Date : 04/07 - 10/07/18

Agar pads with E. coli
AMP - ERY - NAL - CAM - KAN - RIF - NEO -

Results : Good videos of killing with AMP, ERY, NAL and CAM

Date : 19/07/18

Stock of bacteria (storage at -80°C - 9 cryotubes):

- E.coli
- B.megnaterium

Protocols:

1) Prepare an overnight culture of the wanted bacteria
2) Prepare glycerol stock at 50%
3) In a cryotube add 500µL of glycerol 50% and 500µL of the overnight culture
4) Place at -80°C

CODE: Name of the bacteria "A.bcd", date, number of the tube

Date : 20/07/18

Agar pads : E.coli - Neo
Results : Grow.

Overnight culture of E.coli + B.megnaterium

Killing Kinetics, **Mutagenesis**, **Flow Cytometry** preparation

Date: 21/07/18

B.megnaterium don't seem as Bacillus → Staphylococcus

Killing Kinetics E.coli Ampicilin **:**
1 day preparation + 1 day experiment + 1 day results

- Condition : without antibiotic, 0.25xMIC, 0.5xMIC, 1xMIC, 2xMIC, 4xMIC
- Taking samples : 0min, 5min, 10min, 15min, 30min, 35min, 40min, 45min, 2h, 4h, 6h, 8h
- CFU : 2 dilutions per condition and per time
- Centrifugation of sample to remove antibiotic = 1min 6000rpm
- Place at 4°C before continue experiment
- Incubation of liquid culture at 37°C 180 rpm

Protocol:

Ampicillin					
MIC (ug/ml)	10				
Stock concentration (mg/ml)	100				
Volume of media for experiment (mL)	50				
Desired concentration (x MIC, ug/mL))	**0.25**	**0.5**	**1**	**2**	**4**
Desired concentration (ug/mL))	2.5	5	10	20	40
Volume of AB stock in 50 mL of media (**uL**)	**1.25**	**2.5**	**5**	**10**	**20**

Mutagenesis - E.coli Ampicillin**:** during Killing Kinetics

- Condition : without antibiotic, 0.25xMIC, 0.5xMIC, 1xMIC, 2xMIC, 4xMIC
- Taking samples : 0min, 45min, 2h, 4h, 6h, 8h
- 2 plates per condition and per time
- Rifampicin plate at 100µg/mL

Flow Cytometry - E.coli Ampicillin: during Killing Kinetics

Date: 22/07/18

Killing Kinetics :
New dilutions and conditions for the next one

	Control		0.25 x MIC		1 x MIC		2 x MIC	
0 min	*-4*	*-5*	*-4*	*-5*	*-4*	*-5*	*-4*	*-5*
5 min	*-4*	*-5*	*-4*	*-5*	*-4*	*-5*	*-4*	*-5*
10 min	*-5*	*-6*	*-5*	*-6*	*-4*	*-5*	*-4*	*-5*
15 min	*-5*	*-6*	*-5*	*-6*	*-4*	*-5*	*-4*	*-5*
20 min	*-5*	*-6*	*-5*	*-6*	*-4*	*-5*	*-4*	*-5*
25 min	*-5*	*-6*	*-5*	*-6*	*-4*	*-5*	*-4*	*-5*
30 min	*-5*	*-6*	*-5*	*-6*	*-4*	*-5*	*-3*	*-4*
35 min	*-5*	*-6*	*-5*	*-6*	*-4*	*-5*	*-3*	*-4*

40 min	*-5*	*-6*	*-5*	*-6*	*-3*	*-4*	*-2*	*-3*
45 min	*-5*	*-6*	*-5*	*-6*	*-3*	*-4*	*-1*	*-2*
2h	*-5*	*-6*	*-5*	*-6*	*-2*	*-3*	*0*	*-1*
4h	*-6*	*-7*	*-5*	*-6*	*-1*	*-2*	*0*	*-1*
6h	*-6*	*-7*	*-5*	*-6*	*0*	*-1*	*0*	*-1*
8h	*-6*	*-7*	*-5*	*-6*	*0*	*-1*	*0*	*-1*

