Global Salm-Surv

A global *Salmonella* surveillance and laboratory support project of the World Health Organization

Laboratory Protocols

Level 2 Training Course

MIC susceptibility testing of *Salmonella* and *Campylobacter*

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Introduction

The MIC (Minimal Inhibitory Concentration) of a bacterium to a certain antimicrobial agent can be determined and today gives the best quantitative estimate for susceptibility.

MIC is defined as the lowest concentration of antimicrobial agent required to inhibit growth of the bacteria. The principle is simple: Agar plates, tubes or microtitre trays with two-fold dilutions of antibiotics are inoculated with the bacteria and incubated. The next day the MIC is recorded as the lowest concentration of antimicrobial agent with no visible growth.

The MIC tells you about the degree of resistance and might give you important information about the resistance mechanism and the resistance genes involved. MIC-determination performed as agar dilution is regarded as the golden standard for susceptibility testing.

In contrast, diffusion tests are primarily qualitative methods that normally should only be used to report whether a bacterium is resistant or not. Principle: After an agar plate is inoculated with the bacteria, a tablet, disk or paperstrip with antimicrobial agent is placed on the surface. During incubation the antimicrobial agent diffuses into the agar and inhibits growth of the bacteria if sensitive. Diffusion tests are cheap compared to most MIC-determination methods. E-test is a diffusion test, but has been developed to give an approximate MIC-value.

Well standardised methods are essential for all kinds of susceptibility testing, since the methods are highly sensitive to variations in several factors, for example, size of inoculum, contents and acidity of the growth medium, time and temperature of incubation. The agar diffusion methods are also strongly influenced by factors such as agar depth, diffusion rate of the antimicrobial agent and growth rate of the specific bacteria.

The MIC-determination and disk diffusion methods described in this protocol are in accordance with the international recommendations given by the National Committee for Clinical Laboratory Standards (NCCLS). The NCCLS describes how to perform the testing and sets international guidelines for interpretation of the results.

Quality control is regularly performed by running specific control strains as recommended by NCCLS.

2. Antimicrobial susceptibility testing by agar dilution (MIC)

Introduction

Agar dilution susceptibililty testing is regarded as the golden standard for all other susceptibilily testing methods.
It is of course extremely important to be able to prepare the agar plates in such a way that the obtained antimicrobial concentration in the plates are exactly or very close to the desired concentrations
When preparing antimicrobial solutions and agar plates for agar dilution susceptibility testing, we therefore strongly recommend following the international guidelines given by the NCCLS (NCCLS document M7-A5 "*Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*").
Instructions on how to prepare the antimicrobial solution are outlined in table 5 (NCCLS document M100-S12) and further explained in appendices 1 and 2. The dilution procedure might at first seem a little complicated, but this method ensures that there is minimal risk of making out-of-scale-dilutions for the smallest concentrations in the test range.

Materials

Equipment

- McFarland standard 0.5
- Nephelometer or white paper with black lines
- Multi-point inoculator (applies up to 30 inocula to the same agar plate)
  At this course only parts from a multi-inoculator will be used: A stand with inoculation pins, and a well inoculation pot.
- Graduated pipettes (20 µl - 1000 µl)
- Disposable loops (1 µl and 10 µl)

Media

- Sterile normal saline, 4 ml volumes in tubes for nephelometer
- Eppendorf-tubes with 900 µl sterile normal saline
- Mueller-Hinton II agar plates (9 mm) for Salmonella with two-fold dilutions of antibiotic:
  Chloramphenicol (1-64 µg/ml)
  Ampicillin (0.5-32 µg/ml)
  Tetracycline (1-32 µg/ml)
- Mueller-Hinton II agar plates (9 mm) containing 5% cattleblood for camphylobacter with two-fold dilutions of antibiotic:
  Ciprofloaxin (0.125-16 µg/ml)
  Nalidixan (1-128 µg/ml)
  Tetracycline (0.5-32 µg/ml)
  Erythromycin 0.25-32 µg/ml)
  An example of the dilution procedure for preparing agar plates is shown in Appendix 1.
- Mueller-Hinton II agar plates (9 mm) for Salmonella without antibiotic for growth control (2 per test-antibiotic)

- Mueller-Hinton II agar plates (9 mm) containing 5% cattleblood for campylobacter without antibiotic for growth control (2 per test-antibiotic)
- Nutrient agar plates for purity control of inoculum suspension

Bacterial strains

- *Salmonella* strains on non-selective agar.
- *Campylobacter* strains on non-selective agar
- 4 strains for quality control: *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 and Campylobacter jejuni ATCC 33560

Safety

Carry out all procedures in accordance with the local codes of safe practice.

Preparing antimicrobial solutions and agar-plates for agar dilution MIC testing.

Procedure

Day 1

1. Find the highest concentration in your test-range in the column: “Final Concentration at 1:10 Dilution in Agar”. The row where you read the highest concentration will be your starting point in the dilution of the antimicrobials.

2. Find the stock solution for your test-range in the column: “Concentration”.

3. From the number of agar plates you want to prepare for each concentration, calculate the needed volume of agar per concentration.

4. 10 % of this volume will be antimicrobial solution. Bear this in mind when you calculate the needed volume of antimicrobial solution.

5. In the columns “Volume + Distilled Water” you will find the scale of dilution between the stock solution and the solvent.

6. You have to multiply the sum of the two columns by a digit large enough so that you are sure to have enough solution for preparing the agar plates and for further dilution of the antimicrobial solutions for rows 3, 6, 9 and 12.

7. When you incorporate the further dilution in the calculation of the antimicrobial solution for rows 3, 6, 9 and 12 bear in mind that at this step you have to add the needed volumes of antimicrobial solution for the next three rows.

Theory / comments

NCCLS manual M100-S12 page 114

Procedure

Theory / comments

8. You have now finished the first line of plates. Continue with the next concentration using the same procedure. Be aware of the change in the column: "Source". This step number refers to the solution from which the next line of solutions is made. Remember to multiply by a digit large enough so that you have enough of your solution for the agar plates and for preparing the next solutions**.**

9. When you prepare the stock solution remember to multiply so that the amount of antimicrobial to be weighed exceeds more than 100mg (for better accuracy).

10. When you plan your preparation of the antimicrobial solution, it may be an advantage to use the scheme in appendix 2 for the calculation of the solutions. Appendix 1 is an example of a calculation.

How to prepare the agar for producing plates to the agar dilution method

Day 1

1. The Müller Hinton II agar are melted and warmed in a water bath to approximately 50°C.

2. The different solutions (concentrations) of antimicrobials are poured into measuring glasses and labelled.

3. Add the agar to the measuring glasses with the antimicrobials and mixed gently. (If necessary, add blood to the agar before you pour it into the measuring glasses).

