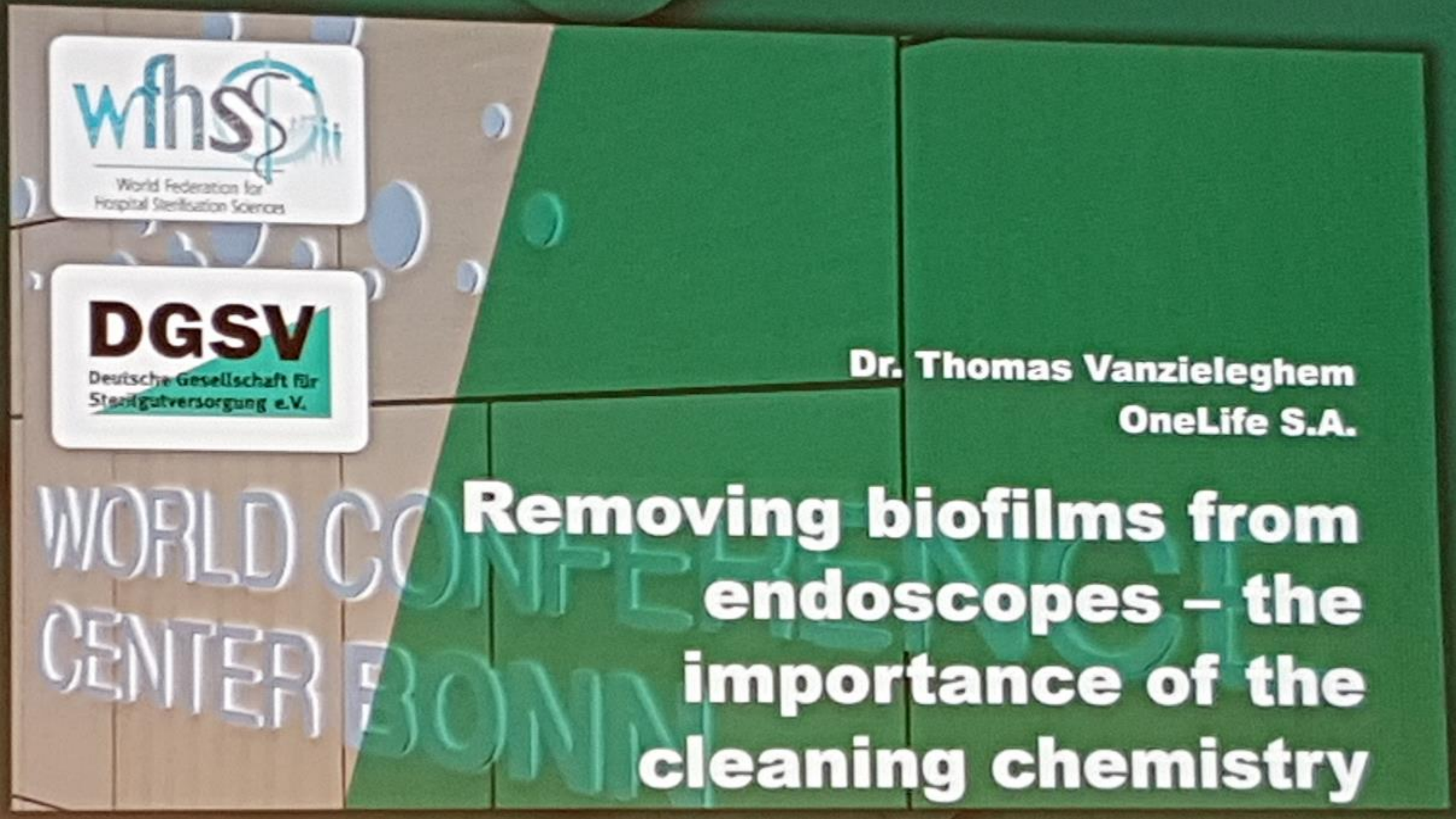


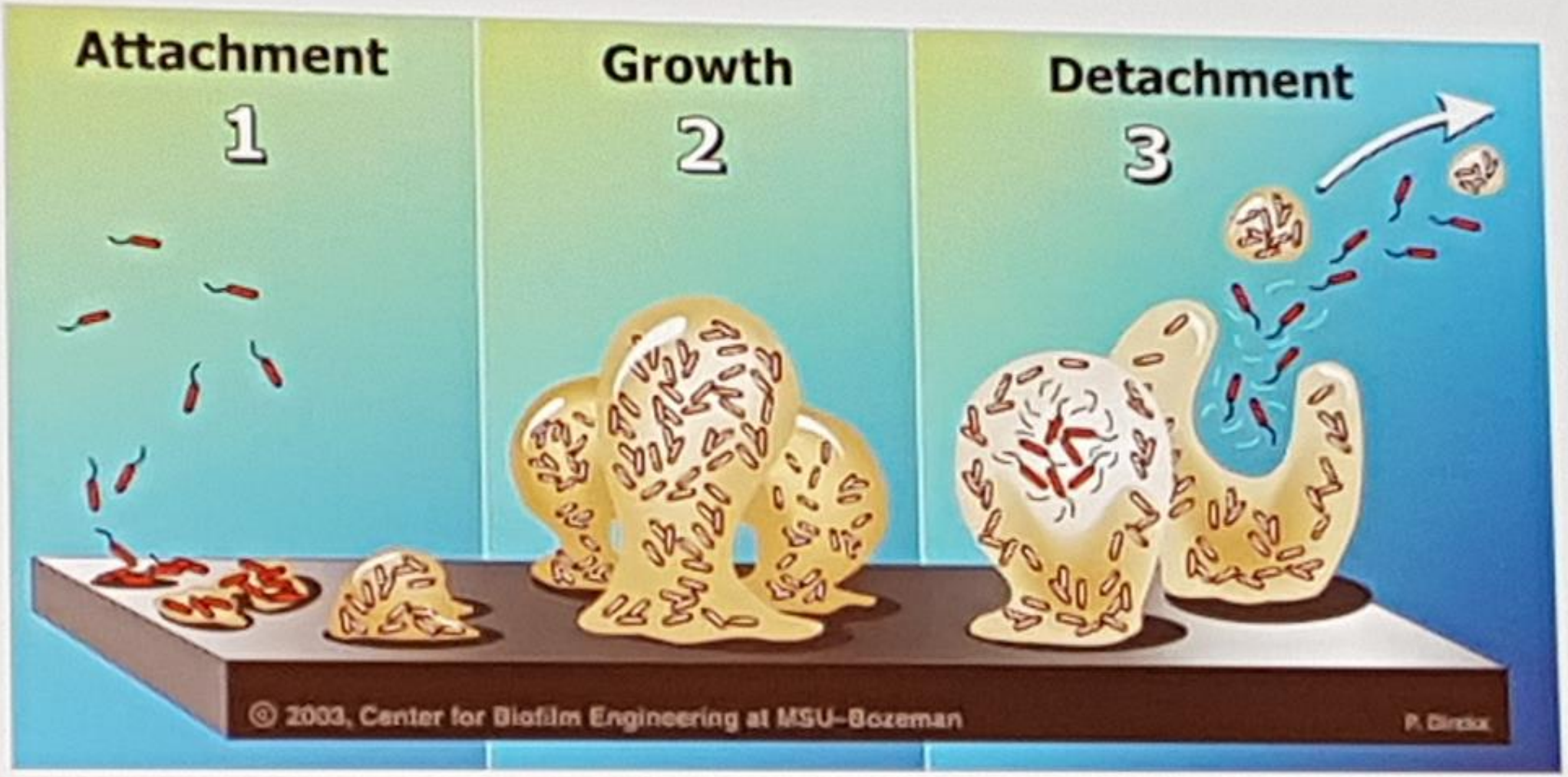
Dr. Thomas Vanzieleghem
OneLife S.A.

Removing biofilms from endoscopes – the importance of the cleaning chemistry

BIOFILM FACTS

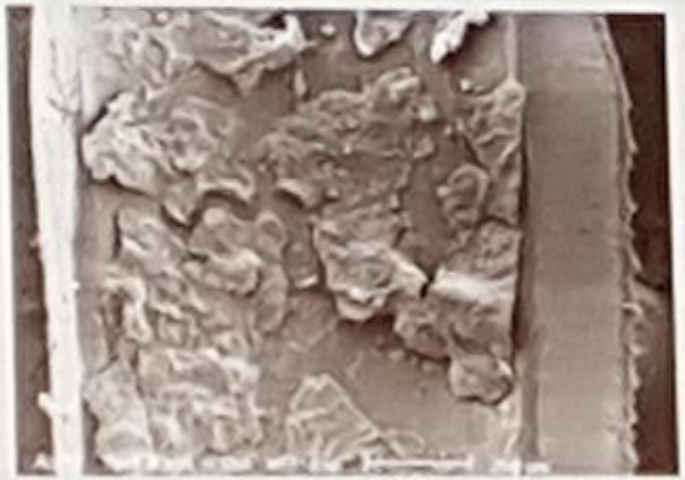
- 99% of bacteria grow as aggregated, sessile communities (biofilm)
- Biofilm are highly protected and highly resistant to antibacterial treatments (antibiotics and disinfectants)
- Biofilm are genetically different than bacteria in the planktonic state
- Biofilm can adhere to stainless steel, even highly polished SS, **within 30 seconds** and can also bind to PTFE
- NIH estimates more than 80% of microbial infections in humans are caused by biofilm

