Vesismin Health
Preventing infections

La importancia del biofilm

“Soñaba que se hacía invisible: verlo todo, escucharlo todo, aprenderlo todo, sin que nada palpable señalara su existencia.”

"Nada se opone a la noche" (2011), Delphine De Vigan

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Preventing infections

Una bonita (demasiado) historia

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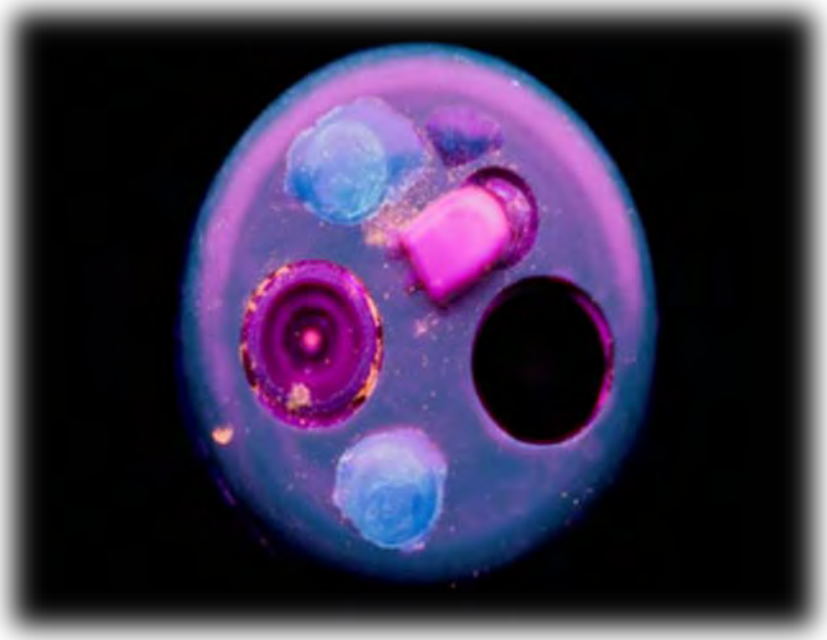
Pero nos tememos que NO es tan fácil…



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¿Qué es el biofilm?

"El biofilm se compone de microorganismos vivos y viables, como bacterias, que existen **en forma de colonia o comunidad**. En otras palabras, los biofilms están vivos y tienen una **estructura social compleja** que científicos e ingenieros están tratando aún de desvelar. Una estructura que, al mismo tiempo, **les protege y les permite crecer**."

Chapter 1: Introduction to Biofilms

Section 2: A Brief Introduction to Biofilms

www.cs.montana.edu/webworks/projects/stevesbook/contents/chapters/chapter001/section002/green/page001.html

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El biofilm YA está presente en nuestras vidas

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El biofilm YA está presente en nuestras vidas

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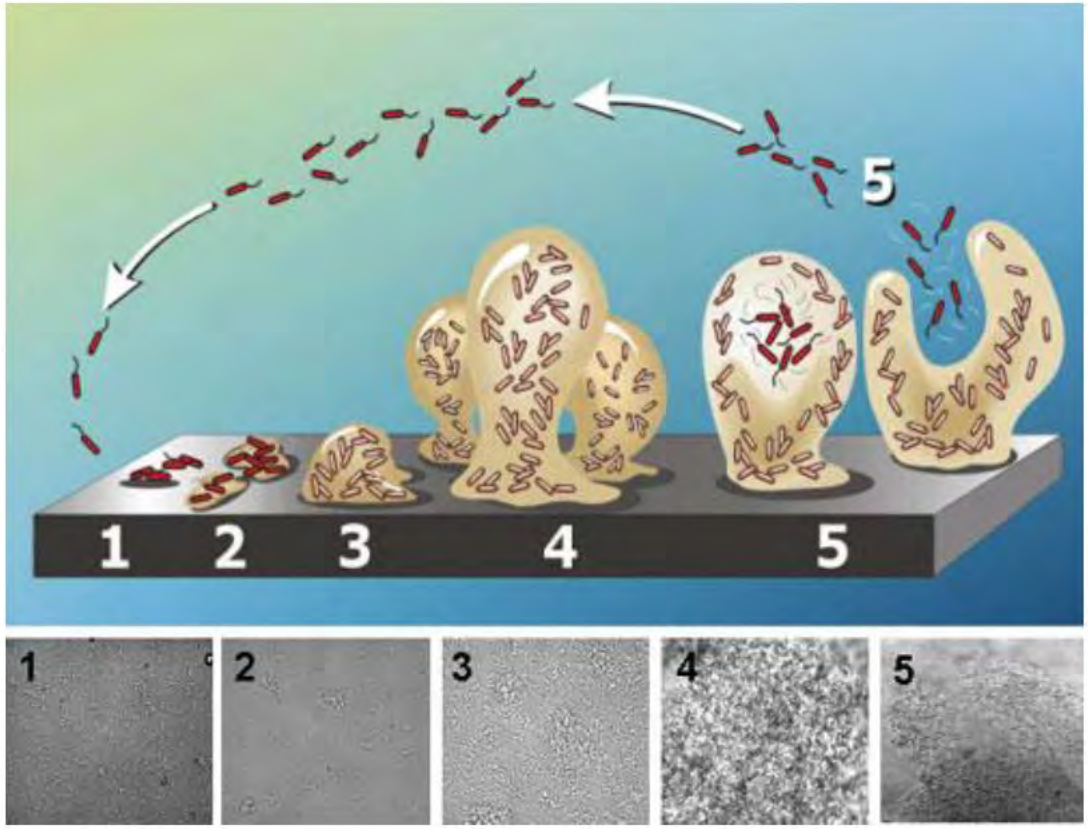
Adherencia, agregación y formación de una matriz polimérica extracelular



(Stoodley et al., 2002)

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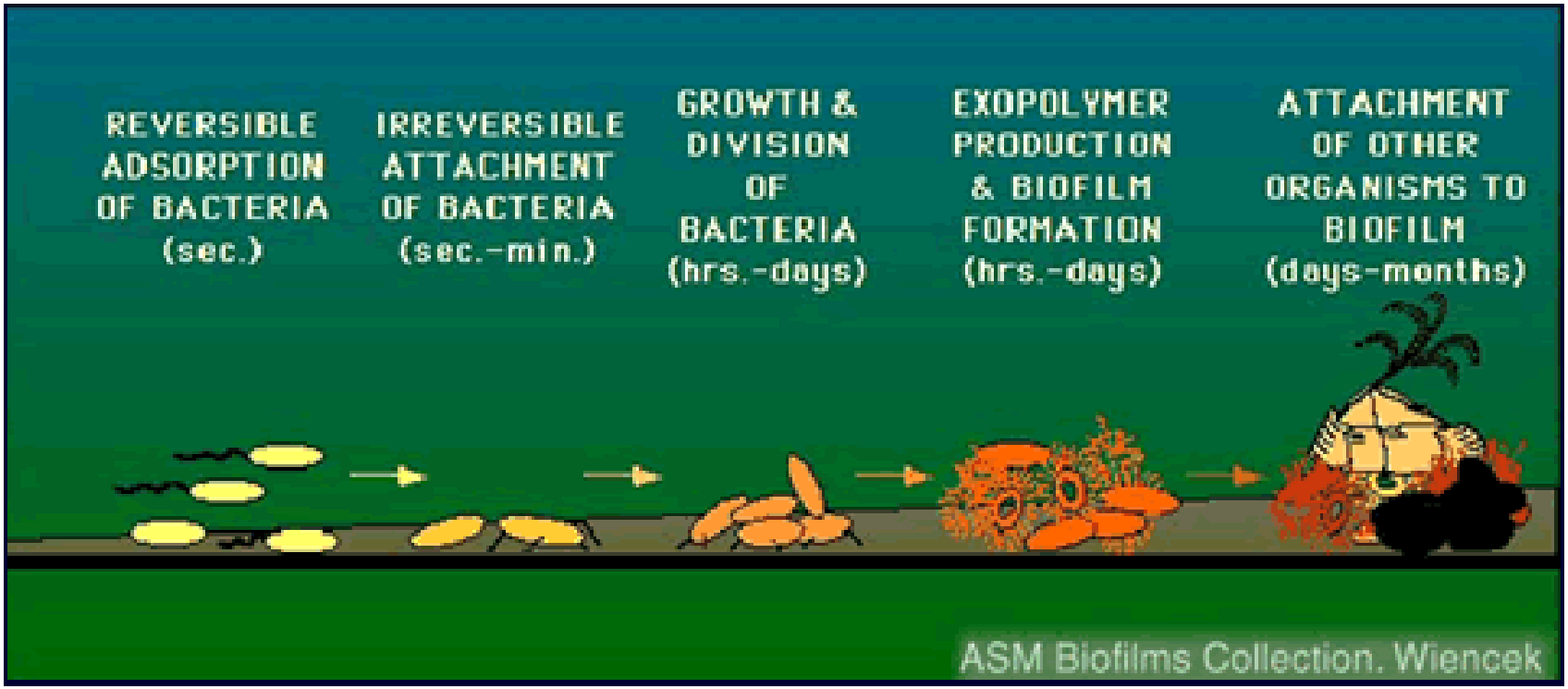
El tiempo importa: cada paso en su momento



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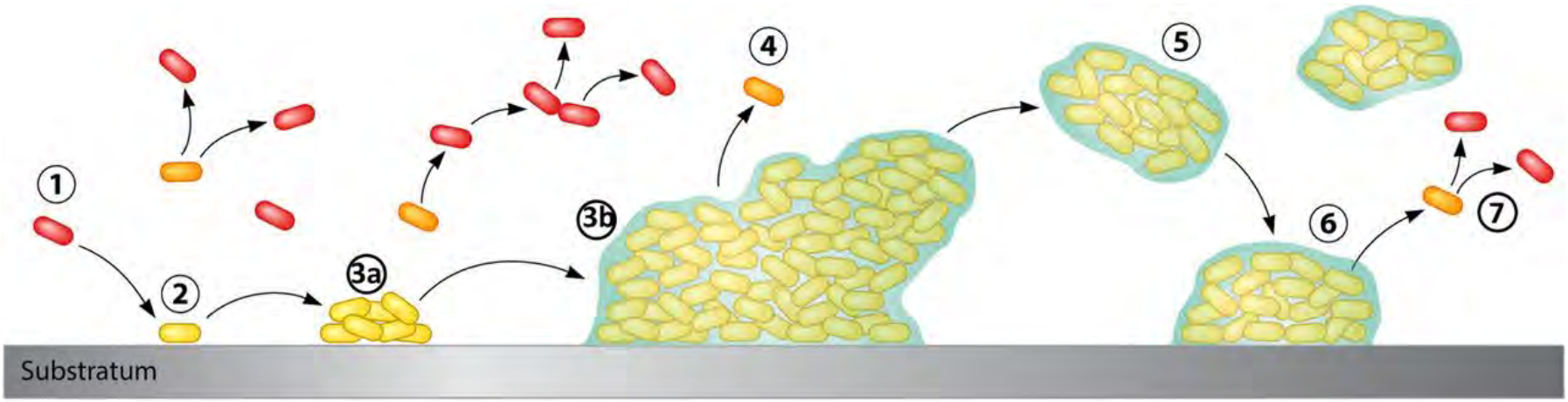
Los microorganismos bentónicos modifican su fenotipo



1) Planktonic phenotype
2) Newly attached planktonic cells – "settler" biofilm phenotype*
3a) Maturing biofilm stage – biofilm phenotype
3b) Fully mature biofilm stage – biofilm phenotype
4) Newly single cells dispersed from the biofilm – newly dispersed phenotype
5) Detached biofilm aggregates – biofilm phenotype
6) Reattached biofilm aggregates – biofilm phenotype
7) Newly dispersed phenotype cells from the biofilm giving rise to planktonic phenotype cells

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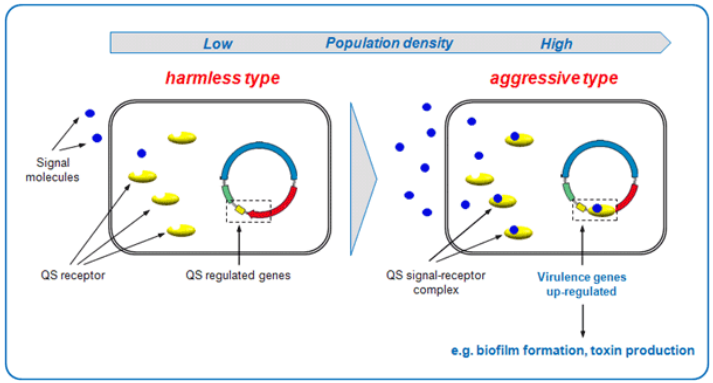
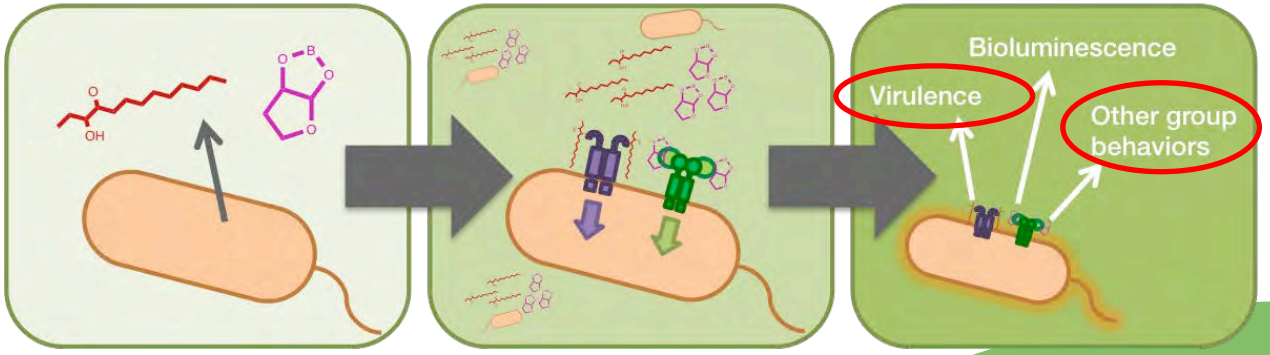
¿Cómo lo hacen?: *Quorum Sensing*

"El *Quorum Sensing* (QS, "percepción de cuórum" o "autoinducción") es un sistema de comunicación molecular que permite a múltiples formas de vida, y en especial a bacterias, modular su comportamiento fisiológico en función de la densidad de población. Entre otros factores, modula su motilidad, la expresión de factores de virulencia y la formación de biofilms. Las bacterias Gram Negativas comparten un mismo sistema QS."
https://solucionesdesinfeccion.com/2018/11/05/cinamaldehido-y-quorum-sensing-una-clave-para-inhibir-la-formacion-de-biofilms/#more-2651





SYNTHESIS RECOGNITION RESPONSE

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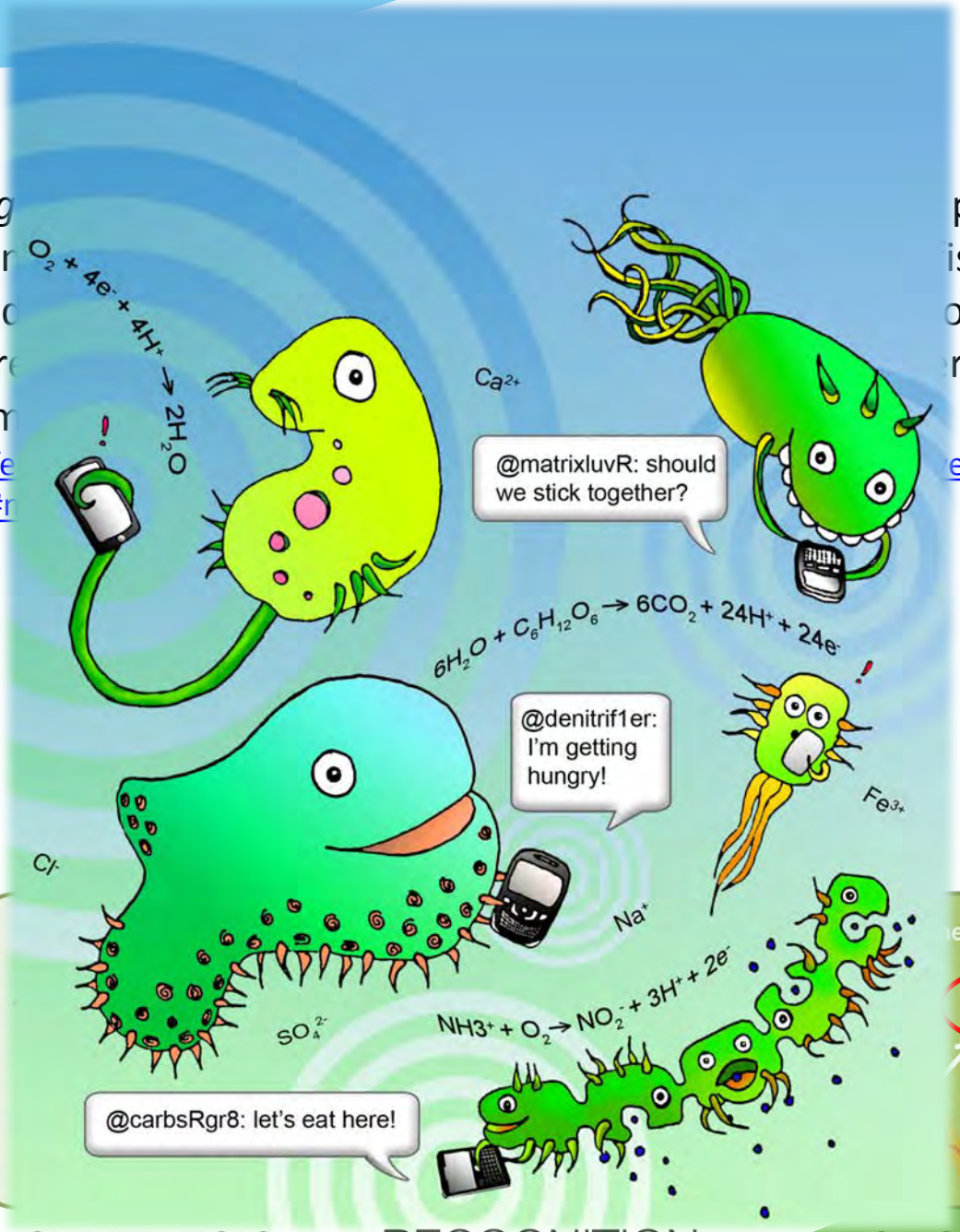
"El *Quorum Sensing* permite a múltiples formas de vida, y en isiológico en función de la densid otilidad, la expresión de factore rias Gram Negativas comparten un mism

https://solucionesdesinfe e-para-inhibir-la-formacion-de-biofilms/#r



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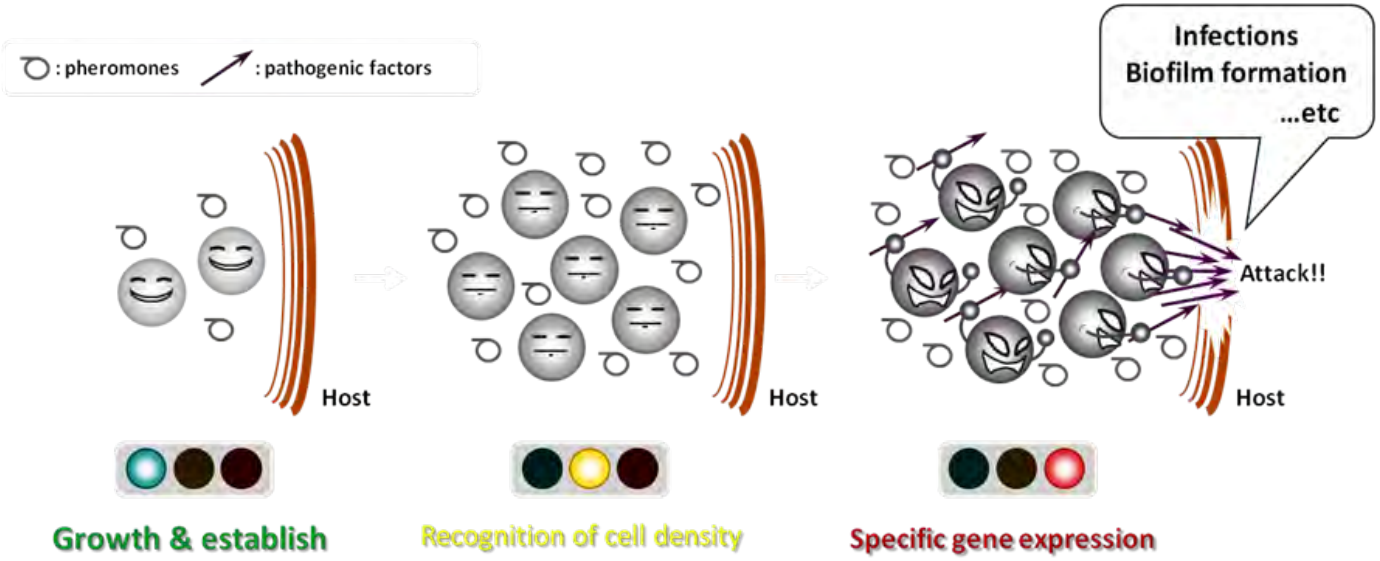

Cell density dependent gene expression in quorum sensing
(e.g. virulence expression)

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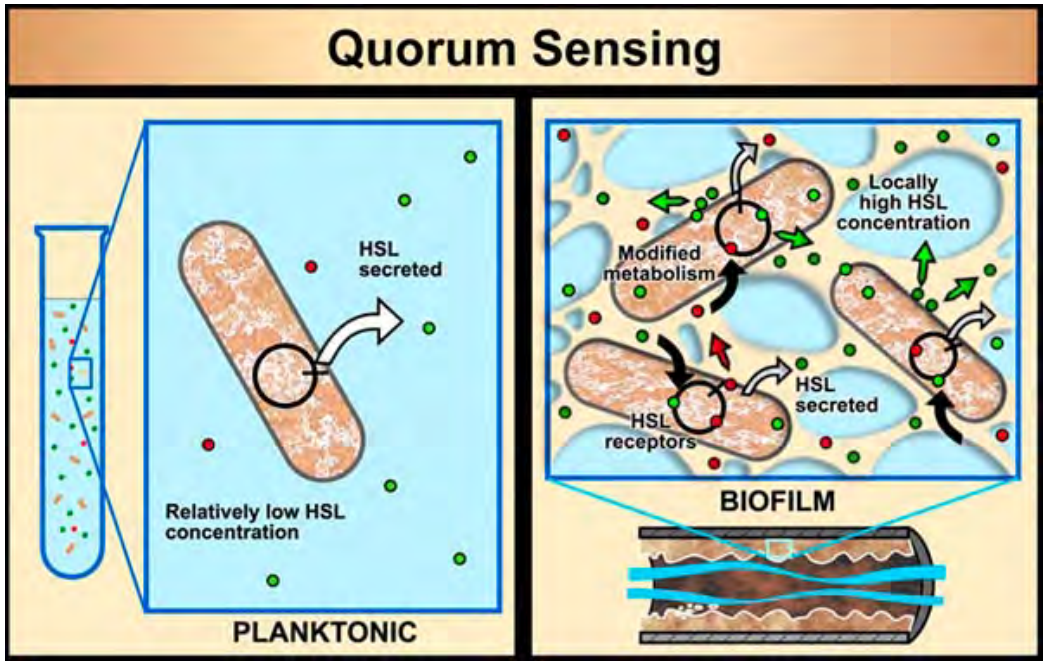

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Un problema añadido: transferencia de resistencias en el seno del biofilm

"Las bacterias Gram Negativas comparten un mismo sistema QS."
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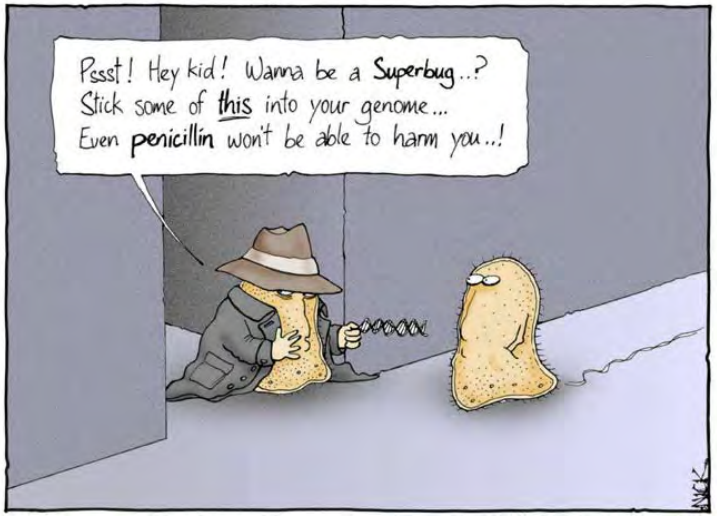
REVIEW ARTICLE FEMS Microbiology Reviews, fux010, 41, 2017, 276–301

Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria



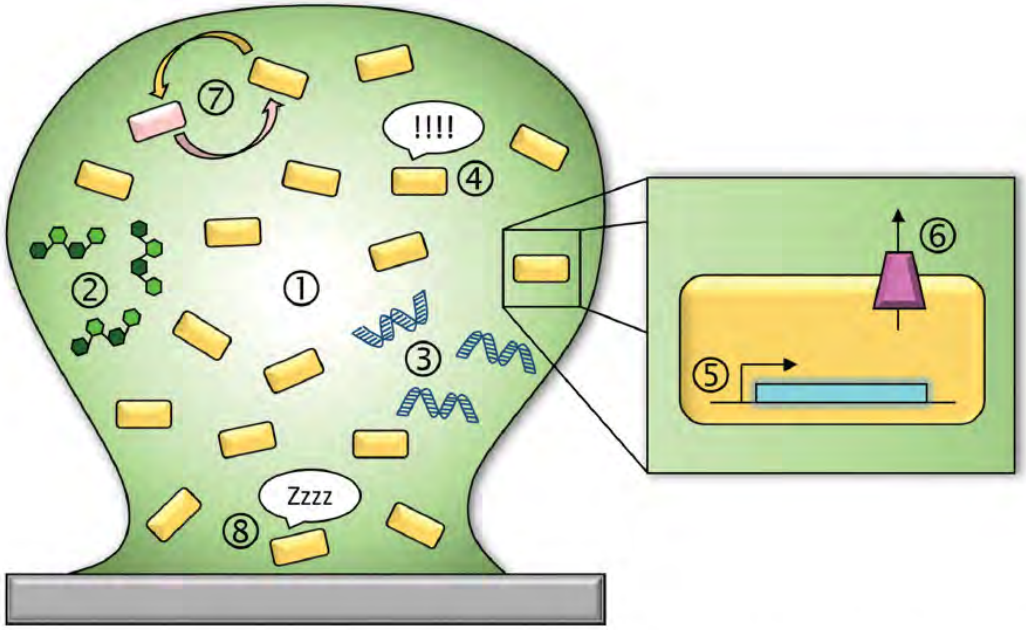


Figure 1. Schematic overview of the major antimicrobial resistance and tolerance mechanisms employed by bacterial biofilms. Biofilm cells (yellow rectangles) are embedded in a mushroom-shaped matrix (shown in green). The biofilm is attached to a surface (grey rectangle), which can be biotic or abiotic. Pictorial representations of the resistance mechanisms are numbered as follows: (1) nutrient gradient (demonstrated here as a colour-intensity gradient) with less nutrient availability in the core of the biofilm, (2) matrix exopolysaccharides, (3) extracellular DNA, (4) stress responses (oxidative stress response, stringent response, etc.), (5) discrete genetic determinants that are specifically expressed in biofilms and whose gene products act to reduce biofilm susceptibility via diverse mechanisms (*ndvB*, *brlR*, etc.), (6) multidrug efflux pumps, (7) intercellular interactions (horizontal gene transfer, quorum sensing, multispecies communication, etc.) and (8) persister cells.

