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Hollow Fiber in-vitro model @Idorsia

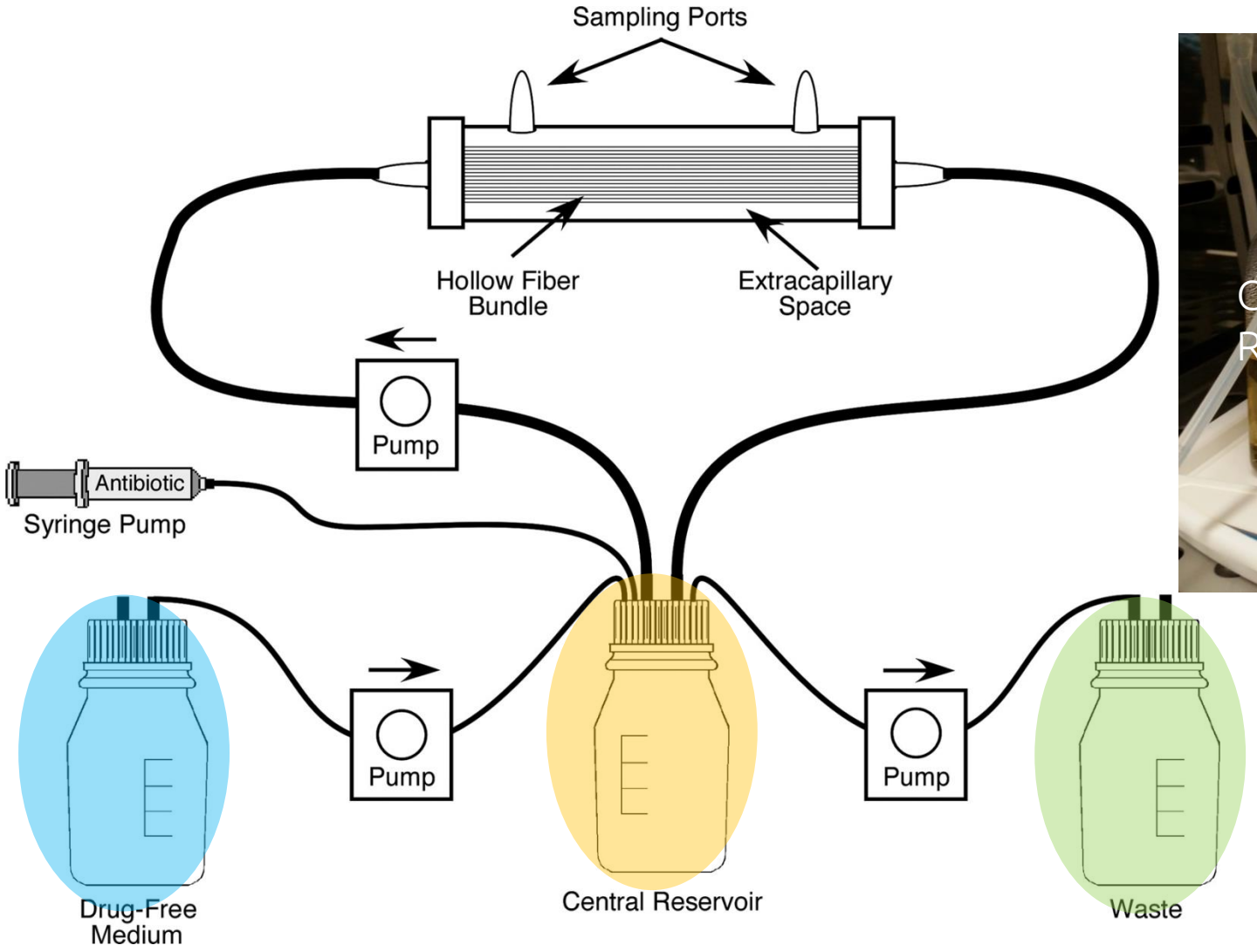
Workshop November 27, 2019 - Jonathan Delers

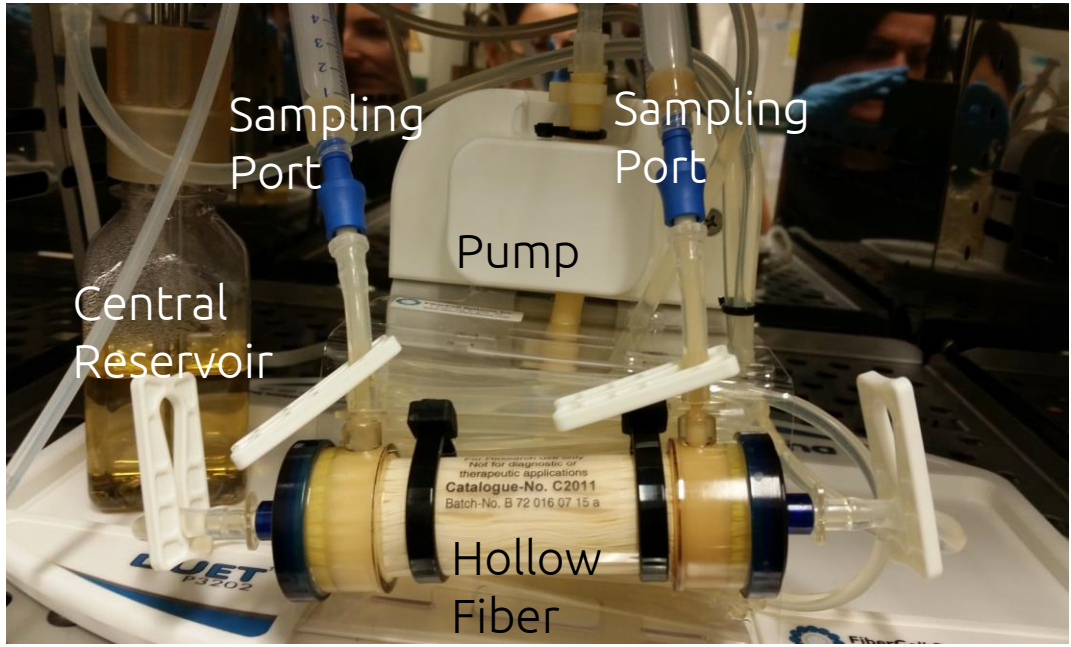
Goals

- Assessing dosing strategy for the control of infections and the reduction of resistance development of in-house compounds
- Testing of XDR strains
- Simulation of predicted human PK
- Evaluation of Emergence of Resistance
- Combination of two drugs

