Modelos para el estudio de biofilms microbianos

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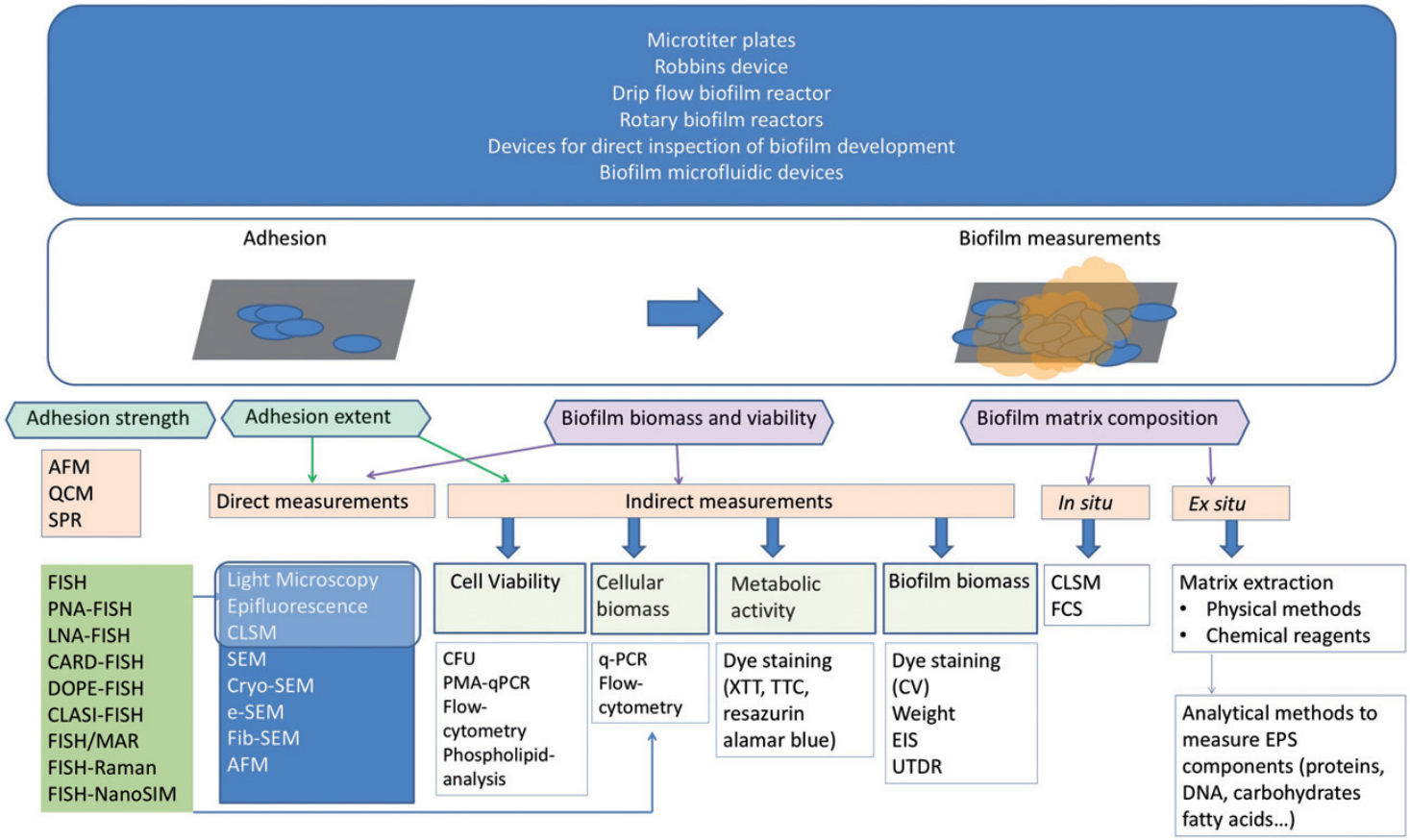
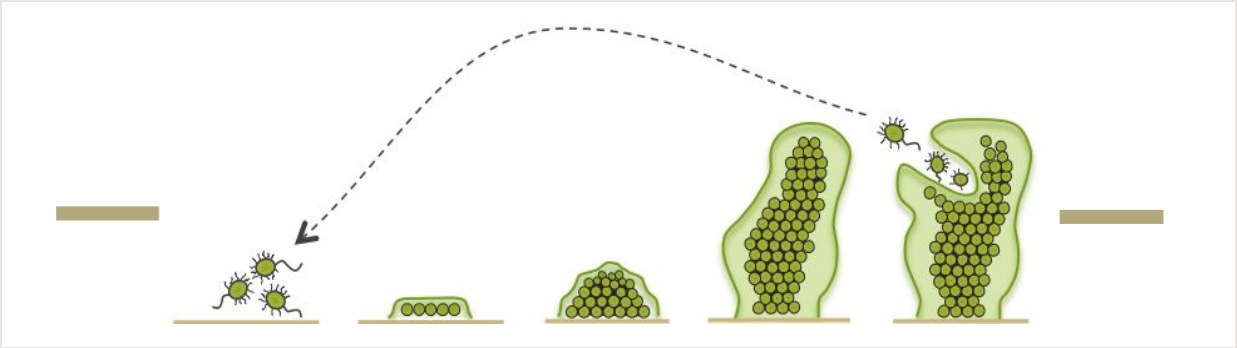


Figure 1. Overview of methods to grow and characterize biofilms, which includes different biofilm devices, methods to assess adhesion extent and strength, and techniques to measure biofilm biomass, viability and matrix composition. © Joana Azeredo.