4. Pour the agar into empty petri dishes, which have been labelled. (The agar depth is crucial using this method).

Procedure

Theory / comments

5. Wait until they are set then reverse them and incubate them overnight for control of the purity.

6. Allow the surface of the agar-plates to dry before use. (Use plates within 14 days).

MIC determination by agar dilution

Day 1

Standardisation of inoculum
From a pure o/n culture, pick material from at least 3-4 colonies. Resolve totally in 4 ml NaCl in tubes. Mix.

This is done to minimize the risk of picking bacteria which have lost their resistance.

Adjust to McFarland 0.5 (nephelometer): Calibrate the nephelometer before use and gently turn all suspensions upside-down before measuring. Adjust turbidity of inoculum to match that of the standard.

McFarland 0.5 ~ approximately 10^8 CFU/ml

If a nephelometer is not available: Compare visually with the McFarland 0.5 standard using white paper with black lines as background.

The McFarland 0.5 suspension is diluted 10-fold to yield the final inoculum suspension: Transfer 100 µl to 900 µl saline in Eppendorf tubes. Turn the tube up-side-down two times.

The inoculum suspension ~ approximately 10^7 CFU/ml.

The inoculum suspension should be used for inoculation within 15 minutes.

To avoid further growth of inoculum.

Inoculation and incubation
Transfer 400 µl of the inoculum suspension to the multi-point inoculator wells.

This procedure must be carried out in a flow bench to avoid contamination

Procedure

Place the control strains as shown on the result sheet (Appendix 3) and write down the orientation of the other isolates too.

Inoculate plates starting with the lowest concentration. Remember to inoculate one of the growth control plates before and after. It is important that all plates are dry before inoculation.

Allow the inoculum-spots to dry upside down before incubation. (37°C for 16-20 h for Salmonella and 42°C for 48 h for Campylobacter).

Purity control: Spread 10 µl of the inoculation-suspension on a non selective agar plate. Incubate at 37°C /42°C overnight.

Day 2

Reading plates/interpretation of results
Check purity of the inoculum suspension. If not OK, results should not be reported.

Read plates as follows on a dark background:

- Use the result sheet (Appendix 3) for orientation of the isolates on the plates.
- Check growth on the two control plates. If growth is weak (faint haze, pinpoint colonies or <10 colonies), results can not be reported.
- The MIC is read as the lowest concentration without visible growth. A faint haze, pinpoint colonies or growth of a single colony should be ignored.

Be aware of special reading for trimethoprim and sulphonamides. In these cases the MIC is recorded as the lowest concentration where a growth reduction of 80-90 % can be seen.

Theory / comments

Most multi-point inoculators apply 1-2 µl of the suspension to the agar surface. The final inoculum on the agar will then be approximately 10^4 CFU per spot.

The MIC is determined from two-fold dilutions of the antimicrobial agent. Be aware that "the true" MIC can be anywhere between the observed MIC and the dilution step below.

The antibiotic trimethoprim and the sulphonamides allow growth of the bacteria for some generations before inhibition occurs.

Procedure

Further interpretation of the MIC is done according to the NCCLS recommendations (breakpoints for Enterobacteriaceae are visualised in the result sheet for microdilution broth testing, Appendix 4 and in Appendix 5 regarding Campylobacter).

The acceptable MIC-ranges for the quality control strains as recommended by the NCCLS for Enterobacteriaceae are shown in Appendix 6. For Campylobacter the MIC-ranges of the quality control strains are based on population-distribution in Appendix 7.

Theory / comments

The NCCLS standard do not include breakpoint-recommendations for all of the compounds and organisms tested. In these cases breakpoints are assigned in accordance to the population-distribution after testing a large number of isolates. (Appendix 5 and Appendix 7).

3. Composition and preparation of culture media and reagents

The media and reagents are available from companies like Oxoid, Merck and Difco. The composition of the dehydrated media given below is an example and may vary a little among the different manufacturers. Also the media should be prepared according to the manufacturers description if it differs from the description given here.

Mueller Hinton II agar (e.g. from BBL)

Beef extract	2.0 g
Acid hydrolysate of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
Distilled water	1000 ml

Preparation:
Dissolve the dehydrated medium in water by heating if necessary. Adjust pH to 7.2 - 7.4, transfer into bottles and autoclave at 110°C for 20 min.

Saline solution

Sodium chloride	8.5 g
Water	1000 ml

Preparation:
Dissolve the sodium chloride in the water, by heating if necessary. Adjust pH ~ 7.0 after sterilisation. Dispense the solution into tubes so 4 ml is obtained after autoclaving at 121°C for 20 min.

Columbia-agar

	25 L
Columbia agar base (Oxoid CM331)	1125 g
Water	25,000 ml
Natriumhydroxid 5N	
Saltsyre 4N	

Preparation:
Dissolve the Agar Base in water, and let it stand for 15 min. Boil the solution for 15 min., and adjust pH~7,1-7,5. The medium is poured into 1000 ml flasks and autoclaved at 121°C for 15 min.

Columbia-agar with cattle blood

Columbia agar	950 ml
Cattle blood	50 ml

Preparation:
Melt the agar and add cattle blood. Pour plates with about 15 ml melted medium in each. Incubate overnight at 37°C.