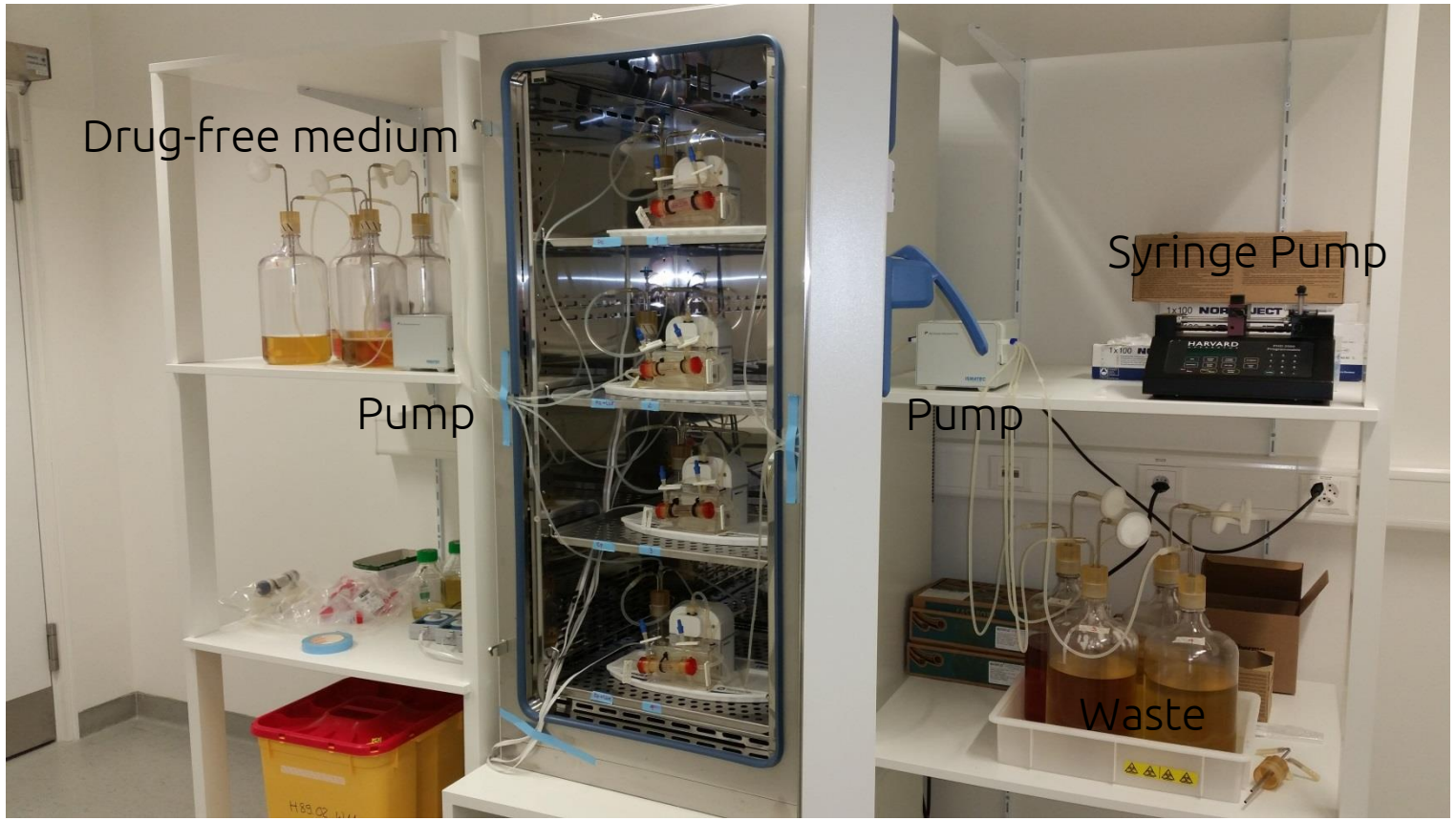
Hollow Fiber Model (Overview)

Hollow fiber model a better fit to study resistance development than *in vivo* model





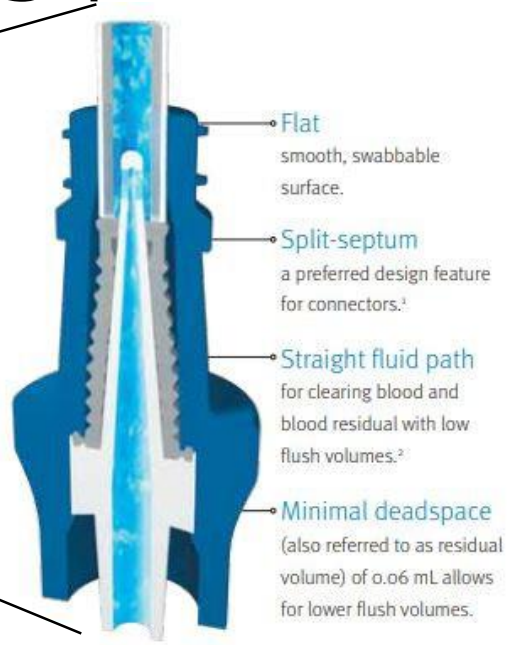
In-house Setup

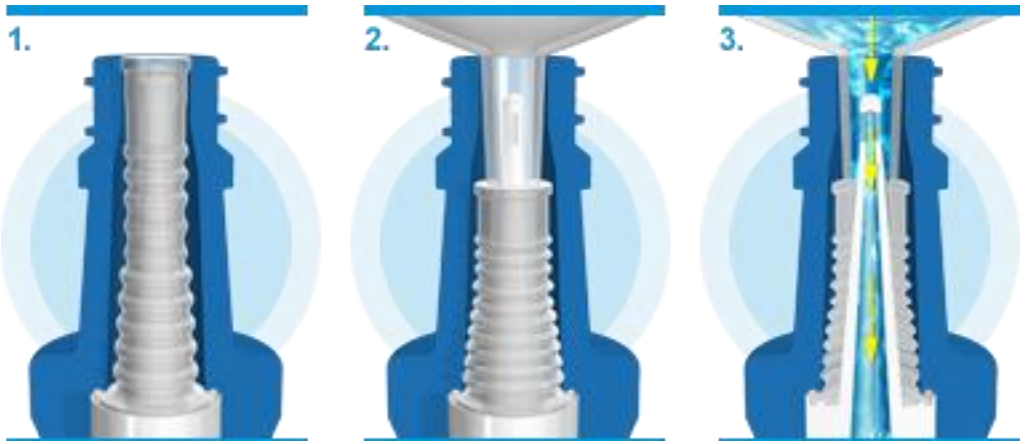


- **Reservoir volume:** 4000ml
- **Central Compartment vol:** 107ml (50ml Reservoir + Cartridge and tubing)
- **Medium:** Mueller Hinton Broth II
- **Strains used:** *P. aeruginosa* ATCC 27853 and PAO1, *K. pneumoniae* ATCC 43816
- **Pump velocity:**
  - central (duet) pump: ~100ml/min
  - peripheral pump: drug dependent
- **Cartridge:**
  - C3008 (low MWCO (5 kd)); cellulosic fiber