BIOFILM LIFE CYCLE



BIOFILMS ON ENDOSCOPES

Shortly after being used, endoscopes develop a conditioning film composed of bodily fluids, proteins, polysaccharides and other components. This alteration of the surface characteristics allows bacteria to commence growth and colonization as biofilms



Pajkos *et al.*, JHI, 2004

BIOFILMS ON ENDOSCOPES

Inadequate Cleaning: soil remains in lumens despite cleaning and disinfection

Source of microorganisms :

- *Pseudomonas aeruginosa* : tap water used for cleaning
- *Enterobacteriaceae* : patient flora
- *Staphylococci* : patient and medical staff flora

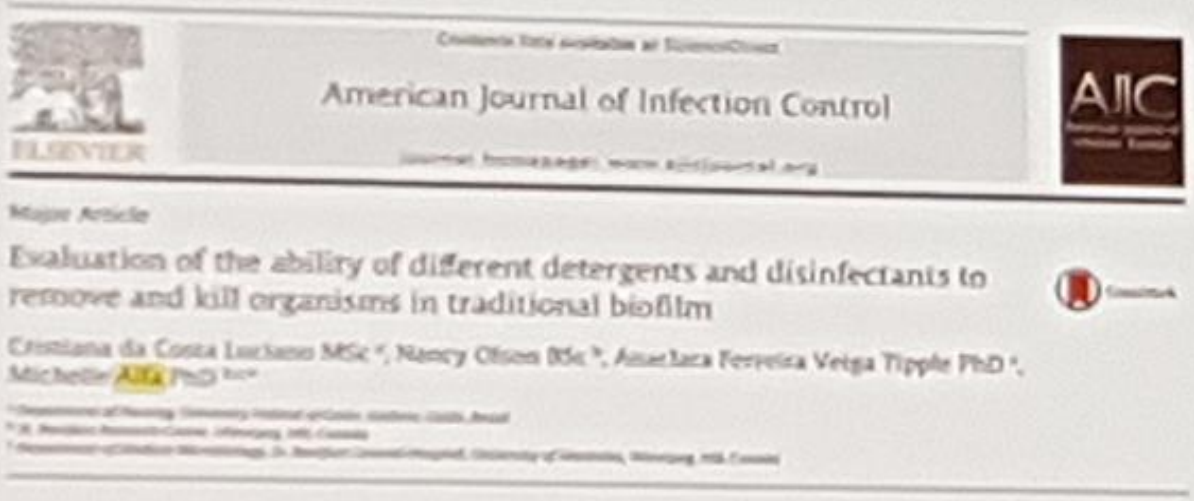
Inadequate reprocessing (missed steps, lack of cleaning) can lead to :

- Formation of buildup biofilm and accumulated soils
- Cross-transmission of same strain to several patients (outbreaks)

Transmission rate: 1,045 cases/10,000 procedures (infections are rarer but are probably underestimated)

CURRENT LIMITS OF REPROCESSING

Once biofilm has developed in an endoscope, it becomes difficult to eradicate and the source of recurrent contamination. Current reprocessing protocols may fail to remove biofilms.