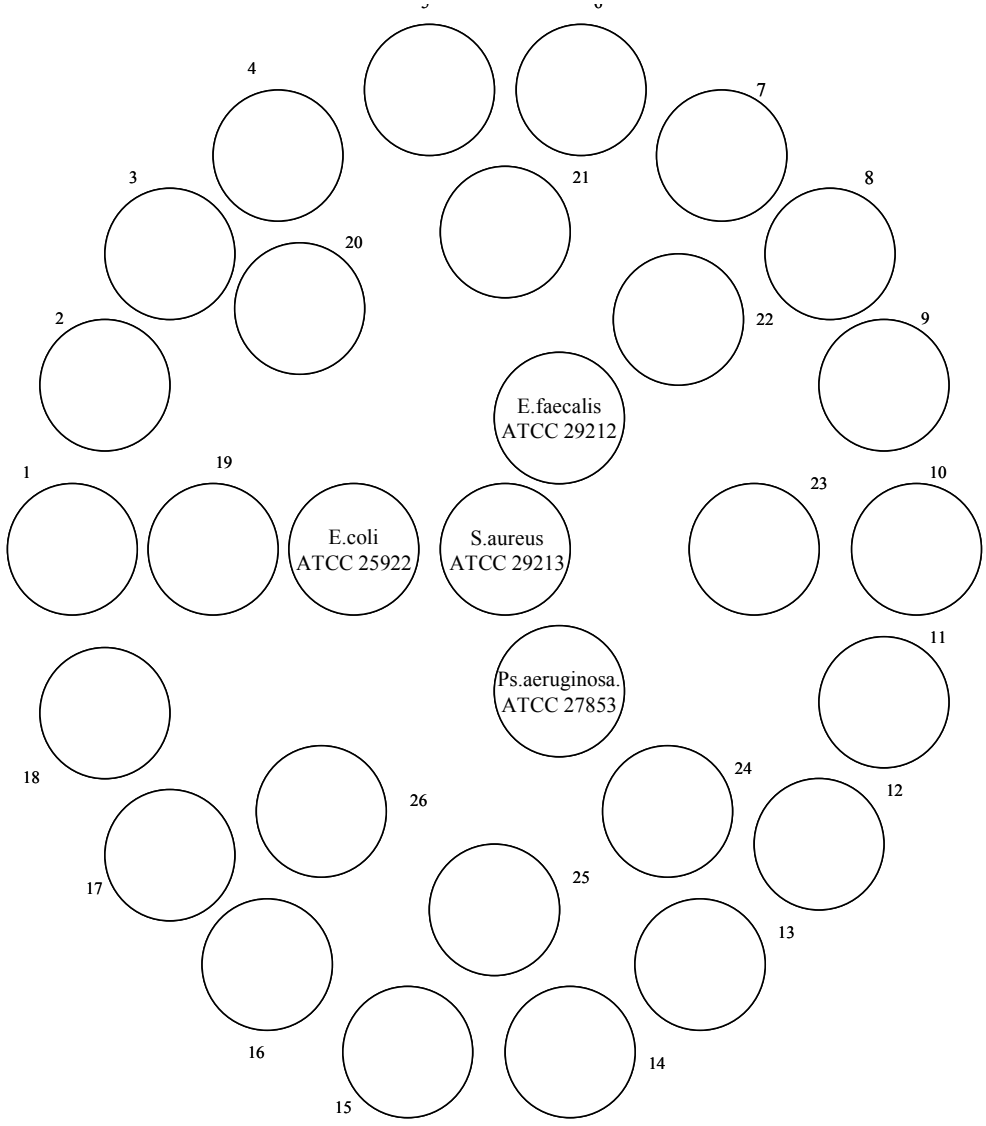
References

1. BARROW & FELTHAM (eds.): *Cowan and Steel's Manual for the Identification of Medical Bacteria,* 3 rd edn.

Date:_____**Record sheet: Salmonella / Chloramphenicol**

Initials:_____**MIC determination by agar dilution**

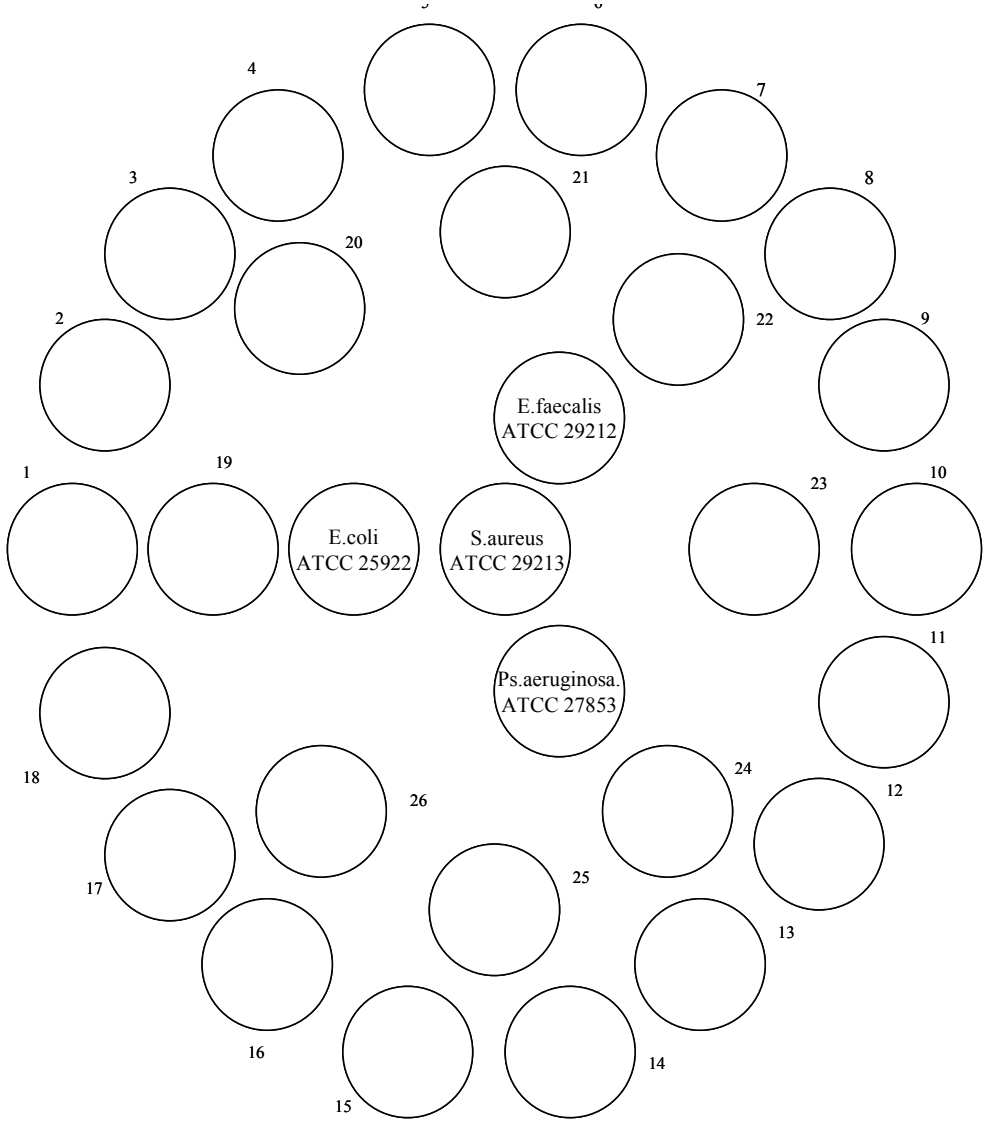
Before inoculation write the ID of the strains 1-26 above each circle.
Write the lowest concentration of antibiotic without growth (the MIC) in each circle.
Fill out the following table.