    formerly C2011 (high MWCO (20 kd)); hydrophilic fiber

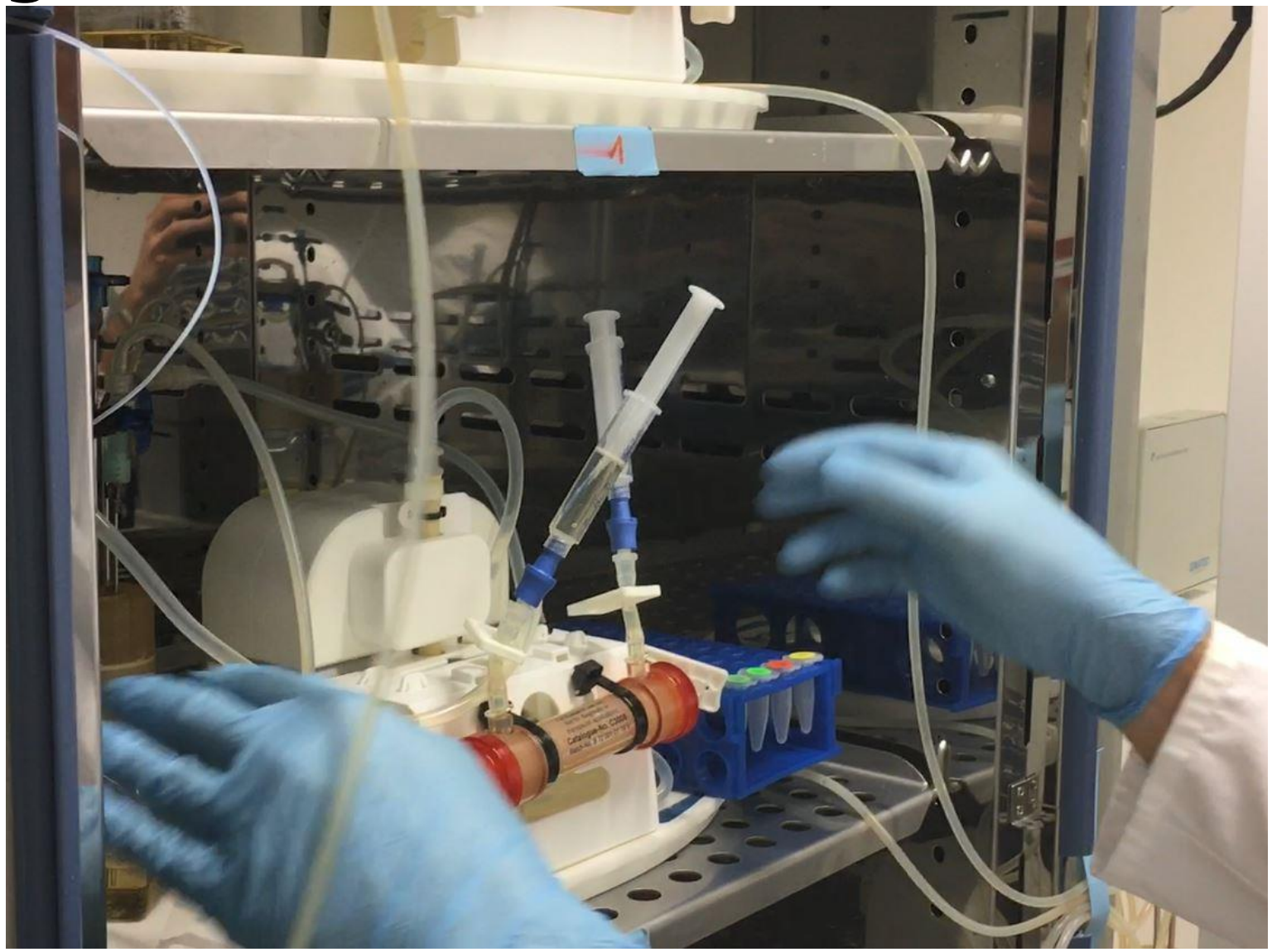
In-house Setup: sampling ports





- Easy to handle
- Reduces the risk of injuries (use of xdr strains less of an issue)
- No issues with contamination

Sampling

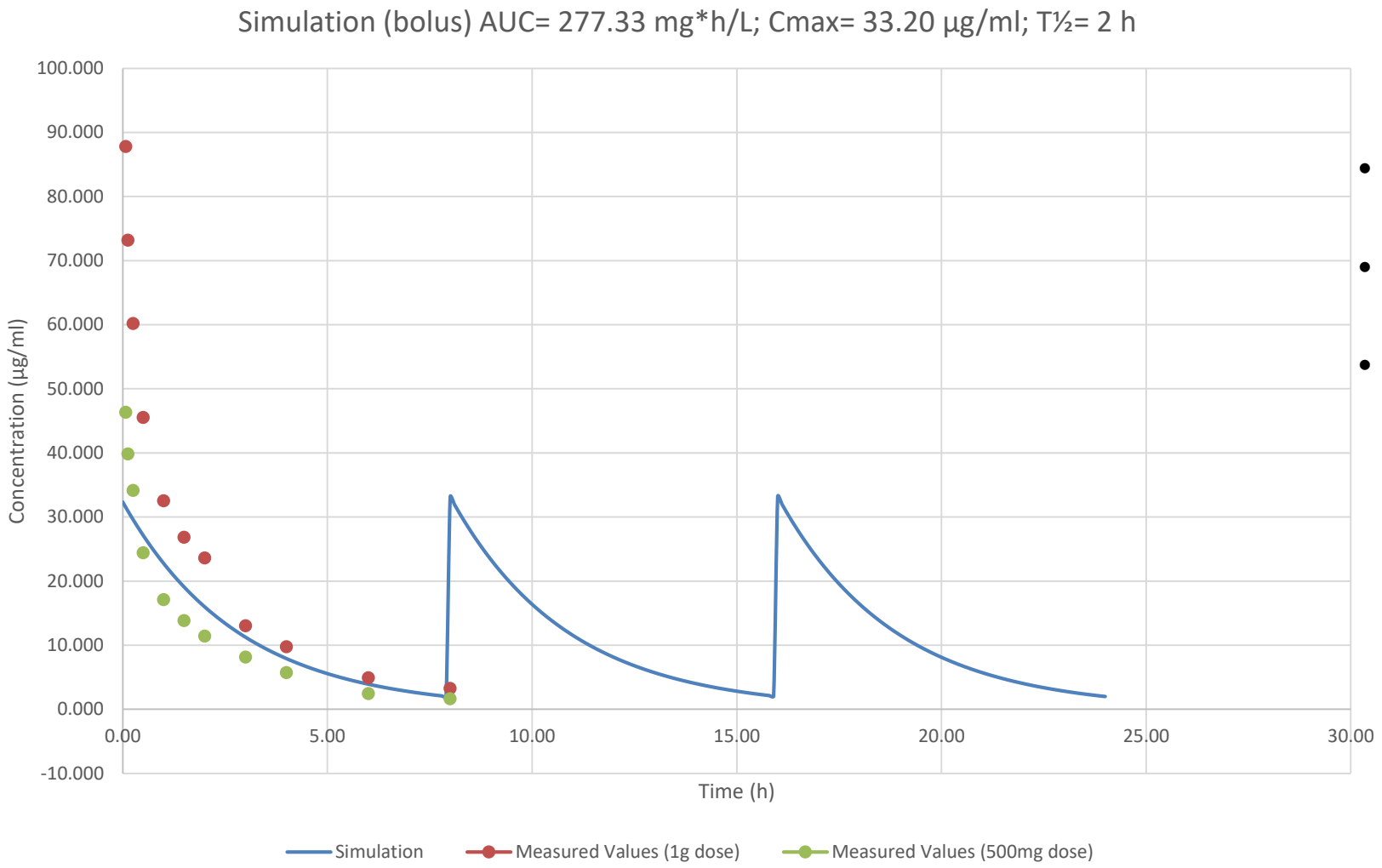


Case study: ceftazidime vs. *P. aeruginosa* ATCC 27853

- Setup:
  - Ceftazidime monograph up to 2g IV: AUC = 280µg*h/ml; Cmax=183µg/ml; $T_{1/2}$ = 1.9h; protein binding 5-23%
  - *P. aeruginosa* ATCC 27853: non ESBL producing ; ampC inducible strain
- Protocol:
  - Regimen used: 3q8 dosing (300 µl injections)
  - Starting inoculum ~1.10^5 CFU/ml. Let grow 4 hours before the 1st injection (1.10^6 CFU/ml)
  - Sampling at: t-4h*, t-2h*, t0h (injection of the drug), t2h, t4h, t6h, t24h, t48h and t72h
  - Samples plated on:
    - Mueller Hinton agar for inoculum size determination
    - Mueller Hinton agar + 4xMIC of ceftazidime (*i.e.* 8 µg/ml) for resistant clones enumeration
  - Estimated consumption of caMHB: 937 ml per 24 hours

