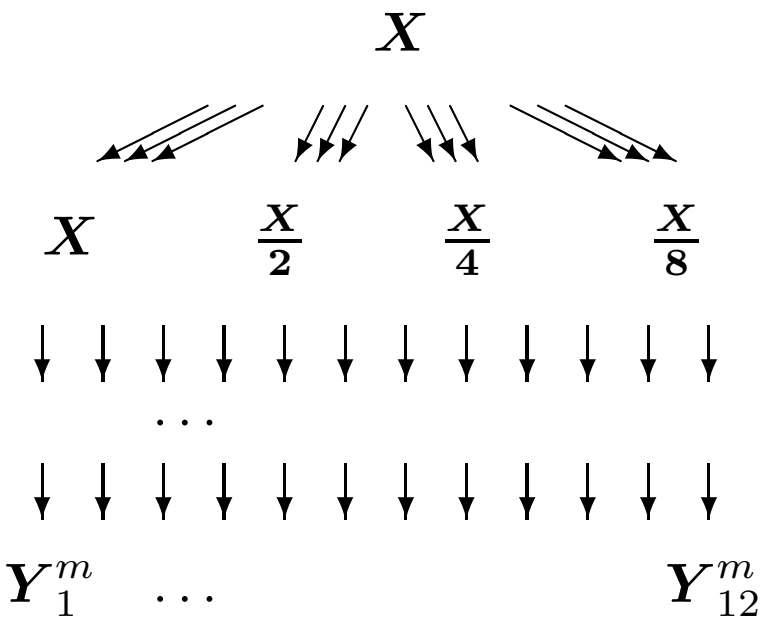

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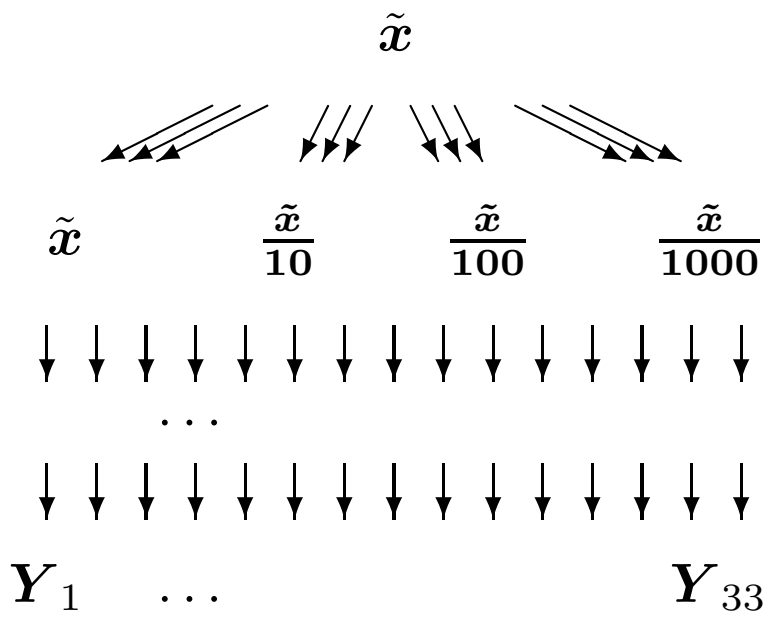

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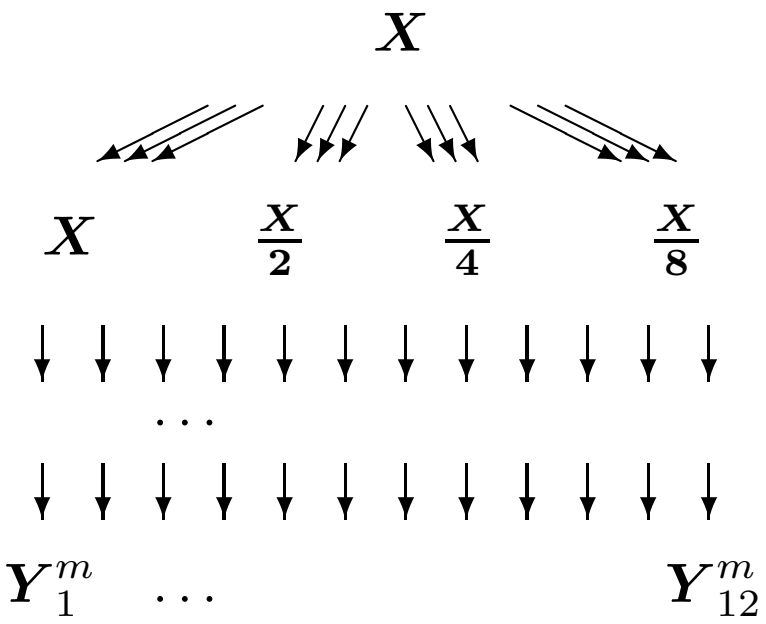

