Standardized, Quality Assured Time-Kill Curve Analysis and Pharmacodynamic Functions of Different Antibiotics for \textit{in-vitro} Evaluation of Treatment Regimens for \textit{Neisseria gonorrhoeae}

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Time-kill curves for *Neisseria gonorrhoeae*

**Challenges:**
- Fastidious bacteria difficult to grow standardized in liquid broth
- Synchronized growth phase for all strains needed
- Interpretation requires expert knowledge
- Normally very low throughput (colony counting!)
Pharmacodynamic analysis of *in-vitro* time-kill data

Estimating pharmacodynamic parameters from time-kill data (Regoes et al., 2004):

- $\psi_{\text{max}}$: maximal growth in absence of antimicrobial
- $\kappa$: slope of Hill function
- $\psi_{\text{min}}$: minimal net growth at high concentrations
- zMIC: concentration that results in zero growth
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Workflow of the novel time-kill assay

1. **Bacterial growth rate** vs. ciprofloxacin concentration (ng/mL)
2. **Bacterial counts** over time (hours)
3. **Image** of bacteria on agar plate
4. **Image** of ciprofloxacin molecule
5. **Image** of bacterial culture in broth
6. **Clock** indicating time progression
Workflow of the novel time-kill assay
Workflow of the novel time-kill assay

1. Bacterial growth rate [h⁻¹]
2. Ciprofloxacin concentration [μg/L]
3. Time [h]
4. Bacteria [CFU/mL]
5. Clock

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Development of the novel time-kill assay
Workflow of the novel time-kill assay
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Development of the novel time-kill assay
Time-kill curves in a susceptible strain (DOGK18)

- **Ciprofloxacin**
- **Ceftriaxone**
- **Tetracycline**

Time-kill assay has improved throughput and distinguishes different antimicrobials.

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Development of the novel time-kill assay

Conc. [xMIC]
Time-kill curves in a susceptible strain (DOGK18)

Time-kill assay has improved throughput and distinguishes different antimicrobials
Pharmacodynamic functions for different antimicrobials in DOGK18

- Ciprofloxacin
- Gentamicin
- Spectinomycin
- Benzylpenicillin
- Ceftriaxone
- Cefixime
- Azithromycin
- Chloramphenicol
- Tetracycline
Pharmacodynamic functions for different antimicrobials in DOGK18

Pharmacodynamic functions quantify the results from rapidly bactericidal to bacteriostatic
Application of the novel assay

Genetic resistance determinants, in vitro time-kill curve analysis and pharmacodynamic functions for the novel topoisomerase II inhibitor ETX0914 (AZD0914) in Neisseria gonorrhoeae

Sunniva Förster, Daniel Golparian, Susanne Jacobsson, Lucy Hathaway, Nicola Low, William Shafer, Christian Althaus and Magnus Unemo
Comparison of mutants resistant to ETX0914

Susceptible strain (WHO O)

Isogenic gyrB mutant (OM-5)

Förster et. al. 2015, submitted
Pharmacodynamic comparison of ETX0914 and ciprofloxacin

ETX0914 concentration [mg/L] vs. Bacterial growth rate [h⁻¹]

Ciprofloxacin concentration [mg/L] vs. Bacterial growth rate [h⁻¹]
Conclusions

The time-kill assay works across susceptible strains, resistant mutants and antimicrobial classes. Pharmacodynamic functions can be used to quantify time-kill data. Evaluation of drug candidates (ETX0914) and mutants (gyrB) is possible. Estimated parameters can be used for pharmacodynamic modelling.
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