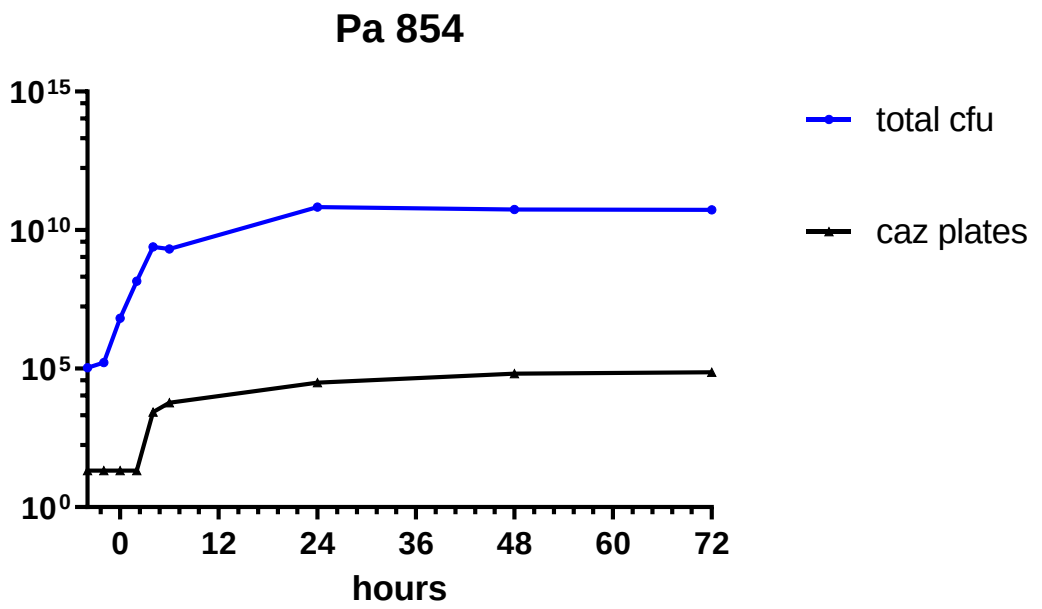
Ceftazidime Drug concentrations: patients vs. HFM

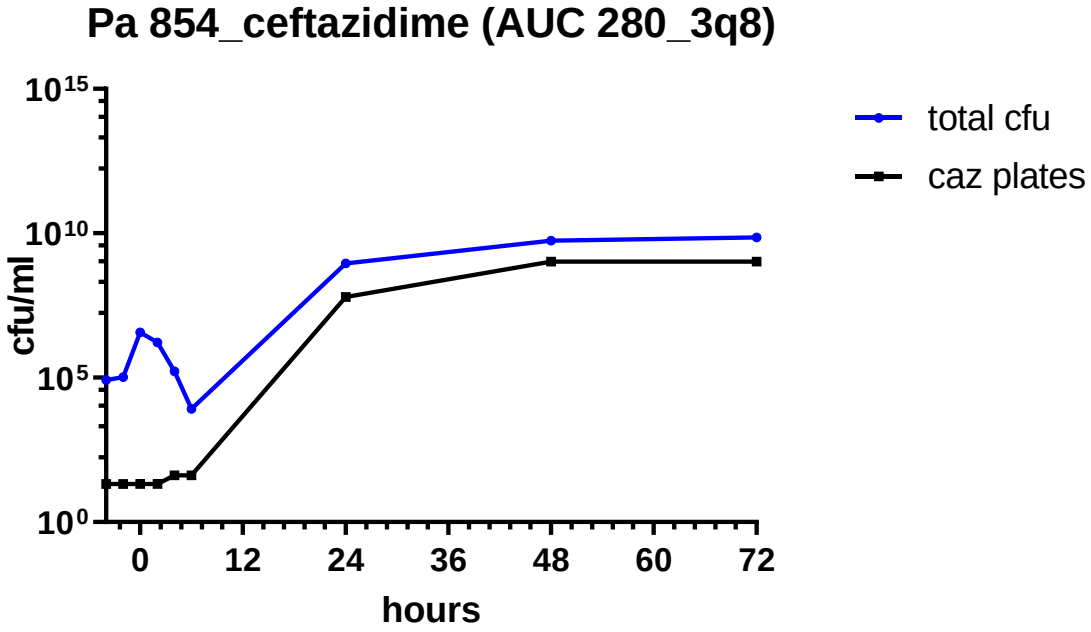
Simulation of one-compartment model with first-order elimination



- HF model approximates human dosing with a 500 mg dose given q8h
- Used in cUTI infections (1.5g total daily dose)
- Max dosing in clinics: 2g q8h for meningitis or septicemia (*e.g.* by PA)

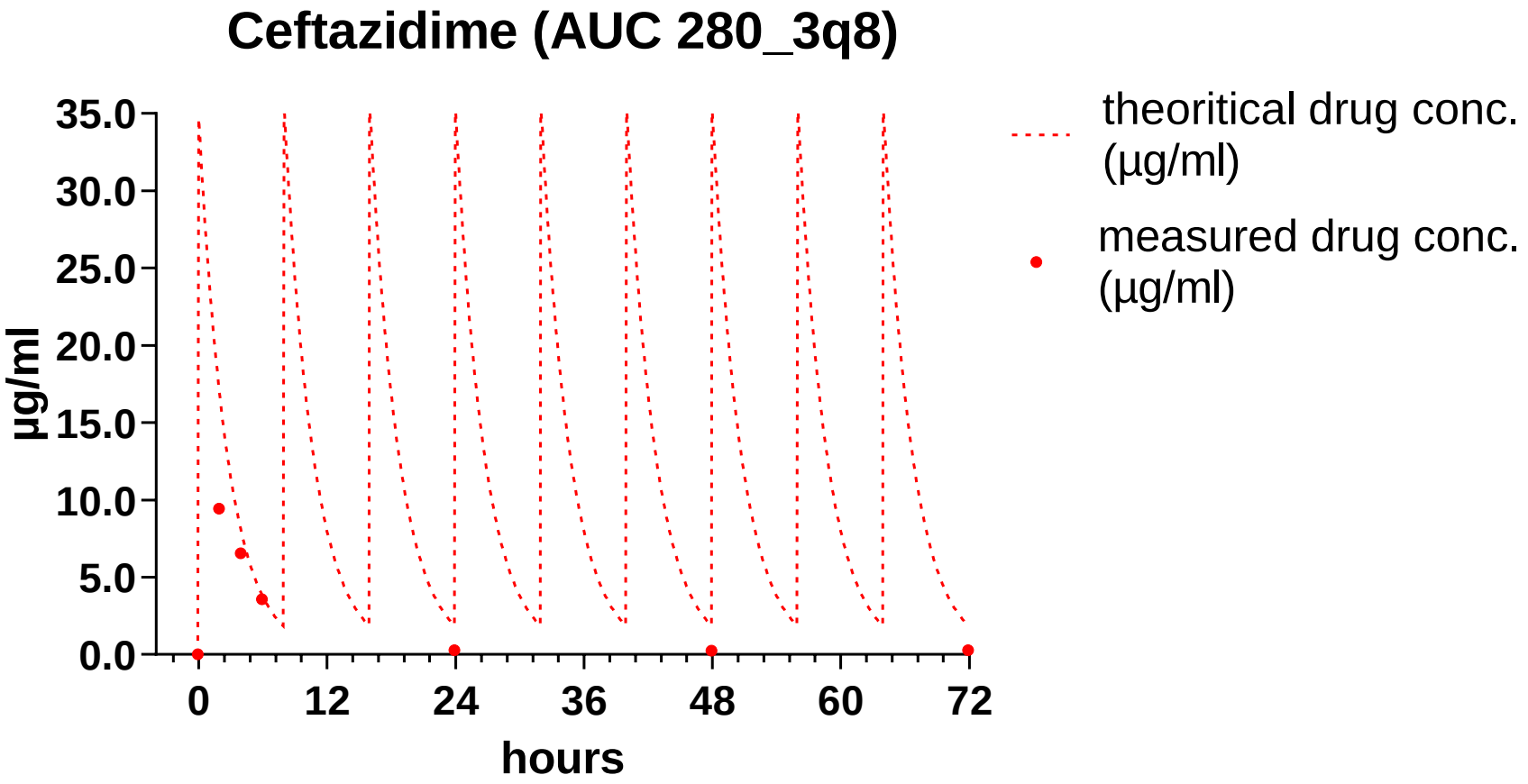
Ceftazidime vs. *P. aeruginosa* ATCC 27853