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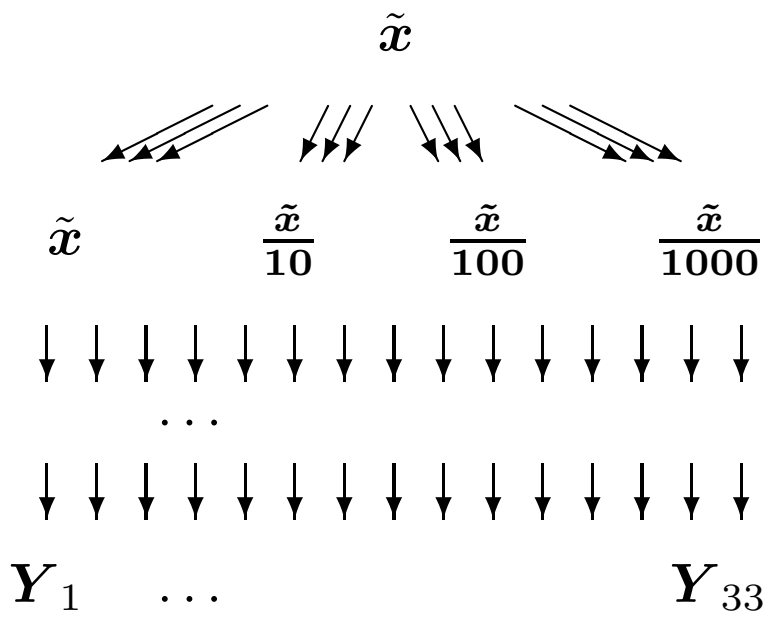

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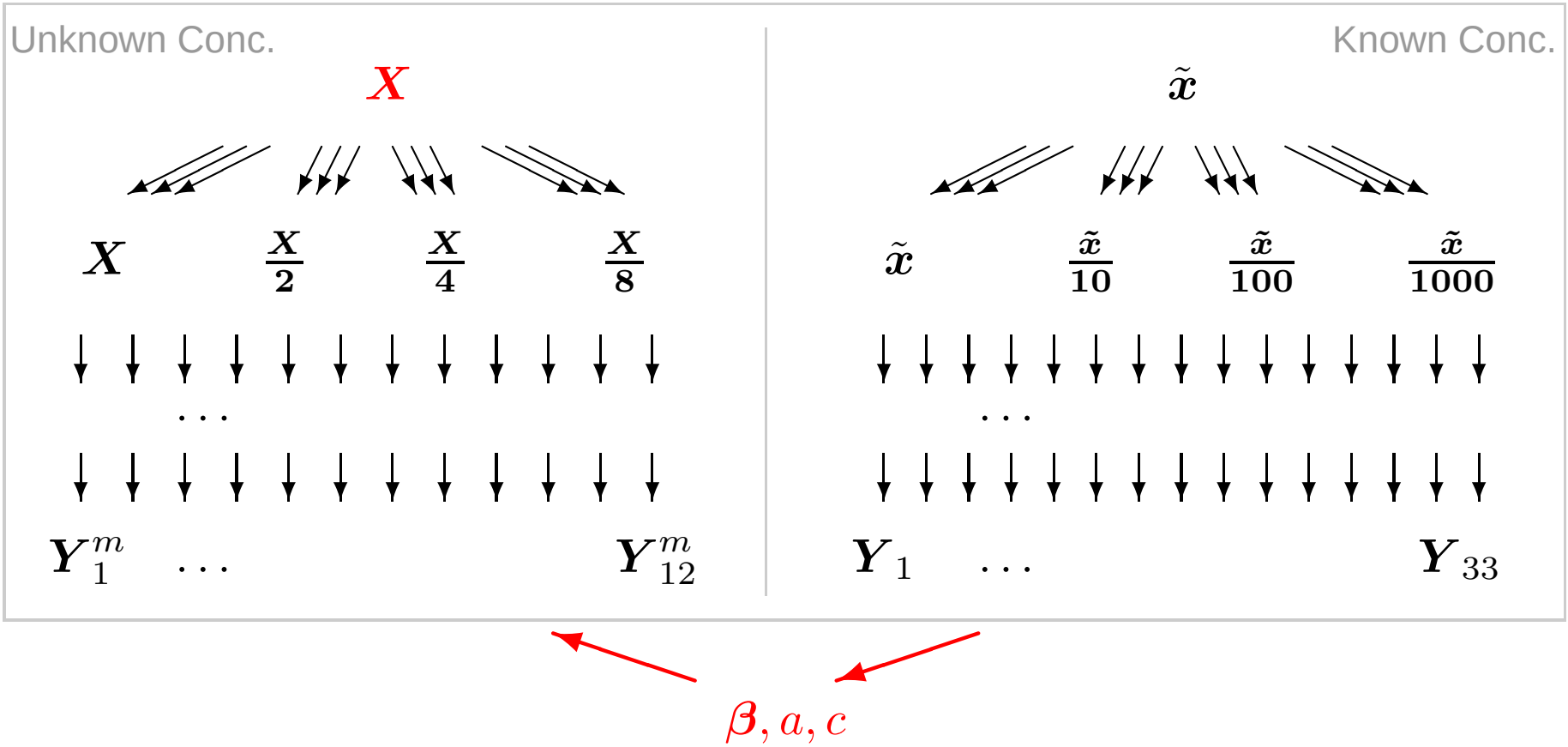