Date:_____ **Record sheet: Salmonella / Tetrecycline**

Initials:_____ **MIC determination by agar dilution**

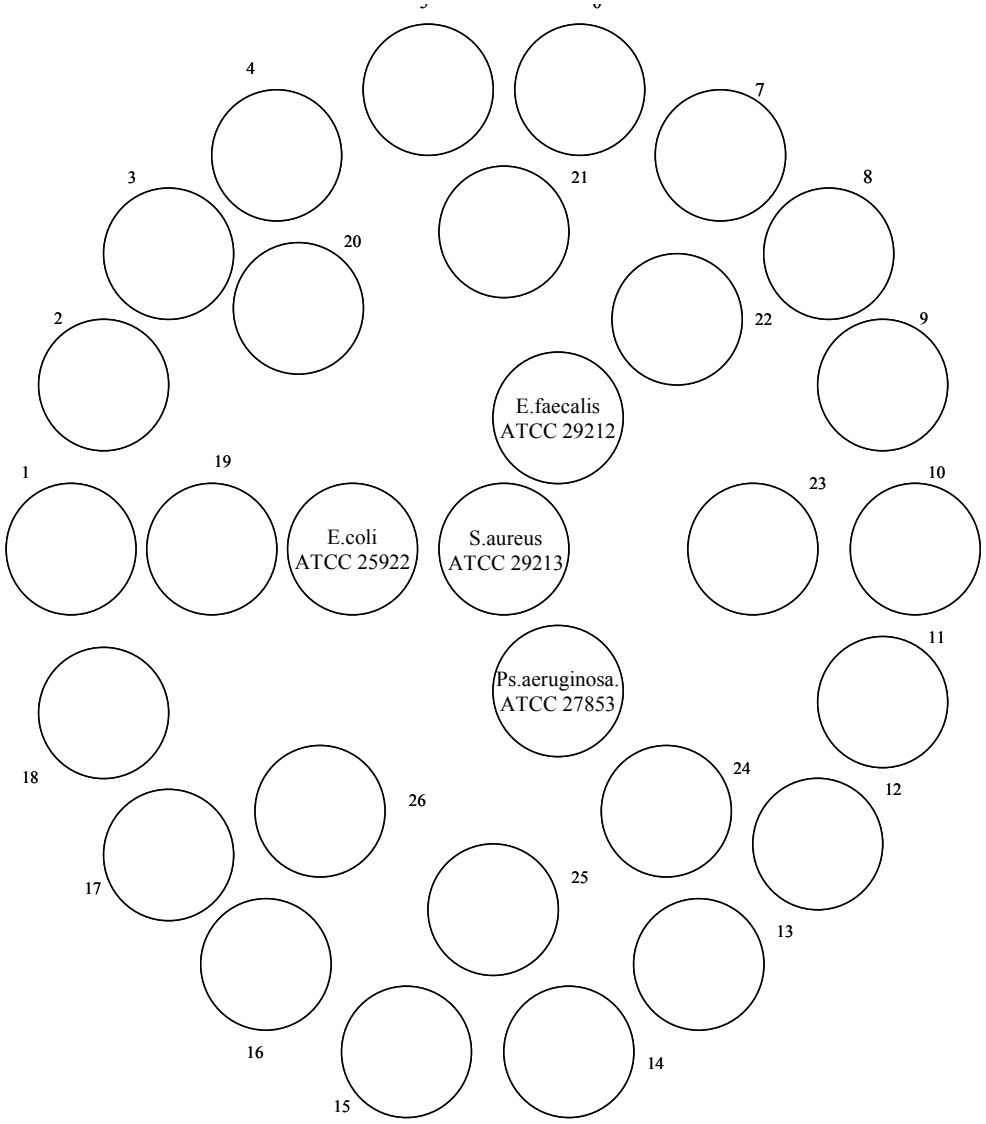
Before inoculation write the ID of the strains 1-26 above each circle.
Write the lowest concentration of antibiotic without growth (the MIC) in each circle.
Fill out the following table.



Date:_____ **Record sheet: Salmonella / Ampicillin**

Initials:_____ **MIC determination by agar dilution**

Before inoculation write the ID of the strains 1-26 above each circle.
Write the lowest concentration of antibiotic without growth (the MIC) in each circle.
Fill out the following table.



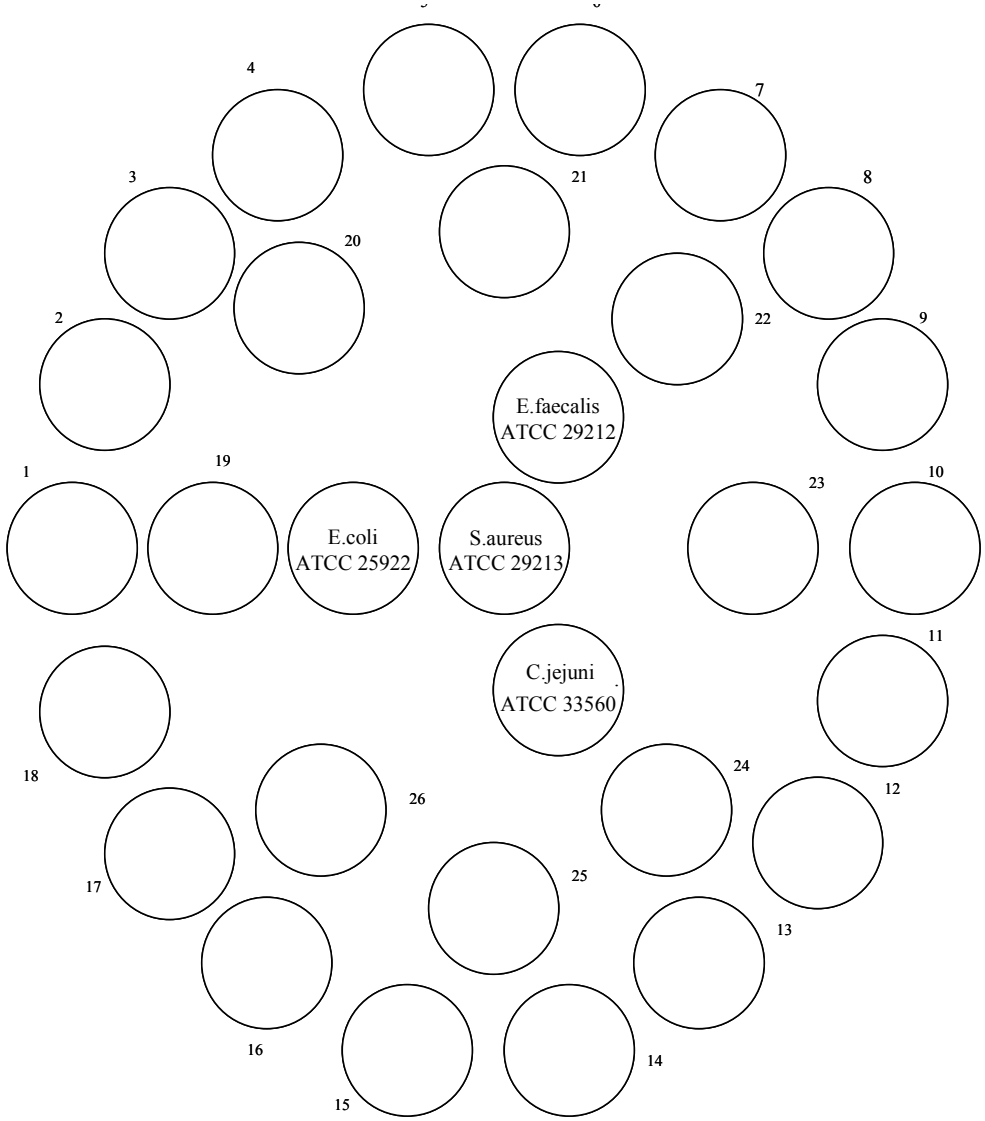
Date:_____ **Record sheet: Salmonella**

Initials:_____ **MIC determination by agar dilution**

No	Strain	Chloramphenicol		Ampicillin		Tetracycline	
		MIC (μg/ml)	Interpretation (R-I-S)	MIC (μg/ml)	Interpretation (R-I-S)	MIC (μg/ml)	Interpretation (R-I-S)
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							

