Hollow Fiber setup – Antimicrobial PK/PD

Workshop at Roche, Basel - November 27th, 2019

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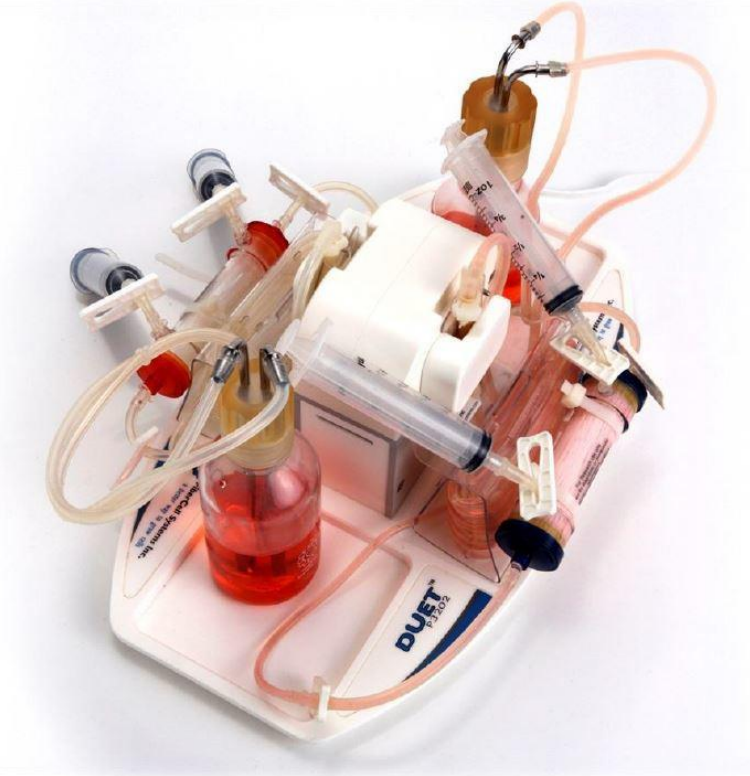
Roche pRED

The Hollow fiber infection model – Objectives

→ Determination of compound efficacy against bacteria while mimicking human PK-PD

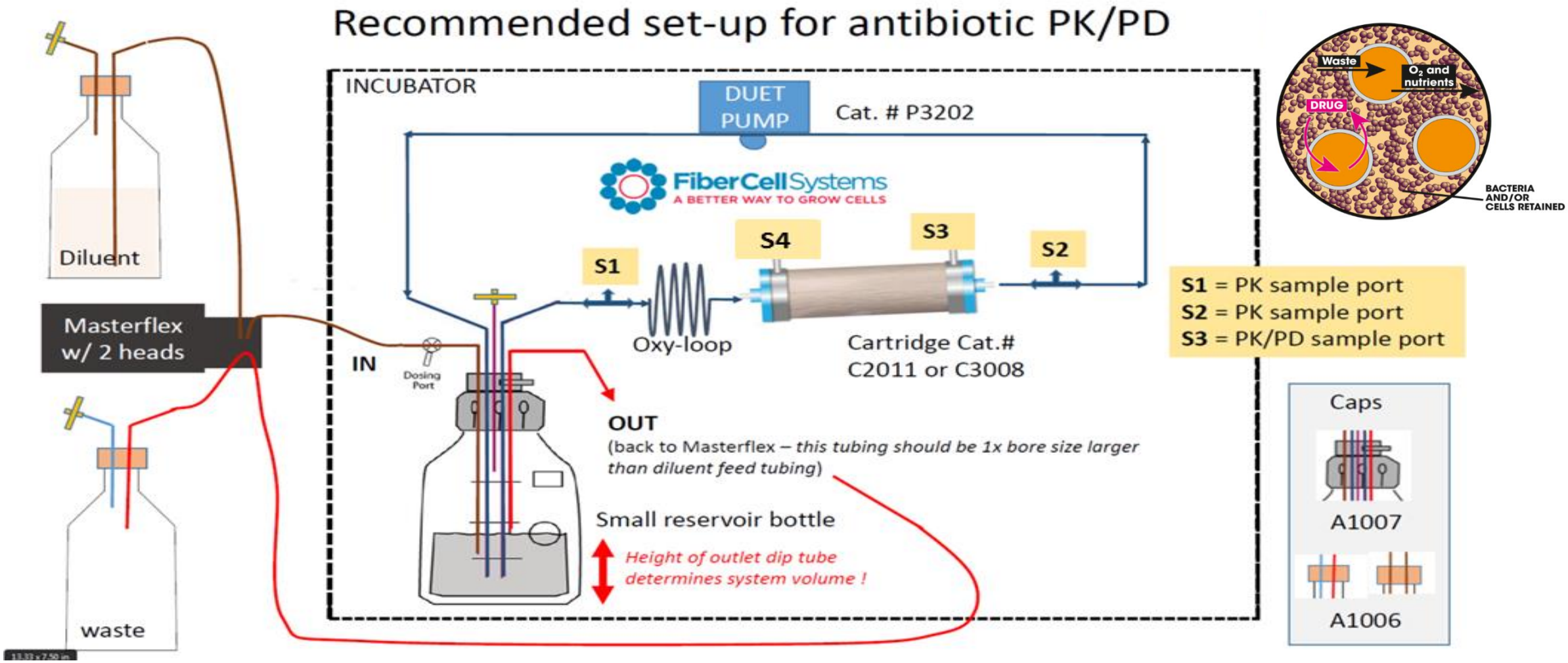
- Evaluation of antibiotic resistance emergence
- Determination of efficacious dosing regimen



Source: KDBIO https://www.kdbio.com/products/fibercell-duet-pump/

SET-UP OF THE SYSTEM

Schema of the system



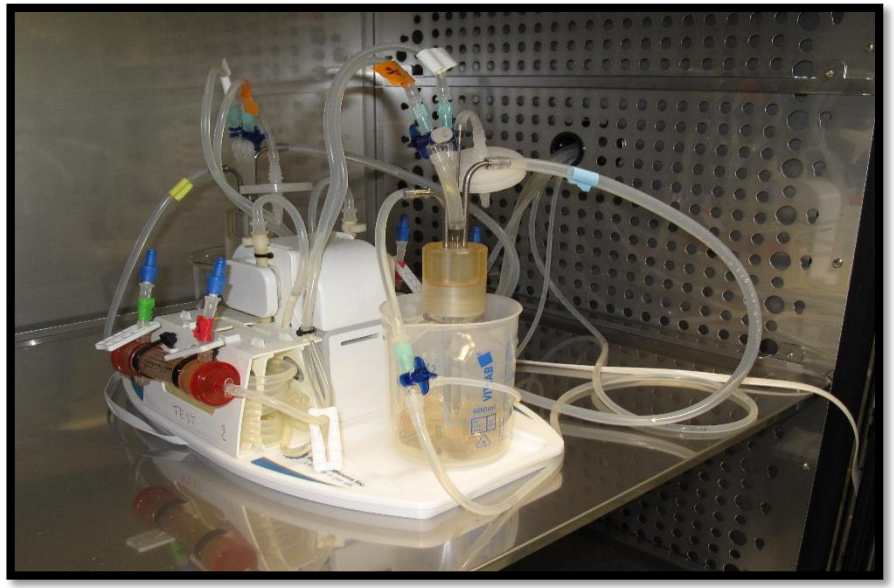
Source: www.fibercellsystems.com

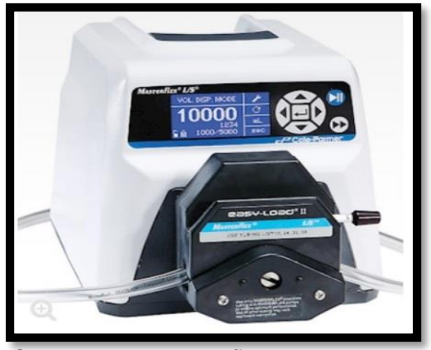
ECS : Extra-capillary space → **S3 – S4**