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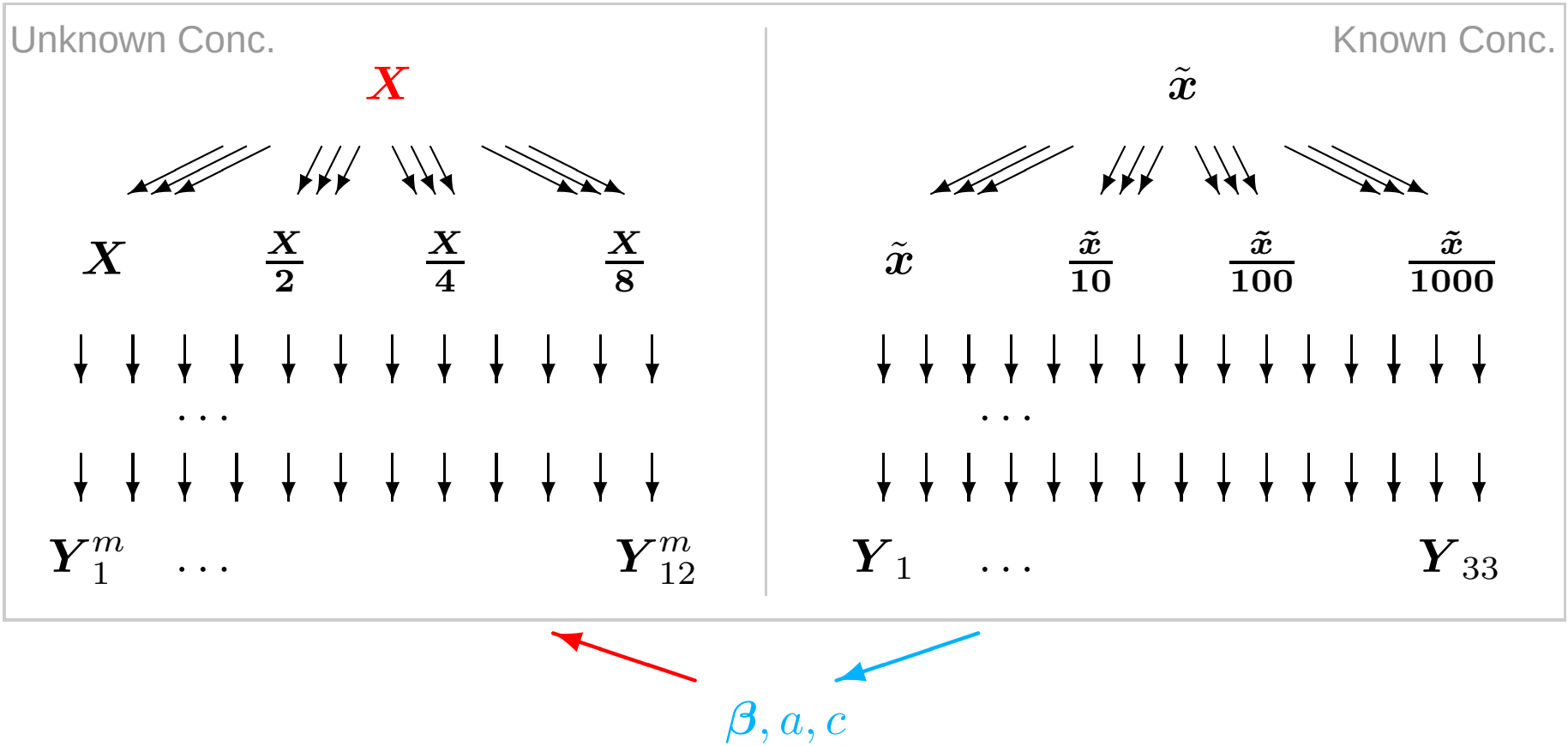

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Bayesian Framework:

$$P(\boldsymbol{\beta}, a, c|\boldsymbol{Y}, \tilde{\boldsymbol{x}}) \propto P(\boldsymbol{Y}|\tilde{\boldsymbol{x}}, \boldsymbol{\beta}, a, c)P(\boldsymbol{\beta})P(a)P(c)$$