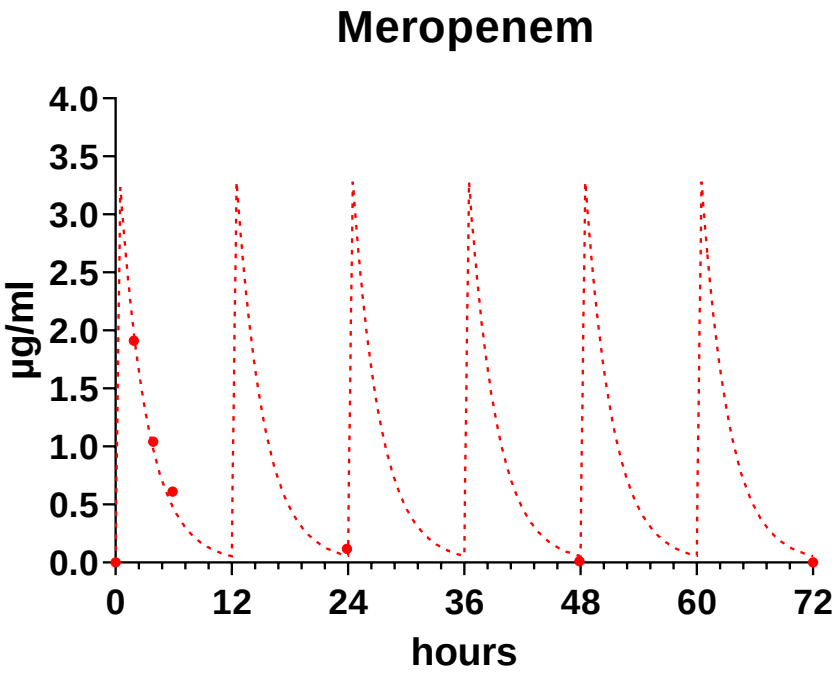
Drug distribution and quantification

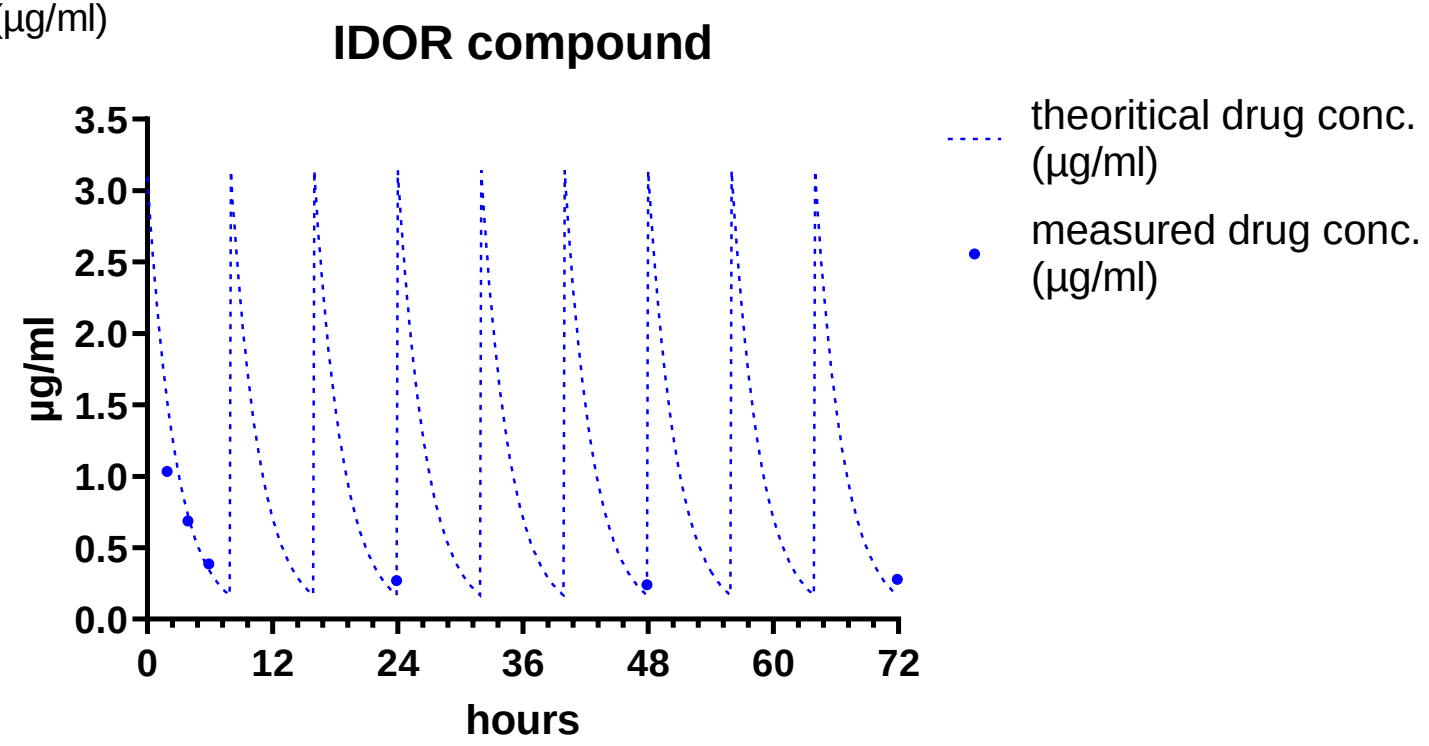
- Done through LC-MS titration



"absence" of the peak of the drug after 24, 48 and 72 hours may be explained by production of ampC ßlactamase

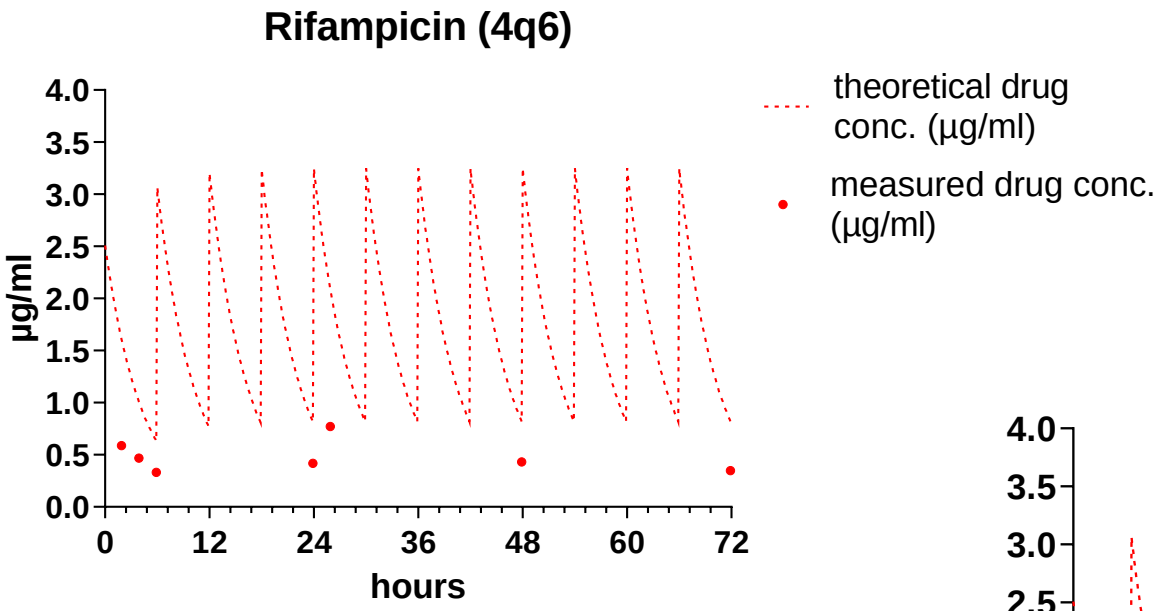
Drug distribution and quantification (meropenem and in-house compound)