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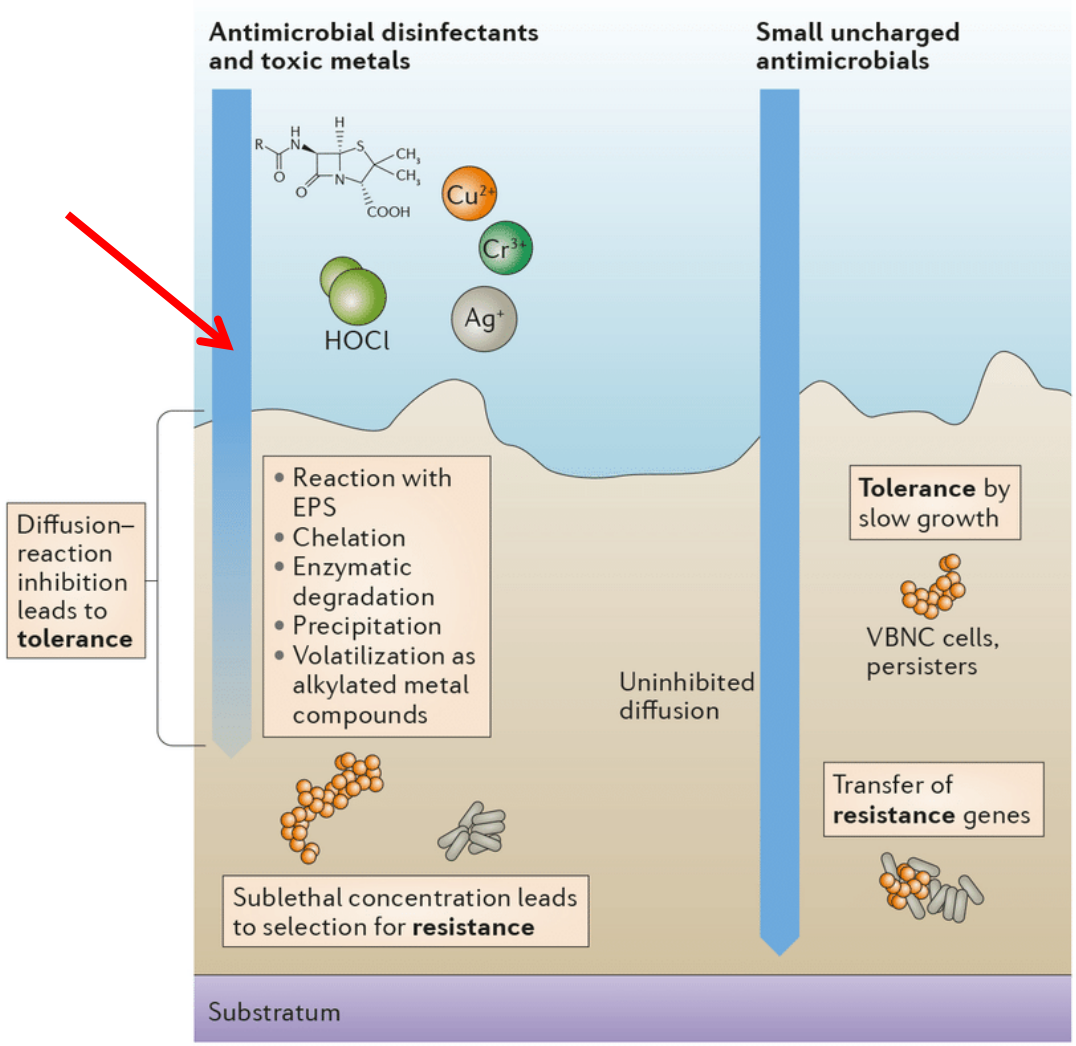
A lo que hay que añadir una baja penetración de los desinfectantes: ¡la tormenta perfecta!

BIOFILM



Nature Reviews | Microbiology

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Reprocesamiento de endoscopios: el reto constante

ECRI Institute

Top 10
Health Technology Hazards

2010. #1: Cross-contamination of endoscopes
2011. #3: Cross-contamination of endoscopes
2012. #4: Cross-contamination from flex. endoscopes
2013. #8: Inadequate reprocessing of endoscopes and surgical instruments
2014. #6: Inadequate reprocessing of endoscopic devices and surgical instruments
2015. #8: Inadequate reprocessing of endoscopes and surgical instruments
2016. #1: Inadequate cleaning of flexible endoscopes before disinfection can spread deadly pathogens
2017. #2: Inadequate Cleaning of Complex Reusable Instruments Can Lead to Infections
2018. #2: Endoscope Reprocessing Failures Continue to Expose Patients to Infection Risk
2019. #5: Mishandling Flexible Endoscopes after Disinfection Can Lead to Patient Infections

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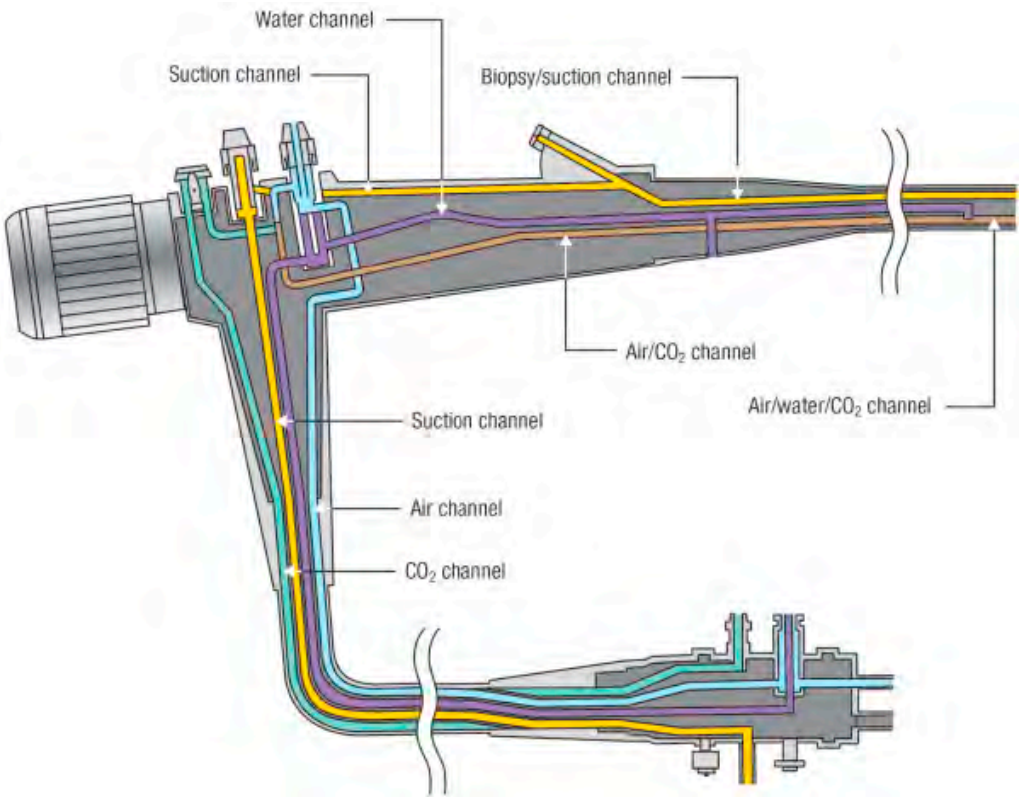
La clave: la complejidad de los endoscopios



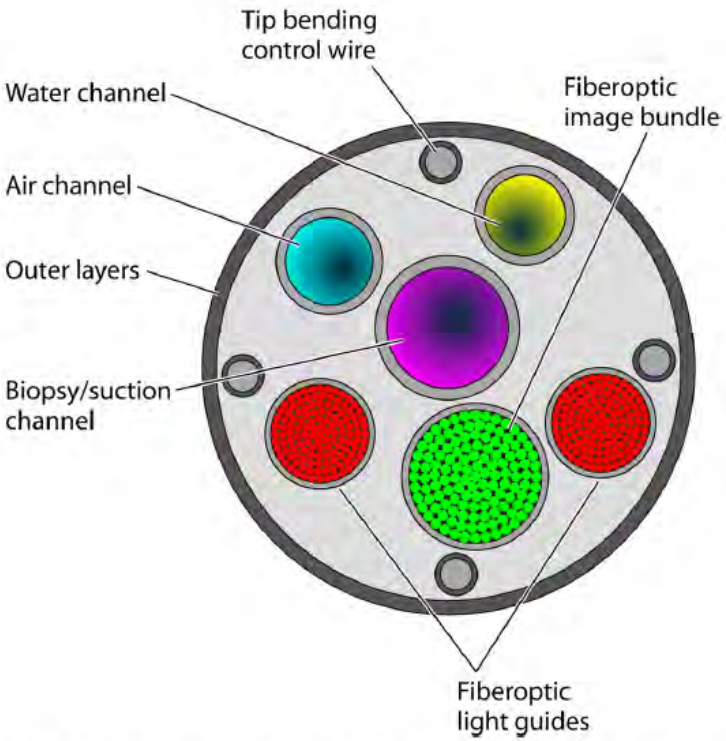


FIG 1 Schematic drawing of a cross section of a flexible endoscope showing the complex design and multiple internal channels (inner diameter, 2.8 to 3.8 mm).

Clin Microbiol Rev. 2013 Apr;26(2):231-54.

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El biofilm se impone a la Desinfección de Alto Nivel

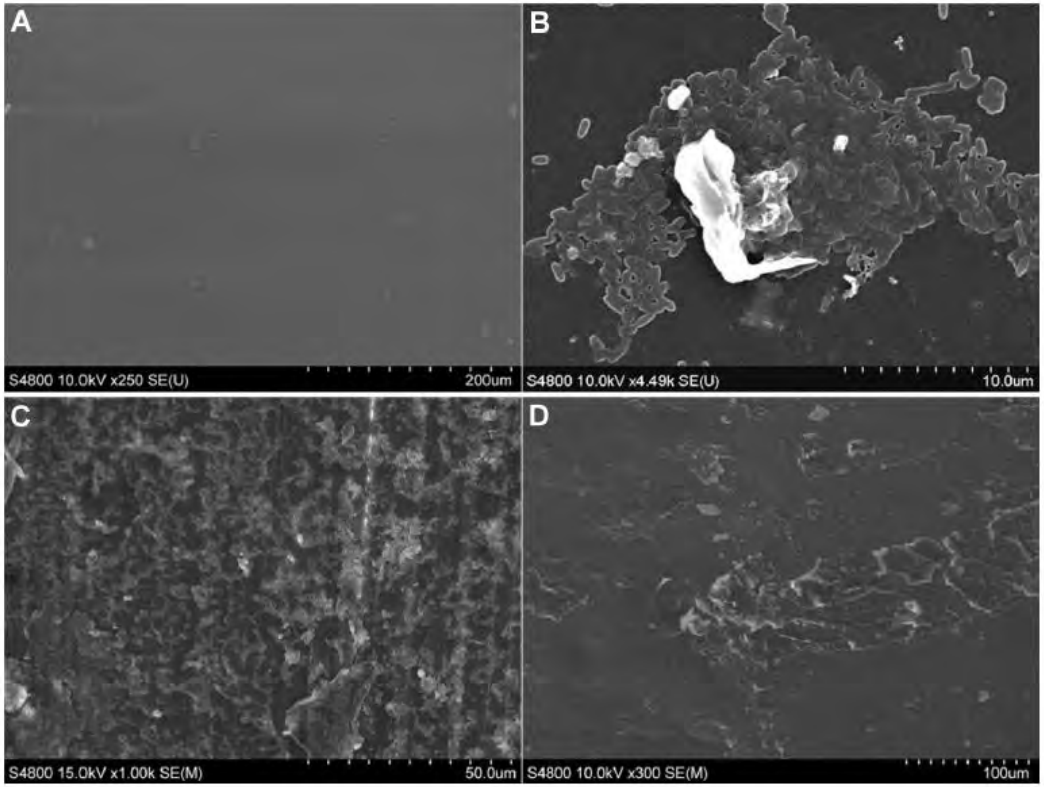


Fig 1. Biofilm growth on the inner surface of suction and biopsy channels of endoscopes used clinically.