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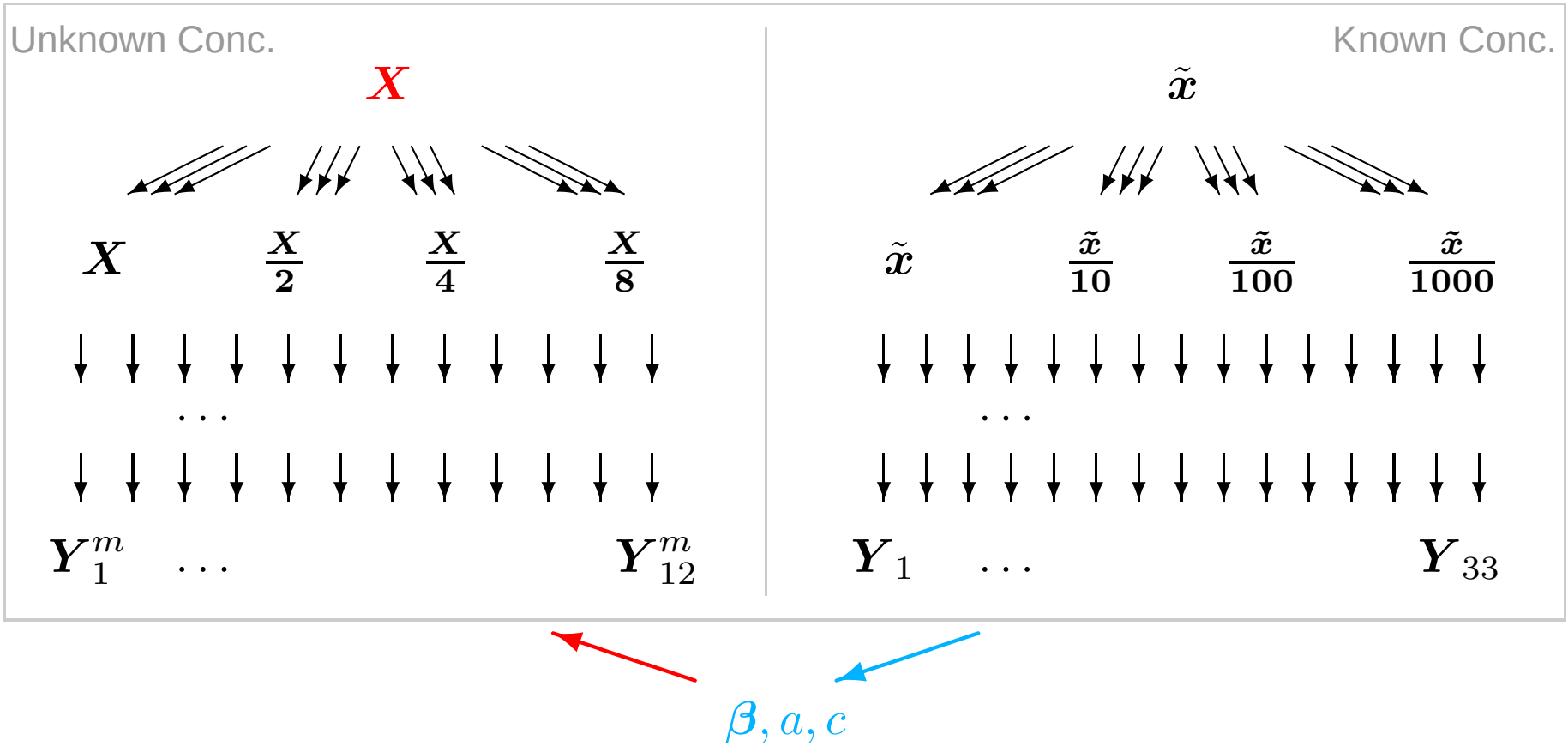

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Bayesian Framework:

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Advantages of Bayesian Approach

- coherent approach for a complex problem
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Metrologists would apply GUM / GUM S1:
([BIPM et al., 2008], MC sampling from error model)