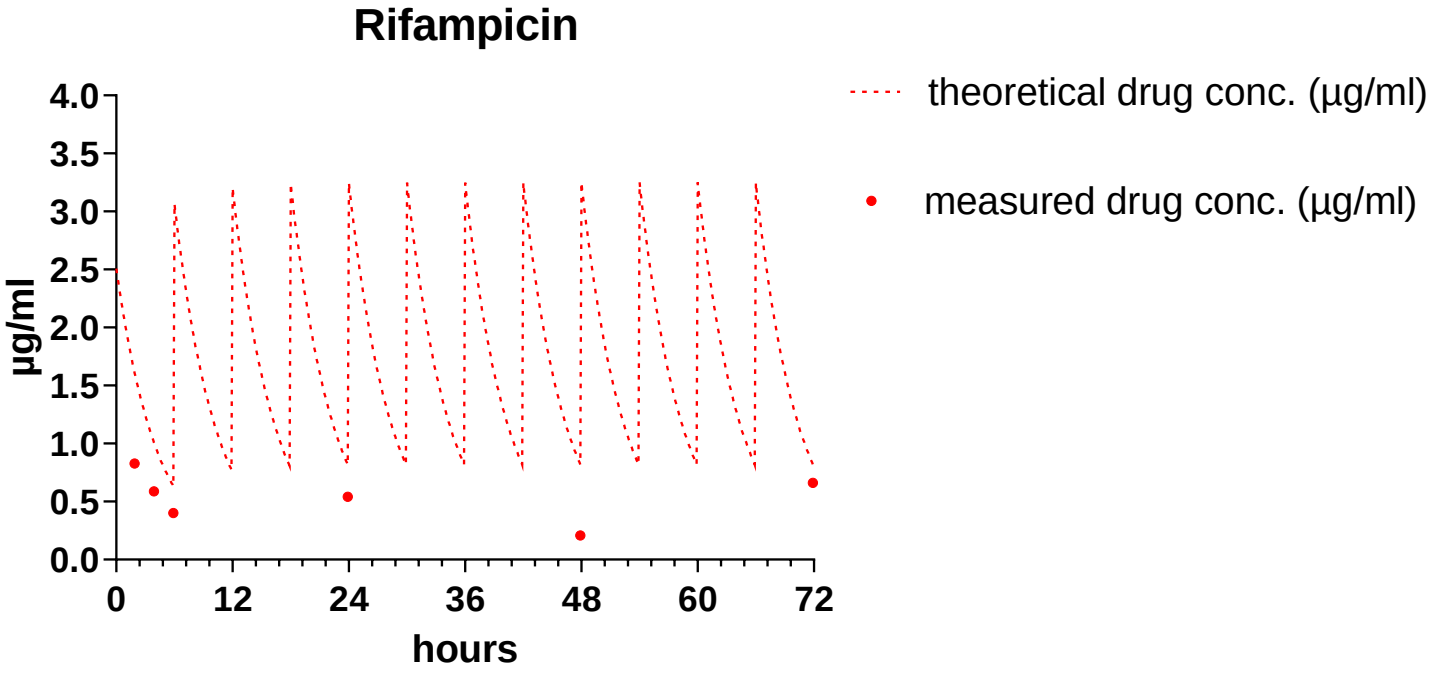




- Overall good fit between the theoretical and practical drug concentrations

Drug distribution and quantification (rifampicin)





- Possible binding to the surface of the fibers

Case study: combination between ceftazidime and IDOR compound vs P. aer ATCC 27853

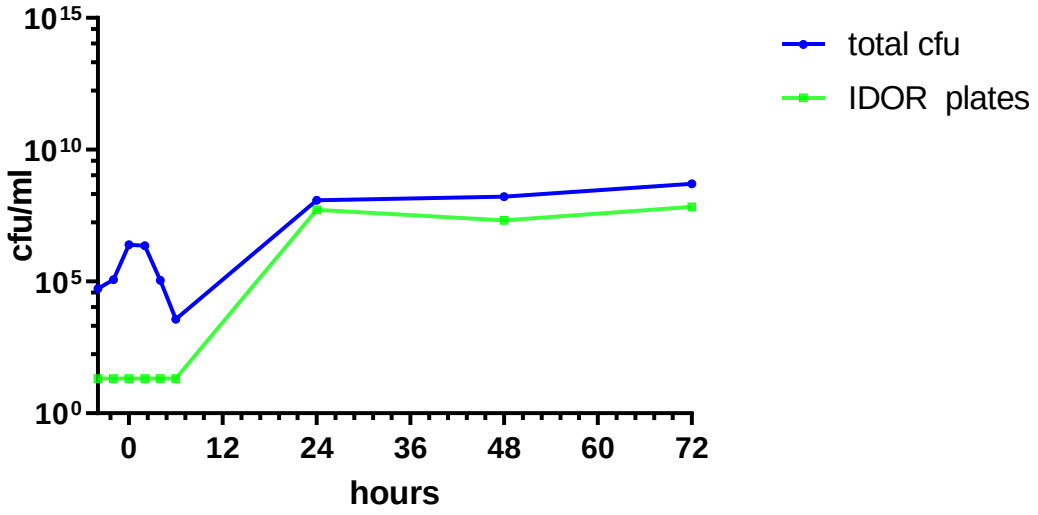
- **Goal**
  - Evaluating the outcome on inhibition and prevention of resistance regrowth of 2 agents combined together and not preventing regrowth on their own

- **Setup**:
  - Ceftazidime AUC = 280 µg*h/ml ; IDOR compound AUC = 25 µg*h/ml. Same half-life has been used for both compounds for technical reason (here $t_{1/2}$ = 1.9 h)

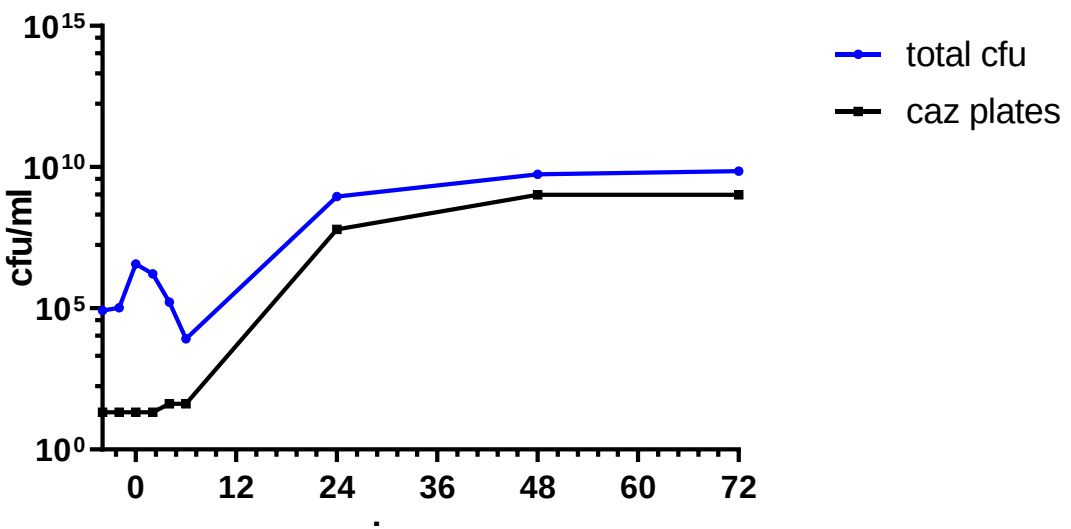
- **Protocol:**
  - Regimen used: 3q8 dosing for both drugs
  - Plating on:
    - Mueller Hinton agar for inoculum size determination
    - Mueller Hinton agar + 4xMIC of ceftazidime (*i.e.* 8 µg/ml) and + 4xMIC IDOR cpd (*i.e.* 2 µg/ml) for resistant clones enumeration
  - Estimated consumption of caMHB: 937 ml per 24 hours