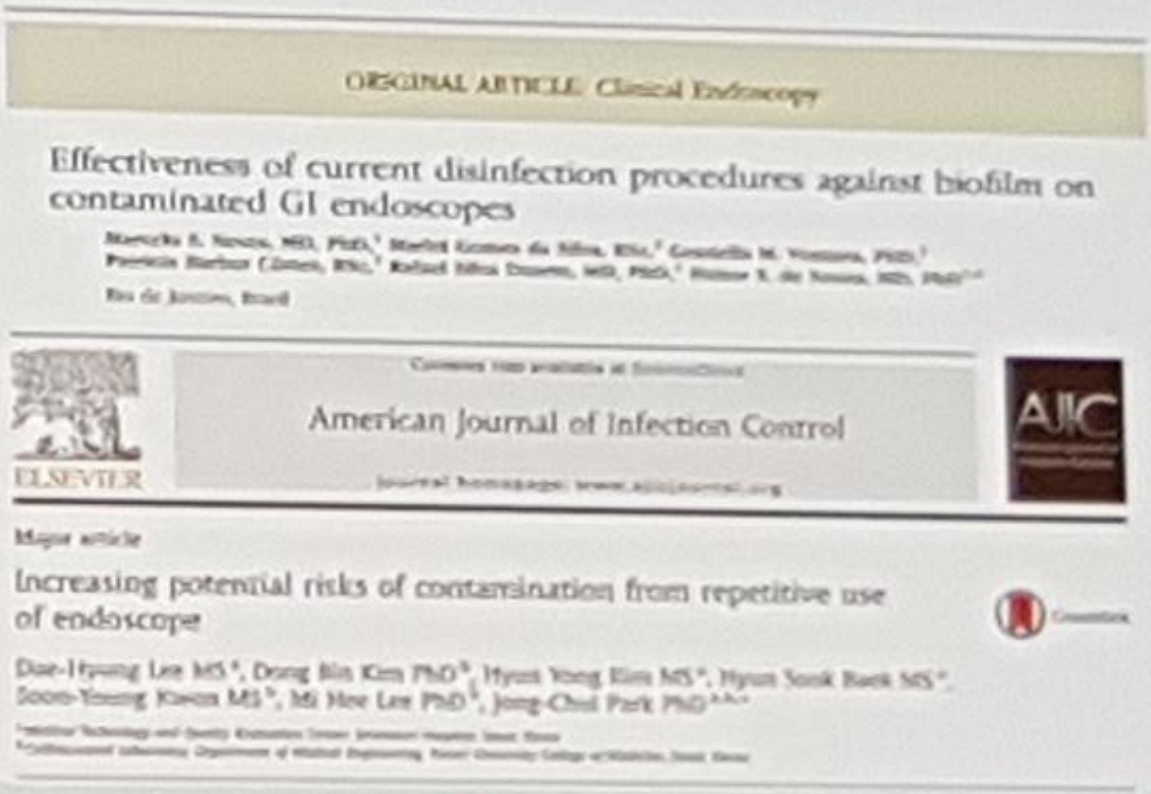


American Journal of Infection Control

Major Article

Evaluation of the ability of different detergents and disinfectants to remove and kill organisms in traditional biofilm

Cristiana da Costa Luciano MSc [a], Nancy Olson BSc [b], Anuradha Ferreira Veiga Tipple PhD [a], Michelle Alfa PhD [b,c]



ORIGINAL ARTICLE: Clinical Endoscopy

Effectiveness of current disinfection procedures against biofilm on contaminated GI endoscopes

American Journal of Infection Control

Major article

Increasing potential risks of contamination from repetitive use of endoscope

Dae-Hyoung Lee MS [a], Dong Bin Kim PhD [b], Hyun Yong Kim MS [a], Hyun Sook Baek MS [a], Soon-Young Kwon MS [b], Mi Hee Lee PhD [b], Jong-Chul Park PhD [a,b,c]

IS CLEANING THE KEY TO BAN BIOFILMS?

The objectives of our study were:

- To assess the biofilm performance of several commercially available cleaners in *in vitro* models
- Document the efficacy of a curative cleaning treatment to endoscopes that displayed non-acceptable culture results after microbiological surveillance (preliminary data)

METHODS

Biofilms were grown in 96-well plates in static conditions in rich medium (TSB or LB)

Isolates used in this study :

- Reference isolates
- Clinical isolates

Bacterial species	Reference isolates	Clinical isolates
S. aureus (MRSA)	ATCC33591	2003/1083 (C1) * : surgical wound 2009/0025 (C2) * : Chronic ear infection
P. aeruginosa	PAO1	PA509 (C3) : surgical bandage PA20 (C4) : arterial catheter
K. pneumoniae	ATCC700603	010 (C5) : Craniopasty 928 (C6) : Central Venous Catheter
E. coli	ATCC25922	8550 (C7) : Urinary catheter 8922 (C8) : Urinary catheter
E. faecalis	ATCC29212	9794 (C9) : Urinary catheter 9781 (C10) : Urinary catheter

METHODS

All cleaning tests were performed in Water of Standard Hardness (3.33 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1.25 mM $MgCl_2$)

Cleaners used in this study

Temperature : 25°C and 40°C

Cleaners	pH at 20°C		Active substances	Dosage (%)		Recommended temperature (°C)
Code	undiluted	1% solution	Enzymes	IFU	Used in this study	
A	8	7.8	Multiple	1	1	40 to 45
B	8	7	Multiple	0.5	0.5	25
C	7.6	7.7	One	0.2 - 0.5	0.5	None
D	8	8	One	0.8 - 1.6	1.6	20 to 40
E	8.5	8.1	Multiple	0.1 - 1	1	20 to 45
F	Powder	10.4	One	0.5	0.5	25
G	8.6	7.5	None	0.5	0.5	"Cold or hot"
H	8.4	7.8	Multiple	0.2	0.2	"All water temperatures"
I	5.95	7.45	None	0.5	0.5	20 to 35
J	10.1	8.7	One	0.2 - 1	1	40 max.
K	8.5	8	Multiple	0.5	0.5	below 35