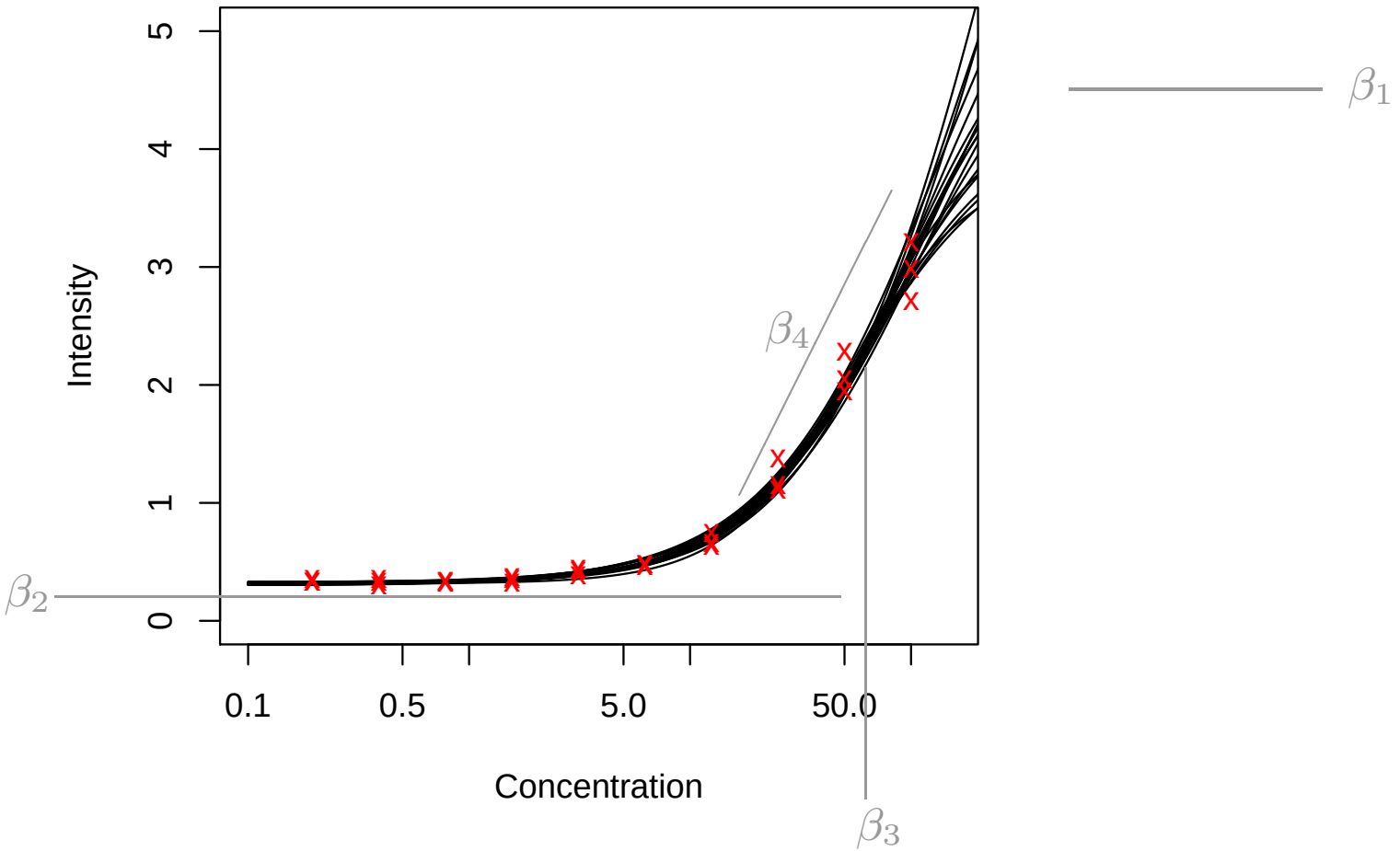
Calibration: Error model needs to be known in advance.

Estimation: Prior knowledge (on concentration) can't be included.

How to join 12 concentration estimates to a single one?

Bayesian Calibration and Concentration Estimation

Sigmoid Calibration Function f



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  - if any well converts all FDP to flourescein it contains at most 2nmol flourescein
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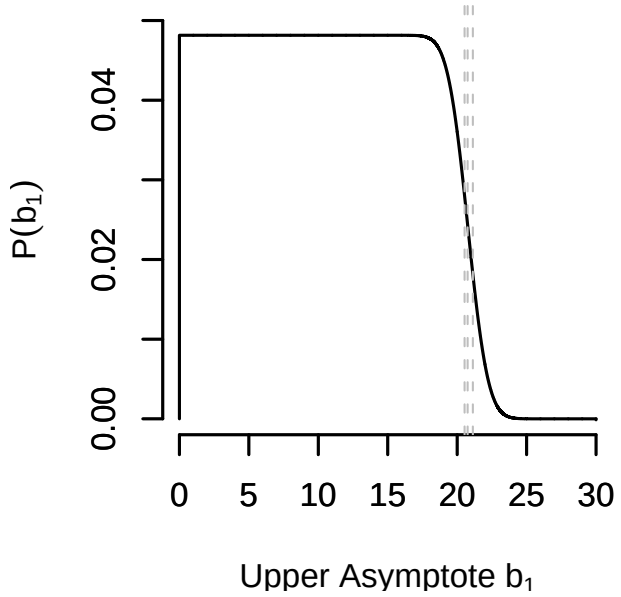
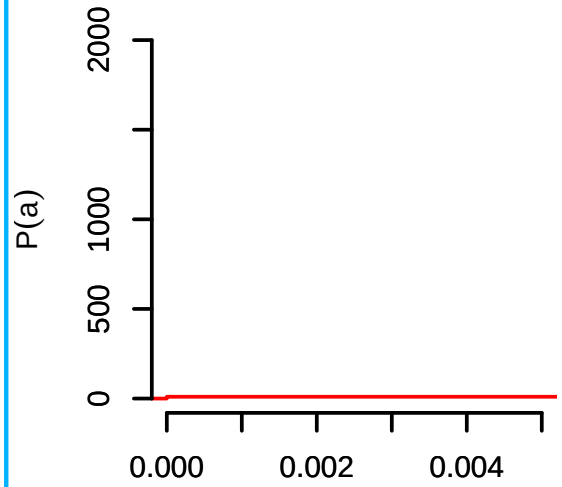
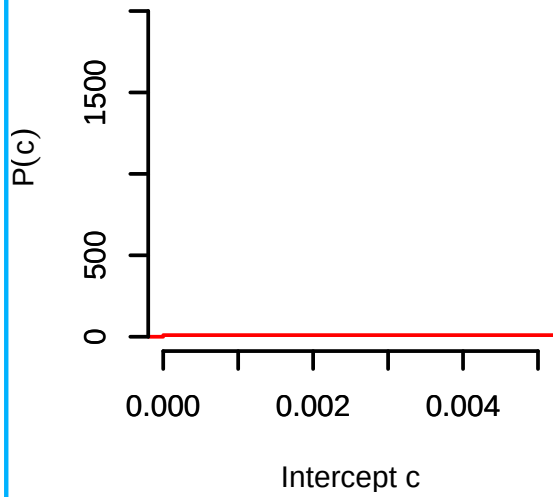