W. Ren-Pei et al. / American Journal of Infection Control 42 (2014) 1203-6

La procrastinación, el mejor amigo del biofilm (I)

American Journal of Infection Control 41 (2013) S77-S80

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

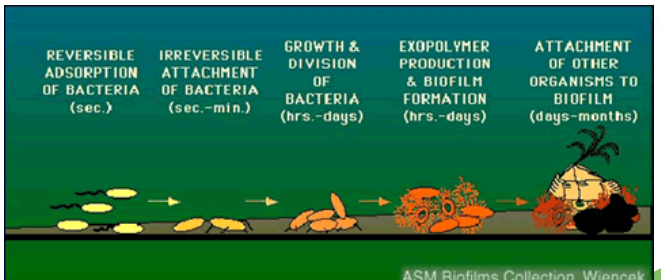
Original research article

The role of biofilms in reprocessing medical devices

Table 1
Conditions required for biofilm formation

Condition	Potable water pipe	Indwelling medical device	Reusable medical device
Colonizing microorganisms present	✔	✔	✔
Surface to be colonized	✔	✔	✔
Sufficient nutrients and water	✔	✔	✔
Temperature conditions for growth	✔	✔	✔
Time required for development of a biofilm	✔	✔	?

"Reusable medical devices like flexible endoscopes that are ***promptly cleaned and disinfected****, rinsed and dried pose little risk to patients. However,* ***bacterial biofilms can develop inside endoscope channels if established reprocessing protocols are not met and these biofilms can be difficult to remove****."*



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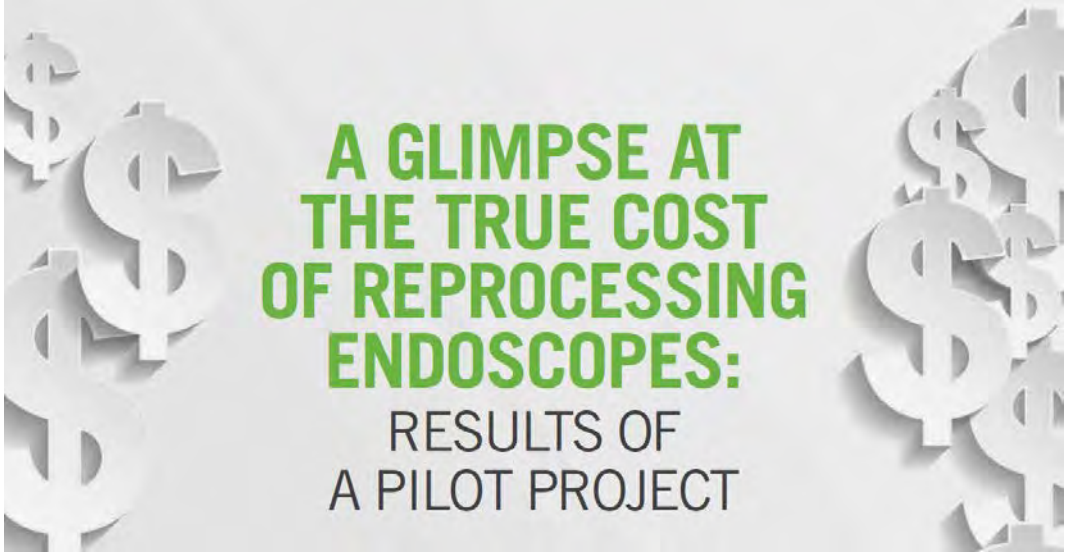
La procrastinación, el mejor amigo del biofilm (II)



Communiqué REPRINTED WITH PERMISSION FROM THE INTERNATIONAL ASSOCIATION OF HEALTHCARE CENTRAL SERVICE MATERIEL MANAGEMENT www.iahcsmm.org

BEDSIDE PRE-CLEANING

The new standards emphasize the importance of immediate bedside pre-cleaning. This involves wiping the outside of the endoscope and flushing channels before transporting the endoscope to the reprocessing room.

The purpose of bedside pre-cleaning

- The number of bacteria on an endoscope can double every 20 to 30 minutes after it is used.
- Pre-cleaning washes away debris and prevents residue from drying out.
- Once biofilm begins to grow, it can be difficult or impossible to remove.
- Outbreaks of infection have been blamed on a failure to pre-clean endoscopes.

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La procrastinación, el mejor amigo del biofilm (III)

Gastroenterology 2016;151:46–50

AGA CLINICAL PRACTICE UPDATE: COMMENTARY

Infection Using ERCP Endoscopes

*"**Lapses in reprocessing** can result in chronic colonization by adherent biofilm that **cannot be eradicated by repeated and thorough cleaning and HLD**. The aggregation of biofilm permits otherwise susceptible microorganisms to defy HLD."*

(…)

*"Evidence supports that there are several important aspects to endoscope reprocessing that combine **to reduce the risk of biofilm accumulation and colonization** and are worthy of reinforcement here. Routine post-procedure manual cleaning of the endoscope—wiping the exterior, flushing the channels, and brushing the elevator lever **immediately after use and before the surfaces have become dried**—is most effective."*

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La procrastinación, el mejor amigo del biofilm (IV)

*"We propose that **immediately after every procedure**, pre-cleaning of the endoscope be performed in the **procedure** room (in accordance with the endoscope manufacturer's instructions) followed by prompt leak testing and **mechanical cleaning** (detergent use, **channel brushings**, channel reprocessing, cleaning accessories, water rinsing) in the reprocessing area."*

Delayed reprocessing of endoscopes

Letters to the Editor

Volume 73, No. 4 : 2011 GASTROINTESTINAL ENDOSCOPY 853

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¡El frotar NO se va a acabar!

Communication from the ASGE Quality Assurance In Endoscopy Committee

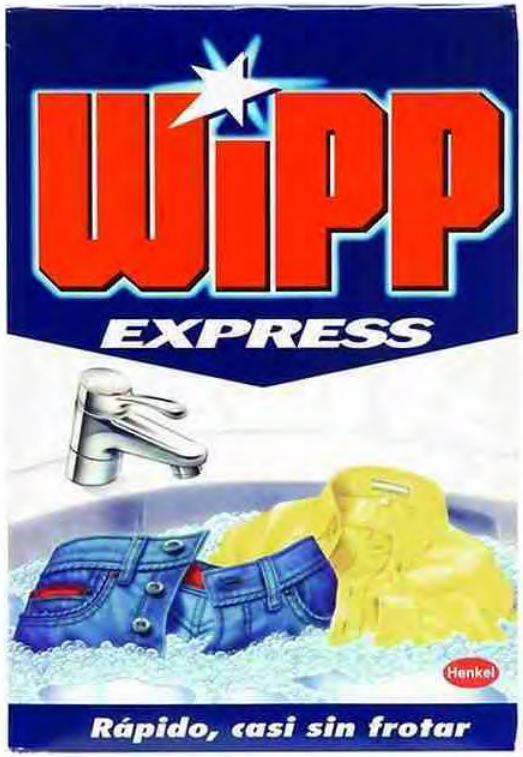
GUIDELINE

AMERICAN SOCIETY FOR GASTROINTESTINAL ENDOSCOPY ASGE

Infection control during GI endoscopy *Gastrointest Endosc. 2018 May;87(5):1167-1179.*

Manual cleaning

"The first, and one of the most important, steps in the prevention of transmission of infection by endoscopy, is ***manual cleaning of the endoscope with detergent solution and brushes****. (…) This should be performed* ***as soon as possible*** *on removal of the endoscope from the patient to prevent drying of material on the surface of the endoscope and within the channels.* ***Manual cleaning minimizes the chances of bacterial biofilm developing within the endoscope channels****."*



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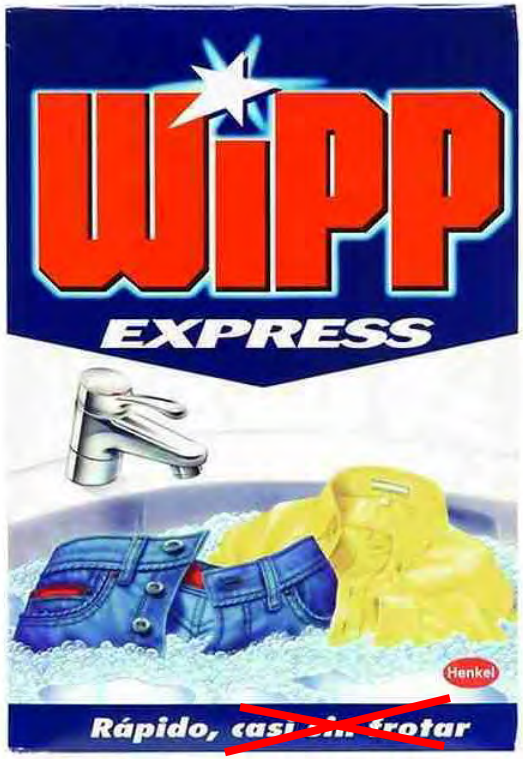

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Algunas guías internacionales más (I)

Essential Elements of a Reprocessing Program for Flexible Endoscopes – Recommendations of the Healthcare Infection Control Practices Advisory Committee

Last update: January 25, 2017
From: https://www.cdc.gov/hicpac/recommendations/flexible-endoscope-reprocessing.html

Recommendations

Essential Steps for Flexible Endoscope Reprocessing

To ensure flexible endoscopes are safe for patient use, all staff involved in reprocessing this equipment must understand and consistently follow a number of steps which have been distilled down to seven essential steps. Ensuring adherence to these steps requires a complete and effective reprocessing program. These recommendations apply to all settings where endoscopic procedures are performed and where endoscopes are reprocessed.

1. **Pre-cleaning**
    a. Pre-clean flexible endoscopes and reusable accessories by following the device manufacturer's instructions for use (IFU). Perform pre-cleaning immediately following completion of the endoscope procedure to help prevent the formation of biofilm.

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Algunas guías internacionales más (II)

Guideline for Use of High-Level Disinfectants & Sterilants in the Gastroenterology Setting

SGNA Society of Gastroenterology Nurses and Associates, Inc.

SGNA Practice Committee 2016 – 17

D. Biofilm

Biofilm can form on endoscopes, within water supply lines, and in automated endoscope reprocessors (AERs). Biofilm forms when bacteria group together on a wet surface and secrete large amounts of polysaccharide, which create a protective mass that cannot be removed with high-level disinfection (Muscarella, 2010). Therefore, prompt, meticulous manual cleaning to remove biologic material and strict adherence to reprocessing guidelines is the best approach to preventing biofilms (Alfa & Howie, 2009; Fang et al., 2010; Ren et al., 2013).

Meticulous manual cleaning of all instruments must precede exposure to any high-level disinfectant or sterilant (Petersen et al., 2011; SGNA, 2015a). Inadequate cleaning of instruments has been reported as one factor responsible for transmission of infection by flexible endoscopes (ASGE Standards of Practice Committee et al., 2008; Rutala et al., 2008). This process significantly reduces the organic and microbial challenge to the high-level disinfectant or sterilant and is a vital step in preventing biofilm (Alfa & Howie, 2009).

Simethicone, often used during endoscopy procedures, may foster microbial growth and biofilm development despite proper reprocessing because it contains sugars and thickeners. Minimize use of simethicone pending further studies (Ofstead et al., 2016).

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Algunas guías internacionales más (III)

Reprocessing of flexible endoscopes and endoscopic accessories used in gastrointestinal endoscopy: Position Statement of the European Society of Gastrointestinal Endoscopy (ESGE) and European Society of Gastroenterology Nurses and Associates (ESGENA) – Update 2018

*"Microorganisms embedded in biofilms are **10 to 100 times more resistant to process chemicals** than planktonic (free-floating) microorganisms and are frequently released from biofilms. Therefore it is important to follow the IFU of the endoscope manufacturer and the national guidelines. Some national guidelines recommend performance of all manual reprocessing steps within 30 minutes after completion of the patient examination. If endoscope reprocessing is delayed, **augmented cleaning steps** may be considered.*

In the endoscopy procedure room	Bedside cleaning rinsing and flushing of all channels function control
	Transport from endoscopy room to reprocessing area and start of manual cleaning steps within approximately 30 minutes

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Position Statement

Thieme

▶ **Table 1** Spaulding classification and reprocessing of medical devices.

Spaulding classification	Examples in GI endoscopy	Reprocessing
Semicritical devices	▪ Flexible endoscopes and their endoscope components	▪ Thorough manual cleaning including brushing is mandatory, followed by: Reprocessing, including cleaning, disinfection (attaining at least a given level of minimum bactericidal, fungicidal, mycobactericidal, and virucidal activity), and rinsing ▪ Automated reprocessing in an EWD is strongly recommended ▪ Thorough drying before storage in closed cabinets or storage cabinets with a drying function Competent staff specially trained in endoscope reprocessing (in line with national laws and regulations) are required.

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¿De qué se compone la matriz extracelular?

Table 1. Biofilm chemical composition [114].

S. No	Components	Percentage of matrix
1	Microbial cells	2-5%
2	DNA and RNA	<1-2%
3	Polysaccharides	1-2%
4	Proteins	<1-2% (including enzymes)
5	Water	Up to 97%

Research & Reviews: Journal of Microbiology and Biotechnology (2015)

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¿De qué se compone la matriz extracelular?

Table 1. Biofilm chemical composition [114].

S. No	Components	Percentage of matrix
1	Microbial cells	2-5%
2	DNA and RNA	<1-2%
3	Polysaccharides	1-2%
4	Proteins	<1-2% (including enzymes)
5	Water	Up to 97%

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¿Y con qué se solubilizan las proteínas y polisacáridos?

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Enzymes Enhance Biofilm Removal Efficiency of Cleaners

Antimicrob Agents Chemother. 2016 May 23;60(6):3647-52

"The addition of enzymes to the base formulation had a clear beneficial effect on the efficiency of biofilm removal. The S. aureus biofilm was removed efficiently if an ***active protease*** *was present, whereas for P. aeruginosa, single enzymes added to the formulation were not sufficient. An* ***optimized enzyme mixture including protease, polysaccharidases, and other enzymes*** *in a selected base formulation was required to achieve efficient removal of P. aeruginosa."*

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¿Pero qué concentración y actividad enzimáticas REALES Y DOCUMENTADAS tiene nuestro detergente?

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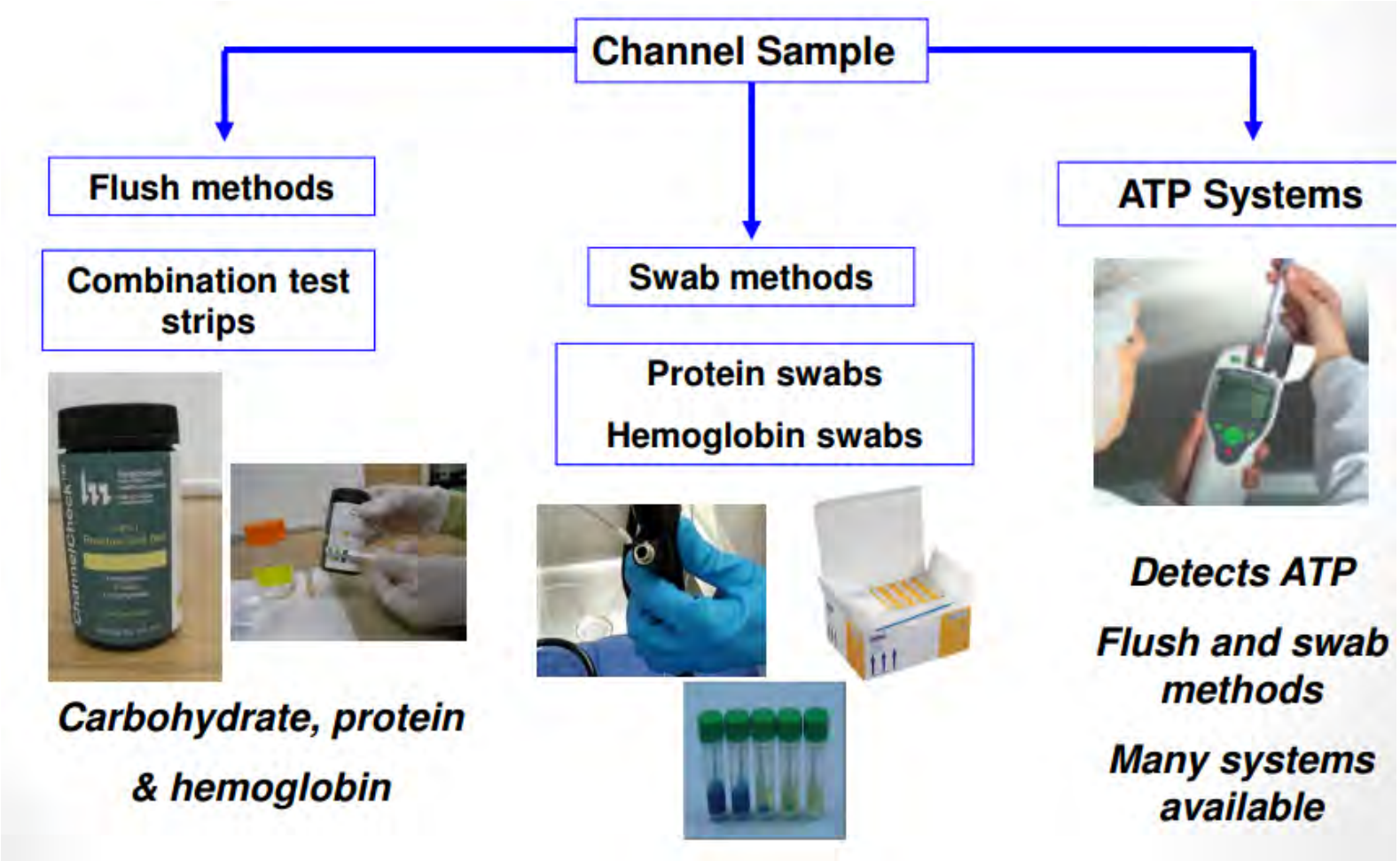
Monitorización del lavado de endoscopios



www.hmark.com/SGNA_review.pdf

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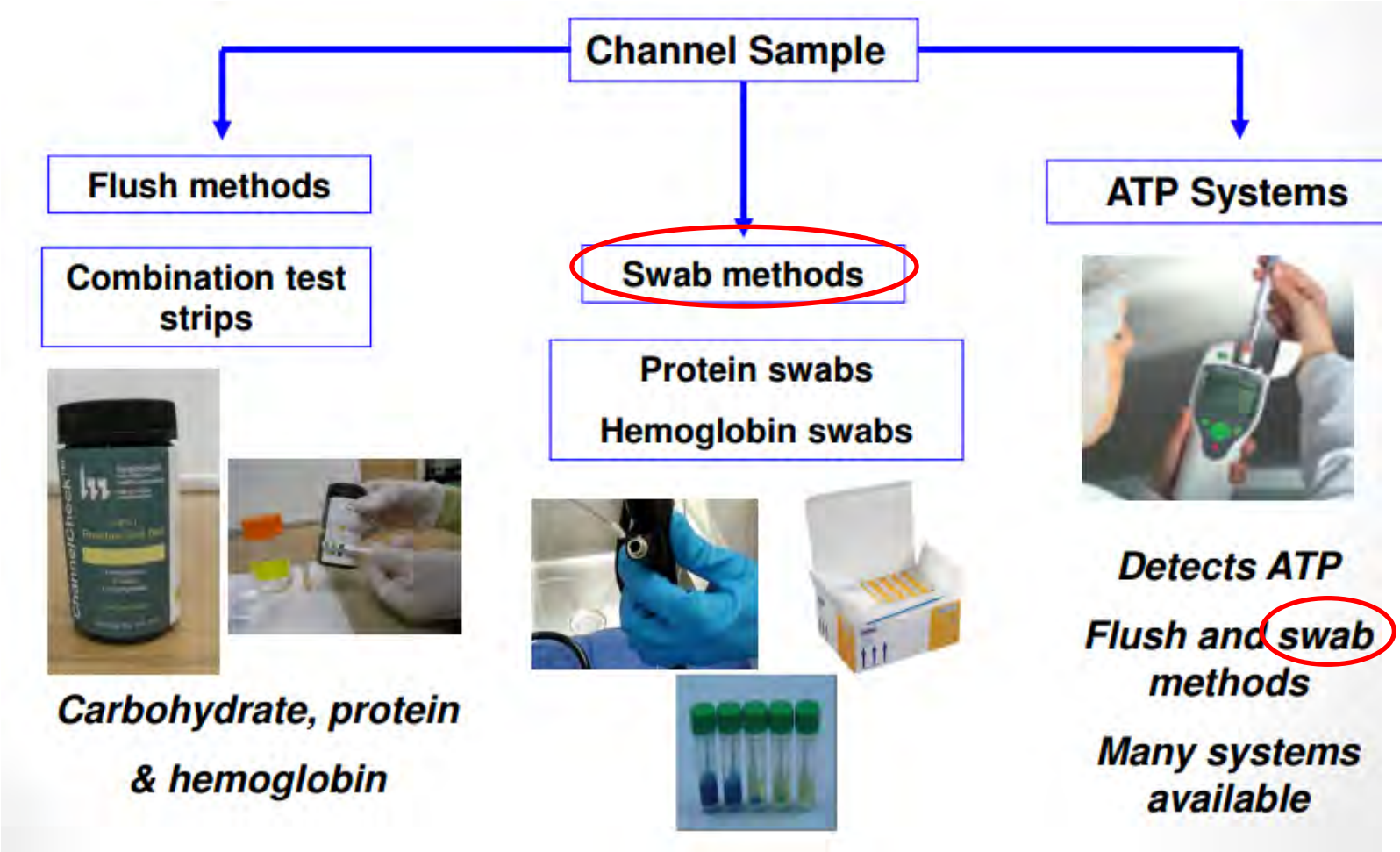

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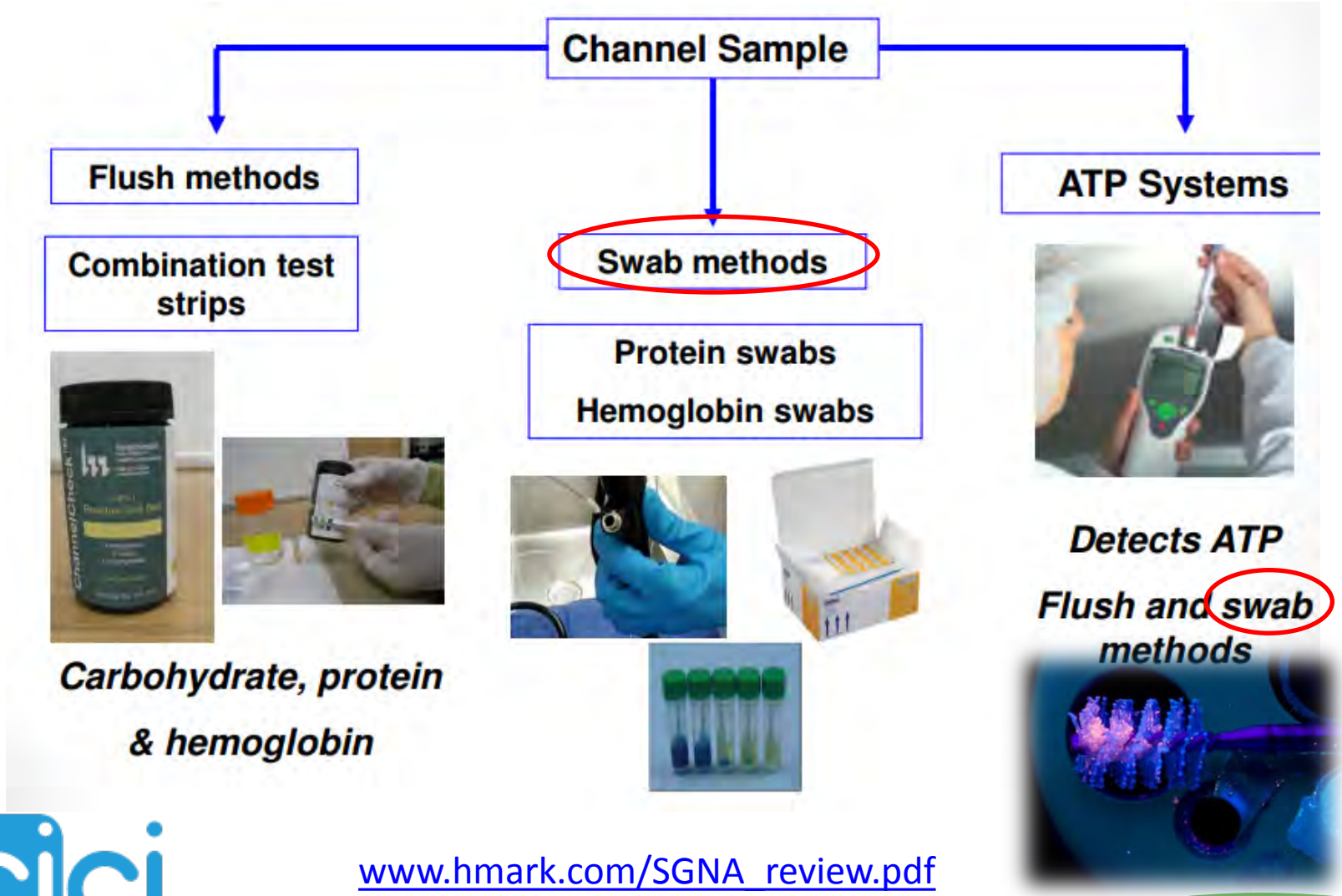
Monitorización del lavado de endoscopios



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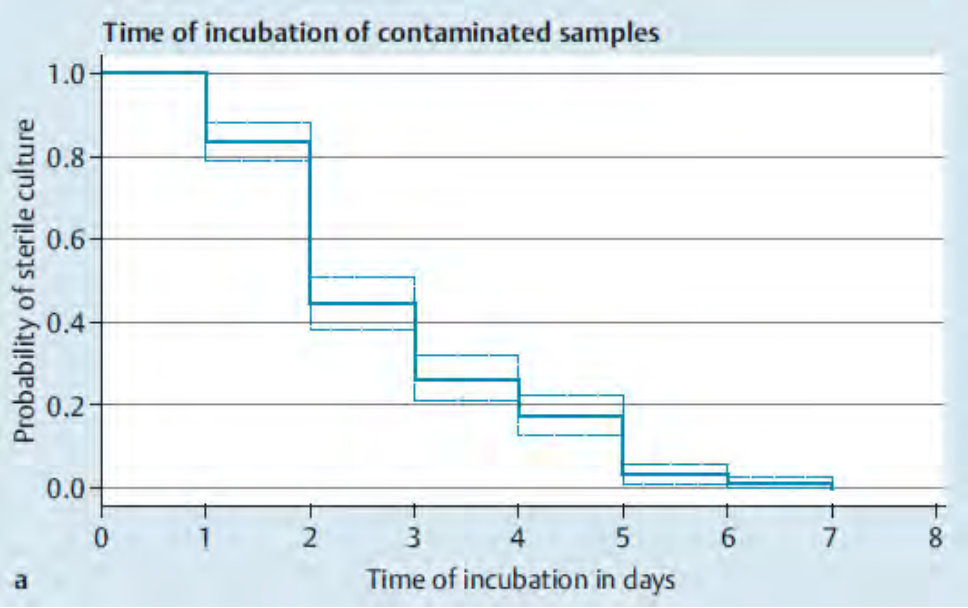
¿Bastan las condiciones normales de incubación para detectar biofilm?

Measures to improve microbial quality surveillance of gastrointestinal endoscopes

Endoscopy. 2016 Aug;48(8):704-10



*"Sampling of channels should be done using solutions that are **able to pick up biofilms, neutralize the peracetic acid, and enable the growth of bacteria**. Moreover, **samples should be incubated for at least 1 week** in order to identify all microorganisms surviving in the endoscope after disinfection. In some published studies, endoscopes were released for use as soon as cultures from channels were negative at 48 hours after plating [8], but this time is definitely too short according to our results. **The risk is to underestimate endoscope contamination and to expose patients to pathogens**.*

We also found that particular attention should be paid to endoscopes that are older than 2 years and those that are not stored in storage cabinets for heat-sensitive endoscopes."

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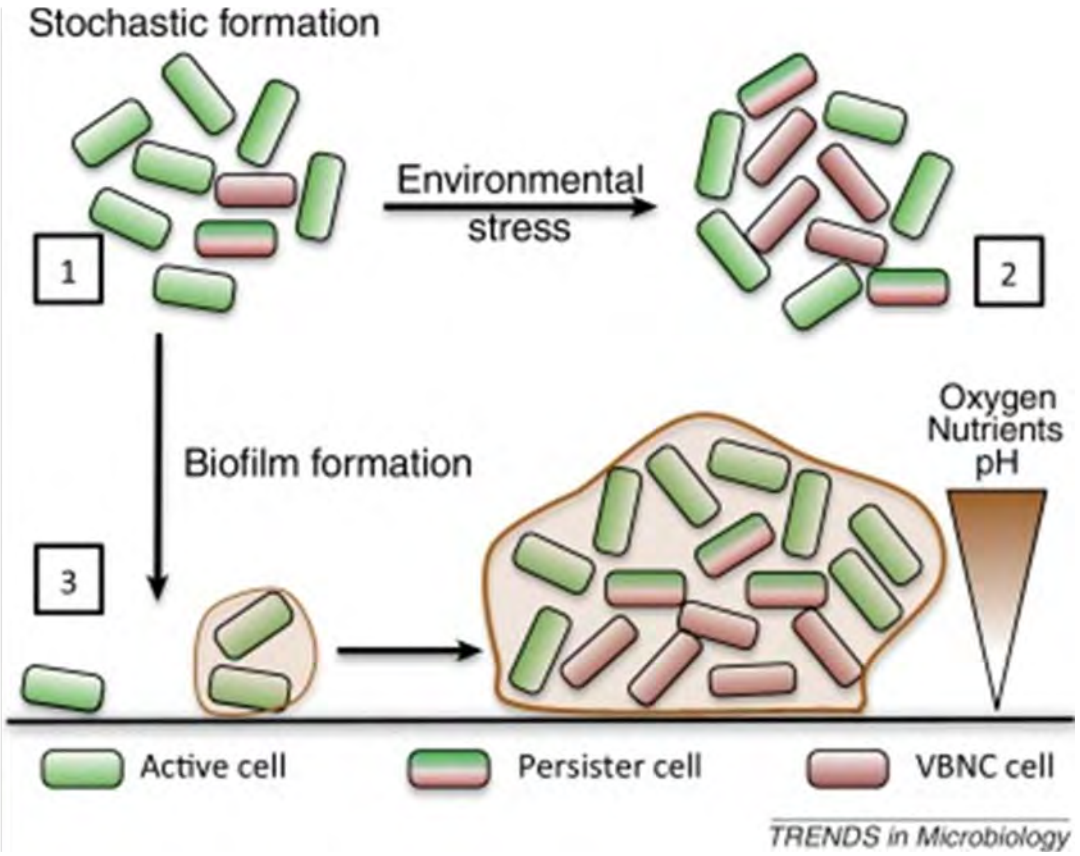
La causa: bacterias VBNC y células “persistentes”

Las bacterias VBNC (*Viable But Non Culturable*) son aquellas que, sometidas a estrés ambiental, entran en un estado de actividad metabólica muy baja, y dejan de dividirse, pero permanecen vivas y pueden revertir a un estado cultivable tras ser “resucitadas”, normalmente en medios enriquecidos y condiciones favorables.



... o detergentes, desinfectantes, etc. !

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¿Conclusiones? (o algo así…)

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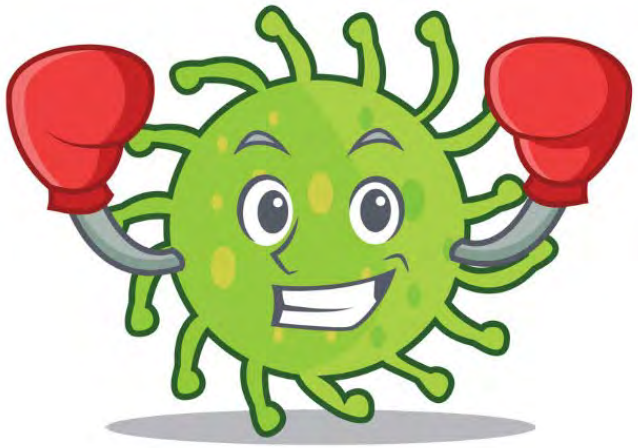
¿Conclusiones? (o algo así…)

Lo que sabemos que NO sabemos…

- La naturaleza y fisiología del biofilm son aún muy desconocidas.
- Ni siquiera hay métodos bien determinados y fiables para su detección.
- Los escasos y recientes modelos de laboratorio disponibles son mono-especie, algo que seguramente nunca encontraremos en un biofilm maduro real.
- A pesar de la publicidad, no sólo no hay detergentes ni desinfectantes con auténticas garantías anti-biofilm: es que ni siquiera existen ensayos normalizados para testar esos efectos.



¿Conclusiones? (o algo así…)

Lo que CREEMOS saber (I) …

- **La remoción MECÁNICA de los residuos es imprescindible y fundamental (énfasis en el cepillado MANUAL de canales internos).**
- **El *timing* es crítico, porque la formación de biofilm puede ser rápida: LAVADO MANUAL o, en su defecto, PRE-PROCESAMENTO INMEDIATOS.**
- **La matriz extracelular del biofilm es básicamente glicoproteica, así que el uso de detergentes enzimáticos CON ALTA CONCENTRACIÓN Y ACTIVIDAD DE PROTEASAS Y AMILASAS parece una buena apuesta.**

¿Conclusiones? (o algo así…)

Lo que CREEMOS saber (II) …

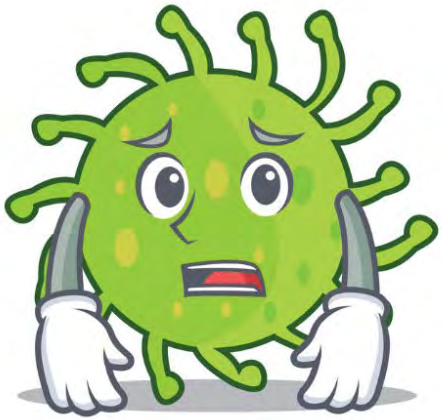
- **El reprocesamiento automático reduce errores, pero JAMÁS SUSTITUYE NI COMPENSA UN LAVADO MANUAL DEFICIENTE EN FORMA Y/O TIEMPO.**
- **Tras el reprocesamiento, es fundamental el SECADO DE LOS CANALES INTERNOS. A falta de un armario de secado con inyección de aire filtrado, la inyección de alcohol (de rápida y total evaporación) puede ser aceptable.**
- **La naturaleza misma del biofilm lo hace difícilmente detectable en canales internos. Parece inteligente priorizar los muestreos con remoción mecánica (*swabbing*, mejor que *flushing*), e incrementar los tiempos de cultivo de los muestreos microbiológicos (VBNC).**

¿Conclusiones? (o algo así…)

Lo que está por venir…

- **Se están desarrollando modelos de laboratorio para la creación y ensayo de biofilms, que algún día podrán dar lugar a ensayos de eficacia normalizados.**

- **Se están estudiando distintos medios de remoción/destrucción de biofilm:**
  - **Materiales de limpieza (ej. cepillos) con mayor capacidad abrasiva**
  - **Formulaciones enzimáticas optimizadas para la matriz polimérica del biofilm**
  - **Desinfectantes y pre-desinfectantes con mayor penetración a través de la matriz polimérica**
  - **Ingredientes (ej. cinamaldehído) que interfieran con el *Quorum Sensing* (¡dejando al biofilm sin WiFi!)**



¡El modelo ya existía!

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¡El modelo ya existía!

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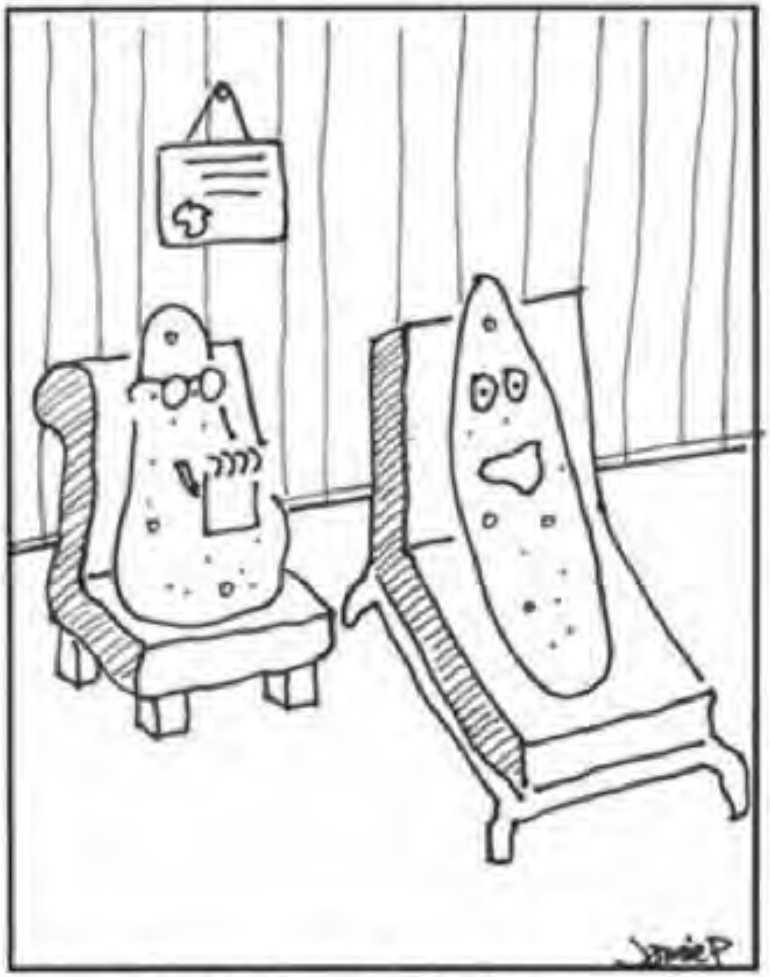
¿Charlamos un rato?



I just can't go with the flow anymore.
I've been thinking about joining a biofilm.

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www.vesismin.com

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https://solucionesdesinfeccion.com

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