BIOFILMS EN SISTEMAS ESTáTICOS



Modelos comerciales

Format/technique	Experimental features	Applications and examples	References
Static biofilms	• Low or no shear • No replacement of medium • No cell washout		
Microtiter plate	• High throughput • Limited biomass	• Phenotypic screening of mutant libraries • Attachment and early biofilm development studies • Biomass quantification with staining	26, 81
Calgary device (MBEC)	• High throughput • Peg material may be modified • Biomass may be recovered from pegs • Limited amount of biomass	• Phenotypic screening of mutant libraries • Antibiotic susceptibility studies • Microscopy with fluorescent probes • Biomass quantification with staining	82
Colony biofilm	• Large biomass in short amount of time • Inexpensive laboratory materials • Low throughput	• Antibiotic susceptibility and penetration studies • Chemical gradient measurements using microelectrodes • Heterogeneity studies using microscopy and fluorescent probes • Cryosectioning studies for gene expression heterogeneity	38, 40, 61, 62, 85, 87

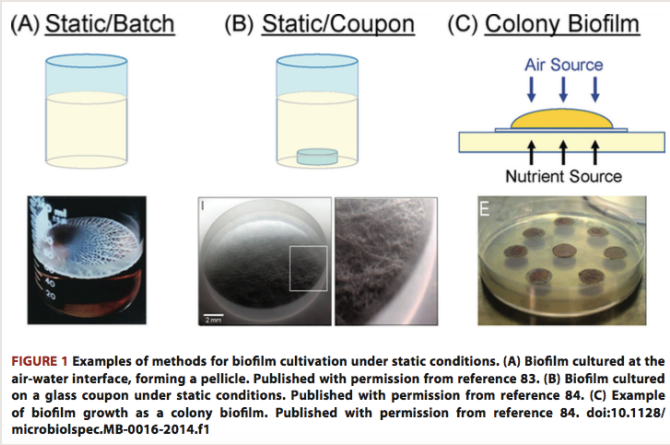


FIGURE 1 Examples of methods for biofilm cultivation under static conditions. (A) Biofilm cultured at the air-water interface, forming a pellicle. Published with permission from reference 83. (B) Biofilm cultured on a glass coupon under static conditions. Published with permission from reference 84. (C) Example of biofilm growth as a colony biofilm. Published with permission from reference 84. doi:10.1128/microbiolspec.MB-0016-2014.f1

Modelo clásico de screening

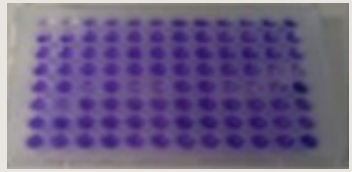
Pre-inóculo de cada aislamiento

https://youtube.com/playlist?list=PLG8B8Uyfh7-D4oBxTzx0JI4-MhBnET3nq

Cultivo de cepas con medio LB

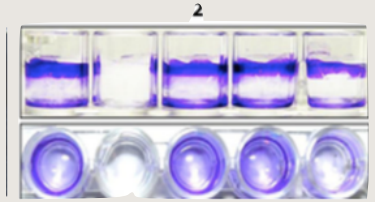
Incubación por 48 hs a 37°C

Remoción de bacterias planctónicas



Medida de absorbancia a 590 nm

El biofilm adherido se tiñe con CV



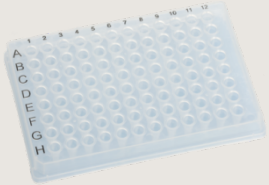
Clasificación

Valores de la absorbancia de acuerdo a la clasificación para la formación de biofilm.

Clasificación	DOc=0,21	Valores
No formador	DO≤DOc	≤0,21
Débil formador	DOc<DO≤2xDOc	0,21<x≤0,42
Moderado	(2xDOc)<DO≤(4x DOc)	0,42<x≤0,84
Gran	(4xDOc)<DO	>0,84

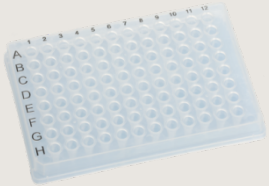
Evaluación de antimicrobianos

Pre-inóculo de cada aislamiento



Cultivo de cepas con medio LB

Incubación por 48 hs a 37°C



Remoción de bacterias planctónicas

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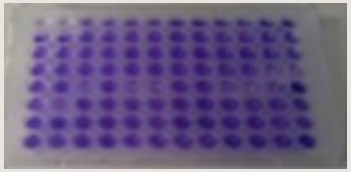


Medida de absorbancia a 590 nm

BIOFILMS EN SISTEMAS DINÁMICOS

Modelos comerciales

Continuous flow biofilms	• Continuous supply of fresh medium • Adjustable shear force • Low to medium throughput		
CDC reactor	• Special surface materials may be used • Multiple biofilms are formed simultaneously • Suitable for time-course study • May be used for anaerobic cultures	• Antibiotic susceptibility/viability studies • Microscopy studies with fluorescent probes • Applicable for omics studies	88
Drip flow reactor	• Special surface materials may be used • High gas transfer • Heterogeneous biofilm • Large biomass in short time	• Antibiotic susceptibility/viability studies • Chemical gradient measurements using microelectrodes • Heterogeneity studies using microscopy fluorescent probes • Cryosection and laser capture microdissection followed by transcriptomic analysis • Biofilm-immune cell interaction	38, 61, 89
Imaging flow cells	• Real-time detection • Surfaces can be modified • Appropriate for short-time experiments	• Real-time imaging • Monitoring attachment, development, and detachment phases • Microscopy with fluorescent tags • Attenuated total reflection Fourier transform infrared spectrometry • Hydrodynamics in biofilm by nuclear magnetic resonance	40, 79, 80, 91

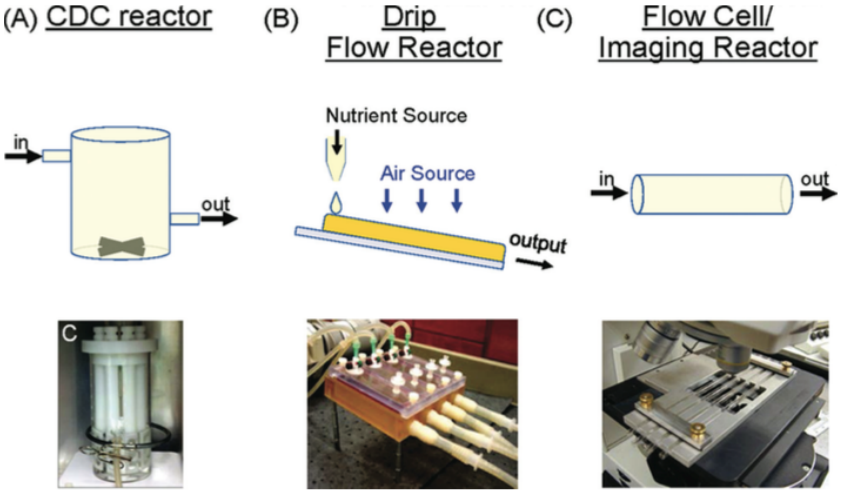
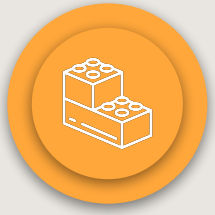


FIGURE 2 Examples of continuous-flow reactors for biofilm cultivation. (A) CDC reactor with medium inlet and outlet ports. Biofilms form on coupons arranged on removable Teflon rods. Published with permission from reference 88. (B) Drip-flow reactor with medium inlet and outlet ports and air exchange ports. Biofilms form on removable slides. Published with permission from reference 89. (C) Capillary flow cell for imaging biofilms. Published with permission from http://centerforgenomicsciences.org/research/biofilm_flow.html. doi:10.1128/microbiolspec.MB-0016-2014.f2

CARACTERÍSTICAS DEL MODELO



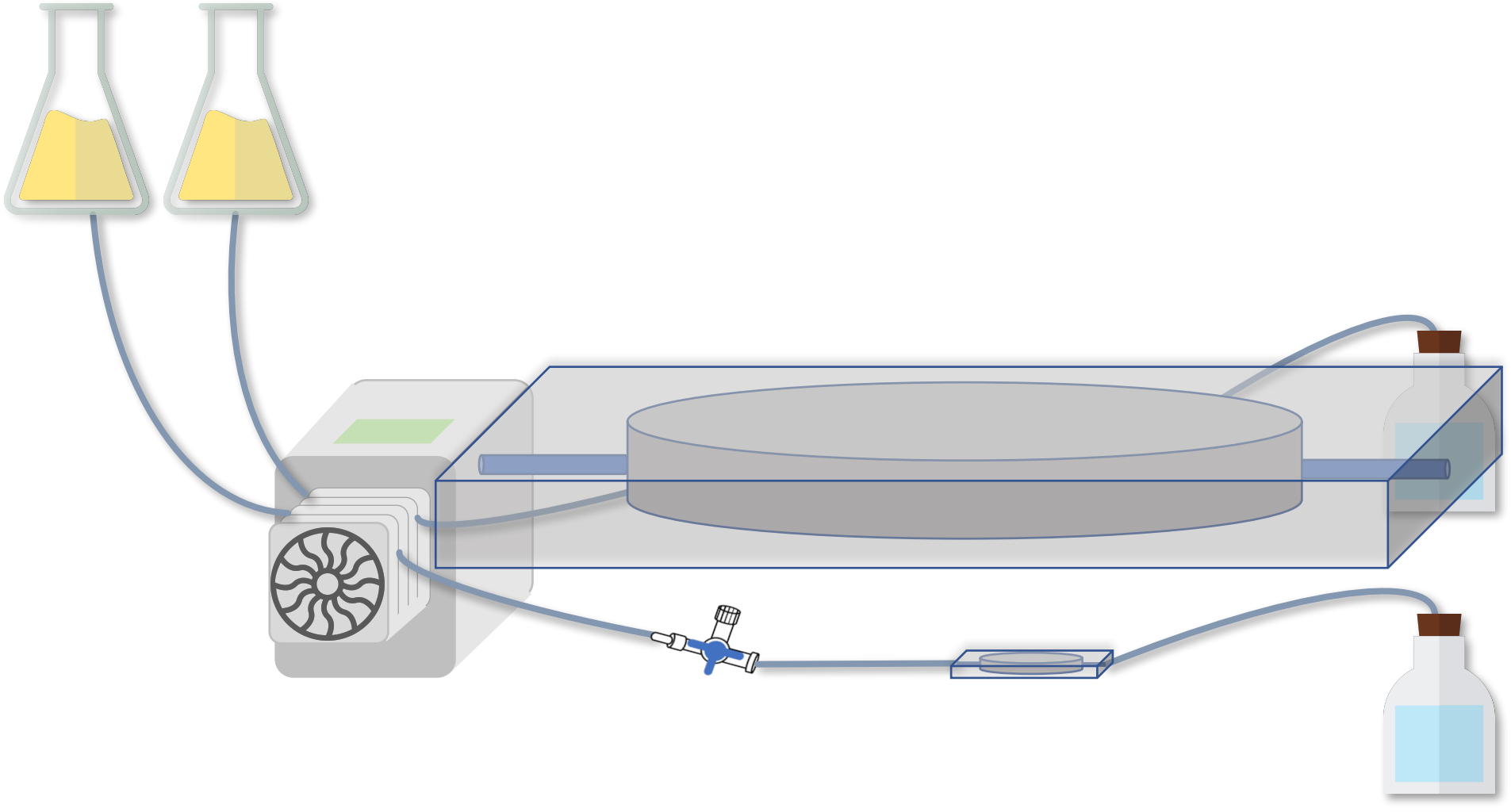
Estructura tridimensional y organización espacial

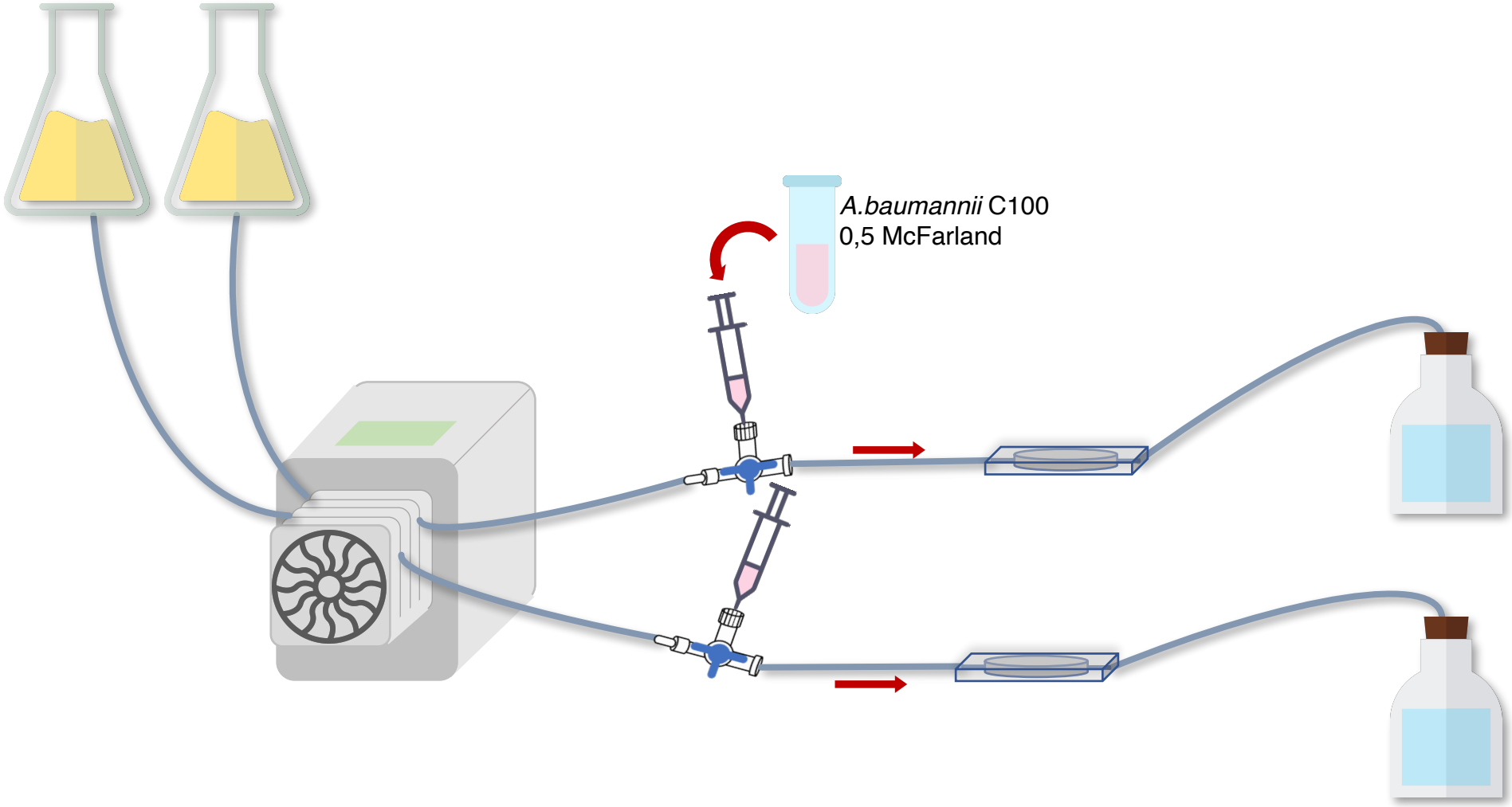
Seguimiento en el tiempo

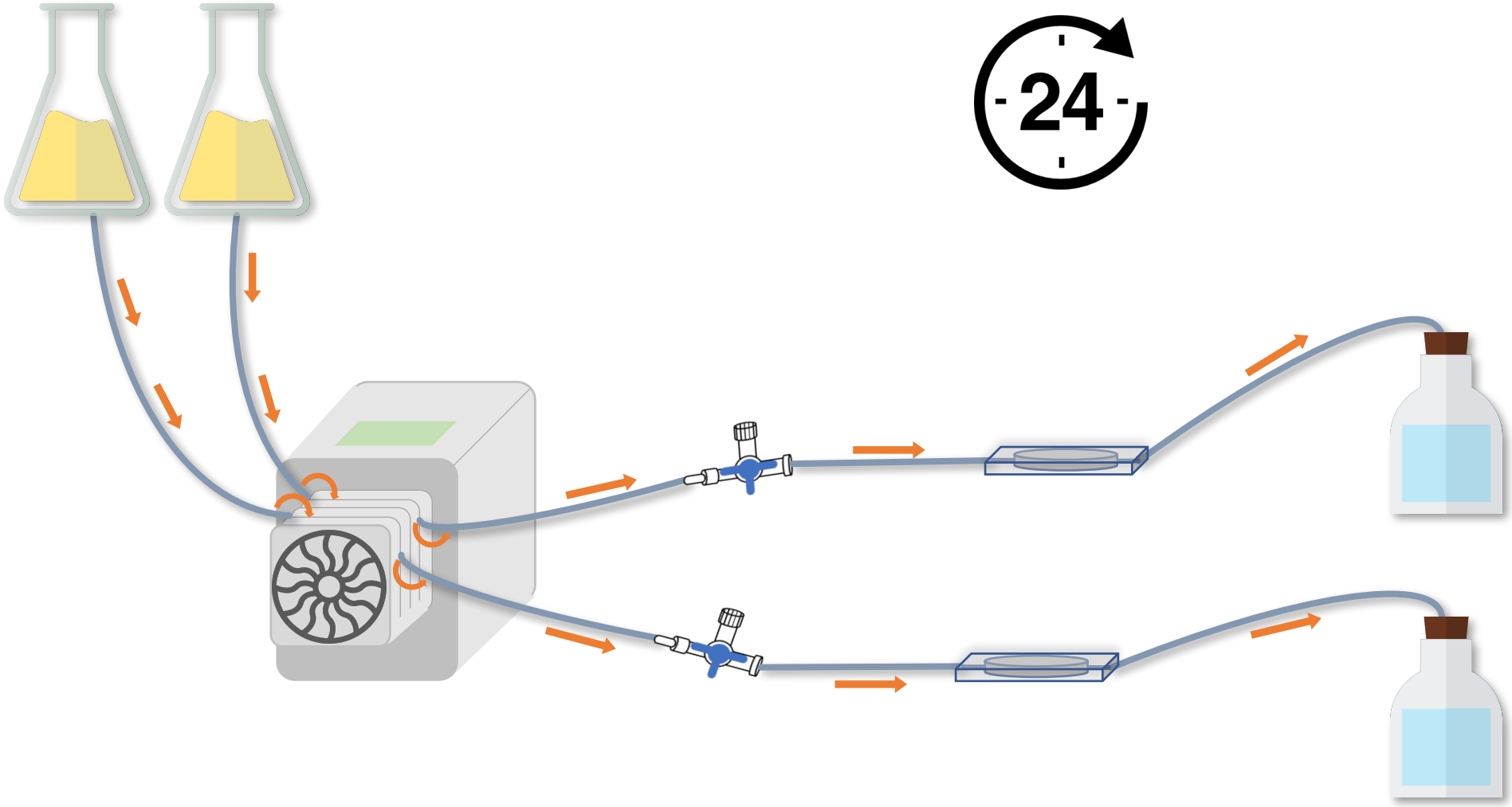
Imitación de características fisiológicas

Microorganismos enfrentados a flujo y turbulencia

No invasivo




A.baumannii C100
0,5 McFarland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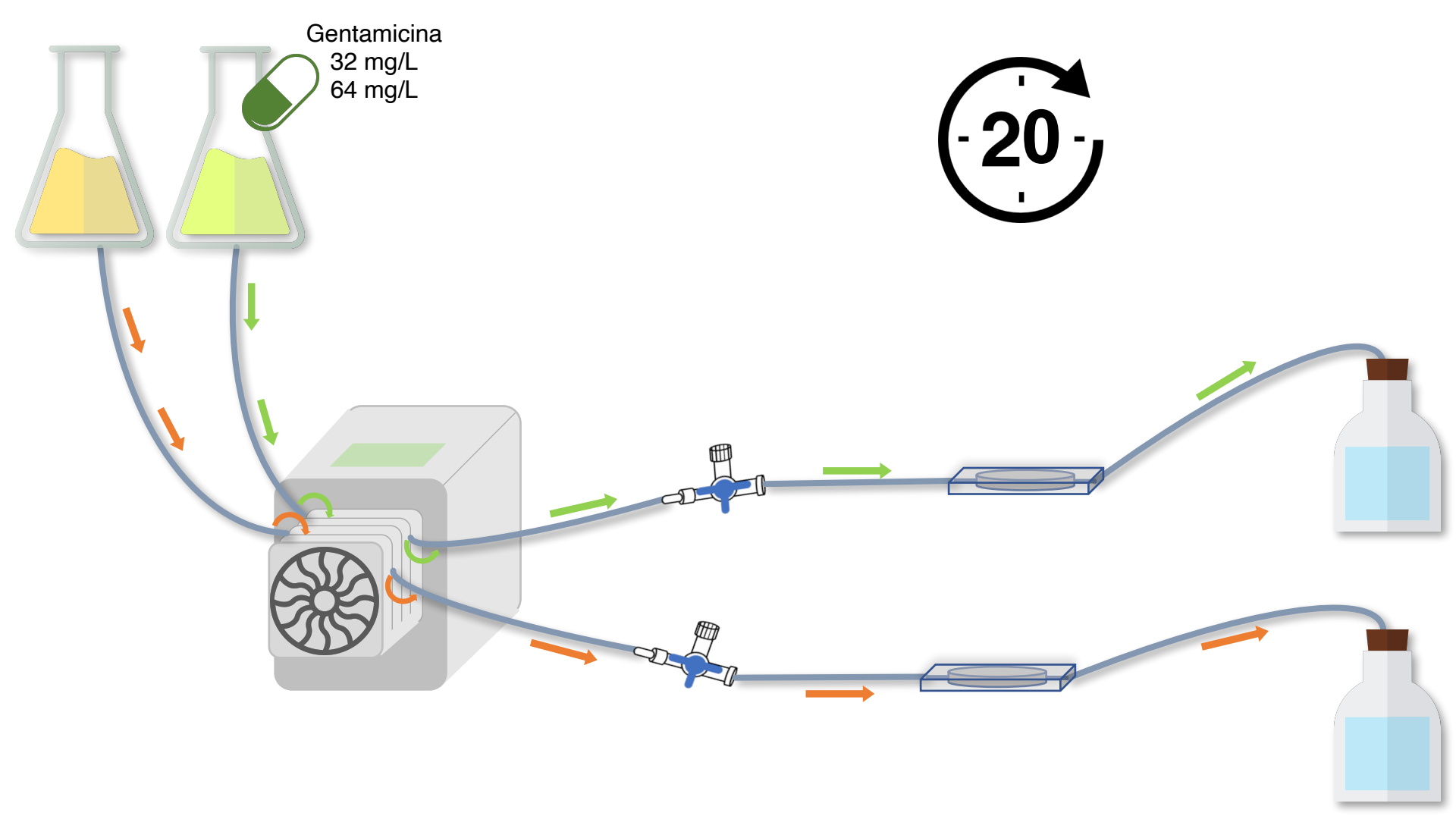
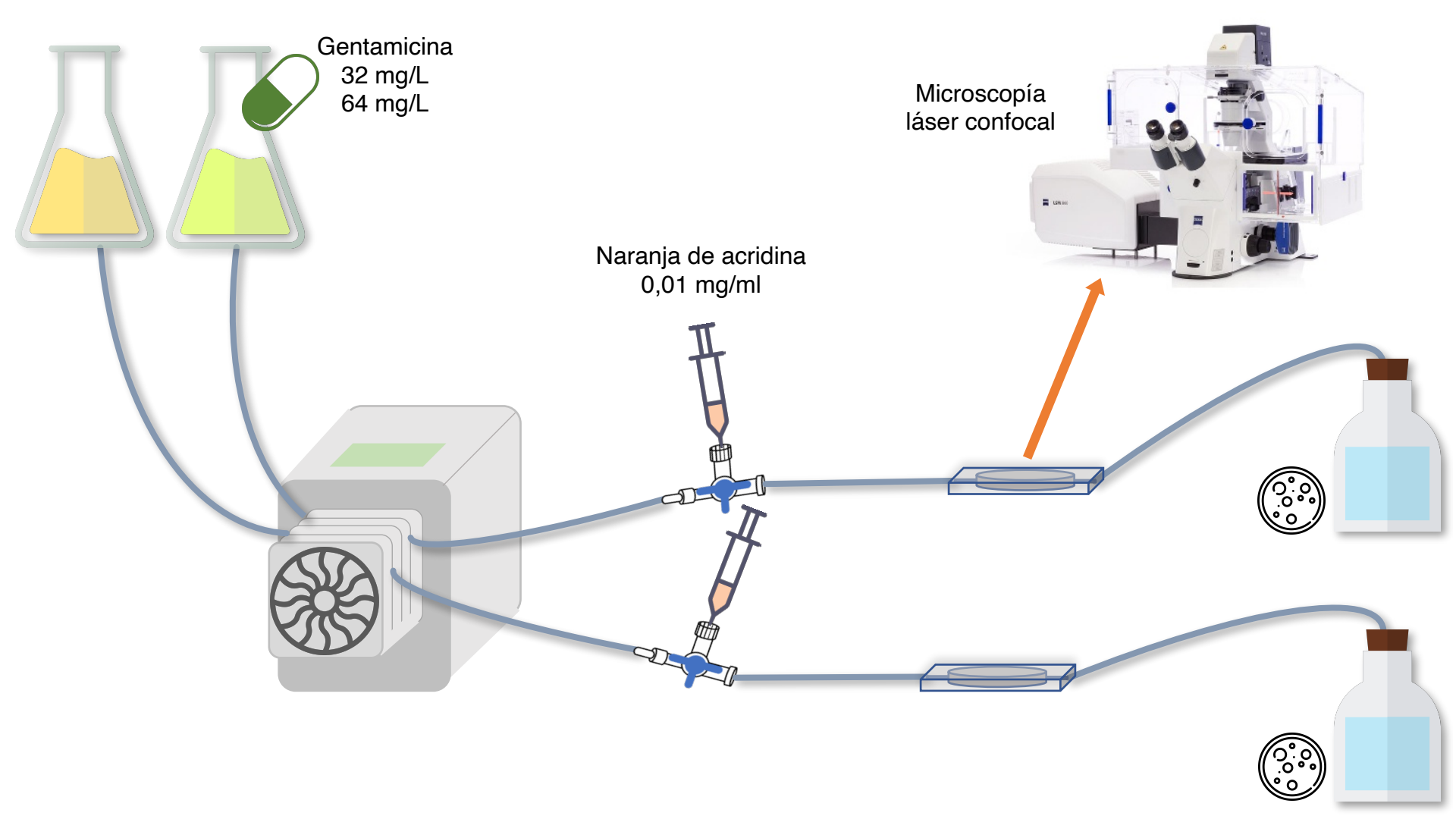
Microscopía
láser confocal

Naranja de acridina
0,01 mg/ml


Gentamicina
32 mg/L
64 mg/L
20


Gentamicina
32 mg/L
64 mg/L
Microscopía
láser confocal
Naranja de acridina
0,01 mg/ml

Tinciones

TABLE 2 Assays applied for biofilm quantification and viability determination[f]

Assay or reagent	Quantification ability	Assay combination	Advantage(s)	Disadvantage(s)	Reference(s)
Fluorescent dyes					
CV	Biofilm matrix biomass		Easy	Dependent on absorption of the dye into the biomass	110, 124, 162
			Inexpensive	Nonspecific to multispecies biofilms	
			Wide applicability	No dimensional information	
				Sample destruction	
				Poor reproducibility	
Congo red	Biofilm matrix biomass		Easy	Low accuracy for biofilm visual analysis	149, 442
			Inexpensive	pH-dependent binding ability	
DMMB	Biofilm matrix biomass	Resazurin, XTT, BTA, FDA	Strain specific (*S. aureus*)	Reagent instability	154, 162
Live/Dead BacLight (Syto 9 and PI)	Semiquantitative	CLSM	Cell viability assessment	Expensive	443, 444
				Intermediate "unknown" population	
				Underestimation of living cells	
				Large no. of samples required	
AO	Apoptotic quantification	Ethidium bromide, epifluorescence microscopy	Time efficient	Lab safety requirements due to high mutagenicity	157, 445
			DNA and RNA labeling		
			Detects apoptotic phenomena		
DAPI	Live-cell biomass	CTC	Feasible combination with other probes	Used only for fixed cells	200, 446
			Nuclear integrity	High concn is required for live-cell staining	
			Cell viability assessment		
XTT	Counts metabolically active cells		Reproducible	Requires highly respirative bacteria	162, 437
			Nondestructive	Variations due to biofilm heterogeneity	
			Cell viability assessment	Time-consuming	
				Large no. of samples required	
AB/resazurin	Counts metabolically active cells		Reproducible	Heat and light sensitive	447, 448
			Cell viability assessment		
CTC	Counts metabolically active cells	DAPI, epifluorescence microscopy	Bright red fluorescence	Detects only highly metabolically active cells	166, 449–451
			Discrimination between active cells and abiotic parts	Toxicity	
			Cell viability assessment	Solute-associated inhibition	

TABLE 2 (Continued)

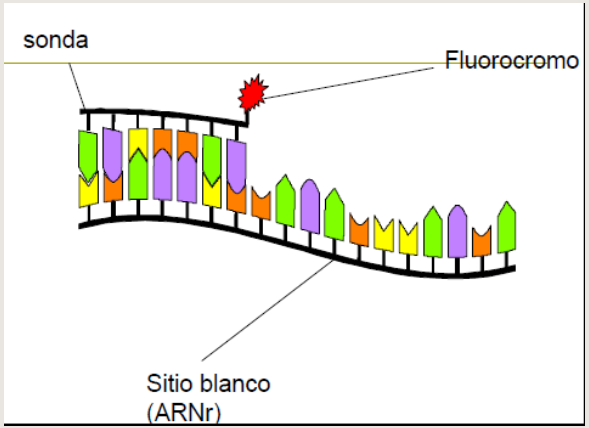
Assay or reagent	Quantification ability	Assay combination	Advantage(s)	Disadvantage(s)	Reference(s)
			Detects bacteria with low metabolic activity		
			Cell viability assessment		
SYBR Green I	Multispecies biofilm cell quantification	Real-time PCR	Reliable and reproducible	Risk of sample contamination	454, 455
	Can synthesize DNA in real time		No specific probes required		
			Cell viability assessment		
Genetic/molecular approaches					
RT-PCR	Multispecies biofilm cell quantification	Gel electrophoresis (DGGE)	Detects uncultivable or challenging-to-culture species, live and dead cells, matrix components	Risk of sample contamination	456
			DGGE detects predominant species, gives early clinical diagnosis	Expensive and complex procedure	
Real-time PCR	Can synthesize DNA in real time	SYBR green I	Easy, rapid, reliable, and reproducible	Risk of sample contamination	455, 457
	Counts cells in multispecies biofilms		High sensitivity		
			Cell viability assessment		
Next-generation sequencing (NGS)	Quantification of genomic sequences	PCR, RT-PCR[e]	High sensitivity	Expensive	458
			Entire transcriptome available in a single analysis (RNA-seq)[e]		
Proteomic analysis	ECM protein component	Mass spectroscopy/NMR	Biofilm phenotype, protein profile determinant, and resistance pattern analysis	Protein expression variations in multispecies biofilms	187, 190
Microscopy					
FISH	Semiquantitative	CLSM	Independent of growth conditions	Low permeability of DNA probes	175, 200, 459, 460
			Applicable to multispecies biofilms	Low sensitivity	
			Detects all viable microorganisms	Hybridization between complementary PNA probes	
			Visualization and spatial distribution	Expensive and lengthy multistep procedure	
IF	Antibody-antigen complexes	Fluorescently labeled antibodies	Simple procedure	Less flexible procedure	461
				Costly	

Assay or reagent	Quantification ability	Assay combination	Advantage(s)	Disadvantage(s)	Reference(s)
CLSM	Quantitative imaging	Fluorescence assay, FISH, FCS	Nondestructive	Probe efficacy dependent on biofilm EPS complexity	162, 204, 205, 462, 463
			3D imaging Cell and EPS spatial distribution Applicable to thick sample	Special equipment required	
SIM	Live-cell biomass imaging	Fluorescent probes	3D imaging of living cells	Specimen instability during multiple-image recording	210, 211, 464
			Enhanced resolution Computational amplification Imaging of thick samples		
OCT	Biomass, structure, and porosity identification	Ultra-broad-bandwidth lasers	Real-time 3D imaging	No cell-level resolution	216, 465–467
			Speedy measurements Noninvasive Label-free	Limited penetration depth	
TEM	Total biofilm matrix biomass imaging		High resolution	Sample prepn required Special equipment required	204, 468, 469
SEM[a]	Synergy with focus ion beam for inner biofilm study	EDS[b]	Surface visualization[a]	Risk of sample distortion due to dehydration[a]	162, 220, 223, 227, 470, 471
ESEM[b]			Detailed 3D visualization[a]	Low resolution[b]	
Cryo-SEM[c]			No structural damage[b]	Artifacts due to sample prepn[c]	
ASEM[d]			No sample prepn[b] Imaging of EPS[b] No dehydration required[c] Nonconductive surfaces[c] Time efficient[c] Nanostructure biofilm surface visualization in liquids[d]	Low resolution[c] Multiple labeling[d]	
STXM	Total biofilm biomass	X-ray fluorescence	Macromolecule distribution	Applicable to thin samples	162, 204, 224, 472, 473
	Chemical biofilm components		Visualization of biological and environmental components and spatial distribution	Special equipment required	
AFM	Chemical biofilm component imaging		Real-time 3D imaging	Artifacts and sample damage due to incorrect tip elections	227, 228, 474–476
			Little/no sample prepn Performed in both air and water Elucidation of molecular interactions High resolution	Deformation of soft samples Poor image quality in water Special equipment required	

