

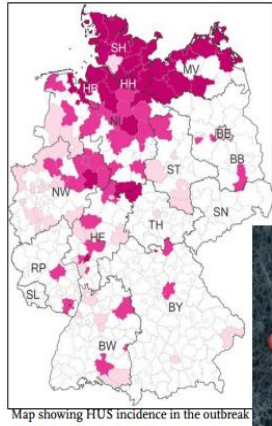
EHEC O104:H4 Outbreak Germany 2011

May 2011, Germany

3950 people infected

855 hemolytic-uremic syndrom

53 death



EHEC bacteria of the outbreak strain O104:H4
Scanning electron microscope. Scale: 1 µm
Source: Holland, Laue (Robert Koch Institute)



Analysis: E.coli outbreak poses questions
for **organic farming**



Organic Bockshornklee = Organic
Fenugreek



Ökologischer Gartenbau
pflanzen* Gemüse und Kräuter
Sprossfit* Keimprossen

	Sample
A	Chicken steak sample 1
B	Tomato salad sample 2
C	Raw chicken meat sample 2
D	Raw chicken meat sample 3
E	Patient 1
F	Patient 2



Antibiotic susceptibility testing

METHODS

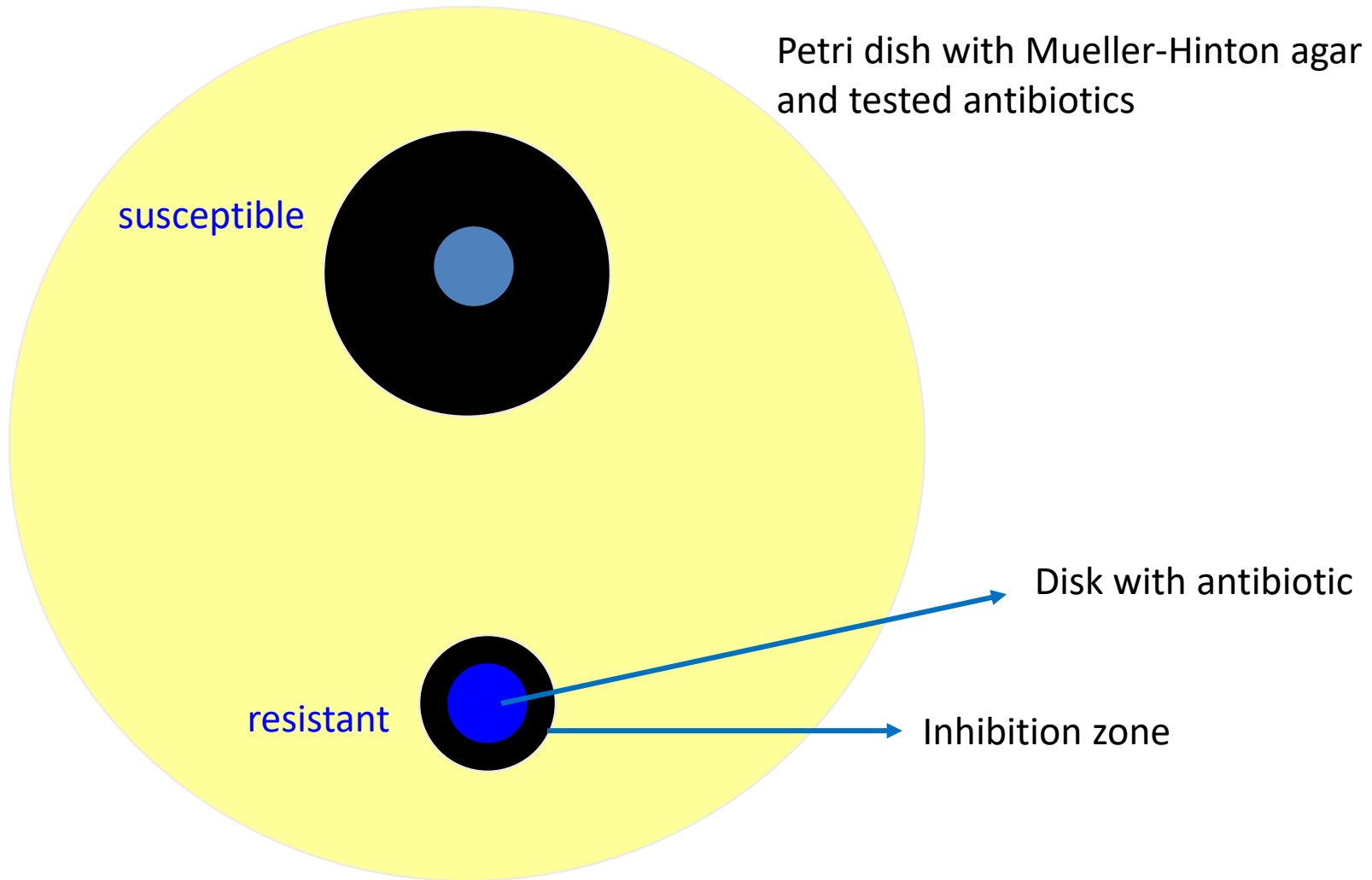
Disk diffusion method

- qualitative method

Dilution methods

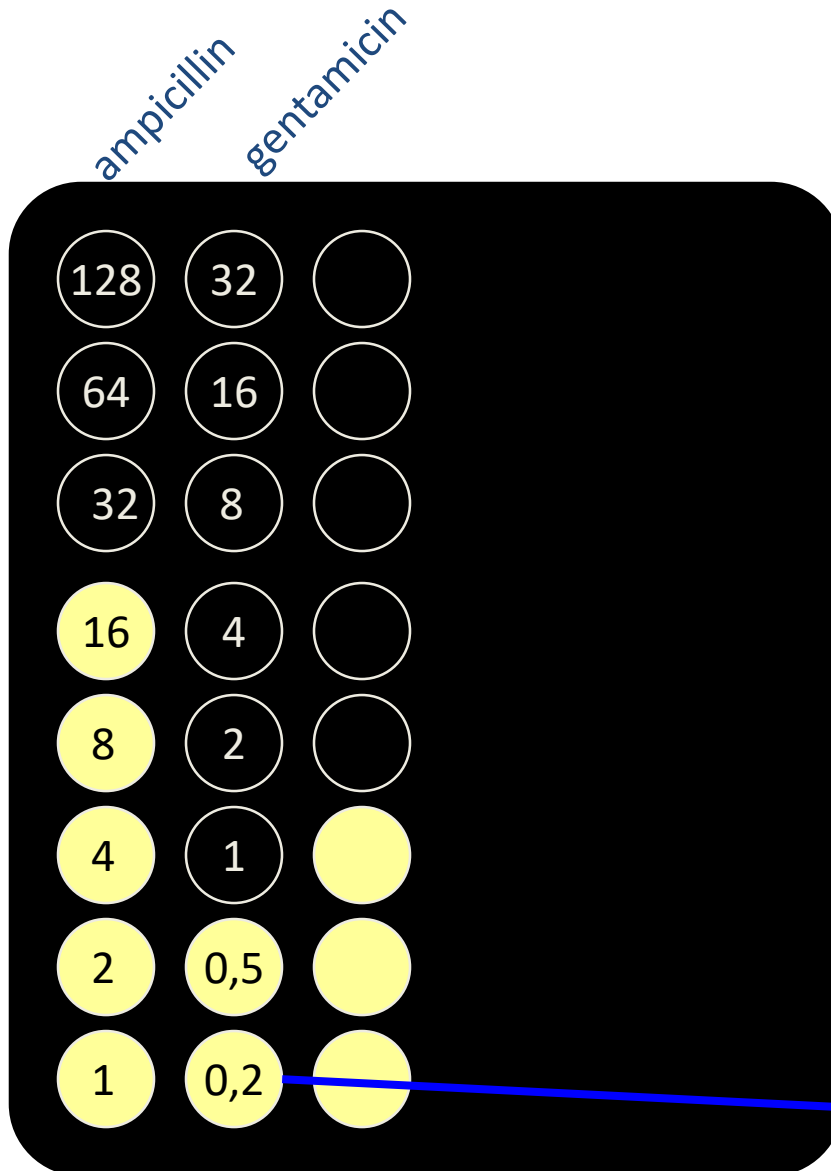
- quantitative method
 - Broth dilution method
 - Agar dilution method

Disk diffusion method



Broth dilution method – MIC testing

MIC – minimal inhibitory concentration (ug/ml, mg/l)