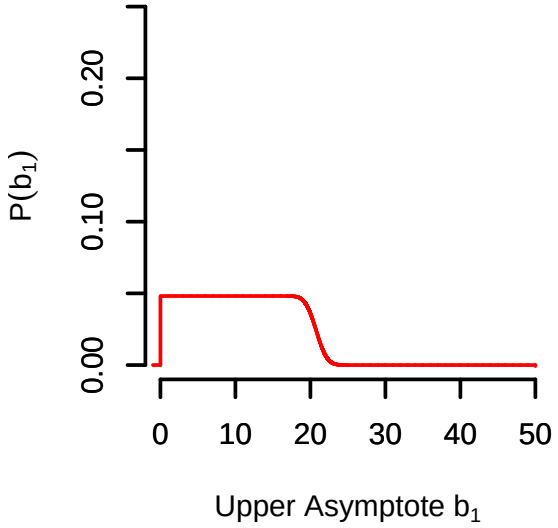
Example: Bayesian Calibration

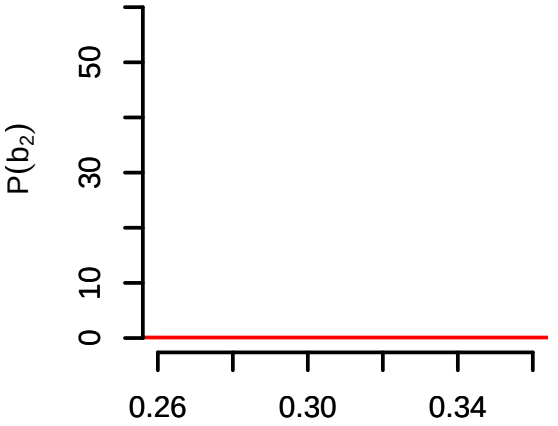
Priors for Error Model

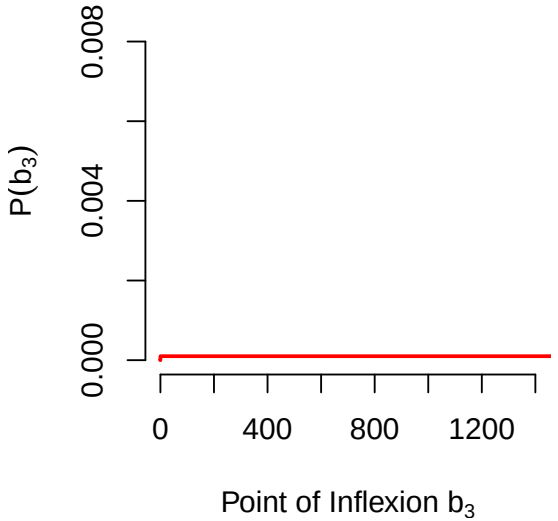


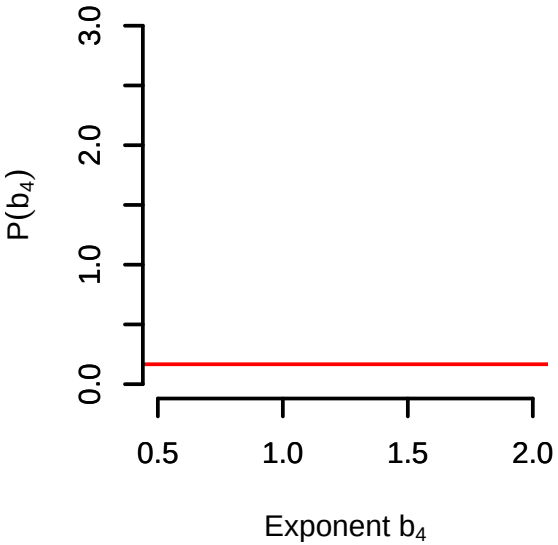


Priors for Parameters of f