Date 23/07/18 → 13/08/18
Waiting for consumables

Date 14/08/18

Time line experiments:

- Ecoli = the whole story
- Bsub = all antibiotics for MIC
- Suppression of Mseg → problem of growth
- Killing kinetics for E.coli + B.sub
- Control Killing Kinetics = Ampicilline + Ovispirine
- Before using production, antibiogram like test

Date 16/08/18

MIC: B.sub - All Antibiotics

Dilution V1:

Antibiotic	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Concentration (µg/mL)	500	250	125	100	75	50	25	10	5	1	0,5	0
Volume of 10x diluted stock of antibiotic (µL)	X	100 of A	100 of B	40 of A	100 of C	100 of D	100 of F	40 of F	100 of H	40 of I	100 of J	0
Volume of MHB (µL)	200 - X	100	100	160	100	100	100	160	100	160	100	200

X = 500 * 200 / Ci
AMP, GENT, KAN, NAL, TMP, OLG : 10 µL
CAM : 33.3 µL
ERY, RIF : 50 µL
Didn't do Antibiotic stock dilution so concentrations are 10 times higher for AMP, CAM, ERY, KAN,RIF, NAL

Date 17/08/18

MIC: B.sub - All Antibiotics

Dilutions V2 :

Antibiotic (mg/mL)	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Concentration (µg/mL)	50	25	12,5	10	7,5	5	2,5	1	0,5	0,1	0,05	0
Volume of x diluted stock of antibiotic (µL)	X	100 of A	100 of B	40 of A	100 of C	100 of D	100 of F	40 of F	100 of H	40 of I	100 of J	0
Volume of MHB (µL)	200 - X	100	100	160	100	100	100	160	100	160	100	200

AMP : 100X dilution, 10 µL
NEO : 10X dilution, 10 µL
KAN : 100X dilution, 10 µL
NAL : 100X dilution, 10 µL
TMP : 10X dilution, 10 µL
OLG : 10X dilution, 10 µL
CAM : 30X dilution, 20 µL
ERY : 20X dilution, 10 µL
RIF : 10X dilution, 10 µL

Results: https://drive.google.com/drive/folders/1s9QjOouQ6TP5e5PrMN84-Hk-To9t_l8l

Date 18/08/18
Killing Kinetics : Do a culture of 50mL in LB for 2h than if OD600=0.02, add the drug

Date 20/08/18

MIC: B.sub - All Antibiotics → New dilutions !

Dilutions V3 :

We should have an antibiotic work solution of 100 µg/mL

	C Stock	Dilution to do	(uL)	V MHB (uL)
AMP,NAL,KAN	100mg/mL	1000	1	999
CAM	30mg/mL	X300	3,3	996,7
ERY, RIF	20mg/mL	x200	5	995
NEO, TMP	10mg/mL	x100	10	990

For ERY, NEO, RIF, TMP :

C diluted : 100 ug/mL	A	B	C	D	E	F	G	H	I	J	K	L
ATB Concentration (ug.mL)	10	5	1	0,1	0,05	0,025	0,01	0,005	0,0025	0,001	0,0005	0
Volume to take (uL)	**30 Diluted ATB**	**150 A**	**60 B**	**30 C**	**150 D**	**150 E**	**120 F**	**150 G**	**150 H**	**120 I**	**150 J**	**0**
MHB Volume (uL)	270	150	240	270	150	150	180	150	150	180	150	300

For AMP, KAN, NAL, CAM :

C diluted : 100 ug/mL	A	B	C	D	E	F	G	H	I	J	K	L
ATB Concentration (ug.mL)	50	25	15	10	5	2,5	1	0,5	0,1	0,05	0,01	0
Volume to take (uL)	**150 diluted ATB**	**150 A**	**180 B**	**200 C**	**150 D**	**150 E**	**120 F**	**150 G**	**60 H**	**150 I**	**60 J**	**0**
MHB Volume (uL)	150	150	120	100	150	150	180	150	240	150	240	300

Date 21/08/18

MIC: B.sub - All Antibiotics (Replicat n°2)
Protocol: Dilutions V3:

Date 22/08/18

Cell Spotting : replacement of spreading cell on plate to count CFU

What would be the settings ?