Microtiter plate
8 x 12 wells

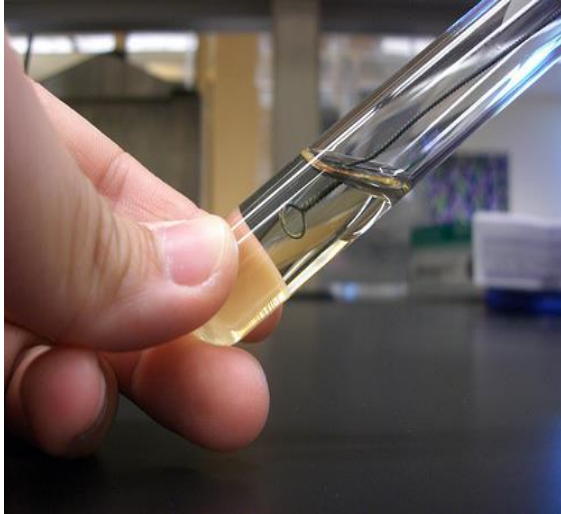
Mueller-Hinton broth with serial dilution of
antibiotics and inoculated bacterial strains

Antibiotic concentrations

Disk diffusion method

Steps:

1. Bacterial culture
2. Inoculum of bacterial culture (1 - 3 x 10⁸ cells/ ml)



3. Inoculation of cultivation plates

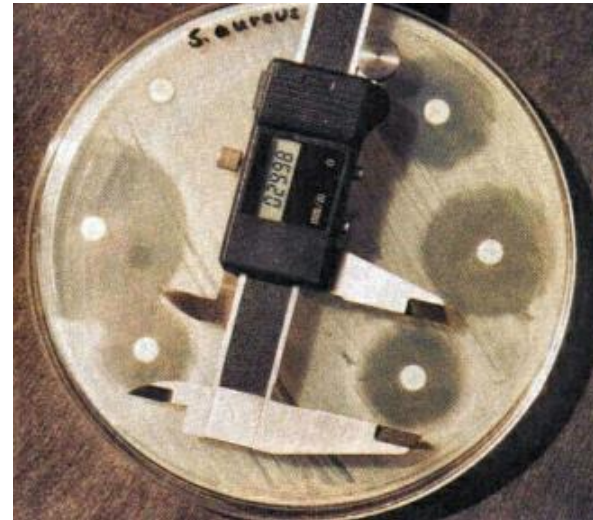


Disk diffusion method

4. Application of antibiotic disks



5. Measurement of inhibition zones



6. Interpretation of results

– sensitive, intermediate, resistant

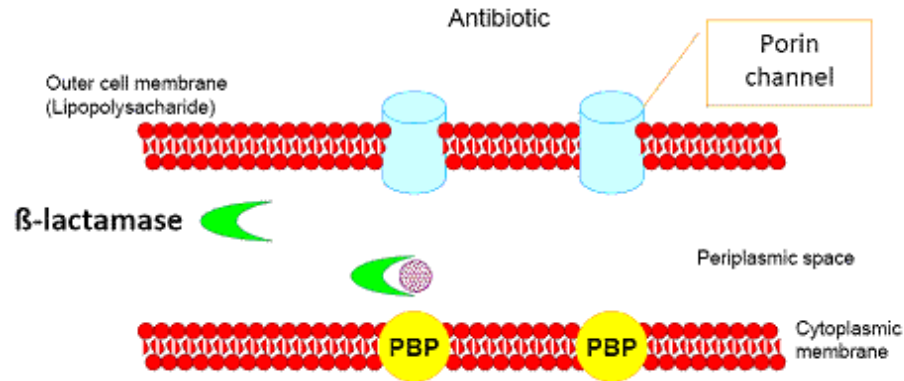
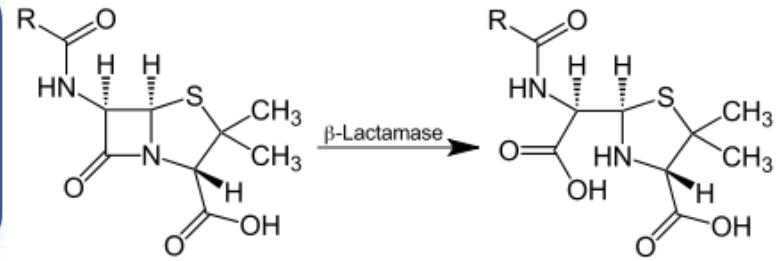
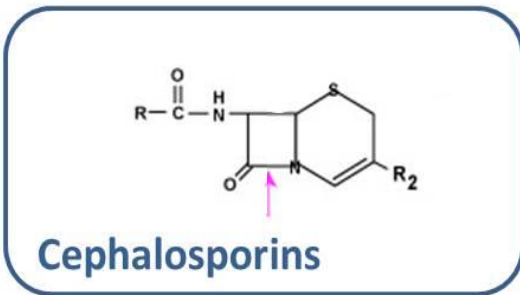
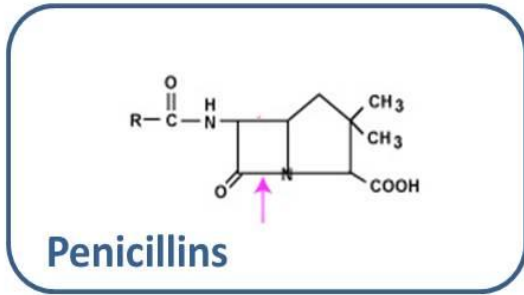