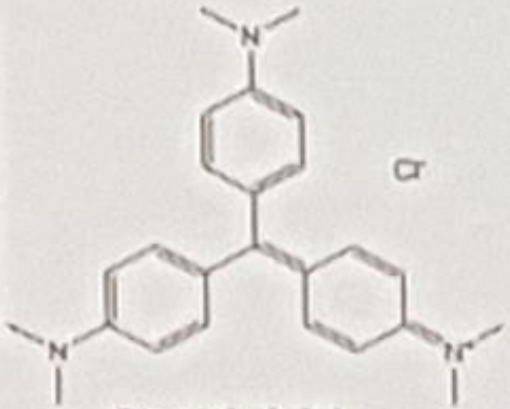
Cleaner A =
OneLife enziQure ®

METHODS

Biofilm analysis methods :

Crystal violet assay (Biomass quantification)

Detection of **matrix and cells** by non-specific staining of all biofilm constituents



Crystal violet

Absorbance λ= 570nm

LIVE/DEAD staining

Detection of **viable** and dead **bacteria** by confocal microscopy

IN VITRO RESULTS

Removal of biofilm biomass at 40°C (%

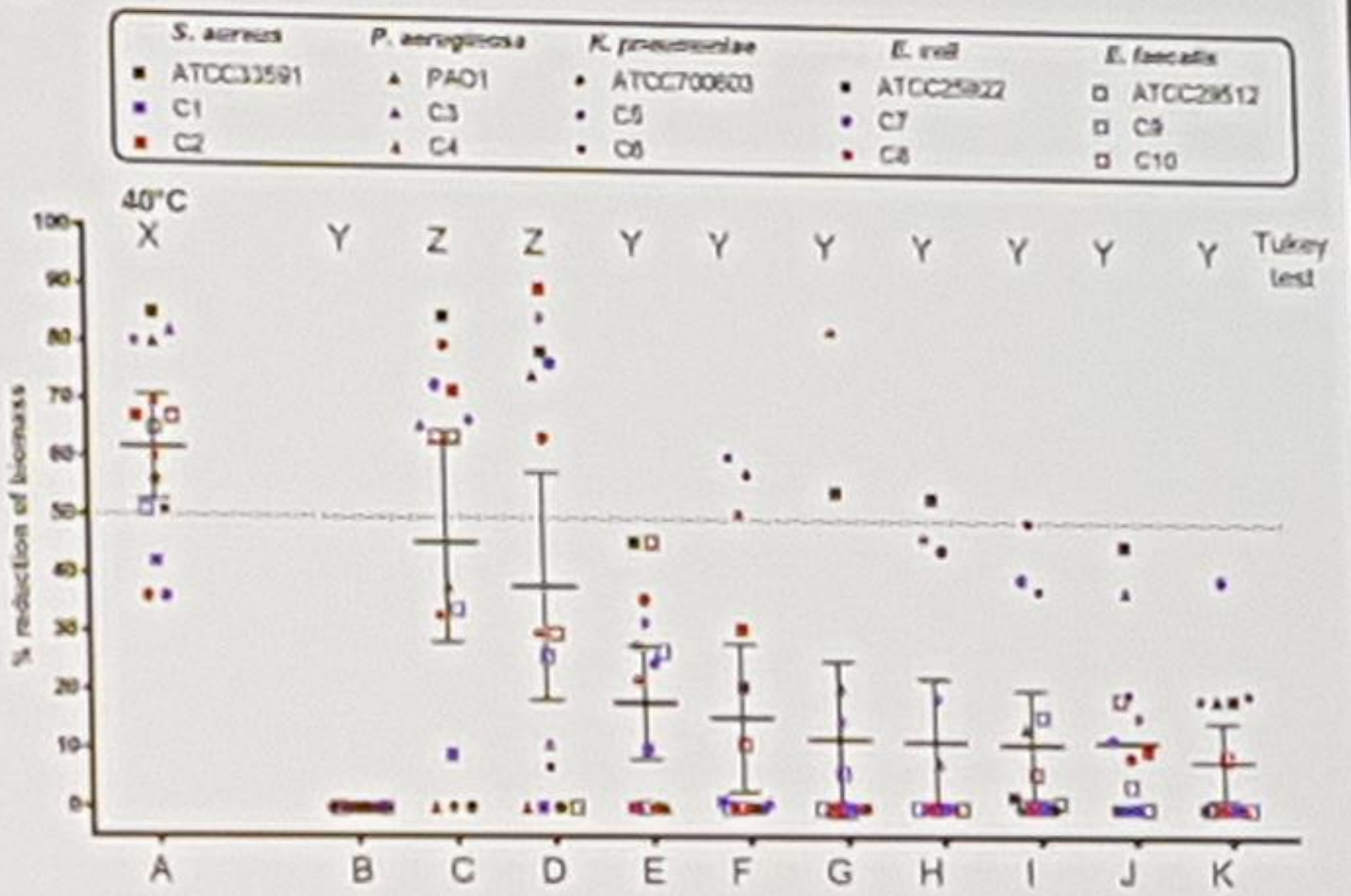
Ranking :