Date:_____**Record sheet: Camphylobacter / Ciprofloxacin**
Initials:_____**MIC determination by agar dilution**

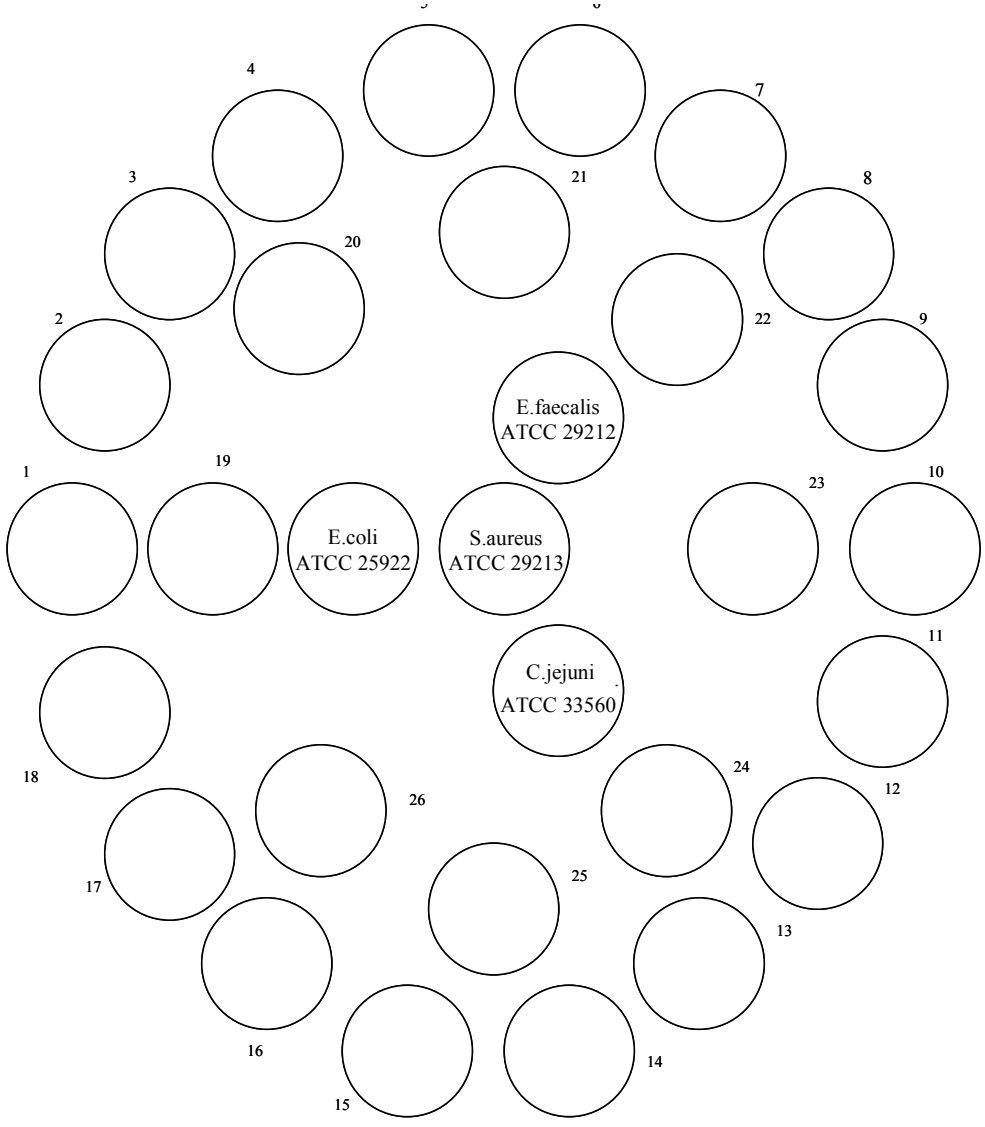
Before inoculation write the ID of the strains 1-26 above each circle.
Write the lowest concentration of antibiotic without growth (the MIC) in each circle.
Fill out the following table.



Date:_____ **Record sheet: Camphylobacter / Nalidixan acid**

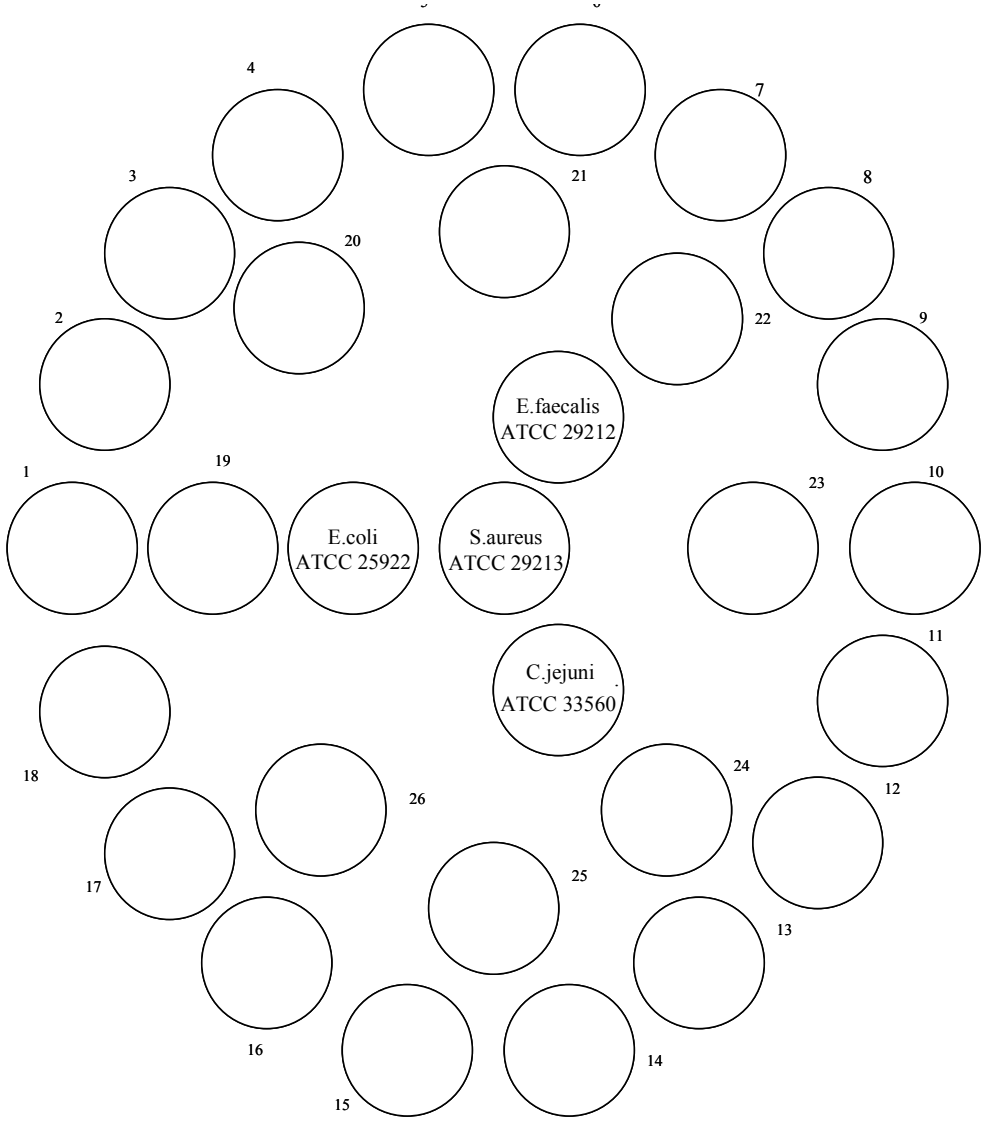
Initials:_____ **MIC determination by agar dilution**

