# Optimal design of in vitro time kill curve experiments for the evaluation of antibiotic effects

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## Background and Objective

In order to integrate information of antibiotic effects from in vitro time kill curve experiments semi-mechanistic pharmacokinetic-pharmacodynamic (PKPD) models are of interest [1,2]. While informative compared to basic MIC measurements, that provide only a point estimate of the antibiotic effect, these experiments are labour intensive as bacteria counts are measured at a range of different concentrations and time points. In addition, autocorrelation (AC) may be of importance when optimizing time-kill curve experiments due to time dependent fluctuations in bacterial counts.

This work aims to optimize a common design for five modelled antibiotics in order to provide an efficient generalized experimental setup.

## Methodology

### •Model implementation

A previously developed model [1] was implemented in the PopED optimal design software [3] for five different antibiotics (Figure 1) that shared bacteria-specific parameters.

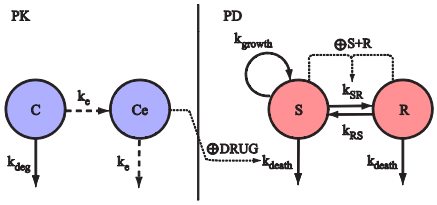


**Figure 1** The antibiotics growth model by Nielsen *et al* feature two bacterial compartments (red), one susceptible growing (S) and one resting drug resistant (R). The drug affect the bacterial death rate through a delay compartment.

### •Autocorrelation

Time dependent AC between sample points was diagnosed by residual error plots (Figure 3) and estimated by implementing AR(1) residual autocorrelation (Equation 1) [4] for the PKPD model using NONMEM 7 [5]. Changes in base model were made accordingly.

**Equation 1** *AR(1) autocorrelation*

$$corr(\varepsilon_{t_1}, \varepsilon_{t_2}) = e^{(-|t_1 - t_2| * k_{corr})}$$

**Equation 2** *D-optimal OFV*

$$OFV = \left| \sum_{i=1}^{n} FIM_i \right|^{-1}$$

**Equation 3** *Efficiency*

$$Efficiency = \frac{OFV_{opt}^{\,1/p_{opt}}}{OFV_{base}^{\,1/p_{base}}}$$

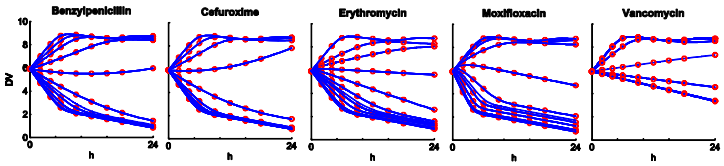


**Figure 2** Model-predicted response curves for the five studied antibiotics. The original design included 9 unique sampling points studied at 9-10 concentrations.

### •Sampling schedule optimization

The optimization was performed simultaneously for all studied antibiotics in order to give a general design. For comparison single antibiotic models were optimized separately.

A D-optimal design criterion with a reduced FIM calculation was utilized with parameters independent across sub-models (Equation 2). Designs were compared based on efficiency (Equation 3).

## Results

### •Autocorrelation

A clear AC between the residuals was evident in the original data (Figure 3), with the AC half-life estimated to 7.5 h.

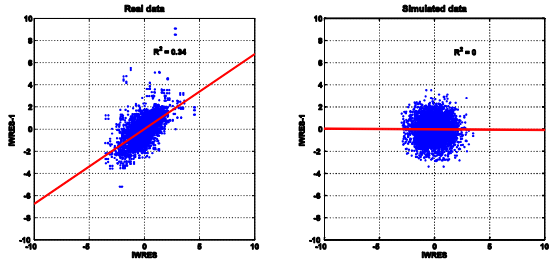


**Figure 3** Plot of IWRES, the sample prediction error weighted by the individual residual standard error, between samples for real and simulated data. The residuals of the real data exhibit a clear AC-pattern ($R^2$=0.34), which is lost when simulating and re estimating with a model lacking AC.

### •Sampling time optimization

The optimal sampling times differed between optimizations lacking and including AC with the former including five unique sampling times and the latter seven. The combined efficiency for the optimal design developed without consideration of AC was 102% when evaluated on a model with AC while the corresponding efficiency for the design that considered AC was 123%. A 33% reduction in the number of sample points (9 → 6) is possible with a small change in efficiency and parameter uncertainty (CV) (Figure 4)

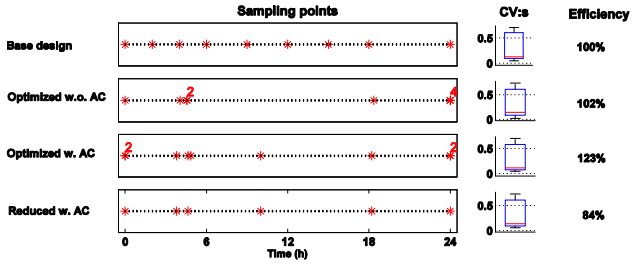


**Figure 4** Sampling points, box-plot of parameter CV:s and efficiency for: base design, optimized design without considering AC, design optimized considering AC , and reduced optimal design without clustered samples. Raised numbers indicate multiple of sample.

### •Efficiency for single antibiotics

When the optimized designs were evaluated for one antibiotic at a time, the common design that included AC increased the efficiency with 18-35% compared to the base designs for 4 of 5 drugs (Benzylpenicillin, Cefuroxime, Erythromycin and Vancomycin) (Figure 5)

For Moxifloxacin, efficiency decreased by 11% compared to the base design, although the design with AC was superior to the design without AC. In all cases the reduced design has a lower efficiency compared to the full design, whereas optimizing the design for one antibiotic at a time provides the highest efficiency for that specific spieces.

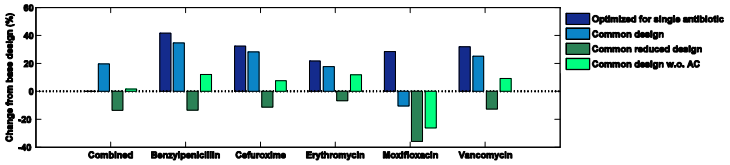


**Figure 5** Change in efficiency compared to base design for single antibiotic design, combined design with AC, reduced combined design with AC, and combined design without AC, for combined and single antibiotic models.

## References

[1] Nielsen EI, Viberg A, Lowdin E, Cars O, Karlsson MO, Sandstrom M. Semimechanistic Pharmacokinetic/Pharmacodynamic Model for Assessment of Activity of Antibacterial Agents from Time-Kill Curve Experiments. Antimicrob Agents Chemother. 2007 January 1, 2007;51(1):128-36.

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[3] Nyberg J, Ueckert S, Karlsson MO, Hooker A. PopED v. 2.11. 2010. http://poped.sourceforge.net/

[4] Karlsson M, Beal S, Sheiner L. Three new residual error models for population PK/PD analyses. Journal of Pharmacokinetics and Pharmacodynamics. 1995;23(6):651-72.

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## Conclusions

- Autocorrelation was evident in original data.
- Lower performance of designs lacking autocorrelation
- Possible to reduce the number of sample points with little reduction in efficiency
- The proposed general design appears to be sufficient for a range of antibiotics with different mechanism of action