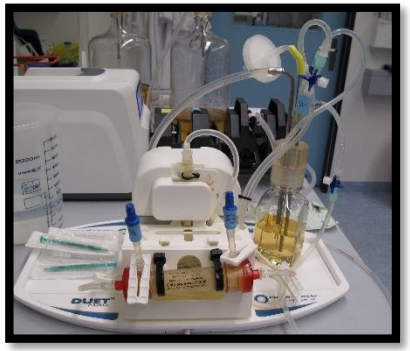
ICS : Intra-capillary space → **S1 – S2**

Installation

- Two cartridges C3008 per Duet pump
- One 4-head Masterflex pump per Duet







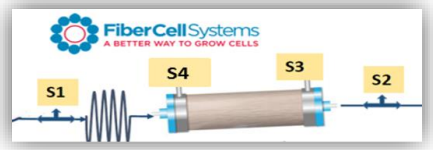
Sources: www.masterflex.com *www.fibercellsystems.com*

Experimental procedure

- **Material preparation**
- **Cartridges washing** *(24h with PBS - 24h with CAMHB)*
- **Inoculum preparation**

Cartridge control (without compound)

Cartridge test (with compound)



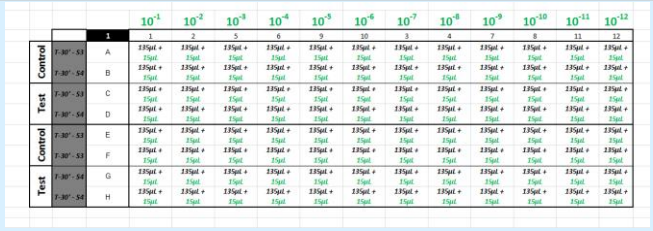
Sampling S3 - S4
(0.5mL each)

Sampling S1 - S2
(0.25mL each)

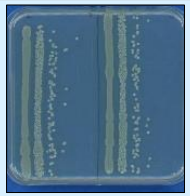
Transfer of the content in **2mL Masterblock - Sampling S3 - S4**

	Control		Test		Control		Test		Control		Test	
	1	2	3	4	5	6	7	8	9	10	11	12
A	T-30' - S3	T-30' - S4	T-30' - S3	T-30' - S4	T6h - S3	T6h - S4	T6h - S3	T6h - S4	T47h - S3	T47h- S4	T47h - S3	T47h- S4
B	T0 - S3	T0 - S4	T0 - S3	T0 - S4	T7h - S3	T7h - S4	T7h - S3	T7h - S4				
C	T30' - S3	T30' - S4	T30' - S3	T30' - S4	T27h - S3	T27h - S4	T27h - S3	T27h - S4				
D	T1h - S3	T1h - S4	T1h - S3	T1h - S4	T28h - S3	T28h - S4	T28h - S3	T28h - S4				
E	T2h - S3	T2h - S4	T2h - S3	T2h - S4	T29h - S3	T29h - S4	T29h - S3	T29h - S4				
F	T3h - S3	T3h- S4	T3h - S3	T3h- S4	T30h - S3	T30h- S4	T30h - S3	T30h- S4				
G	T4h - S3	T4h - S4	T4h - S3	T4h - S4	T31h - S3	T31h- S4	T31h - S3	T31h- S4				
H	T5h- S3	T5h - S4	T5h- S3	T5h - S4	T32h - S3	T32h- S4	T32h - S3	T32h- S4				

Transfer in **96well Microtiter plate - Serial dilution in PBS**



Plating on a MHE square plate (**40µL** in duplicate)



PD samples

Transfer of 300µL in **Eppendorf tubes**

Centrifuge **5' - 13 000rpm - 4°C**

Transfer the supernatant (2x100uL) in **0.5mL Masterblock**

Dilute 1/4 in Internal Standard Solution in a **Matrix Plate** and store at -20°C for **PK analysis**

P1	1	2	3	4	5	6	7	8	9	10	11	12
A	T0 - S1$_1$	T0 - S1$_2$	T0 - S2$_1$	T0 - S2$_2$	T0 - S3$_1$	T0 - S3$_2$	T0 - S4$_1$	T0 - S4$_2$	T30' - S1$_1$	T30' - S1$_2$	T30' - S2$_1$	T30' - S2$_2$
B	T30' - S3$_1$	T30' - S3$_2$	T30' - S4$_1$	T30' - S4$_2$	T1h - S1$_1$	T1h - S1$_2$	T1h - S2$_1$	T1h - S2$_2$	T1h - S3$_1$	T1h - S3$_2$	T1h - S4$_1$	T1h - S4$_2$
C	T2h - S1$_1$	T2h - S1$_2$	T2h - S2$_1$	T2h - S2$_2$	T2h - S3$_1$	T2h - S3$_2$	T2h - S4$_1$	T2h - S4$_2$	T3h - S1$_1$	T3h - S1$_2$	T3h - S2$_1$	T3h - S2$_2$
D	T3h - S3$_1$	T3h - S3$_2$	T3h - S4$_1$	T3h - S4$_2$	T4h - S1$_1$	T4h - S1$_2$	T4h - S2$_1$	T4h - S2$_2$	T4h - S3$_1$	T4h - S3$_2$	T4h - S4$_1$	T4h - S4$_2$
E	T5h - S1$_1$	T5h - S1$_2$	T5h - S2$_1$	T5h - S2$_2$	T5h - S3$_1$	T5h - S3$_2$	T5h - S4$_1$	T5h - S4$_2$	T6h - S1$_1$	T6h - S1$_2$	T6h - S2$_1$	T6h - S2$_2$
F	T6h - S3$_1$	T6h - S3$_2$	T6h - S4$_1$	T6h - S4$_2$	T7h - S1$_1$	T7h - S1$_2$	T7 - S2$_1$	T7 - S2$_2$	T7h - S3$_1$	T7h - S3$_2$	T7h - S4$_1$	T7h - S4$_2$
G	T27h - S1$_1$	T27h - S1$_2$	T27h - S2$_1$	T27h - S2$_2$	T27h - S3$_1$	T27h - S3$_2$	T27h - S4$_1$	T27h - S4$_2$	T28h - S1$_1$	T28h - S1$_2$	T28h - S2$_1$	T28h - S2$_2$
H	T28h - S3$_1$	T28h - S3$_2$	T28h - S4$_1$	T28h - S4$_2$								