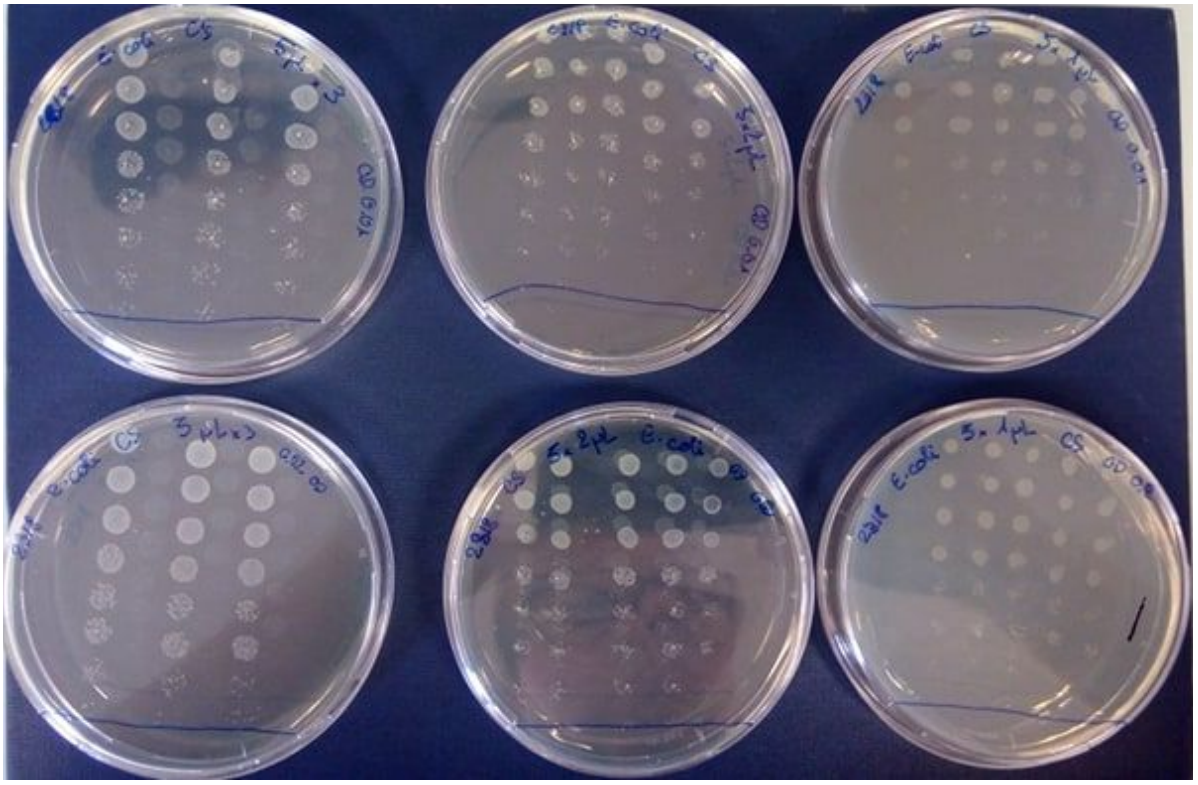
→ how many dilution on one plate (drop in a line) ?
→ how many replicat on one plate (number of row) ?
→ which volume in one drop to count easily the CFU and don't mix the dilutions ?