Combination data

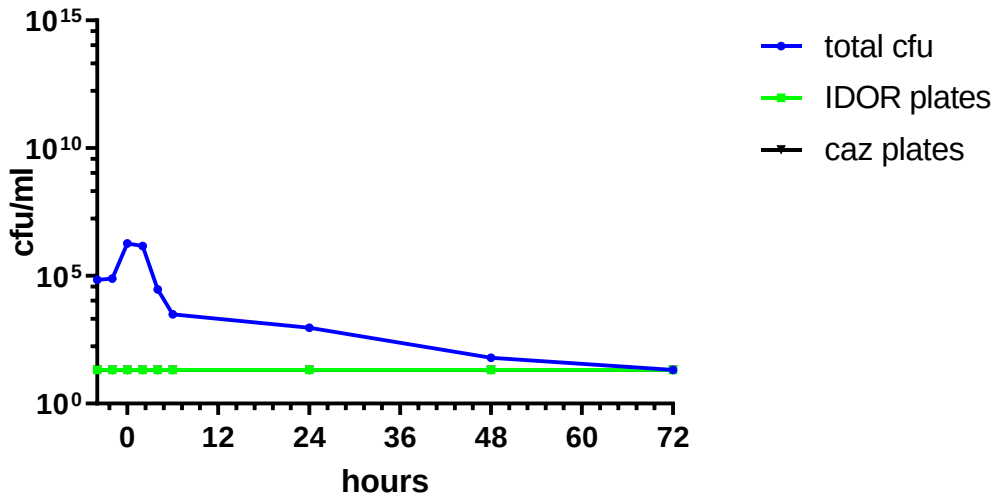
Pa 854_IDOR compound (AUC 25_3q8)



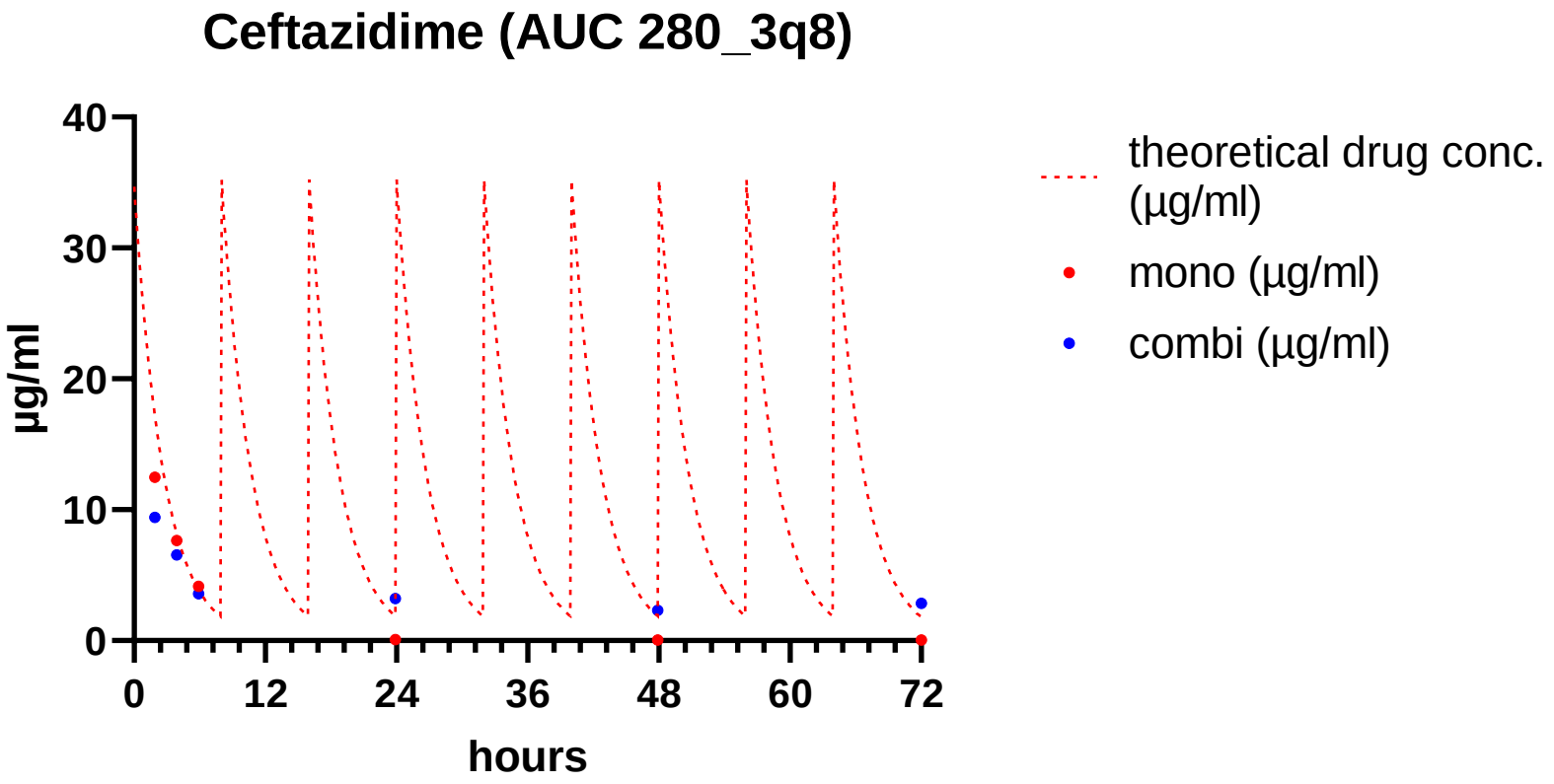
Pa 854_ceftazidime (AUC 280_3q8)



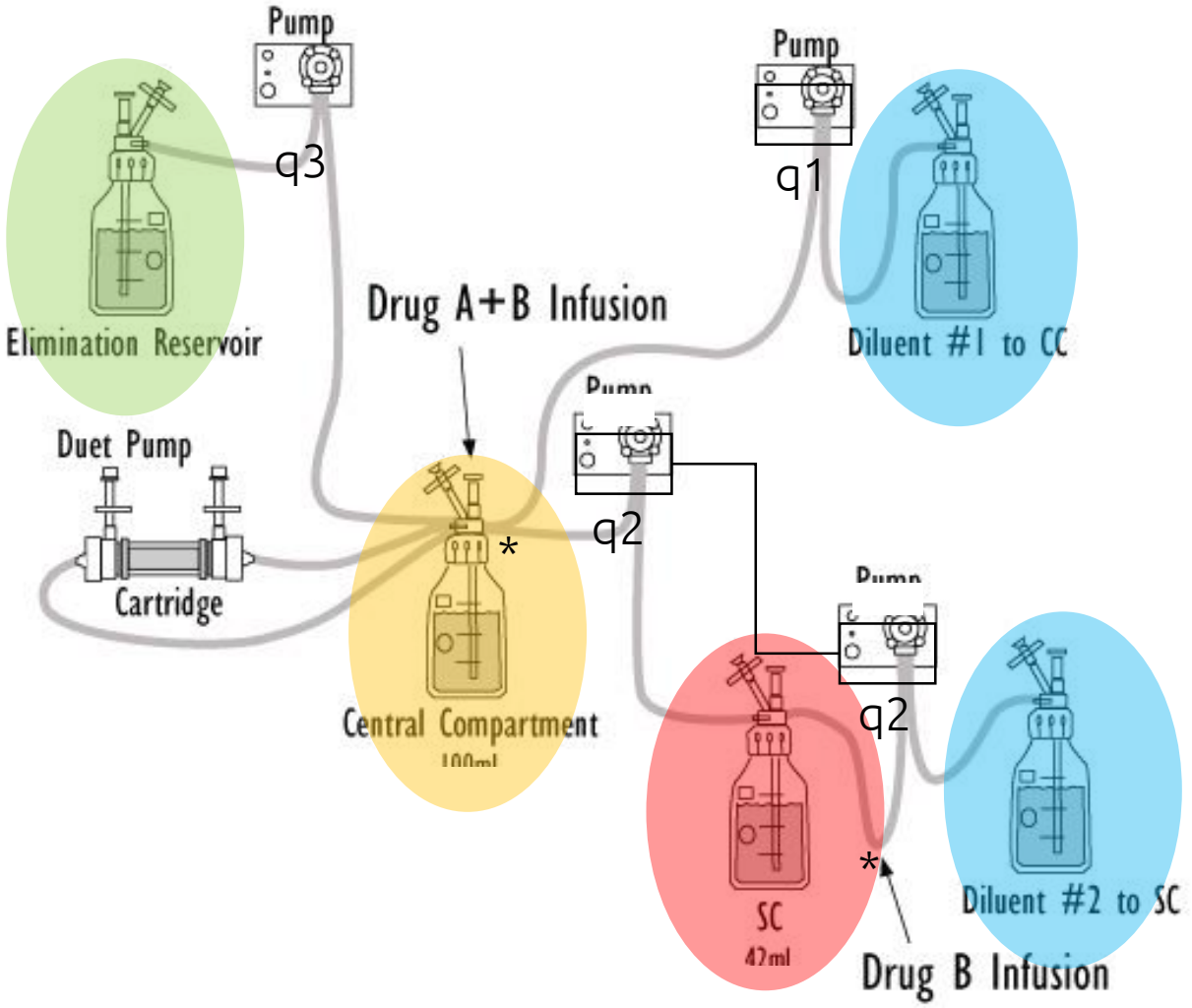
Pa 854_IDOR compound (AUC 25_3q8) + ceftazidime (AUC280_3q8)



Drug distribution and quantification



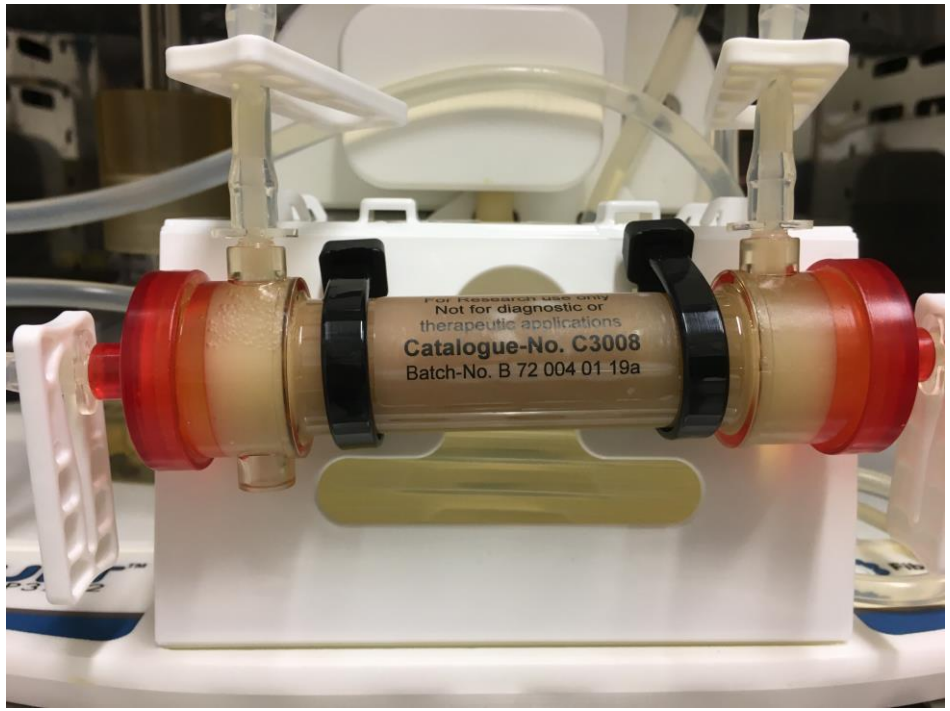
Combination next step: 2 drugs with 2 different half-lives



* Y-connector or 4 way stop-cock; drug added via infusion pump

Issues

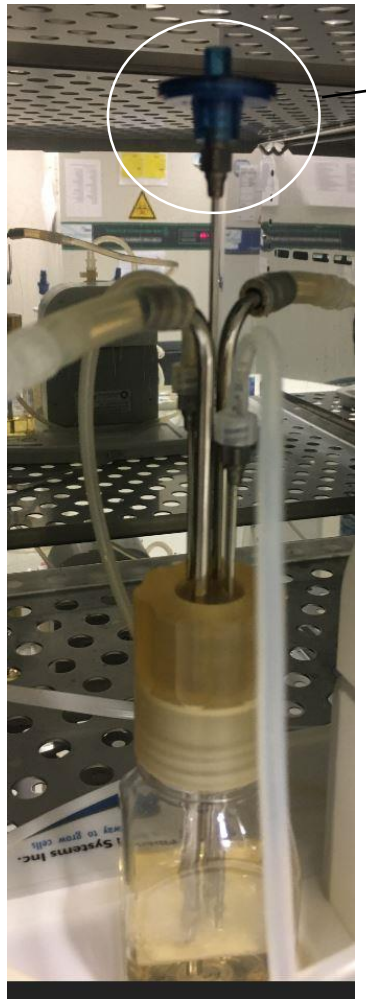
- Emptying of the cartridge over time when absence of growth



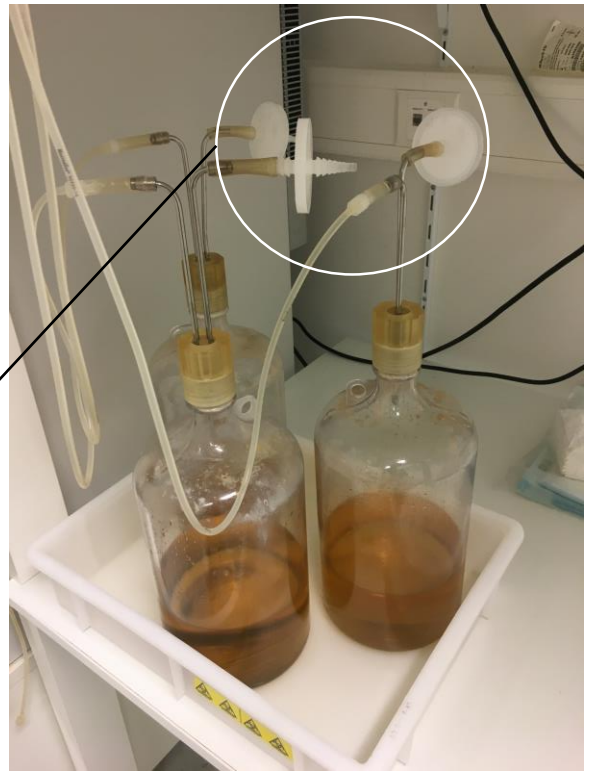
Volume in the ECS is decreasing over time

Problem possibly linked to the pressure inside the system

Issues (2)



25 mm 0.2 µm Millex filter (Hydrophobic Fluoropore Membrane) - Millipore



55 mm 0.2 µm Millex venting filters (Hydrophobic Fluoropore Membrane) - Millipore

Learning/discussion on HFM

- Easy to set-up
- It allows us to test XDR strains (not tested *in vivo* for safety reasons)
- Reduction of animals needed to perform similar studies: ethical and practical aspects
- Simulation of human PK in HFM is possible (only applied to first order elimination kinetics) thanks to the use of syringes pump allowing infusion and/or multiple doses (3q8, 4q6, etc…)
- Resistance development can be followed via plating on agar containing 4xMIC of the tested drug

Learning/discussion on HFM

Limitations/issues

- Emptying of the ECS becomes problematic on longer assays
- Experiment duration is limited as far as drugs with short half-lives are concerned (*eg.* $t_{1/2}$ ≤2 hrs = >4 L of fresh medium are needed for experiment longer than 72 hours)
- Combination of 2 antibiotics/drugs with fairly different half-lives is technically challenging to set-up