PK samples

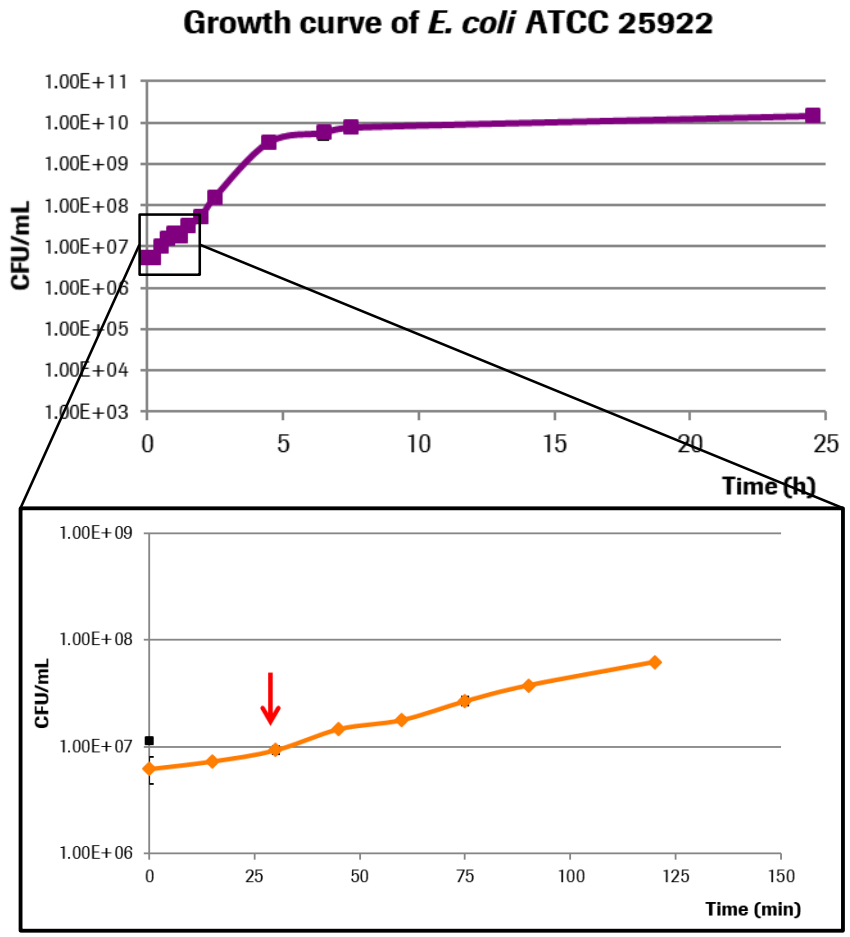
Hollow fiber procedure for the set-up

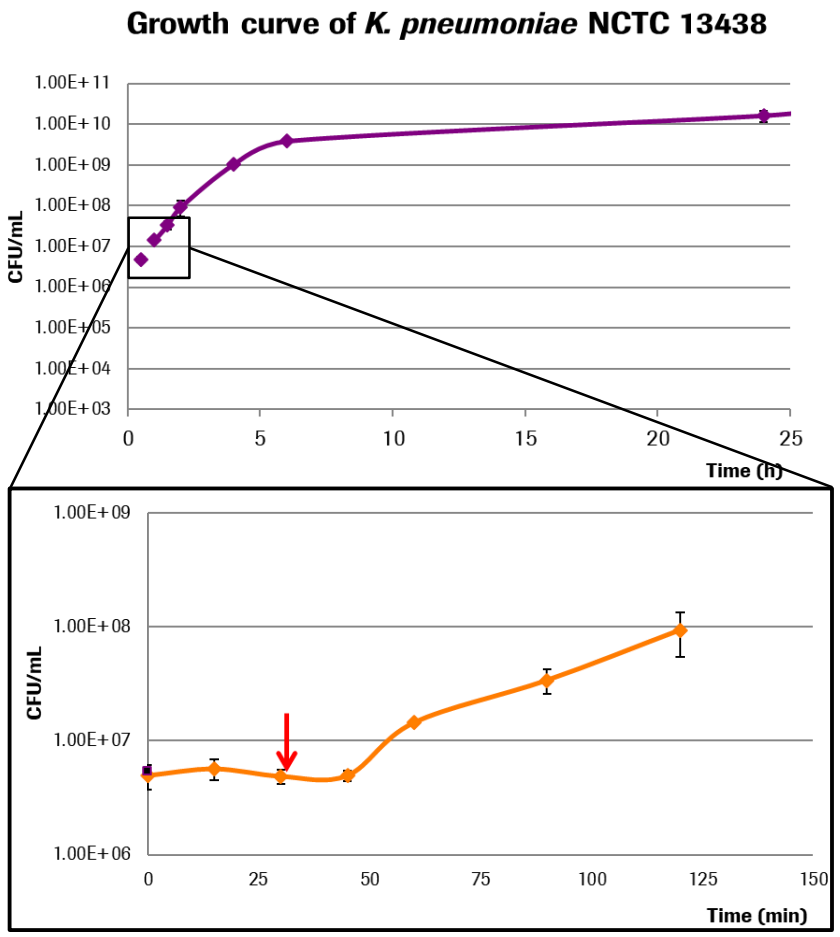
- **Bacterial aspect**
  - Bacterial growth in the cartridge - Lag phase
  - Cartridge inoculation
- **PK aspect**
  - Compound distribution in the system
  - Absorption and elimination of the compound administered by bolus injection or infusion
- **PK/PD studies**
  - Meropenem / *E. coli* ATCC 25922 (w/o elimination)
  - Meropenem / *K. pneumoniae* NCTC 13438 (w/o elimination)
  - Multiple injections over 48h for both strains (with elimination)

BACTERIAL ASPECT

Bacterial growth in ECS

- Starting inoculum: 1.10^7 CFU/mL (mid-log exponential phase culture)





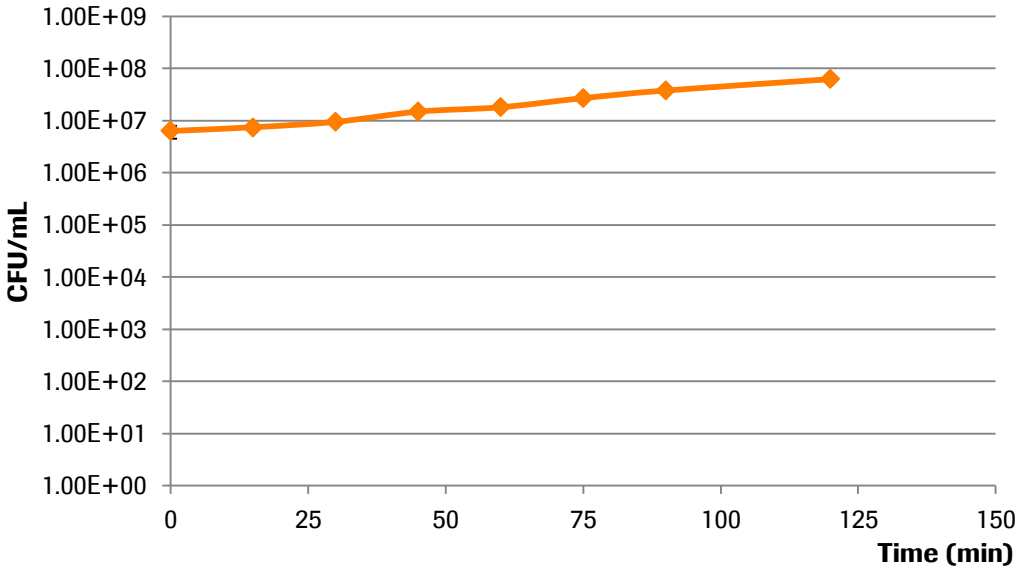
→ Lag phase between 30 and 40 minutes

→ **30 min** selected as starting point for treatment

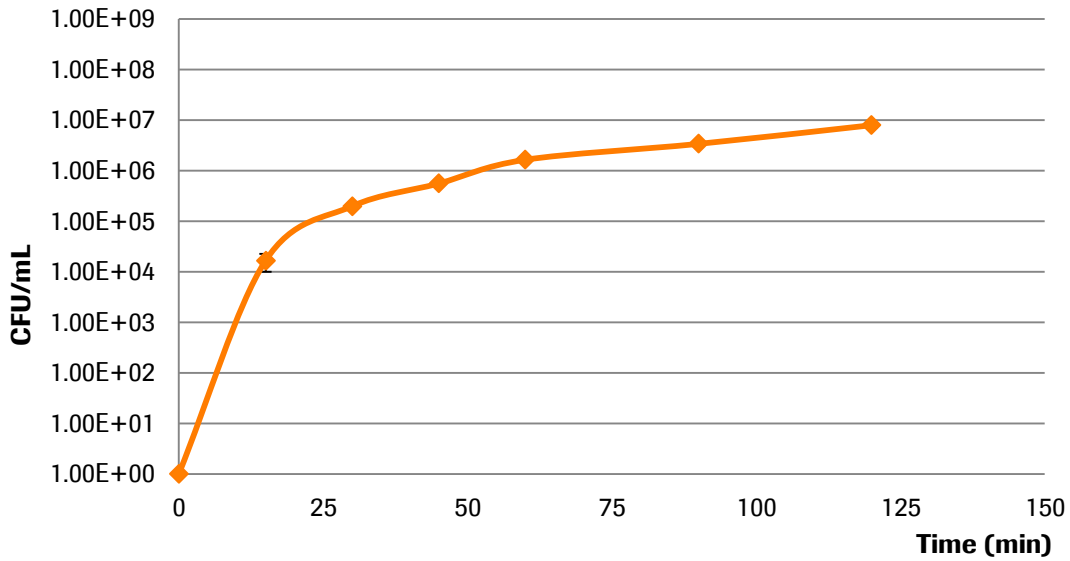
Growth curve – Inoculation volume

→ **Inoculation of *E. coli* ATCC 25922 in ECS**

- Inoculum prepared from a mid-log exponential phase culture
- Target Inoculum size in ECS: **1.10^7 CFU/mL** (in 14 mL)
- Sampling at opposite port of injection