A > C, D > B, E, F, G, H, I, J, K

Max. biofilm removal :

62 % - across all isolates (cleaner A)

92 % - Cleaner D on isolate C2



IN VITRO RESULTS

Removal of biofilm biomass at 25°C (%)

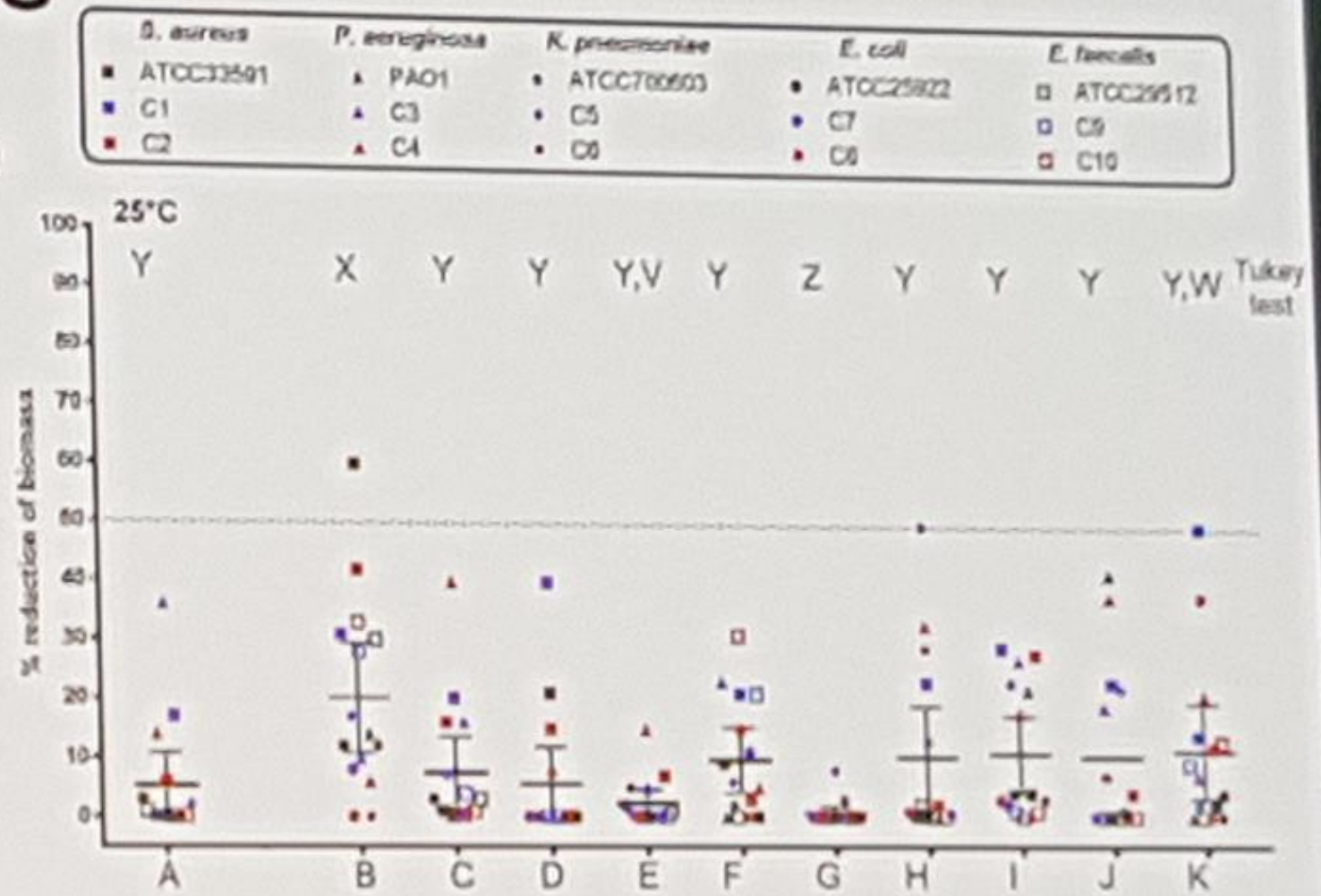
Ranking :