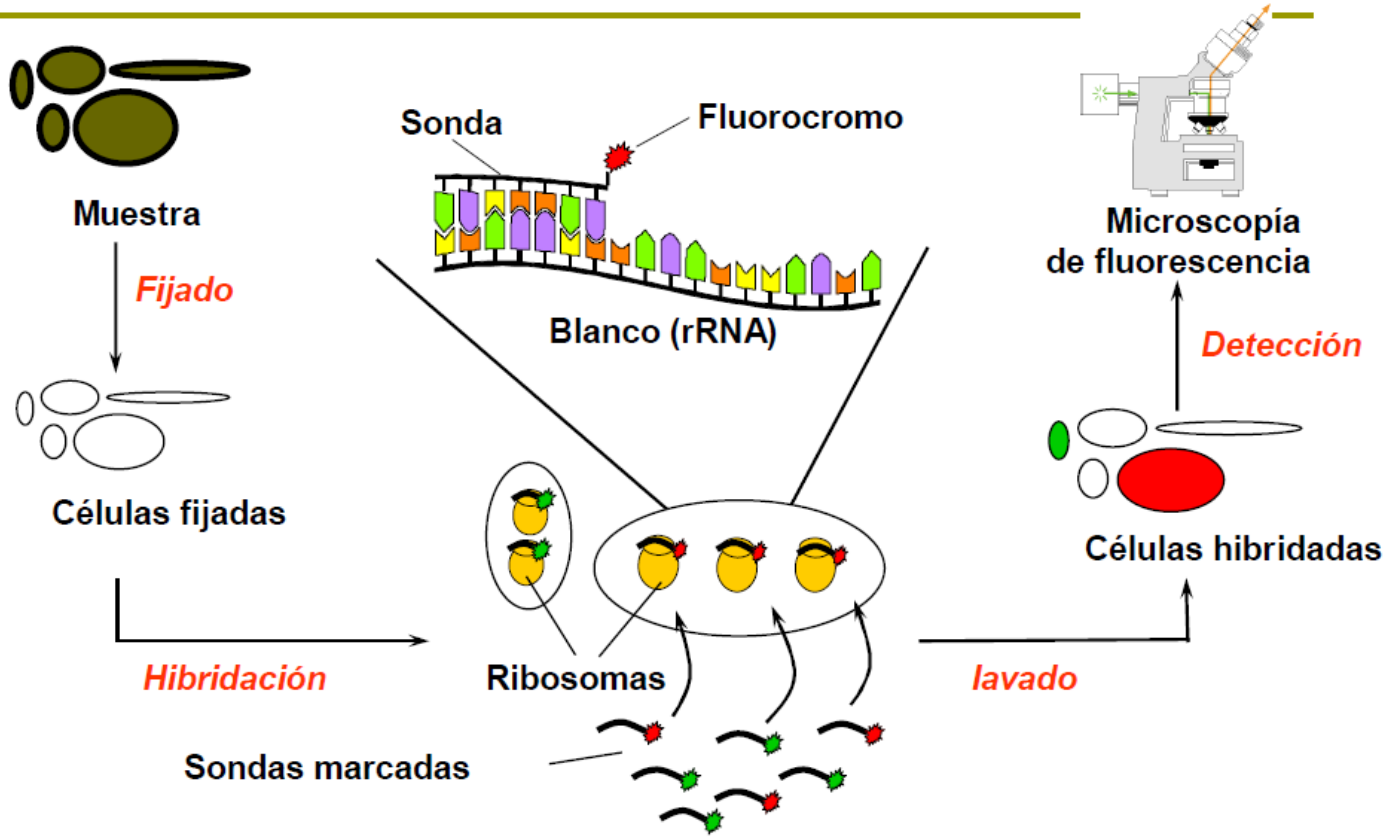
FISH

Técnica de tinción que permite identificar bacterias en muestras complejas usando Microscopía de fluorescencia y confocal.

Empleo de sondas para ADN o ARN marcadas fluorescentemente



Protocolo para FISH



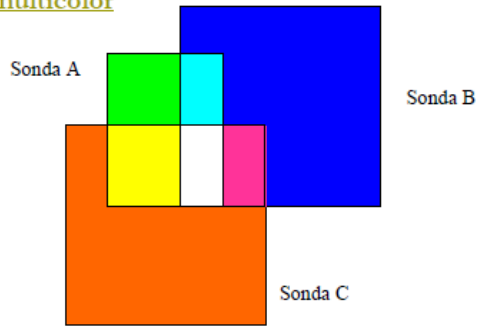
Sondas: aproximación jerárquica

sonda	blanco	Domi-nio	Clase	Sub-clase	Género
ARCH915	16S	*Archaea*			
EUK516	18S	*Eucarya*			
EUB338	16S	*Bacteria*			
CF319a	16S		CFB		
HGC69a	23S		*Actinobacteria*		
PLA30	23S		*Planctomycetes*		
ALF1b	16S		*Proteobacteria*	α	
BET42a	23S			β	
GAM42a	23S			γ	
SRB385	16S			δ	
DNMA657	16S			ε	*Desulfonema* sp.
ARC94	16S				*Arcobacter* sp.

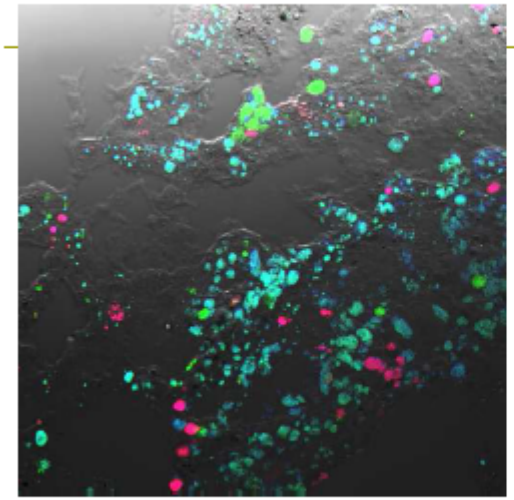
Fluorocromos para FISH

	Excitation	Emission
Alexa488	490 nm	520 nm (verde)
Fluos	495 nm	523 nm (verde)
Cy3	550 nm	570 nm (rojo)
Alexa546	555 nm	570 nm (rojo)

FISH multicolor



Aplicación múltiples sondas



Composition of the AOB Community

NSO1225 (β-subclass AOB)

Nc. mobilis — NEU653 (*Nitrosomonas europaea/eutropha/halophila*) — *N. europaea/eutropha*

NmV (*Nitrosococcus mobilis*)