Before inoculation write the ID of the strains 1-26 above each circle.
Write the lowest concentration of antibiotic without growth (the MIC) in each circle.
Fill out the following table.



Date:_____**Record sheet: Camphylobacter / Tetracycline**
Initials:_____**MIC determination by agar dilution**

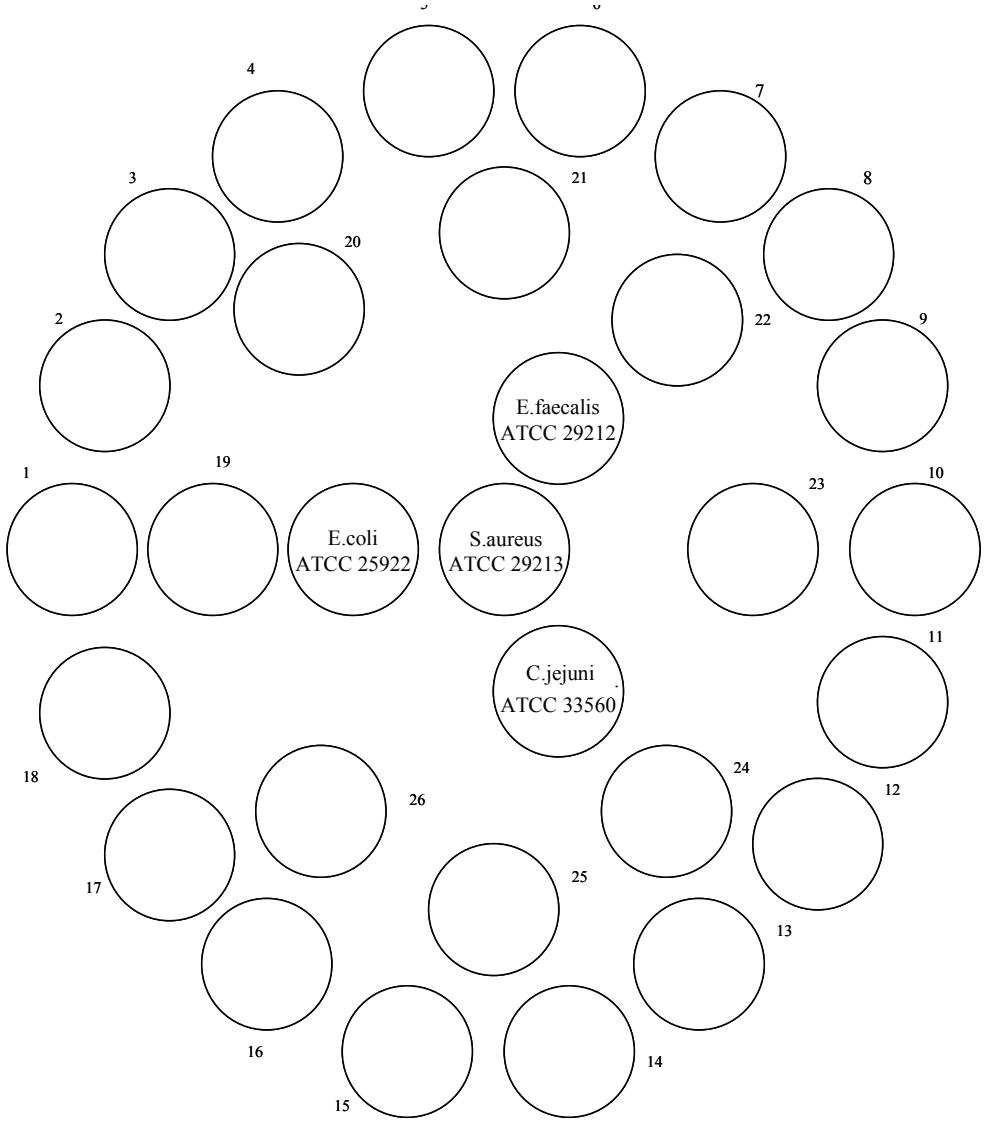
Before inoculation write the ID of the strains 1-26 above each circle.
Write the lowest concentration of antibiotic without growth (the MIC) in each circle.
Fill out the following table.



Date:_____**Record sheet: Camphylobacter / Erythromycin**

Initials:_____**MIC determination by agar dilution**

Before inoculation write the ID of the strains 1-26 above each circle.
Write the lowest concentration of antibiotic without growth (the MIC) in each circle.
Fill out the following table.