B > A, C, D, E, F, H, I, J, K > G

Max. biofilm removal :

20.5 % - across all isolates (cleaner B)

63 % - Cleaner B on isolate ATCC33951

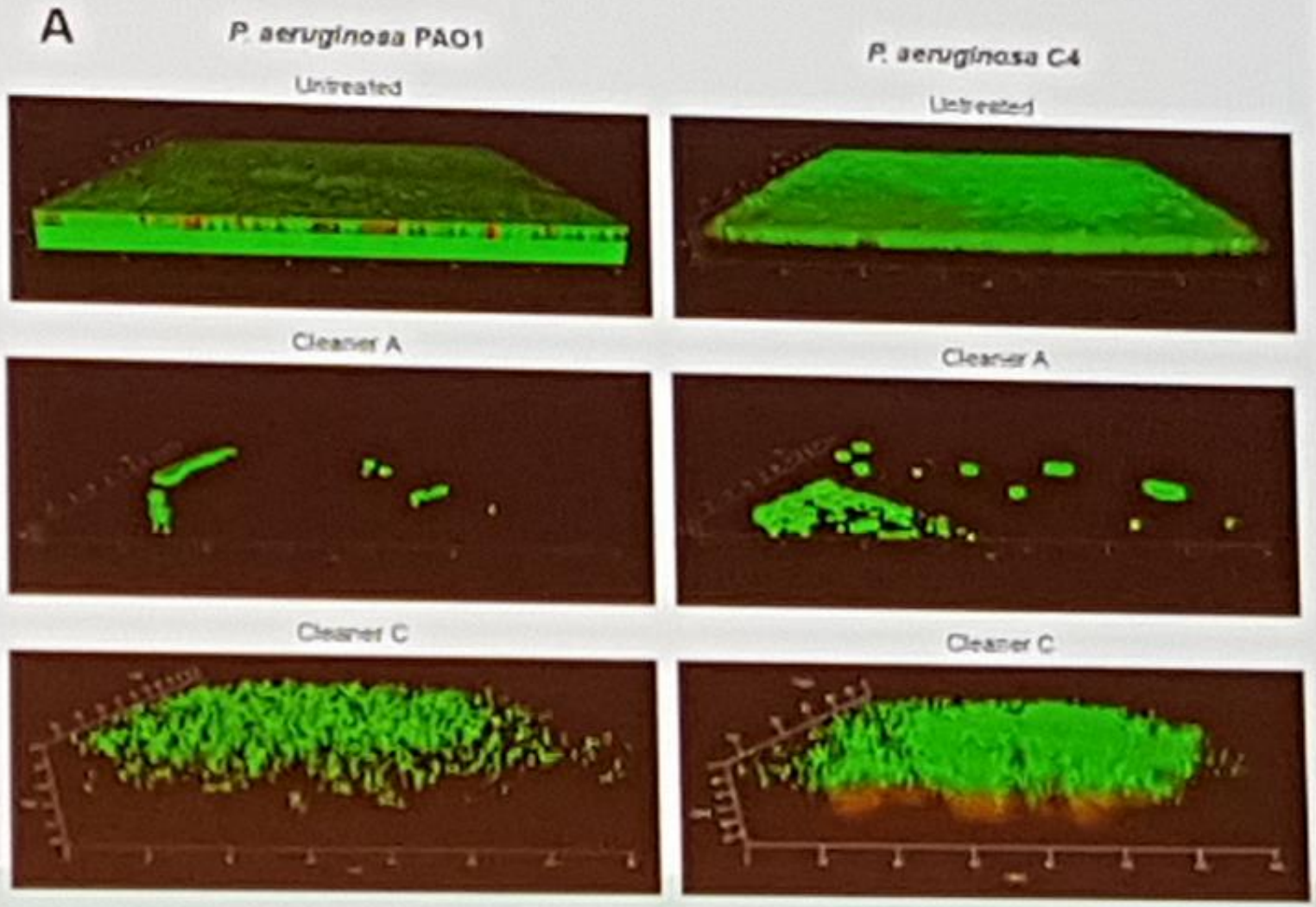


IN VITRO RESULTS

Confocal microscopy

The two best cleaners were tested:

Cleaner A and C at 40°C



CONCLUSIONS ON IN VITRO RESULTS

Statistical analysis revealed that :

- Biofilm removal was more efficient at 40°C than at 25°C (p-value < 0.0001)
- Enzymatic cleaners are more active than non-enzymatics ones at 40°C (p-value < 0.0001) but no difference was observed at 25°C (p-value > 0.05)
- Biofilm removal by cleaners is strongly dependent on the isolate that formed the biofilm (p-value < 0.0001)
- Within the group of (multi-)enzymatic cleaners, large discrepancies were observed

  → Efficacy depends on formulation and is not apparent to the end-users

TESTS IN THE FIELD WITH ENDOSCOPES

Curative cleaning protocol with enziQure® (cleaner A),
Cleaner with the best results in *in vitro* static models.

Protocol :

- 3 brushing and flushing steps
- Soaking temperature : 40°C
- Soaking time : 60 min
- Cleaning is followed by a standard AER cycle
- Sampling is performed during storage

Step	Action	Accessories	Time (min)
1	Prepare a 15 L bath at 45°C	Add Cleaner as per manufacturer instructions	3
2	Connect the endoscope for a leak test	Leak test device	0.5
3	Immerse the endoscope		
4	Flush every channels with detergent solution	Syringes, Octopus flushing system (optional)	2
5	Brush all channels that can be brushed three times	Appropriate endoscope channel cleaning brushes	3
6	Soak		25
7	Flush every channels with detergent solution	Octopus flushing system (optional), syringes	2
8	Brush all channels that can be brushed three times	Appropriate endoscope channel cleaning brushes	3
9	Soak		25
10	Drain the cleaning solution		1
11	Pour 15L of tap water and rinse the endoscope (channels and outside)	Syringes, Octopus flushing system (optional)	5
12	Drain the rinse water		
13	Place endoscope in AER and launch a cleaning + HLD cycle	AER	15 – 45 *

TESTS IN THE FIELD WITH ENDOSCOPES

Hospital	Endoscope type	First microbiological control (in 100 ml)	Intensive cleaning and HLD as per hospital procedures	Second microbiological control (in 100 ml)	Corrective cleaning with Cleaner A and HLD	Microbiological control after corrective procedure (in 100 ml)
U1	Echo-endoscope	>150 CFU + *Stenotrophomonas maltophilia*	Yes (double 5 min manual cleaning + AER)	> 150 CFU + *S. maltophilia*	Cleaner A + AER	< 1 CFU Absence of *S. maltophilia*
U1	Echo-endoscope	75 CFU + *P. aeruginosa* + *Streptococcus spp.*	Yes (double 5 min manual cleaning + AER)	16 CFU + *S. maltophilia*	Cleaner A + AER	0 CFU Absence of *S. maltophilia*
U1	Echo-endoscope	Return from maintenance / Not tested	Yes (double 5 min manual cleaning + AER)	> 150 CFU + *S. maltophilia* + *P. aeruginosa*	Cleaner A + AER	< 5 CFU Absence of *S. maltophilia* and *P. aeruginosa*
U2	Gastroscope	>350 CFU	Yes (No manual cleaning, longer AER cycle)	> 350 CFU	Cleaner A + AER	0 CFU
NU1	Gastroscope	> 100 CFU + *P. aeruginosa*	Yes (5 min manual cleaning + AER)	> 100 CFU + *P. aeruginosa*	Cleaner A + AER	0 CFU Absence of *P. aeruginosa*
NU2	Duodenoscope	1.000 CFU + *P. aeruginosa*	Yes (5 min manual cleaning + AER)	10.000 CFU + *P. aeruginosa*	Cleaner A + AER	< 20 CFU Absence of *P. aeruginosa*
NU3	Duodenoscope	5.000 CFU + *P. aeruginosa*	Yes (5 min manual cleaning + AER)	5.000 CFU + *P. aeruginosa*	Cleaner A + AER	< 20 CFU Absence of *P. aeruginosa*

CONCLUSIONS ON IN VITRO RESULTS

Preliminary data on 7 endoscopes from 4 hospitals show that :

- Recurrent microbial contamination can be difficult to remove with regular reprocessing procedures
- Curative manual cleaning followed by high level disinfection allowed eradication of microbial load from the endoscope
- Further data are needed to confirm the efficacy of this approach to solve issues associated with recurrent contamination of endoscopes

THANK YOU FOR YOUR ATTENTION

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One Life

DETECT & DISSOLVE BIOFILM MATRIX