<http://www.clsi.org/>

Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Third Edition (M 31-A3)

Disk No.	Antibiotic disk		Amount of antibiotic in the disk (µg)	Size of inhibition zone		
	Abbreviation	Active compound		R resistant	I intermediate	S sensitive
1	AMP	ampicillin	10	≤ 13	14 - 16	≥ 17
2	S	streptomycin	10	≤ 11	12 - 14	≥ 15
3	S3	sulphonamides cp.	300	≤ 12	13 - 16	≥ 17
4	TE	tetracycline	30	≤ 14	15 - 18	≥ 19
5	SXT	trimethoprim/sulfamethoxazole	1,25/23,7	≤ 10	11 - 15	≥ 16
6	C	chloramphenicol	30	≤ 12	13 - 17	≥ 18
7	KF	cephalothin	30	≤ 14	15 - 17	≥ 18
8	NA	nalidixic acid	30	≤ 13	14 - 18	≥ 19
9	CAZ	ceftazidime	10	≤ 14	15-17	≥ 18
10	CN	gentamicin	10	≤ 12	13 - 14	≥ 15
11	AMC	amoxicillin/clavulanic acid	30(20/10)	≤ 13	14-17	≥ 18
12	CIP	ciprofloxacin	5	≤ 16	17 - 22	≥ 23

Resistance to beta-lactams – production of beta-lactamase



Extended-spectrum beta-lactamase (ESBL)



E. coli sensitive to cephalosporins

E. coli resistant to cephalosporins



Penicillin



Penicillin binding protein (PBP)



Extended Spectrum Beta-Lactamase





Inhibitor



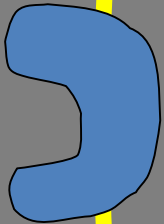
Penicillin



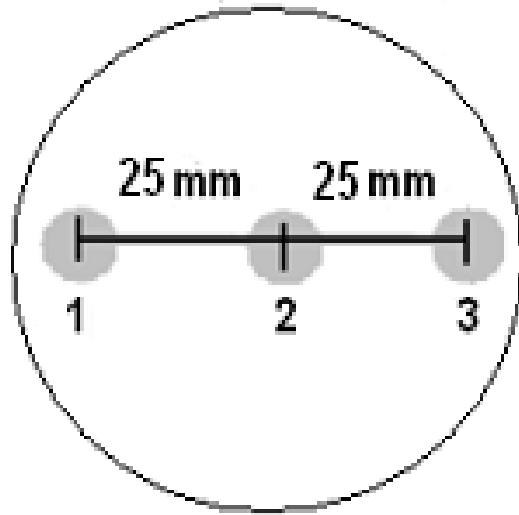
Penicillin binding protein (PBP)