Date:_____ **Record sheet: Camphylobacter**

Initials:_____ **MIC determination by agar dilution**

No	Strain	Ciprofloxacin		Nalidixan acid		Tetracycline		Erythromycin	
		MIC (μg/ml)	Interpretation (R-I-S)	MIC (μg/ml)	Interpretation (R-I-S)	MIC (μg/ml)	Interpretation (R-I-S)	MIC (μg/ml)	Interpretation (R-I-S)
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
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15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									

APPENDIX 1

Example of preparing the dilutions of antimicrobial agents used in agar dilution. (NCCLS M100-S12 table 5)

Step	Concentration ug/ml.	Source	Volume + Solvent ml. ml.	Upscale to user vol. ml. ml.	Final vol. of solvent ml.	Final concentration At 1:10 dilution i agar	Vol. media ml.	Vol. solution ml.
1	5120	Stock	- -			512		
2	5120	Step 1	1 1			256		
3	5120	Step 1	1 3			128		
4	1280	Step 3	1 1			64		
5	1280	Step 3	1 3	***3 + 9***	***12***	***32***	***90***	***10***
6	1280	Step 3	1 7	***3 + 21***	***24***	***16***	***90***	***10***
7	160	Step 6	1 1	***6 + 6***	***12***	***8***	***90***	***10***
8	160	Step 6	1 3	***3 + 9***	***12***	***4***	***90***	***10***
9	160	Step 6	1 7	***3 + 21***	***24***	***2***	***90***	***10***
10	20	Step 9	1 1	***6 + 6***	***12***	***1***	***90***	***10***
11	20	Step 9	1 3	***3 + 9***	***12***	***0.5***	***90***	***10***
12	20	Step 9	1 7	***3 + 21***	***24***	***0.25***	***90***	***10***
13	2.5	Step 12	1 1			0.125		

Antimicrobial: ***Erythromycin.***
Antimicrobial gradient: ***0.25 – 32 ug/ml.***
Concentration of the stock solution: ***1280ug/ml.***
Volume of antimicrobial to be weight: (*1280ug/ml * 6ml) 1280ug/ml * 80 = 102.4mg*
Volume of Agar: ***90ml***
Volume of antimicrobial solutions (10% of agar vol):***10ml.***

APPENDIX 2

Scheme for preparing dilutions of antimicrobial agents used in agar dilution. (NCCLS M100-S12 table 5)

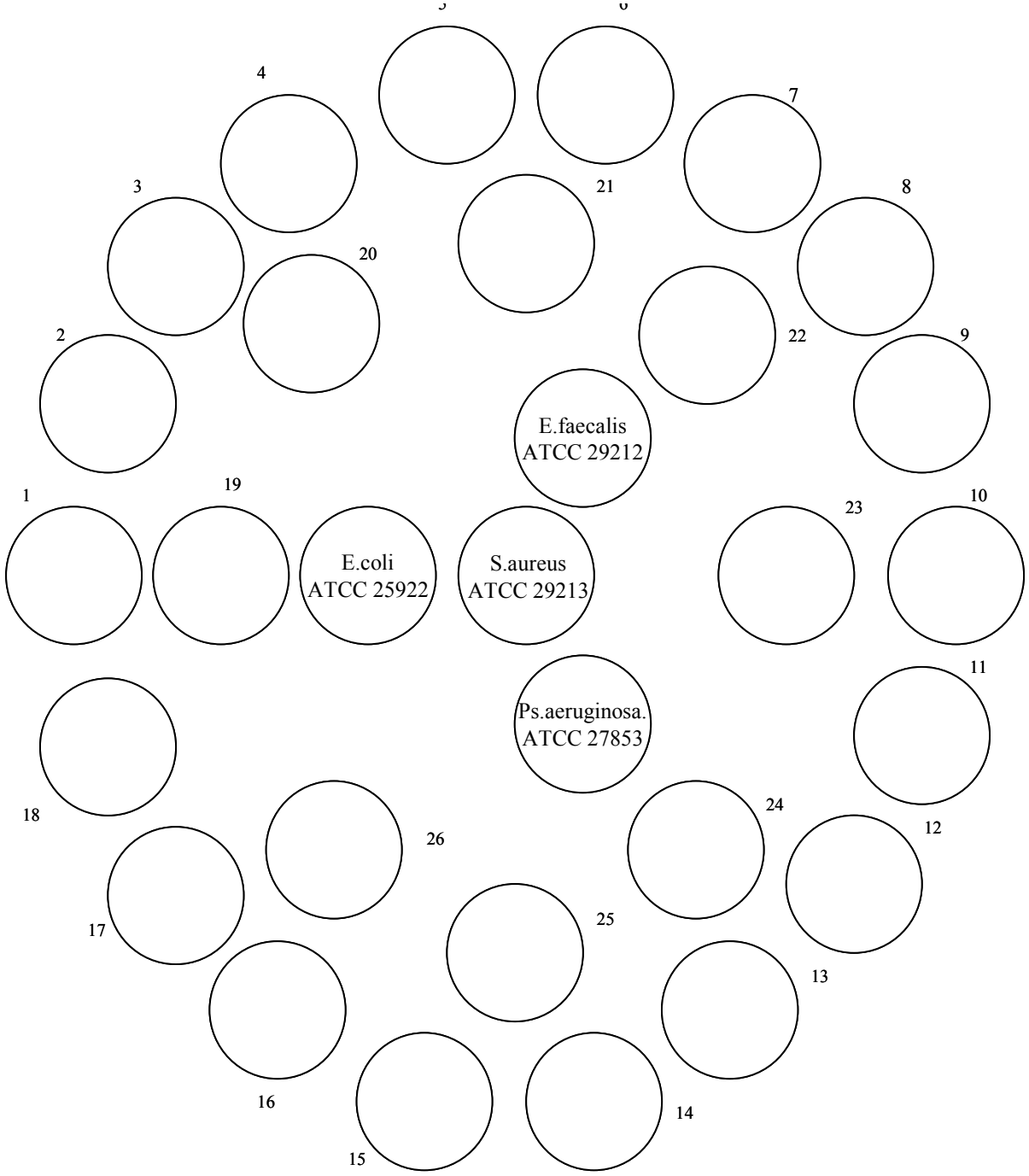
Step	**Concentration** ug/ml.	**Source**	**Volume + Solvent** ml. ml.	**Upscale to user vol.** ml. ml.	**Final vol. of solvent** ml.	**Final concentration** At 1:10 dilution i agar	**Vol. media** ml.	**Vol. solution** ml.
1	5120	Stock	- -			512		
2	5120	Step 1	1 1			256		
3	5120	Step 1	1 3			128		
4	1280	Step 3	1 1			64		
5	1280	Step 3	1 3			32		
6	1280	Step 3	1 7			16		
7	160	Step 6	1 1			8		
8	160	Step 6	1 3			4		
9	160	Step 6	1 7			2		
10	20	Step 9	1 1			1		
11	20	Step 9	1 3			0.5		
12	20	Step 9	1 7			0.25		
13	2.5	Step 12	1 1			0.125		

Antimicrobial:
Antimicrobial gradient:
Concentration of the stock solution:
Volume of antimicrobial to be weight:
Volume of Agar:
Volume of antimicrobial solutions (10% of agar vol):

APPENDIX 3

Record form for MIC determination by agar dilution

APPENDIX 4



Record form for MIC-determination by microdilution broth testing.