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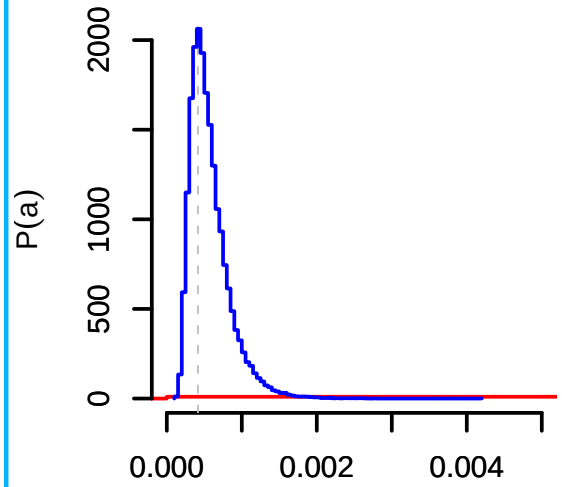
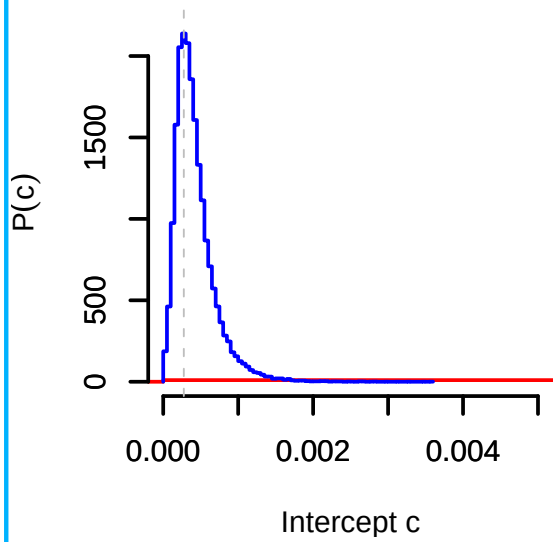
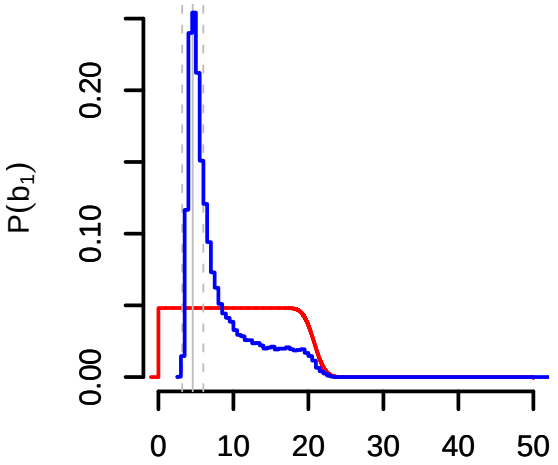
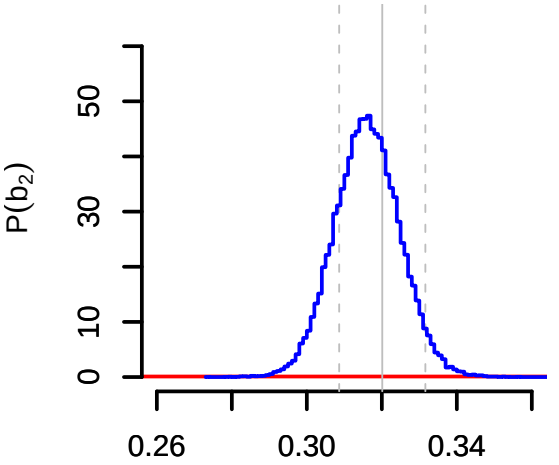
Post. for Error Model