Extended Spectrum Beta-Lactamase

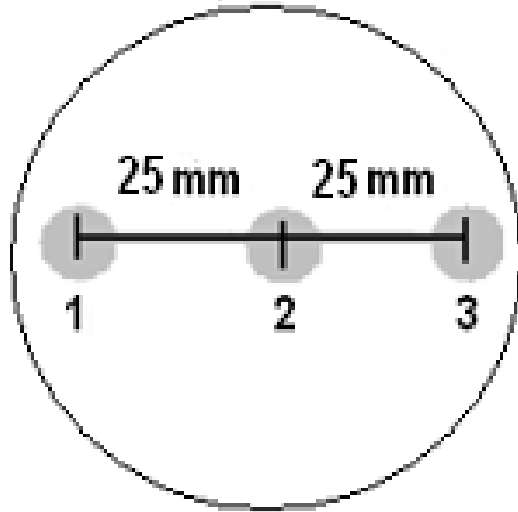


Double disk synergy test – detection of ESBL production



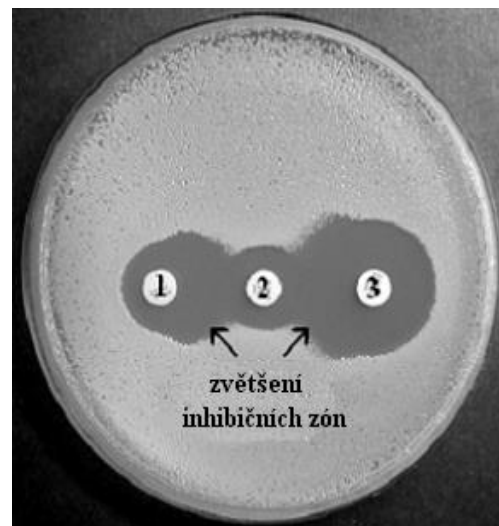
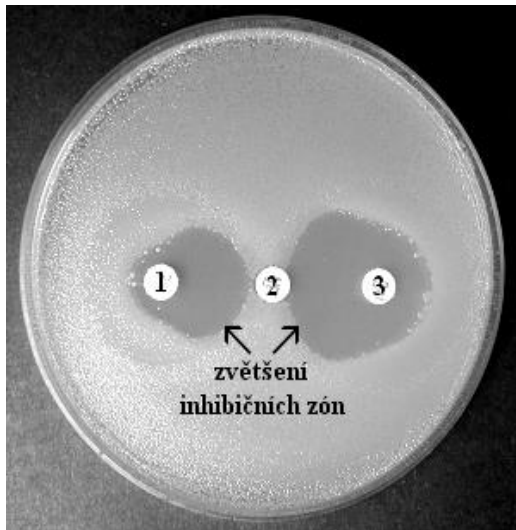
- 1...cefotaxime (CTX, 30 μ g)
- 2...amoxicillin-clavulanic acid (AMC, 30 μ g)
- 3...ceftazidime (CAZ, 30 μ g)

Double disk synergy test – detection of ESBL production

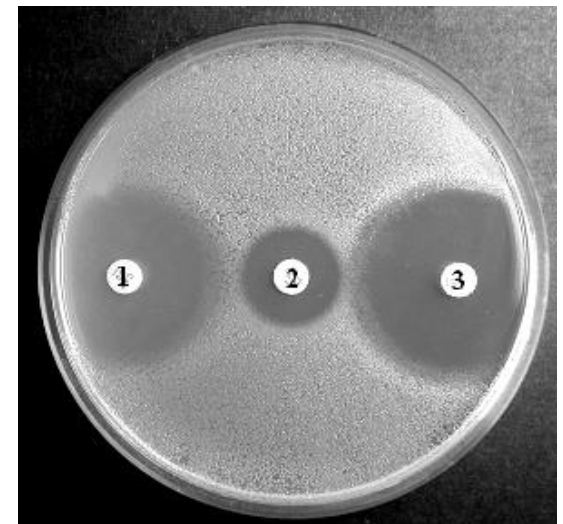


- 1...cefotaxime (CTX, 30 μ g)
- 2...amoxicillin-clavulanic acid (AMC, 30 μ g)
- 3...ceftazidime (CAZ, 30 μ g)