Test with E.coli without antibiotic
Test dilution 0 to -7 = 8 drop on a line
Test 3 replicats on a plate
Test volume drop: 1µl, 2µL, 5µL

Protocol of the running test:

1) Grow cell for 2h
2) When OD600=0.02 make the different test
3) Grow plate at room temperature

Results:



⇒ 3 rows of 8 drops of 4µL

Date 24/08/18

MIC: B.sub - Tetracycline / Erythromycin / Ampicillin / Rifampicin

Dilution V3:

Date 28/08/18

MIC: E.coli - Tetracyclin
Dilution V3 :

Killing Kinetics: E.coli - Ampicilin

- Condition : without antibiotic, 0.25xMIC, 1xMIC, 2xMIC,
- Taking samples : 0min, 30min, 2h, 4h, 6h, 8h

⇒ For ctrl & 0.25*MIC, dilutions will be : 0, -2, -4, -6, -7, -8, -9, -10

Mutagenesis - E.coli Ampicillin**:** during Killing Kinetics

- Condition : without antibiotic, 0.25xMIC, 1xMIC, 2xMIC,
- Taking samples : 0min, 30min, 2h, 4h, 6h, 8h
- 2 plates per condition and per time
- Rifampicin plate at 100µg/mL

Flow Cytometry - E.coli Ampicillin: during Killing Kinetics

Date 04/09/18

Chemical AMP preparation :
- 20min room temperature before making a solution
- Add ultra pure water
- Make aliquots

MIC: E.coli - Maganin 2 / Trimetoprime

Dilution V3 :

Dilution V4 - AMP:

Cwork solution = 1 mM = 2,4669 mg/mL

4-5/09/18	A	B	C	D	E	F	G	H	I	J	K	L
C Magainin (ug/mL)	300	150	75	37,5	18,75	9,38	4,69	2,34	1,17	0,59	0,29	0
Volume to take from work solution (uL)	36,48	150	100	0	0	0	0	0	0	0	0	
MHB Volume (uL)	263,52	100	150	150	150	150	150	150	150	150	150	300

MIC: B.sub - Maganin 2 / Ampicillin / Erythromycin

Dilution V3 :

Dilution V4 - AMP & Ery:

AMP: 100 ug/mL	A	B	C	D	E	F	G	H	I	J	K	L
ATB Concentration (ug.mL)	50	37,5	25	18,75	12,5	9,375	6,25	3,125	1,5625	0,78125	0,390625	0
Volume to take (uL)	100	75	100A	100B	100C	100D	100E	100G	100H	100I	100J	0
MHB Volume (uL)	100	100	100	100	100	100	100	100	100	100	100	300

TMP 04/10												
C : 100 ug/mL	A	B	C	D	E	F	G	H	I	J	K	L
ATB Concentration (ug.mL)	25,	12,5	6,25	3,13	1,56	0,78	0,39	0,20	0,10	0,05	0,02	0,01
Volume to take (uL)	50	100A	100B	100C	100D	100E	100F	100G	100H	100I	100J	0
MHB Volume (uL)	150	100	100	100	100	100	100	100	100	100	100	300

ERY : Cstock = 30mg/mL - 37µL ERY stock dans 963

C diluted : 100 ug/mL	A	B	C	D	E	F	G	H	I	J	K	L
ATB Concentration (ug.mL)	250	175	125	87,50	62,50	43,75	31,25	21,88	15,63	10,94	7,81	
Volume to take (uL)	100	70	100A	100B	100C	100D	100E	100G	100H	100I	100J	0
MHB Volume (uL)	100	130	100	100	100	100	100	100	100	100	100	300

Date 05/09/18

Killing Kinetics: E.coli - Ampicilin

- Condition : without antibiotic, 0.25xMIC, 1xMIC, 2xMIC,
- Taking samples : 0min, 30min, 2h, 4h, 6h, 8h

Date : 06/09/18

MIC MAG

06/09/2018	A	B	C	D	E	F	G	H	I	J	K	L
C Magainin (ug/mL)	1000	875	750	625	500	437,5	375	312,5	250	175	125	0
Volume to take from work solution (uL)	100	87,5	75	62,5	50	43,75	37,5	31,25	25	17,5	12,5	
MHB Volume (uL)		12,5	25	37,5	50	56,25	62,5	68,75	75	82,5	87,5	100