1.4 x10^8 CFU injected in 14 mL



1.4 x10^8 CFU injected in 1.5 mL

→ Small volume of injection is not appropriate for a good homogenization in the cartridge

PK ASPECT

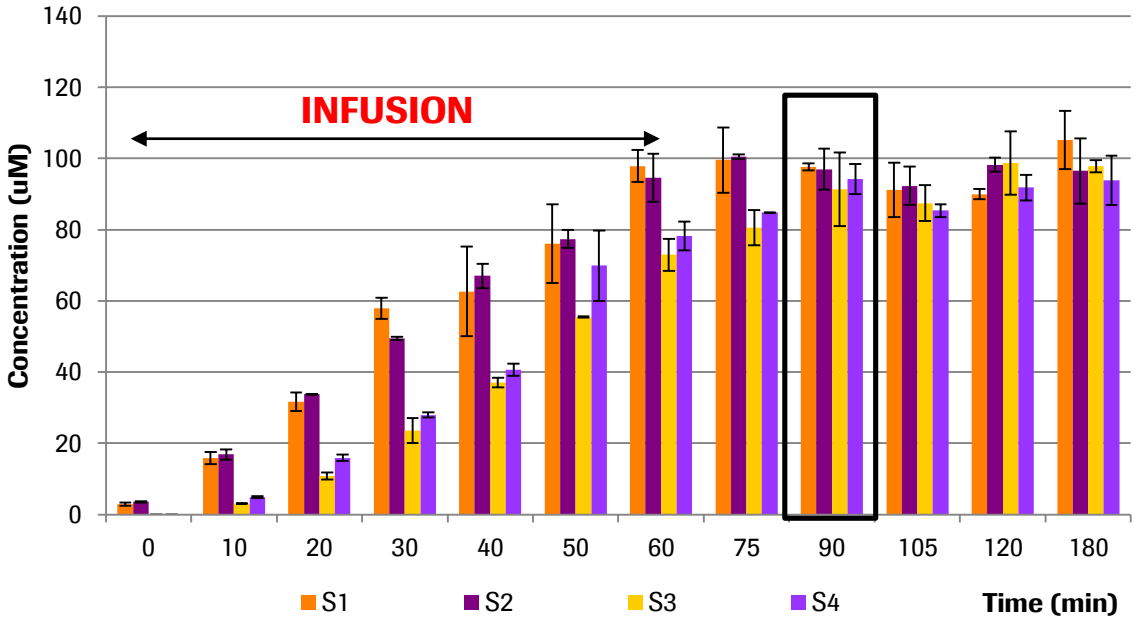
Meropenem distribution in the system

- Simulation of 2g dosage by 1h infusion – without elimination
- Tests of two flow-rates of the Duet pump: 22 and 30

S1 – S2 : ICS

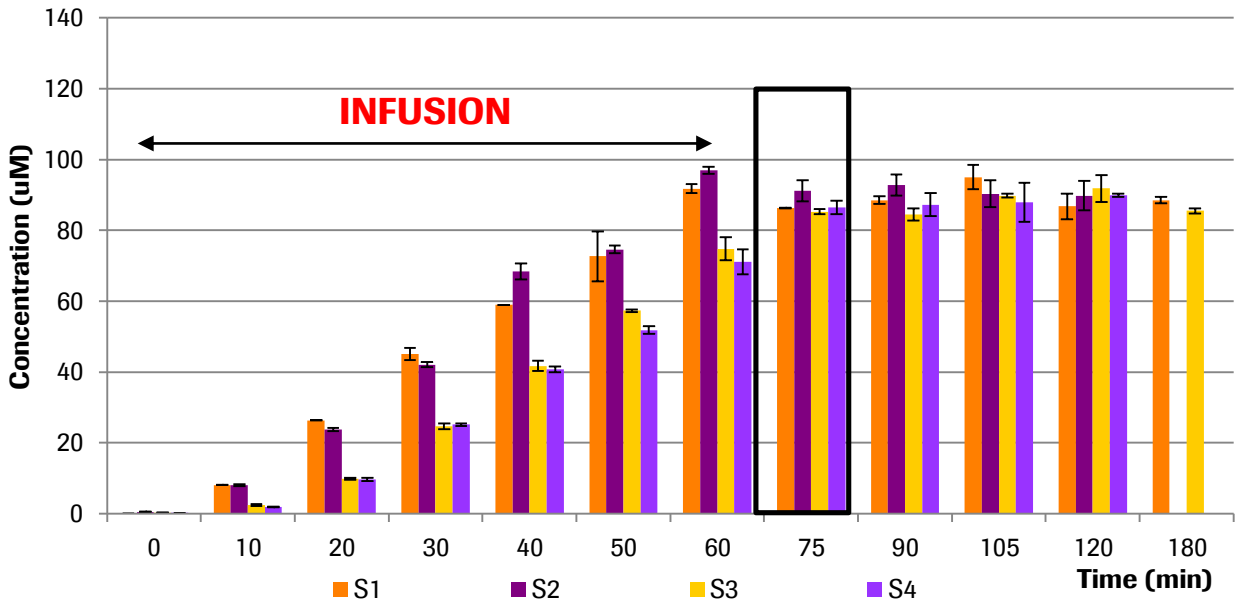
S3 – S4 : ECS

Duet at 22



Equilibrium observed **30min** after end of injection

Duet at 30

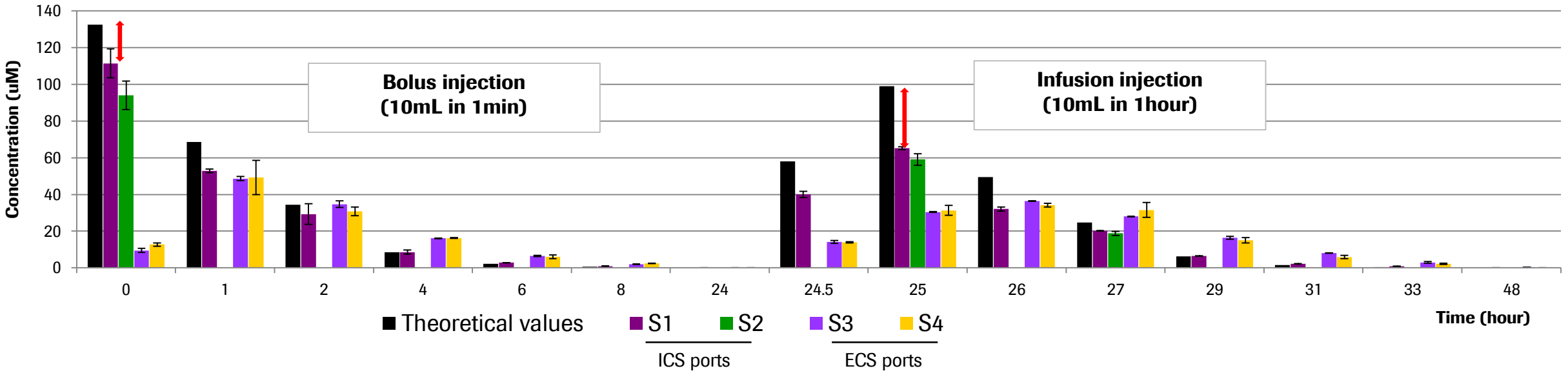


Equilibrium observed **15min** after end of injection

→ Reduction of the time needed for equilibrium after compound injection with a higher flow-rate