Interpretation of results:

MIC in white area:	SENSITIVE
MIC in light grey area:	INTERMEDIARY
MIC in darker grey area:	RESISTANT

Interpretation is in accordance to the NCCLS recommendations, except for a few of the agents, where the breakpoints are assigned by studying the population distributions of MICs.

Sensititre plate code: DKSVSN1

Enterobacteriaceae (E. coli, Salmonella, Yersinia e.g.), Pseudomonas og Bordetella

	1	2	3	4	5	6	7	8	9	10	11	12
A	**CIP 4**	**SPE 128**	**CEP 64**	**AMP 32**	**CHL 64**	**FFN 64**	**GEN 32**	**NEO 32**	**AUG2 32/16**	**TET 32**	**STR 64**	**SMX 1024**
B	**CIP 2**	**SPE 64**	**CEP 32**	**AMP 16**	**CHL 32**	**FFN 32**	**GEN 16**	**NEO 16**	**AUG2 16/8**	**TET 16**	**STR 32**	**SMX 512**
C	**CIP 1**	**SPE 32**	**CEP 16**	**AMP 8**	**CHL 16**	**FFN 16**	**GEN 8**	**NEO 8**	**AUG2 8/4**	**TET 8**	**STR 16**	**SMX 256**
D	**CIP 0.5**	**SPE 16**	**CEP 8**	**AMP 4**	**CHL 8**	**FFN 8**	**GEN 4**	**NEO 4**	**AUG2 4/2**	**TET 4**	**STR 8**	**SMX 128**
E	**CIP 0.25**	**SPE 8**	**CEP 4**	**AMP 2**	**CHL 4**	**FFN 4**	**GEN 2**	**NEO 2**	**AUG2 2/1**	**TET 2**	**STR 4**	**SMX 64**
F	**CIP 0.125**	**SPE 4**	**CEP 2**	**AMP 1**	**CHL 2**	**FFN 2**	**GEN 1**	**TMP 4**	**TMP 8**	**TMP 16**	**TMP 32**	**POS KON**
G	**CIP 0.06**	**COL 4**	**COL 8**	**COL 16**	**COL 32**	**COL 64**	**NAL 8**	**NAL 16**	**NAL 32**	**NAL 64**	**NAL 128**	**POS KON**
H	**CIP 0.03**	**XNL 0.5**	**XNL 1**	**XNL 2**	**XNL 4**	**XNL 8**	**APR 4**	**APR 8**	**APR 16**	**APR 32**	**APR 64**	**POS KON**

Kode	**Antimikrobielt stof**	**Testinterval (µg/ml)**
AUG2	AMOXICILLIN+CLAVULANAT (AM+CL)	2/1-32/16 (forholdet 2:1)
AMP	AMPICILLIN	1-32
APR	APRAMYCIN	4-64
CEP	CEFALOTIN	2-64
CHL	CHLORAMPHENICOL	2-64
CIP	CIPROFLOXACIN	0.03-4
COL	COLISTIN	4-64
FFN	FLORFENICOL	2-64
GEN	GENTAMICIN	1-32
NAL	NALIDIXAN	8-128
NEO	NEOMYCIN	2-32
SPE	SPECTINOMYCIN	4-128
STR	STREPTOMYCIN	4-64
SMX	SULPHAMETHOXAZOLE	64-1024
TET	TETRACYKLIN	2-32
TMP	TRIMETHOPRIM	4-32
XNL	CEFTIOFUR	0.5-8

APPENDIX 5

Ranges for MIC-determination on *Camphylobacter* by Agar-dilution testing.

Interpretation is based on breakpoints assigned by studying the population distributions of MICs.
No international breakpoints for *Campylobacter* have been developed yet.

ANTIMICROBIAL AGENT	Campylobacter.
Chloramphenicol.	≥ 64 μg/ml.
Nalidixic acid.	≥ 64 μg/ml.
Ciprofloxacin.	≥ 4 μg/ml.
Enrofloxacin.	≥ 2 μg/ml.
Erythromycin.	≥ 32 μg/ml.
Gentamicin.	≥ 16 μg/ml.
Neomycin.	≥ 16 μg/ml.
Streptomycin.	≥ 16 μg/ml.
Tetracycline.	≥ 16 μg/ml.

APPENDIX 6

Quality control ranges for MIC determinations on Enterobacteriaceae.

ANTIMICROBIAL AGENT	*Enterococcus faecalis* ATCC 29212	*Staphylococcus aureus* ATCC 29213	*Pseudomonas aeruginosa* ATCC 27853	*Escherichia coli* ATCC 25922
Ampicillin	0,5-2	0,25-1	>32	2-8
Carbadox	8-32	≤ 8	> 128	≤ 8
Chloramphenicol	4-16	2-8	> 64	2-8
Ciprofloxacin	0,25-2	0,12-0,5	0,25-1	0,004-0,015
Colistin	>32	>16	≤2	≤2
Florfenicol	2-8	2-8	>16	2-8
Gentamicin	4-16	0,12-1	0,5-2	0,25-1
Kanamycin	16-64	1-4	>128	1-4
Nalidixic Acid	>128	16-64	≥ 128	1-4
Nitrofurantoin	4-16	8-32	>128	4-16
Streptomycin	32-128	2-8	16-64	4-16***
Sulphamethoxazole	>512	>512	>512	8-32
Tetracycline	8-32	0,25-1	8-32	0,5-2
Trimethoprim	≤1	1-4	>64	0,5-2

Grey area: *NCCLS recommendations*
White area: *Quality control range assigned by the Danish Veterinary Laboratory*
****:* *Quality control range assigned to the Sensititre system by Trek Diagnostic Systems Ltd.*

APPENDIX 7

Quality control ranges for MIC determination on Campylobacter.

Antimicrobial Agent	Enterococcus *faecalis* **ATCC 29212**	Staphylococcus *aureus* **ATCC 29213**	Pseudomonas *aeruginosa* **ATCC 27853**	Escherichia *coli* **ATCC 25922**	Campylobacter *Jejuni* **ATCC 33560**
Ampicillin	1	≤1	>32	8	
Chloramphenicol	8	16	>64	8	
Ciprofloxacin	1	0,5	2	≤0,03	0,12 - 1
Colistin	>64	>64	0,5	≤0,25	
Erythromycin	2	≤0,25	>32	>32	1 - 8
Gentamicin	2	≤0,5	4	1	0,5 - 4
Nalidixic acid	>128	64	128	8	8 - 32
Neomycin	8	≤1	>64	4	
Streptomycin	64	4	64	8	
Sulphamethoxazole	512	128	>512	256	
Tetracycline	16	≤0,5	32	2	1 -4
Doxycycline					0,5 - 2
Meropenem					0,004 – 0,015

Grey area: *NCCLS recommendations (tentative QC ranges approved by the NCCLS-VAST in October 2000*
White area: *Quality control range assigned by the Danish Veterinary Laboratory*