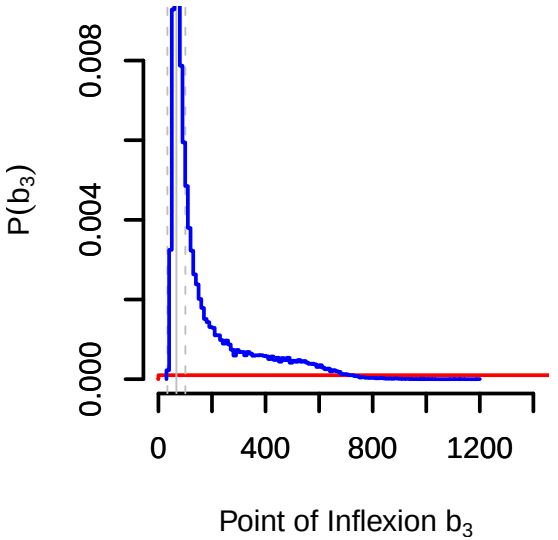


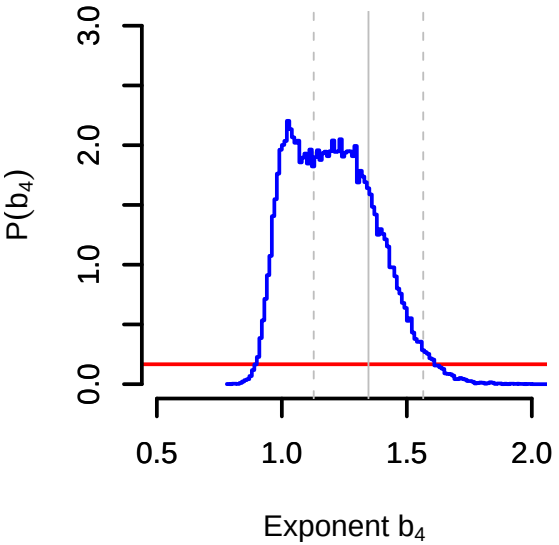


Posteriors for Parameters of f

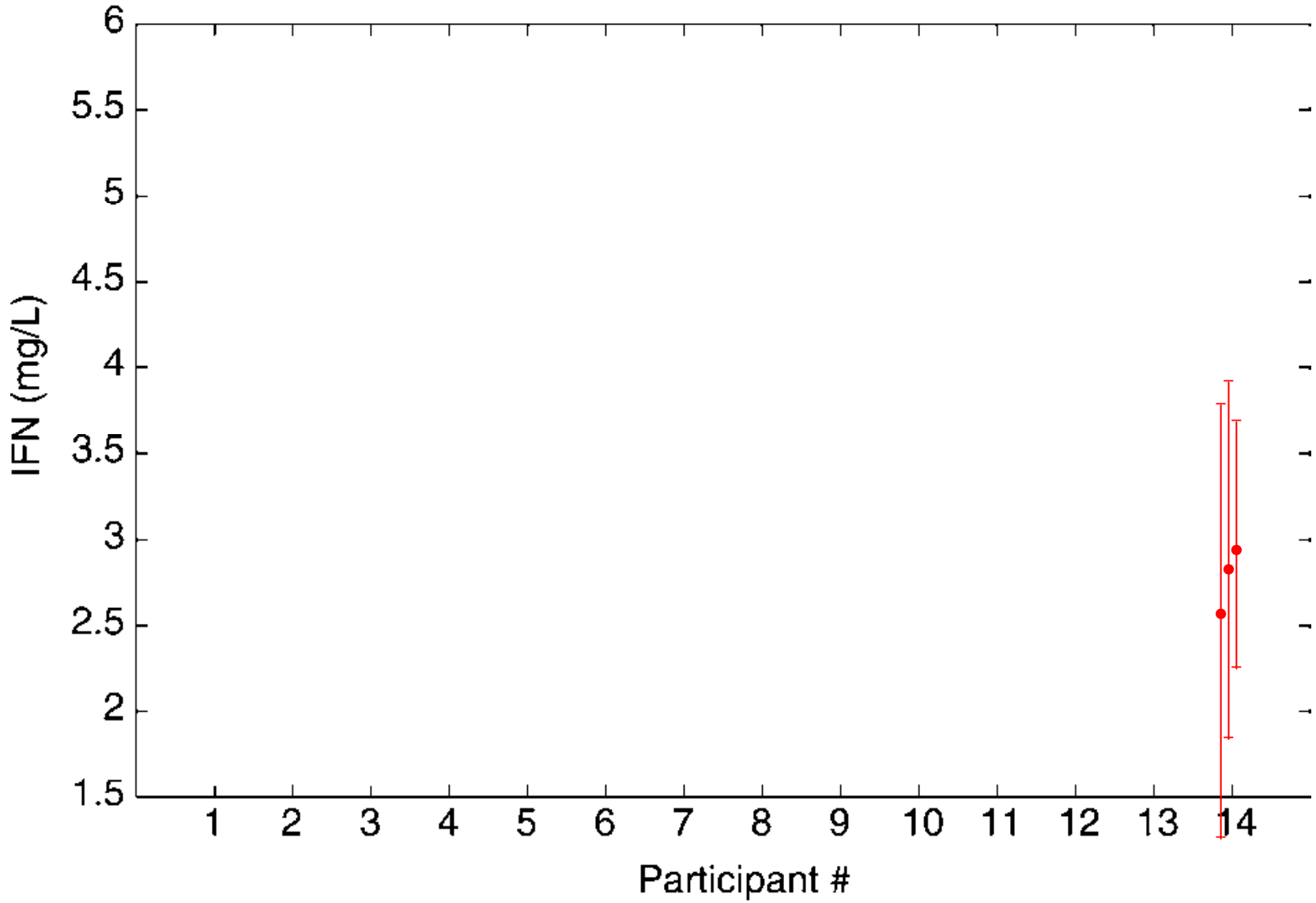








Bayesian Concentration Estimation



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Bayesian approach:

- allows coherent calibration and estimation of ELISA measurements
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- gives (overall) similar results to GUM S1 (under equivalent assumptions)
- gives coherent concentration estimates for PTB data

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Thank you!

[BIPM et al., 2008] BIPM, IEC, IFCC, ILAC, IUPAC, I., IUPAP, and OIML (2008). Evaluation of measurement data – Supplement 1 to the 'Guide to the expression of uncertainty in measurement' – Propagation of distributions using a Monte Carlo method. Joint Committee for Guides in Metrology, Bureau International des Poids et Mesures, JCGM 101:2008, 1st edition. [online] http://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf.

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Add On

sigmoid function:

$$f(\boldsymbol{x}, \boldsymbol{\beta}) = \beta_1 + \frac{(\beta_2 - \beta_1)}{(1 + (\boldsymbol{x}/\beta_3)^{\beta_4})}$$