Simulation Meropenem elimination

- Simulation of 2g dosage by bolus and 1h infusion
- Half-life: 1h
  - ✓ Cmax: **60 µg/mL ⇔ 137 µM**



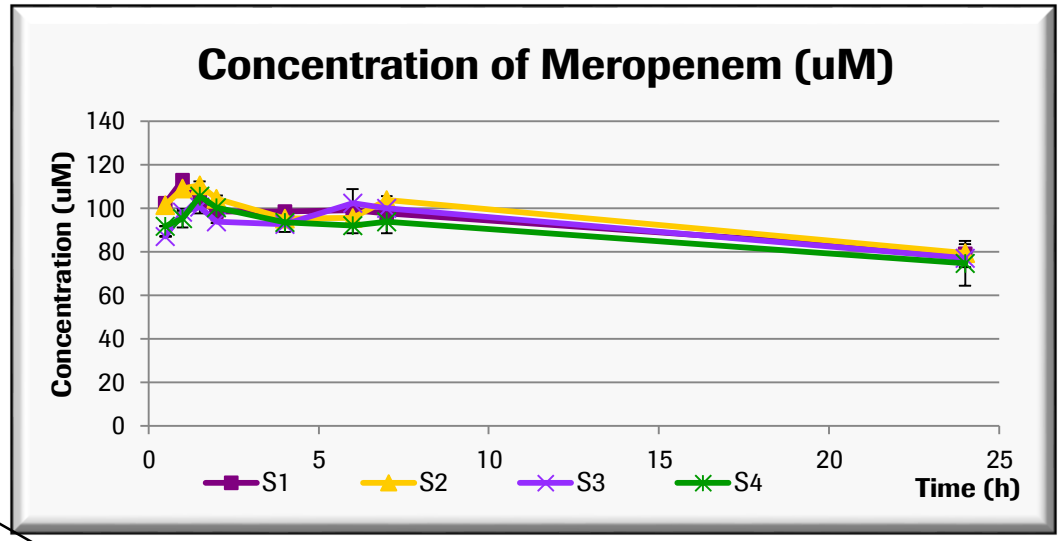
→ Different concentrations are observed in the system between ICS and ECS
→ Cmax lower than expected
→ Degradation of Meropenem at room temperature

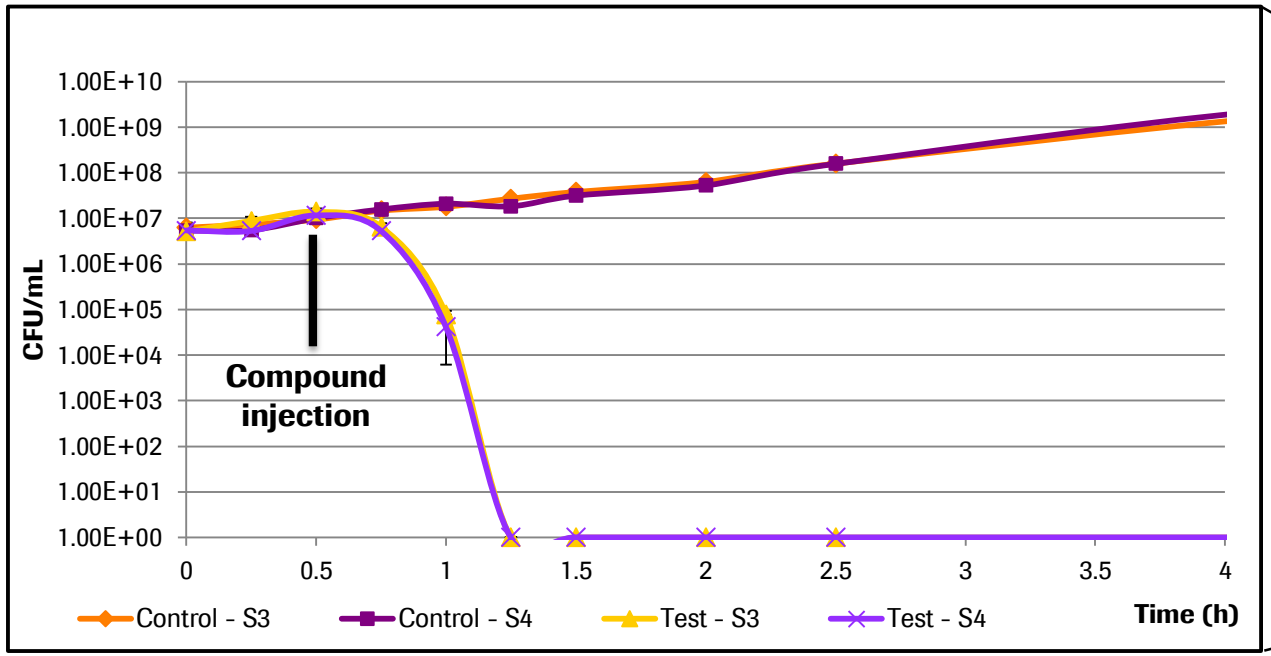
PK/PD STUDIES

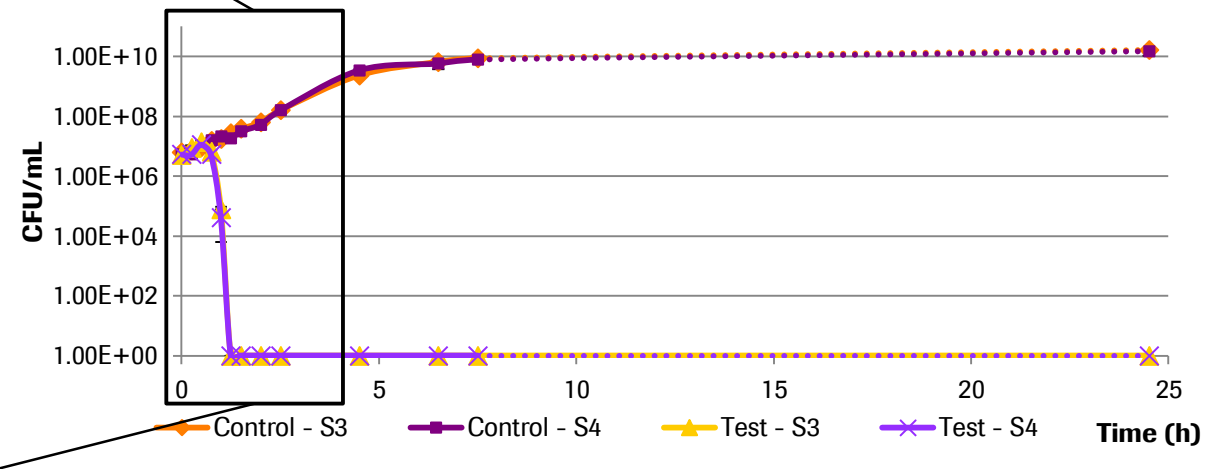
Meropenem / *E. coli* ATCC 25922 (w/o elimination)

→ **Simulation Meropenem 2g Bolus**

- 24h incubation **w/o elimination**
- Inoculum size: **1e7 CFU/mL**
- **Theoretical compound concentration : 60µg/mL = 137µM**
- **MIC = 0.03 µg/mL**