Positive results

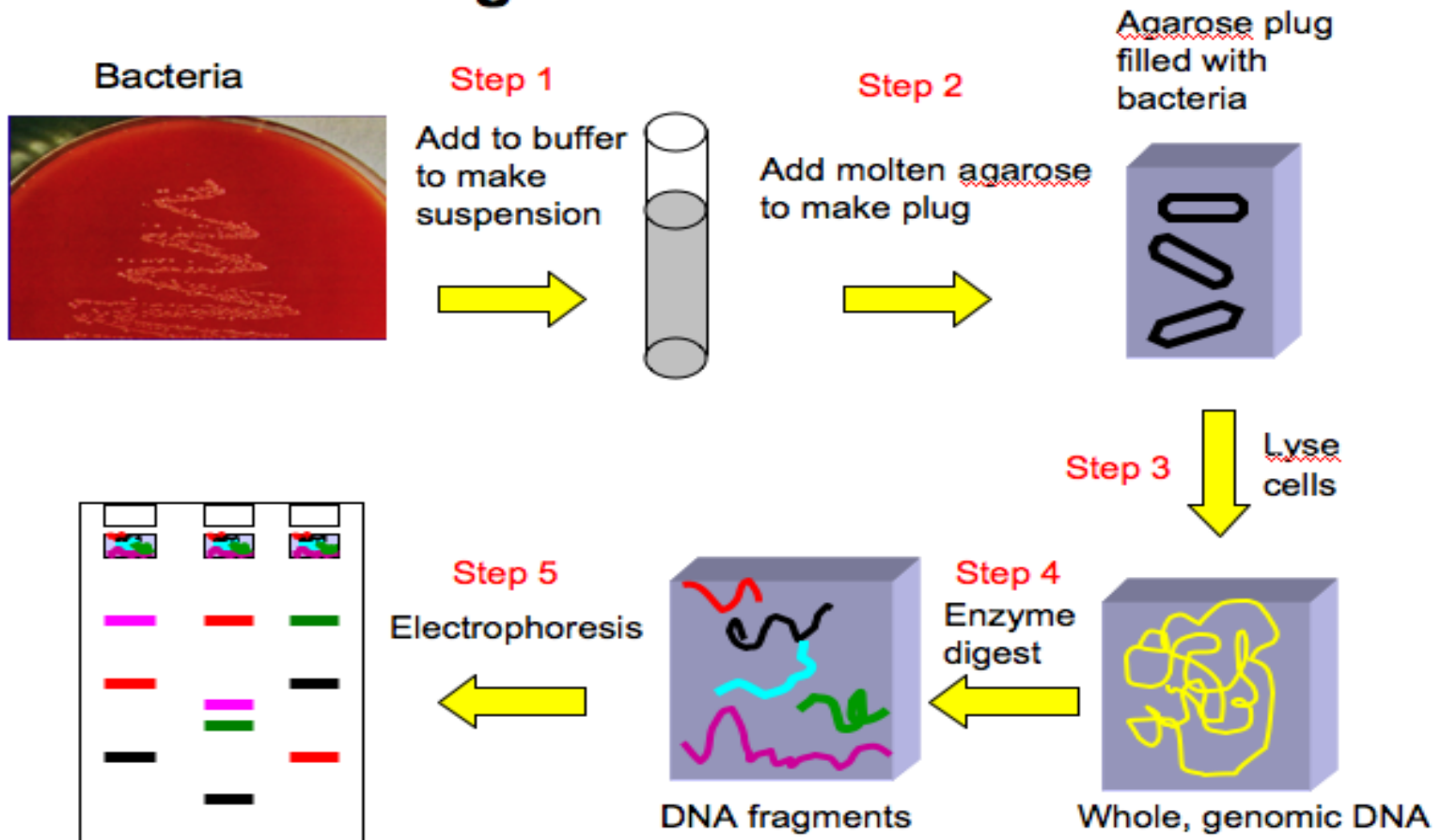


Negative result

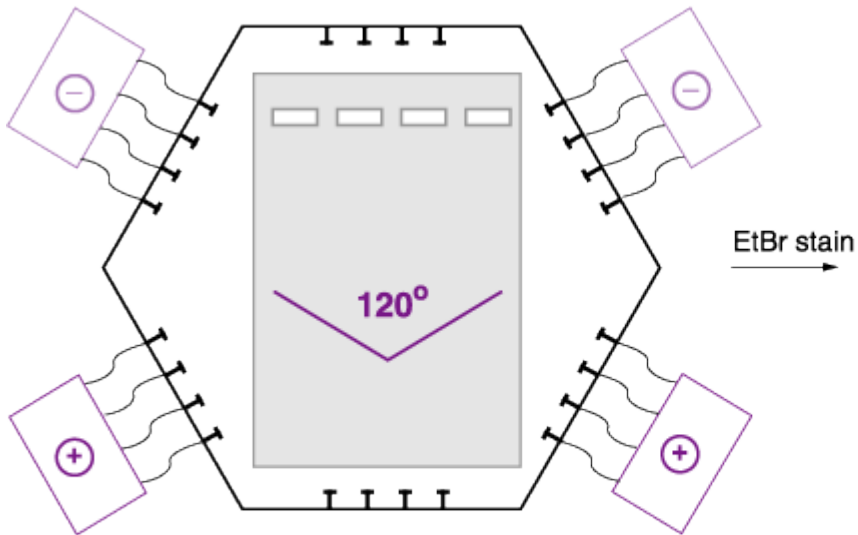


Pulse-field gel electrophoresis

Figure 1 - PFGE



Pulse-field gel electrophoresis



Electric field alternates 120° every 90 seconds for 18 to 24 hours at 14°C