Results : No MIC determined

Date : 07/09/18

07/09/2018	A	B	C	D	E	F	G	H	I	J	K	L
C Magainin (ug/mL)	200	150	100	75	50	37,5	25	18,75	12,5	6,25	3,13	0
Volume to take from work solution (uL)	16,21	12,16	100 A	100 B	100 C	100 D	100 E	100 F	100 G	100 I	100 J	
MHB Volume (uL)	183,79	187,84	100	100	100	100	100	100	100	100	100	300

Results : No MIC determined

Dilution of stock of ovispirin to have 1 uM stock :
Stock = 100 uL à 4,4191 mM
22,6 uL stock + 77,4 uL H2O

MIC Ovispirin

07/09/2018	A	B	C	D	E	F	G	H	I	J	K	L
C Ov1(ug/mL)	183,46	137,6	91,73	68,8	45,87	34,4	22,93	17,2	11,47	5,73	2,87	0

Volume to take from work solution (uL)	16,21	12,16	100 A	100 B	100 C	100 D	100 E	100 F	100 G	100 I	100 J	
MHB Volume (uL)	183,79	187,84	100	100	100	100	100	100	100	100	100	200

Results : MIC is at 11,47 ug/mL for e coli and b sub

Date 08/09/18

Killing Kinetics: B.sub - Ampicilin

- Condition : without antibiotic, 0.25xMIC, 1xMIC, 2xMIC,
- Taking samples : 0min, 30min, 2h, 4h, 6h, 8h
- MIC : 10µg/mL

Mutagenesis - B.sub Ampicillin**:** during Killing Kinetics

- Condition : without antibiotic, 0.25xMIC, 1xMIC, 2xMIC,
- Taking samples : 0min, 30min, 2h, 4h, 6h, 8h
- 2 plates per condition and per time
- Rifampicin plate at 100µg/mL

Flow Cytometry - B.sub Ampicillin: during Killing Kinetics

Date 11/09/18

MIC: B.sub / E.coli - Ovispirin 1

Dilution AMP :

Take from 1µM stock.

11/09/2018	A	B	C	D	E	F	G	H	I	J	K	L
C Ovispirin(ug/mL)	30	22,5	15	11,25	7,5	5,63	3,75	2,81	1,88	0,94	0,47	0
Volume to take from work solution (uL)	26,51	19,89	100 A	100 B	100 C	100 D	100 E	100 F	100 G	100 I	100 J	
MHB Volume (uL)	173,49	180,11	100	100	100	100	100	100	100	100	100	200

Results:

E coli : MIC is 11,25 µg/mL , MIC is 1,88 µg/mL for B.sub

Date 23/09/18

BacTiter Glow:
Storage
→ Lyophilized & Buffer = -20°C
→ Buffer = 4°C or Room Temperature for 48H

→ Reagent = Room Temperature for 8h
= 4°C for 4 days
= -20°C for 1 week
= -80°C for 1 month

Reagent preparation : 100mL of Buffer into the brown bottle containing

Date 24/09/18

Sensitivity test:
Which quantity of bacteria do we need into a plate ?
Which volume of drug do we need to add ?

Volume tested : 1µL / 2µL / 3µL / 4µL / 5µL
Quantity of bacteria tested : 500µL of bacteria solution at OD600 = 1 / 0.1 / 0.0

Drug tested: Ampicilline 20µg/mL (2*MIC)

1) Prepare the solution of bacteria by diluting the overnight culture
2) Spread 500µL of the bacteria solution
3) Wait the plate is dried
4) Add up to 5 drops of drug
5) Incubate at 37°C for the night

Results :

- Volume of 4µL and 5µL are to big ; 3µL is too big if there is 5 drops ; 1µL and 2µL are good
  → Test 0.75µL / 0.5µL / 0.25µL
- B.sub, OD of 0.1 to 0.01 is good / OD of 1 is too high and we see som resistant bacteria
- E.coli, for every concentration bacteria grow but there is too much bacteria to see colored colonies
  → Try again with same parameter but using a new E.coli stock

Date 26/09/18

Sensitivity test:
Which quantity of E.coli do we need into a plate ?
Which volume of drug do we need to add ?