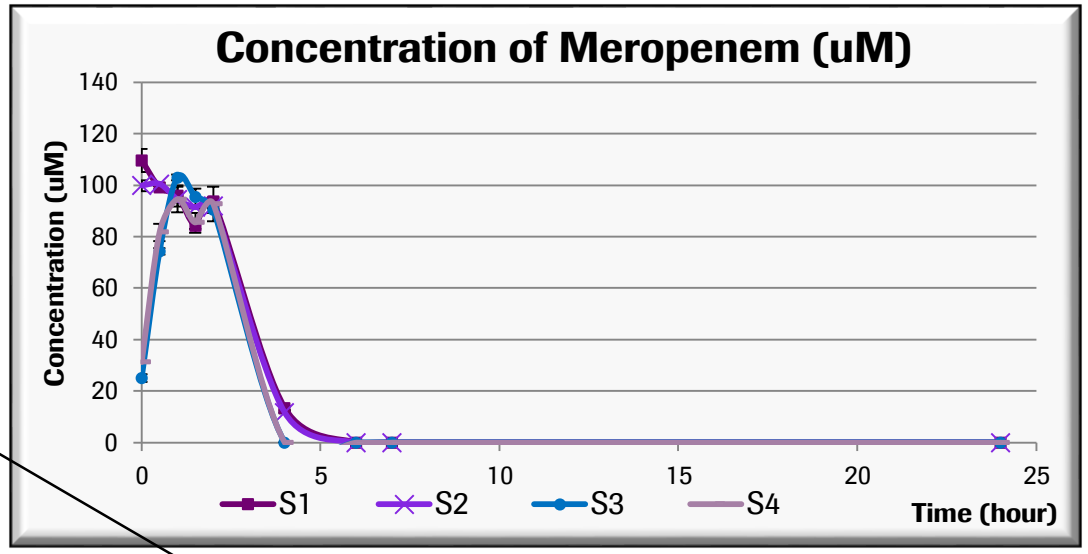


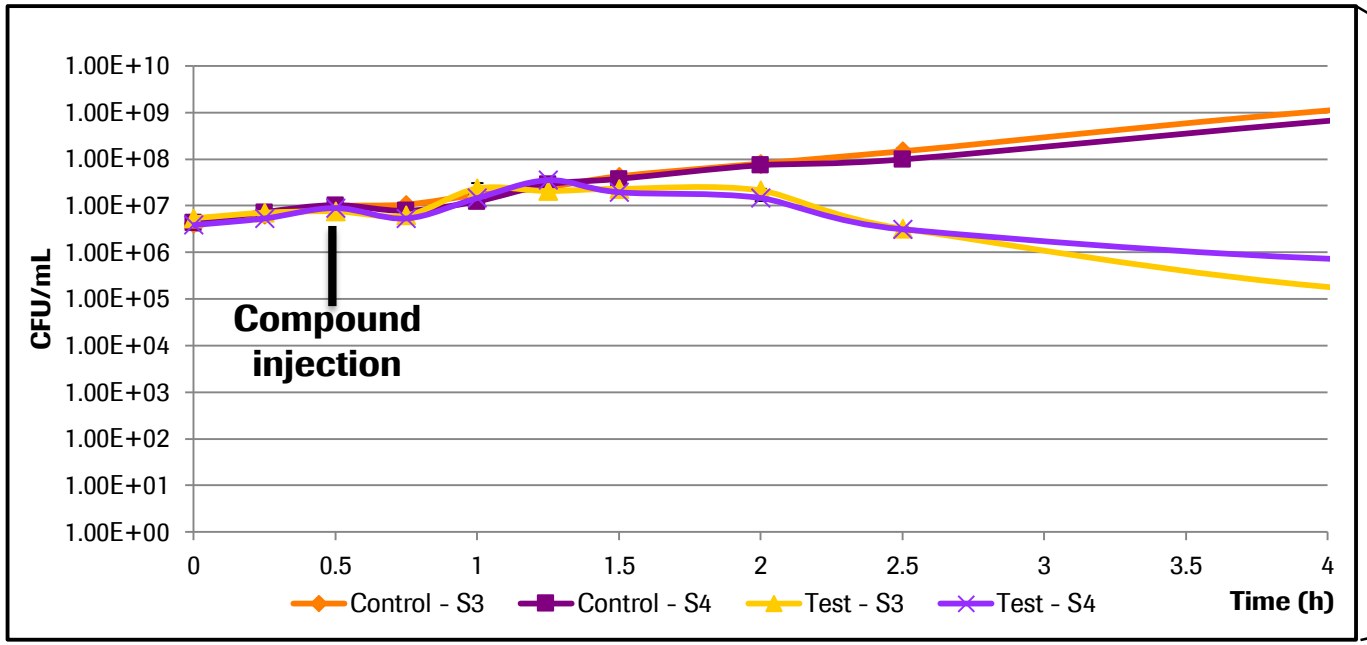
→ Bactericidal effect (>3 log reduction) observed within 45 min after compound injection

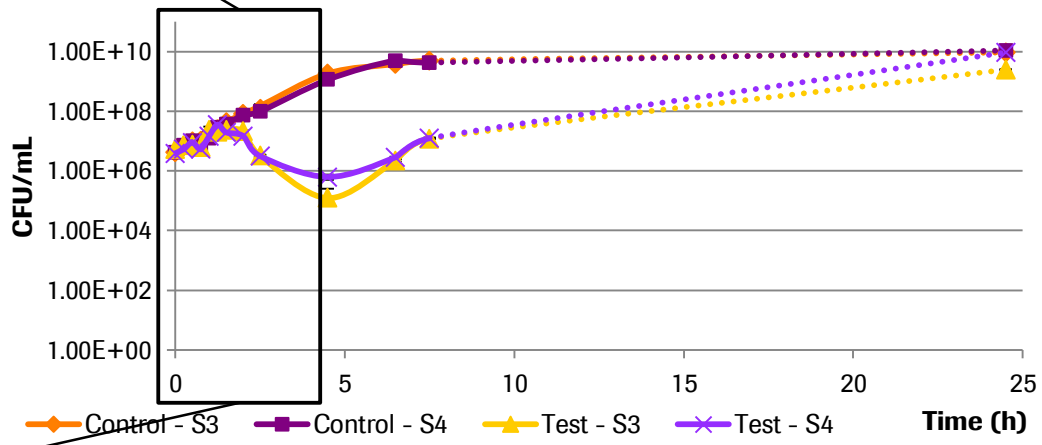
Meropenem / *K. pneumonia* NCTC 13438 (w/o elimination)

→ **Simulation Meropenem 2g Bolus**

- 24h incubation **w/o elimination**
- Inoculum size: **1e7 CFU/mL**
- Compound concentration in the system: **60 µg/mL = 137µM**
- **MIC = 64-256 µg/mL** ***KPC-3***







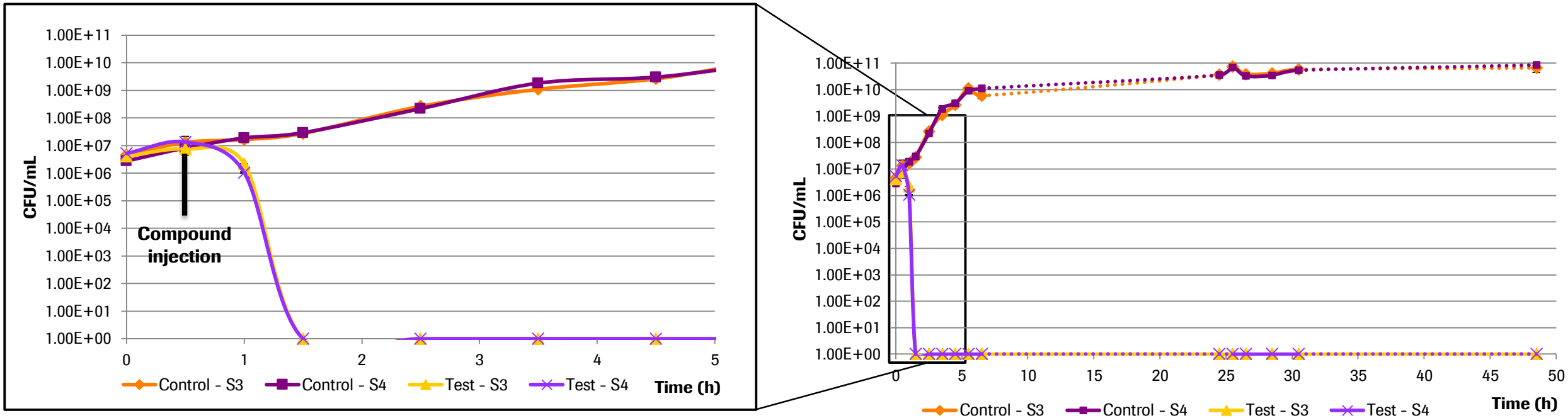
→ No bactericidal effect, regrowth observed after 6h
→ Meropenem degradation between 2h and 5h after injection

Meropenem / *E. coli* ATCC 25922 – 2g q8h over 48h

→ **Simulation Meropenem 2g 1h infusion – 6 injections**

- 48h incubation **with elimination**
- Inoculum size: **1e7 CFU/mL**
- **Theoretical compound concentration : 66µg/mL = 150µM**

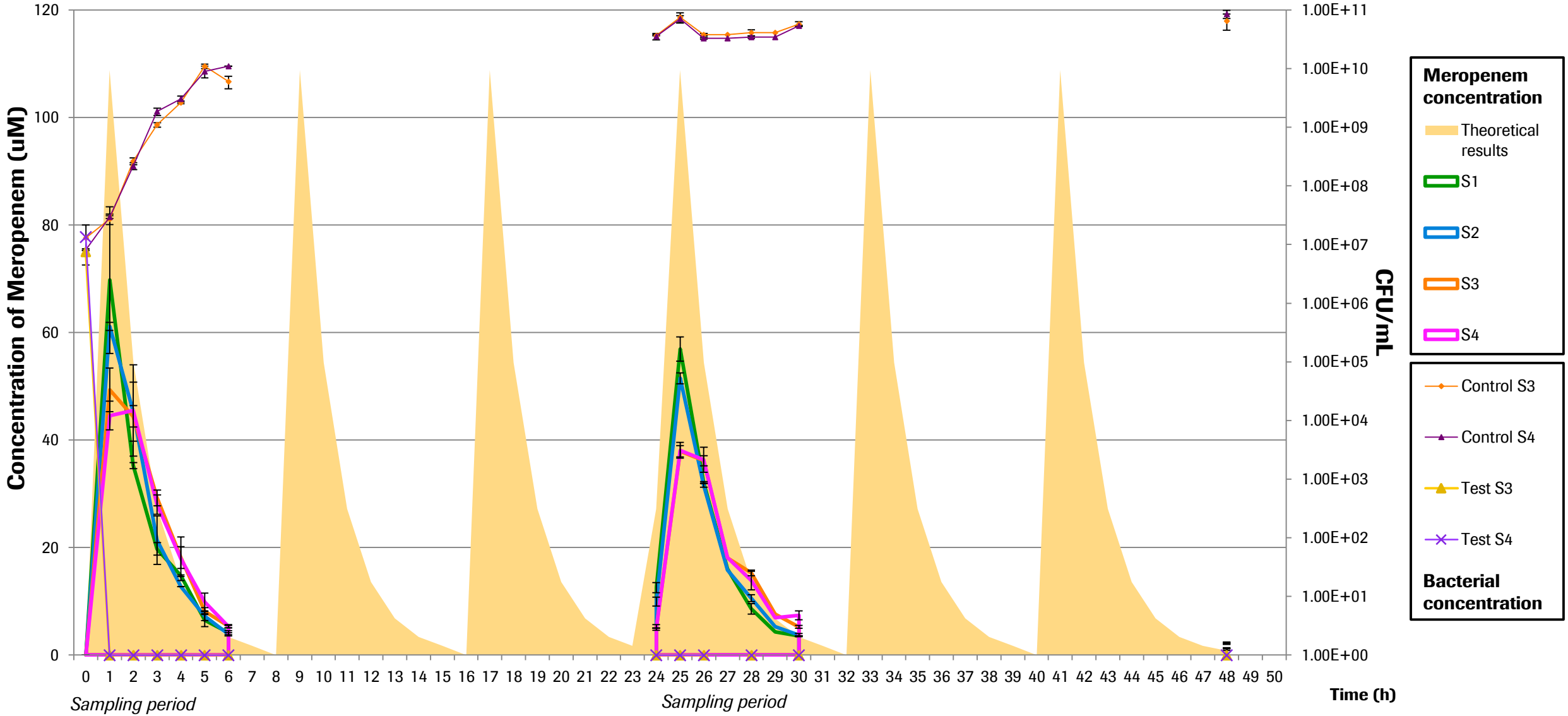
MIC = 0.03 µg/mL



→ Bactericidal effect (>3 log reduction) observed within 60 min after compound injection

→ No resistance emergence observed

Hollow Fiber experiment Simulation 2g Meropenem every 8h + *E. coli* ATCC 25922

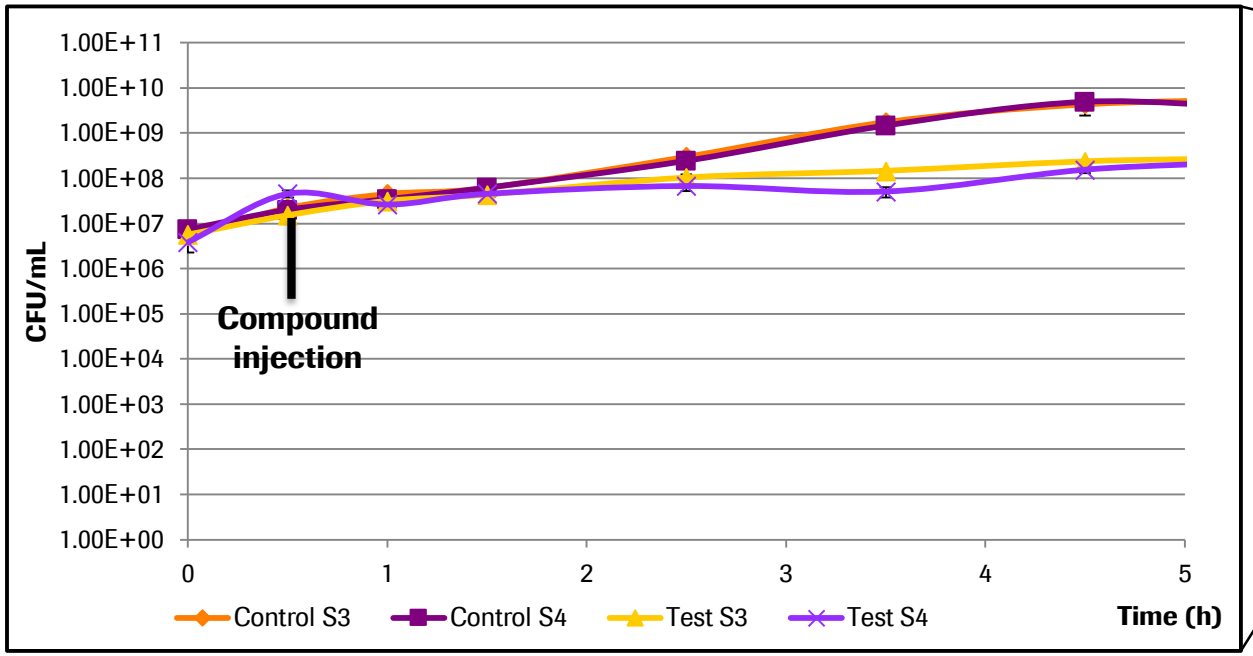


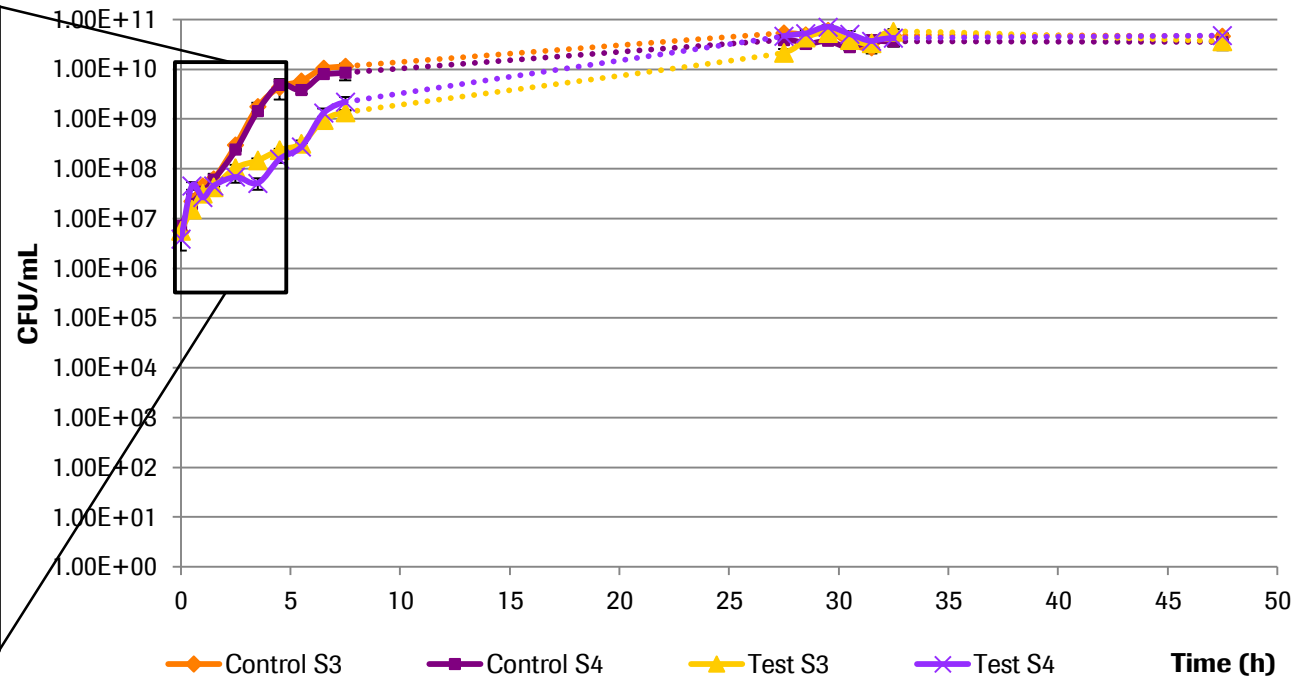
Meropenem / *K. pneumonia* NCTC 13438 – 2g q9h over 48h

→ **Simulation Meropenem 2g 1h infusion – 6 injections**

- 48h incubation **with elimination**
- Inoculum size: **1e7 CFU/mL**
- Compound concentration in the system: **66 µg/mL = 150µM**

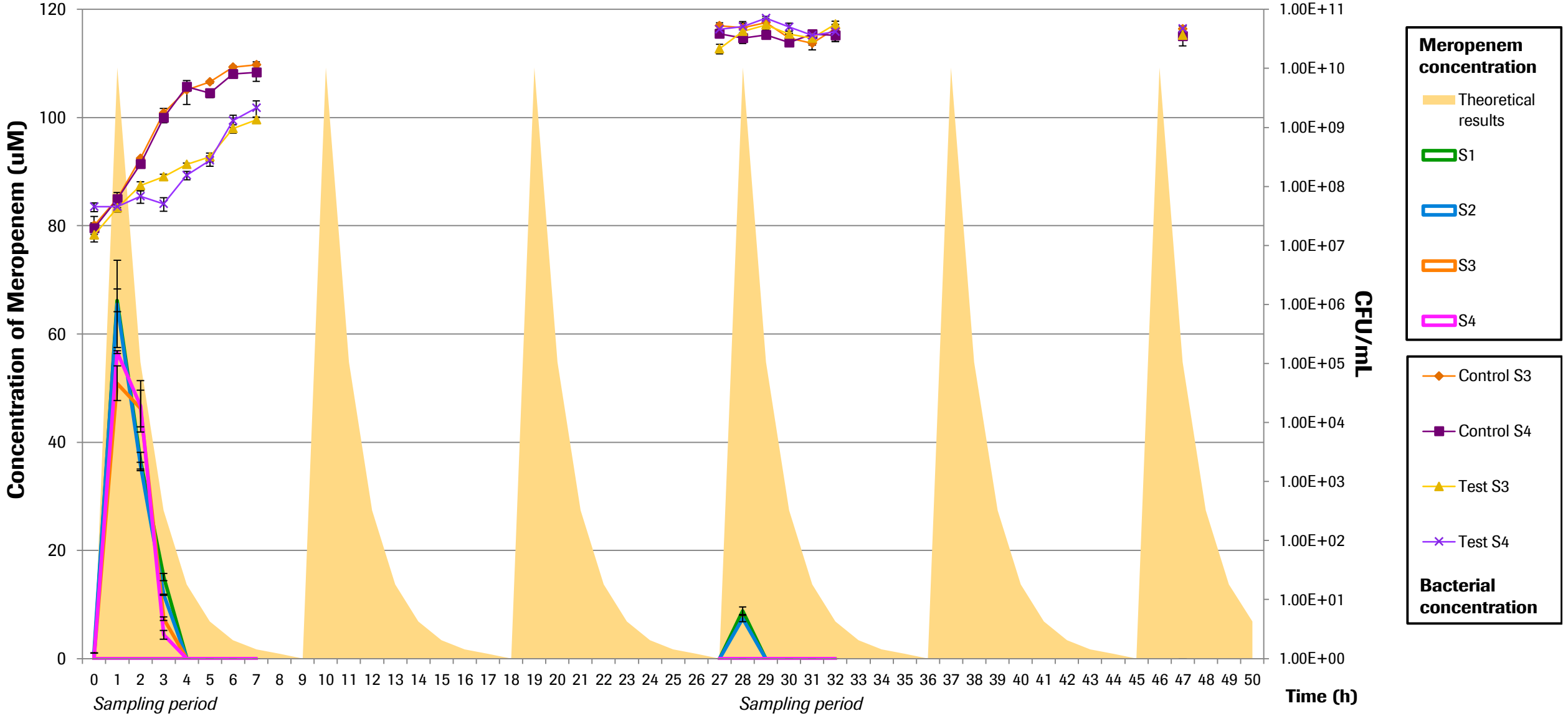
MIC = 64-256 µg/mL





→ No bactericidal effect, slow down of the growth after the first injection
→ Degradation of the compound by the β-lactamases present in the system

Hollow fiber experiment Simulation 2g Meropenem every 9h + *K. pneumoniae* NCTC 13438



SUMMARY

Summary

➢ **PD aspect**

- Lag phase for bacteria to adapt in the system of around 30 min
- Large volume of inoculation needed for homogenization of bacteria in the cartridge

➢ **PK aspect**

- 2-fold elimination kinetic observed
- Reproducible variation of concentrations between ECS and ICS
- Reduction of the time needed to reach equilibrium between ICS and ECS with a higher flow-rate
- Instability of Meropenem – Need to work with freshly made stock solution (injection / calibration)
- Deviation observed between theoretical vs observed Cmax

→ **Importance of proper experimental parameters set-up before studying new molecule in HFIM to determine the feasibility of testing and the parameters setting**

Acknowledgements

- Antony Rutt (KDBIO)
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- Pauline Misson (I2O)
- Séverine Louvel (I2O)
- Solen Pichereau (PS)

Doing now what patients need next