Volume tested : 2µL / 1µL / 0.75µL / 0.5µL / 0.25µL
Quantity of E.coli tested : 300µL of bacteria solution at OD600 = 1 / 0.1 / 0.01
B.sub settings : 300µL of bacteria solution at OD600 = 0.07

Drug tested: Ampicilline 20µg/mL (2*MIC)

Results:

- All volume works on B.sub
- E.coli, for every concentration bacteria grow but there is too much bacteria to see colored colonies
  → Try OD behind 0.01 with volume of 300µL

Date 28/09/18

Sensitivity test:
Which quantity of E.coli do we need into a plate ?

Volume tested : 1µL / 0.5µL / 0.25µL
Quantity of E.coli tested : 300µL of bacteria solution at OD600 = 0.01 / 0.001 / 0.0001

Drug tested: Ampicilline 20µg/mL (2*MIC)

Results:

- We can see colored colony even if it is not a complete carpet of bacteria but the concentration is enough to see if the drug works or not

Date 29/09/18

Sensitivity test:
29 constructs : Core 1 & Core 14

Settings:

- Volume of bacteria =300µL
- E.coli OD600 = 0.007
- B.sub OD600 = 0.014
- Volume of drug = 1.5µL
- Overnight incubation at 37°C

60 plates of MHB agar : 1 plate per construct * 29 constructs * 2 strains + 1 plate control * 2 strains

Results :

- There is a nice carpet of bacteria, so construct don't kill bacteria
  → Are the construct producing ? Is AMP efficient for enough time to kill bacteria ?
  → Make positive control with chemical AMP

Date 2/10/18

Sensitivity test:
Positiv control ovispirin 1 1mM & maganin 2 1mM on E.coli & B.sub

Settings:

- Volume of bacteria =300µL
- E.coli OD600 = 0.004
- B.sub OD600 = 0.025
- Volume of drug = 1.5µL
- Overnight incubation at 37°C

Results:
There is no diffusion of tha AMP into the agar so the area of dead bacteria correspond to the size of the drop

Date 3&4/10/18

MIC : E.coli - all AB 1 ligne

Dilution N°4:

Results:

Antibiotic	MIC 1	MIC 2
Ampicilline	10	9,4
Naladixic Acid	3,125	6,25
Neomicin	0,8	1,5
Trimetoprim	0,4	0,2
Tetracyclin	1,5	1,5
Rifanpicin	6,25	12,5
Erythromycin	37	31,25
Chlorophenicol	3	6,25

Date 10&11/10/18

MIC: E.coli & B.sub - Bioneer Product

Dilution N°5:

	A	B	C	D	E	F	G	H	I	J	K	L
C constructs (ug/mL)	150,0	75,0	37,5	18,75	9,38	4,7	2,3	1,17	0,59	0,29	0,15	0
Volume to take from work solution (uL)	37,07	75 A	75 B	75 C	75 D	75 E	75 F	75 G	75 H	75 I	75 J	
MHB Volume (uL)	112,9	75,0	75,0	75,0	75,0	75,	75,	75,0	75,	75,	75,	150

Growth Curve: E.coli & B.sub - Bioneer Product

Dilution N°5:

Date 12/10/18

Agar Pads: E.coli & B.sub - Bioneer Product 006 & 104

Resultat : video was not stable

Date 13/10/18

Agar Pads: E.coli & B.sub - Bioneer Product 006 & 104 & control
Resultat : Good video, we can see different phenotype depends of the construct and the strain

Date 14/10/18

Agar Pads: E.coli & B.sub - Bioneer Product 81 & 89 & Ovispirin 1

Growth Curve:

Dilutions N°5: