

Biofilm's cleaning : We should use DNAse!



IDI Study Days March 2024



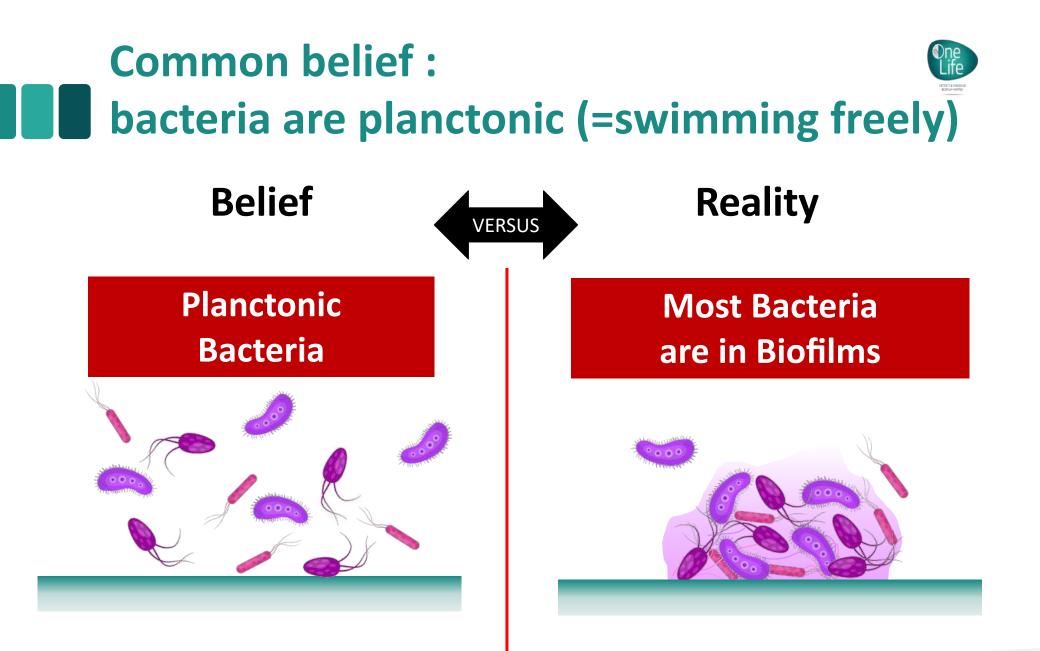


Wendy Glénisson PhD



There are bacteria everywhere...



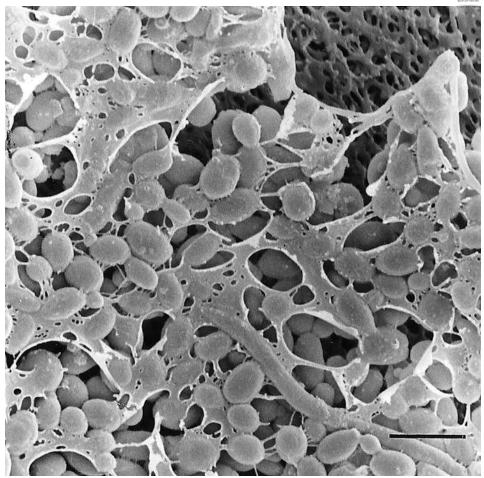






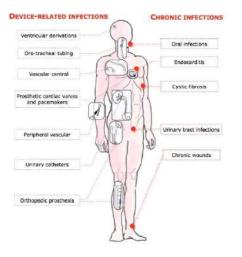
Biofilm is a complex microorganism comunity, attached to a surface and anchored by the adhesive and protective matrix they produce.

It's a dynamic structure, constantly reshaping.





Effects of Biofilm :







The effects of Biofilms are often said to be harmfull :

- Medical field (antibiotic resistant infections on implanted medical devices, nocosomial infections, ...)
- Technologic field (sludge clogging pipes, decreased slide on boat hulls)

They allso have some positive effects (fermentation, wastewater treatment)...



Well known Biofilms ...

Every where...

Dental plaque



Sink residus



... and since ever!

Stromatolites

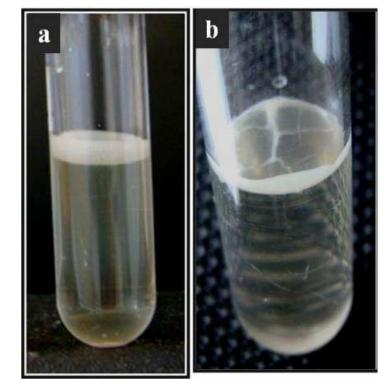




Broader definition :

Biofilm concept was extended to non solid to surfaces interfaces :

- Bacterial community at the interface between air – liquid / gel / mucus (Ferguson BJ, Stolz DB. 2005. Am.J.Rhinol., 19:452-457)
- Biofilm can stick to mobile surfaces like mucous membranes (Akimbekov et al, 2022, Front Nutr.)



=> Colonize many places in Human Body!



Biofilms are deadly!

60 to 80% of bacterial infections consists in biofilms (NIH) (Jama et al *J Chin Med Assoc.* 2018)

Nocosomial Infections :

- Europa : 4 millions/year, => 37 000 deaths (Szabo et al J. Clin Med. 2022)

- World : > 100 million/year

(Zewdu Wasie Taye et al. Front. Public Health, 2023)

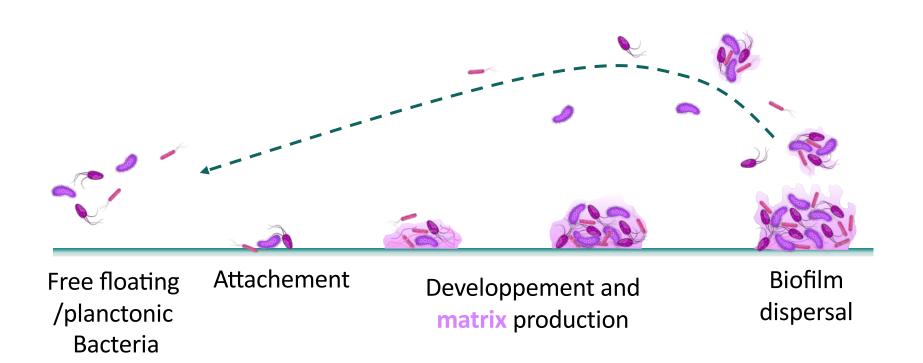
Most nocosomial infections are related to implanted medical devices (urinary catheter, intubation cannula, vascular probe, orthopaedic prosthesis, contact lenses, intrauterine devices, ... etc)



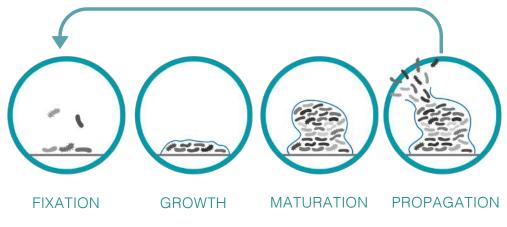
Femoral, peri-prosthetic ostemyelyt

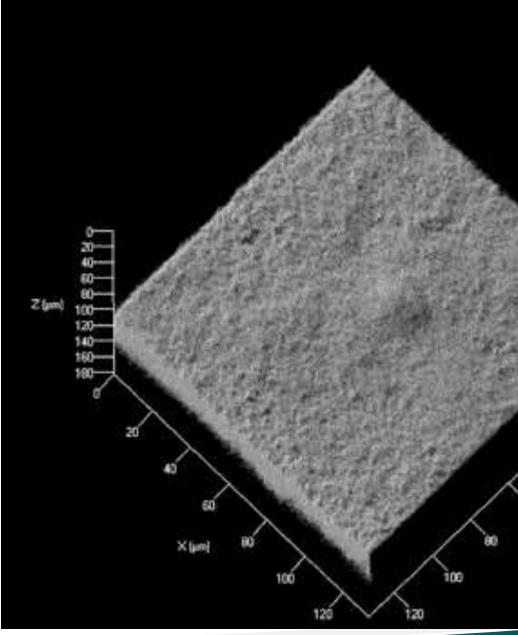


Biofilm life cycle :



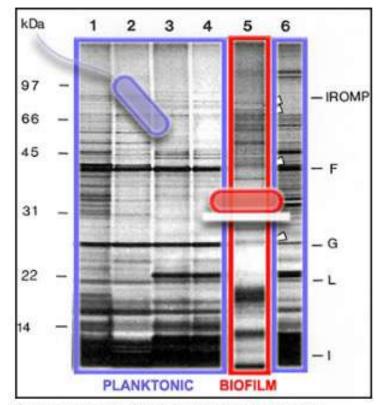
Biofilm growth Electronic Microscopy



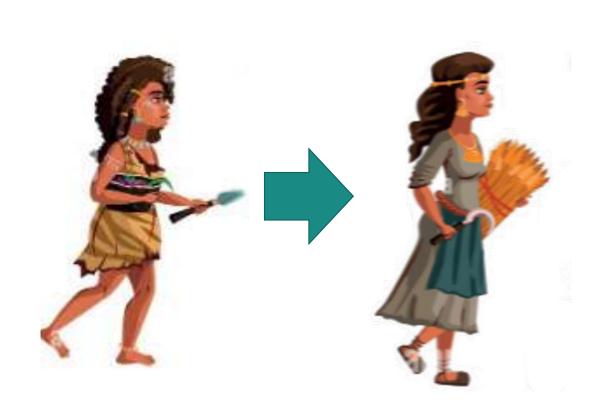


A huge step : from nomadic to sendentary life style





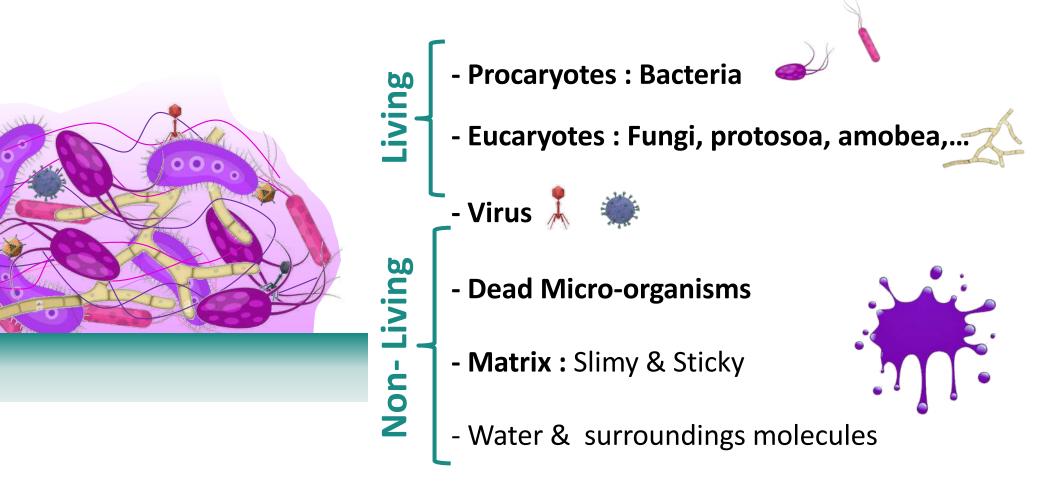
SDS PAGE preparation of the outer membrane proteins (OMPs) of Pseudomonas aeruginosa cells in planktonic and biofilm states. *Courtesy, Hongwei Yu*



OneLife S.A. proprietary confidential



Biofilm composition :





Micro-organismes found in Biofilms :

Bacteria

Staphylococcus aureus MRSA Pseudomonas aeruginosa Yersinia enterocolitica Vibrio cholerae Mycobacterium tuberculosis Salmonella enterica Yersinia pestis Listeria monocytogenes Cronobacter sakazakii

Fungi

Candida albicans Aspergillus fumigatus Fusarium solani Trichophyton rubrum Cryptococcus neoformans Microsporum canis Paecilomyces variotii Rhizopus oryzae Penicillium chrysogenum Virus COVID-19 SARS-CoV-2 Bacteriophage T4 Influenza virus Hantavirus Adenovirus Hepatitis A virus Herpes simplex virus Norovirus

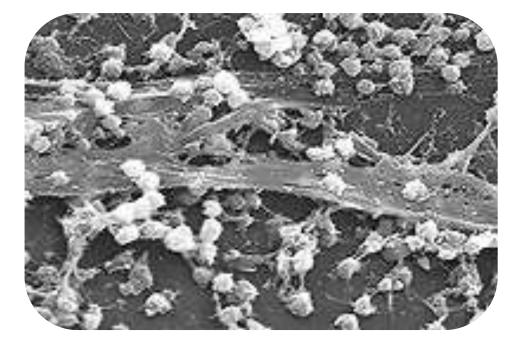
List growing longer each year ! => Infinite variability?

Lactobacillus acidophilus Neisseria gonorrhoeae Propionibacterium acnes Shigella flexneri Stenotrophomonas maltophilia Streptococcus pneumoniae Treponema pallidum Legionella pneumophila Streptococcus mutans Escherichia coli Bacillus subtilis IVIalassezia jurjur Exophiala dermatitidis Candida glabrata Sporothrix schenckii Penicillium digitatum Paecilomyces variotii Trichophyton rubrum Aspergillus niger Histoplasma capsulatum Paecilomyces Illacinus Candida tropicalis ... Etc Vesicular stomatitis virus Human papillomavirus (HPV) Epstein-Barr virus (EBV) Cytomegalovirus (CMV) Herpesvirus 6 (HHV-6) Human immunodeficiency virus (HIV) Human T-lymphotropic virus (HTLV) Respiratory syncytial virus (RSV) Coxsackievirus Norovirus GII.4 Rotavirus group A

... Etc



Matrix : Biofilm's structure and support



Staphylococcus aureus biofilm in a catheter Monroe, PLOS Bio, 2007



Faizan et al., ILVO, 2023. ????

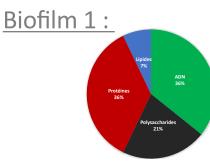


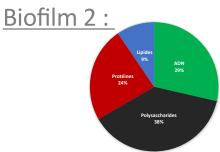
Polysacch.

DNA

Biofilm's matrix composition :

Proteins





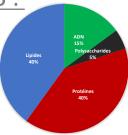
Variable proportions depending on :

➢ Bacterial species composing the biofilm

Lipids

- Nutrients availibility
- ➢ Surface on which the Biofilm grows
- Environnement (stress, desinfection, dryness, ...)

Biofilm 3 :

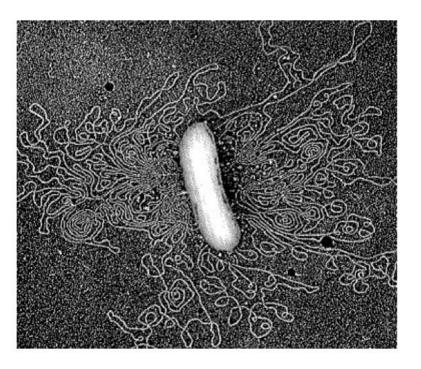


=> INFINITY OF COMBINAISONS ?

Detergents used should have a broad activity spectrum !



Role of extracellular DNA in Biofilms :



An under-estimated role?

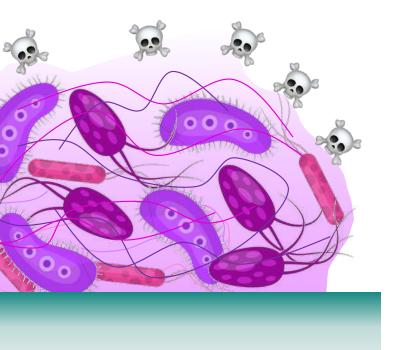
- > Either actively secreted or released from dead bacteria
- \succ DNA is negatively charged ;
- \succ It gathers arround bacteria ;
- It protects bacteria against cationic bactericids!
- > May be used as food in oligotrophic conditions
- 1 DNA molecule (5 millions base pairs)
 = 1,7 mm long. = 500x > 1 bacterium length
 => Huge scaffolding for the Biofilm!



Whitchurch CB et al. 2002 Science, 295:1487 - Flemming HC et al. 2007. J.Bacteriol., 189:7945-7947



Antibiotics and desinfectant resistances :



Minimal Inhibition Concentration increased from 10 to 1000x in a Biofilm when compared to the planctonic state.

Hypothesis :

- Reduced accessibility (Matrix acts like a barrier)
- Metabolic heterogeneity
- Phenotypes specific to Biofilms may appear (persister cells)
- Stress resistance genes activated



Where are Biofilms ?

BIOFILMS grow nearly everywhere!

All they need is :

- Micro-organisms
- Surface / interface
- Nutrients
- Water



How can we decontaminate a surface with Biofilm?





Clean or desinfect?





- Removes stains
- Detergent (for ex. soap) or enzymes
- Decrease the number of bacteria and thus decrease the risk of infection.

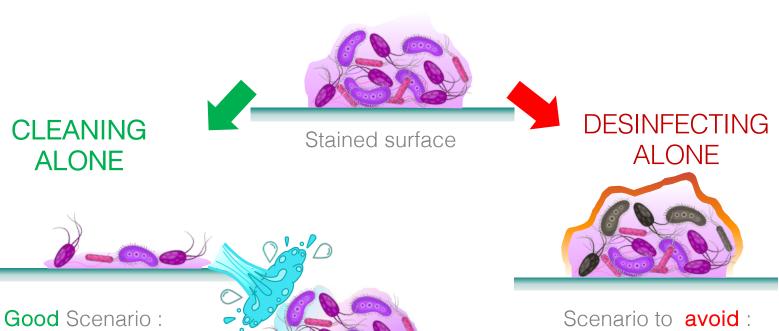


- Kills accessible bacteria
- Desinfectant (for ex. alcohol or acid)
- Often has an dehydrating action and thus fix stains

What's their efficiency on a Biofilm?



Cleaning alone is more efficient than desinfecting alone!



Scenario to **avoid** : Many bacteria remain alive under a fixed layer.

The majority of bacteria are removed

Cleaning alone is more efficient than desinfecting alone!



CLEANING ALONE

<u>OneLife's Enzymatic solution :</u> is efficiently disolving several layers of stains in a few minutes

Preferably use a disinfectant after.



STF load check indicator (Browne)

DESINFECTING ALONE

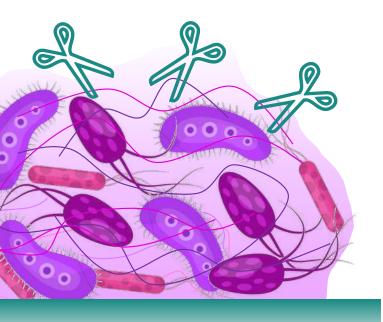
Peracetic acid + H_2O_2 :

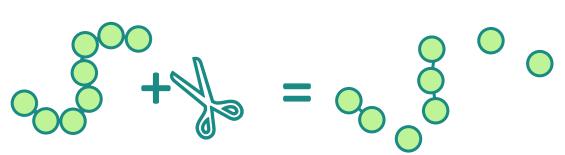
desinfectant that kills bacteria on the surface but has little efficiency on removing the stain... and even fix the stain!

Avoid desinfection alone!



How to clean a Biofilm ?





Disolve matrix ... with enzymes !

- Proteins

=> Proteases

- Lipids

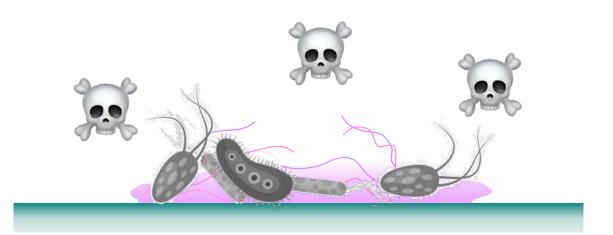
- => Lipase
- extracellular DNA => Dnase
- Exo-polysaccharids => Saccharidase
 - Cellulase, Amylase... Etc...
 - And then rinse!



The Best decontamination :

Clean then **Desinfect**

6





Profil

Enzyms

- Non-living
- Naturally present in the body and environnement

Avantages

- 100% biodegradable
- Natural activity

Qualities

- Cut up to 3 millions
 molecules per second
- Dissolve organic stains and biofilms
- Team work with other cleaning products

OneLife's technology



helps Medical & Health care institutions to :

Detect Biofilm (in endoscopes, on surgical instruments, ...etc)
 Dissolve Biofilms to optimize medical devices's decontaminations
 Prevent formation of new Biofilm



Detect : Visualise Biofilm using DETECT®2



See the invisible proteins and biofilms by staining them in blue!

- Quick and efficient : 5 min
- 360° quality control
- In situ method
- Visible from 10 μ g/cm2





Detect : Flexible inspection camera for medical devices with lumens



Dissolve : Corrective cleaning with enziQure



- For heavily soiled medical devices : such as: endoscopes and surgical instruments.
- Multi-enzymatic formula : 7 enzymes
- Patented enzymatic complex that dissolves biofilm matrix of multiple pathogens.
- Manual or ultrasound cleaning.
- pH Neutral
- Excellent materials compatibility
- Registered Medical Device Class I
- Biodegradability \geq 97 % (OCDE 302B).



Prevent : EnziMed prevent Max



Exclusive Applications

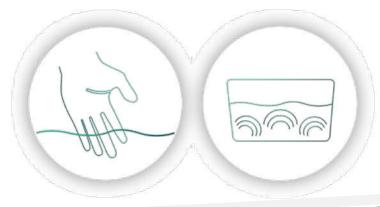
• 1L, 5L or 10L



Endoscopes & surgical instruments



Manual or ultrasound cleaning



Prevent : EnziMed prevent Max



- Targets the e-DNA (extracellular DNA)
- Multi-enzymatic formula : 7 enzymes
- Optimal deep cleaning of Medical Devices such as: endoscopes and surgical instruments.
- Patented enzymatic complex that dissolves biofilm matrix of multiple pathogens.
- pH Neutral
- Outstanding materials compatibility
- Registered Medical Device Class I
- Biodegradability \geq 99 % (OCDE 302B).





Study: Evaluation of biofilm removal



*Iglesias, Y. D. (2019) Antimicrobial Agents and Chemotherapy, 63(7)

Method used

The model used to assess biofilm removal was described by Iglesias* and al. (2019) and adapted to include other bacterial strains encountered in healthcare environments

Strains were grown for 24h or 48h :

- Staphylococcus aureus (Gram-Positive)
- Pseudomonas aeruginosa (Gram-Negative) and
- Escherichia coli (Gram-Negative)

at 37°C in 96-well microplates

Six cleaning solutions, one of which including DNase, were then applied to selected wells

Method: from biofilm incubation to cleaning









Crystal violet staining was finally applied to measure biomass reduction. Inside the same plate, all enzymatic solutions were tested six times and each plate was tested four times.

The results obtained were processed graphically. The colour's intensity is directly proportional to the quantity of residual biofilm.



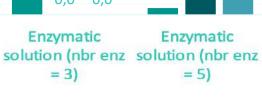
Results

■ S.aureus, ■ P.aeruginosa and ■ E.coli biofilms reduction 70,0 65,5 61,2 60.0 50,0 **Biofilm reduction %** 43.2 40,0 29,3 29,9 30,0 P.aeruginosa 20,0 S.aureus 16,7 11,6 E.coli 9,8 8,7 10,0 5,5 1,2 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,0 0,0 Alkaline

enziMed Prevent enziMed Prevent MAX



Alkaline Enzyr enzymatic solution solution (nrb = 1 enzyme = 1)



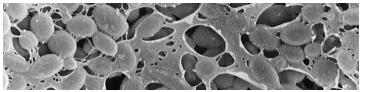
Enzymatic Non-enzymatic solution with chemical solution quaternary ammonium (nbr enz = 3)





Conclusion :

Biofilms are :



- On every humid surface / interface
- Complex, heterogenous, infinitely variable
- Often the cause of serious or fatal illnesses

- Very efficient to disolve Biofilm's matrix
- Natural, biodegradable, safe
- Your allies in surface decontamination

Do you have questions?

Please join us on our booth...



TRINITY MEDICAL

PATIENT CENTRIC TECHNOLOGIES



DETECT & DISSOLVE BIOFILM MATRIX







DETECT®2

Detects proteins and biofilms on instruments

- Quick and efficient (5 min)
- 360° quality control
- In situ method
- No false negatifs
- Visible from 10 µg/cm2





1250

DETECT[®]2



10' 50' 250' Decontemination protocol to be reviewed

Indication of protein concentration (ag/or/)

0.



Examples of results with DETECT®2













DETECT®2





Detect 2

Quality control of the surgical instrument cleaning process.

360°





EN

- Quality control tool of the surgical instruments' cleaning process.
- · Patented and validated technology to detect biofilm and organic soil on instruments.
- Quick result, visible directly (only 5 minutes) on the instrument surface.

.

Exclusive Applications

Product Characteristics

Dental & surgical instruments

- Quick and simple to use: 5 minutes for a basket of instruments (DIN 1/1).
- Evaluates the cleaning quality of instruments washer-disinfectors (WD) or ultrasound.
- High sensitivity (from 10µg/cm² protein . . A single reagent for detecting residual proteins (dye process).
- Compatible with stainless steel, PP. PTFE, POM, aluminium, titanium.
- Registered Medical Device Class L
- Biodegradability ≥ 90 % (OCDE 302B)
- Very low toxicity (reposts available on
- demand)
- Odorless

Enzymes for patients' safety



Chemical Properties

Instructions for use

Appearance: dark blue liquid Density: 1.013 +/- 0.01 pH: 2,25 +/- 0,25

Citric acid: 1-5% COLORANT/Dye: 0,1-1% Alcohol free * Does not contain carcinogens, mutagens, reproductive toxicants or endocrine disruptors

Delivery Units

Main Components*

The color is natural and may vary from one batch to another with no impact on the performance of the product.

Precautions

- Wear gloves and a protective clothing against the dye. Refer to the safety data sheet before use.
- Protect the working area.
- Store in the original container, closed, between +4°C and +25°C
- End of life: 36 months after production



Ref OL22215 6 x 950ml 2 x 51 KIT

Natural Eco-friendly onelife-bf.com



enziDent

High level enzymatic detergent with biofillm treatment for dental instruments



Ref OL20807 2 x 1L

Ref OL20808 6x 1L



Ref OL20806 1 x 5L

Ref OL20809 2x 5L





enziDent[®]

One Life

High-level enzymatic detergent with biofilm treatment for dental instruments





- · Patented multi-enzymatic compound
- · Enzymes dissolve organic soil and biofilm matrix
- Prepares instruments for efficient sterilization

Exclusive Applications

Product Characteristics

Dental rotary & non rotary instruments, Implant healing caps



Neutral, multi-enzymatic compound
 Restores instruments to their original

- condition
 Independent tests (available on request) prove superior efficacy on incrusted soil and biofilm matrix
- Full materials compatibility
- Concentrated for economical use
- High enzymatic activity and stability
 Registered Medical Device Class I
 - Biodegradability ≥ 95 % (OCDE 302B)

Instructions for use

Use enziDent* each time instruments are cleaned for complete removal of soil and to prevent build-up of incrusted soil and biofilm matrix.

- A. Standard dosage : 1%^{*}. Increase dosage to 2%^{*} in case of hard water or heavy soiling or temperature of water < 30°C</p>
- B. Contact time : 15 minutes

EN

C. Ideal temperature : 40 to 45°C (minimum 30°C and maximum 55°C) *See dilution table below

Bath volume (I)	0,5	1	2	3	4	5	10	15
enziDent [®] 1%-2% (in ml)	5 - 10	10 - 20	20 - 40	30 - 60	40 - 80	50 - 100	100 - 200	150 - 300

Waste treatment methods: Remove to an authorized waste treatment plant. Sevage disposal recommendations: May be discharged to wastewater treatment installation. Product/Packaging disposal recommendations: Dispose of contents/container to special waste collection point. When totally empty, containers are recyclable.

Chemical Properties

 Appearance :
 Yellow liquid

 pH unverdünnt: :
 8.5 +/-0.5

 pH verdünnt 1%:
 7.8 +/-0.5

 Density:
 1053+/-0.01

The color is natural and may vary from one

batch to another with no impact on the performance of the product

 Instruments should be rinsed prior to immersion in enziDent® only if product used for initial soak has an extreme pH (>10) or contains oxidizing agents (peracetic acid, hydrogen peroxide or chlorine-based products) or phenols.

 Respect recommended temperatures for optimal performance; efficacy is not guaranteed > 55°C.

Rinse abundantly with water before disinfection and/or sterilization.

Enzymatic activity is maintained for 8h following dilution.
Renew baths frequently according to soil levels.

Precautions

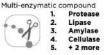
- Wear gloves. In case of prolonged use, gloves covering forearms are recommended. Refer to full safety data sheet before use.
- Store in the original container, closed, between +4°C and +25°C. For optimal performance respect the « Best Before » date on the label.
- End of life: 36 months after production

(ontakt

Main components*

- 1 5% non-ionic surfactants; 1 - 5% anionic surfactants;
- <1% sequestrants; <5% corrosion inhibitors;

 Does not contain carcinogens, mutagens, reproductive toxicants or endocrine disruptors.



Delivery Units



Ref OL20806 5L Ref OL20809 2 x 5L





enziDent Flow

High level detergent with biofilm treatment for the cleaning and maintenance of dental unit, waterlines and suction systems.



Ref OL20907 2 x 1L

Ref OL20908 6x 1L



Ref OL20909 2 x 5L





enziDent® FLOW

High-level detergent with biofilm treatment for the cleaning and maintenance of dental unit, waterlines and suction systems.





- Patented enzymatic compound
- · Breaks down organic soil and biofilm matrix for a more effective decontamination
- Enables in-depth cleaning of waterlines and suction systems. preventing plugs formation on filters
- Fresh menthol fragrance

Exclusive Applications

Product Characteristics

- Neutral multi-enzymatic compound Independent tests (available on request) prove superior efficacy on incrusted soil and biofilm matrix. Full materials compatibility
 - Highly concentrated for economical use High enzymatic activity and stability
 - Biodegradability a 95 % (OCDE 302B)
 - waterlines and suction system
 - Respect recommended temperature quaranteed ≥ 55°C.
 - before disinfection.
 - following dilution. For automatic systems, respect the
 - instructions for use of the manufacturer

Instructions for use - 1x/day (evening)

Use enziDent® Flow each time dental waterlines and suction systems must be cleaned, for complete removal of soil and to prevent the build up of microbial biofilms.

PREPARE: Dilute 25ml in 2.5L of warm water (Ideal temperature: 40 à 45°C - min, 30°C & max, 55°C) CLEAN:

Pour approx. 0.5 to 1L of detergent solution into the sink

Immerse the suction hoses in the bath to drain completely* install the hoses back to its system 3

WASH/RINSE:

EN

Contact time for routine use: 10 minutes minimum (ideally overnight)

Following morning, drain approx. IL of water and rinse the sink DISINFIECT: 1x/Week at least - Disinfect with a disinfectant of your choice** and follow the instructions for use. Clean the installation with water

* Do not immerse fully -2 to create a mixture of ad/detargent solution. Compatible with Drotol cap and other subtering systems. * Comprise with standards, EN 19727 EN 19534, EN 19348, EN 19449, EN 1959, EN 1952, EN 19583.

Waste treatment methods: Remove to an authorized waste treatment plant. Sewage disposal recommendations: May be discharged to wastewater treatment installation. Product/Packaging disposal recommendations: Dispose of contents/container to special waste collection point. When totally empty, containers are recyclable

Chemical Properties

Appearance : Transparent, green Density: 1158 +/- 0.010 pH undiluted product: 85+A 05 pH diluted at 1% : 80+/-05

Wear gloves. In case of prolonged use, gloves

Store in the original container, closed, between

covering forearms are recommended. Refer to full

+4°C and +25°C. For optimal performance respect

- Main components
 - 1 5% non-ionic surfactants. <1% anionic surfactants:
 - <1% sequestrants:
 - <1% perfume.
- Patented multi-enzymatic compound

Down not contain cardinogens, mutagens, reproductive toxicants or endoorine dampton Patented multi-enzymatic compound



Delivery Units



Ref OL20907 2 x 1L Ref-OL20909.2 x 5L Ref OL20907A 2 x 1L Ref OL20909A 2 x 5L Ref OI 20908 6 x 1L Ref OL20908A 6 x 1L



Enzymes for patients' safety

Dental units & suction systems



Registered Medical Device Class I

- Suitable for the cleaning of dental units: Refer to full instructions for Use and Safety Data Sheet before using
- for optimal performance: efficacy is not
- Rinse abundantly with water after use and
- Enzymatic activity is maintained for 8h

Precautions

safety data sheet before use.

the + Best Before + date on the label.

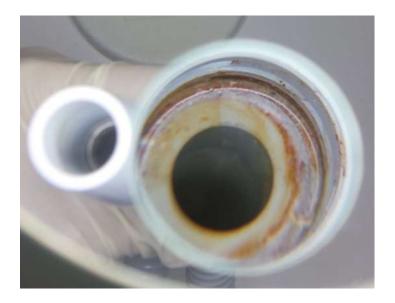
End of life: 36 months after production



Dental Unit Waterlines

Studies described infection or colonisation in susceptible hosts with Legionella spp., Pseudomonas spp. and environmental mycobacteria isolated from dental unit waterlines. *Pankhurst & Coulter 2007 J. Dent. 35(9):712-20.*







enziMed[®] Pre-cleaner

Prevents drying of bio-burden.

Enzymes break down organic matter.

Pre-cleans without intervention; let the enzymes do the work!



 Ref OL20304
 Ref OL203

 6 x 750 ml
 6 x 750 ml

 [EN, NL, FR, DE]
 [EN, PT, ES,



enziMed[®] Pre-Cleaner

Multi-enzymatic foam spray for surgical instruments and rigid or flexible endoscopes.

Proven Efficiency





EN

- Prevents drying of bio-burden.
- Enzymes break down organic matter.
- Pre-cleans without intervention; let the enzymes do the work!

Exclusive Applications

Endoscopes & surgical instruments

Product Characteristics

- Neutral, multi-enzymatic compound.
 - Prepares instruments for decontamination. without need for brushing. Directional foam spray covers instrument
 - surface; high efficiency, reduced waste. No spiils during transport.
 - 1 Full material compatibility.
 - . Ready to use

141

- . Odorless 10
 - High enzymatic activity and stability.
- Registered Medical Device Class I
- . Biodegradability ≥ 95 % (OCDE 302B)

Appearance: pale yellow liquid Density: 1,005 +/- 0,05 Kg/I 8 +/- 0.5

Chemical Properties

Instructions for use

2. Contact time: 15 minutes

Use enziMed® Pre-Cleaner each time instruments need to be pre-cleaned.

Regional legislation (waste): Disposal must be done according to official regulations. Product/Packaging disposal recommendations : Do not empty into drains, dispose of this

1. Use for manual pre-cleaning of surgical instruments and scopes

3. Rinse in clear water before passing through automated washer-disinfector in order to avoid foam forming. 4. Duration of activity: up to 72 hours in closed containers

material and its container at special waste collection point.

The color is natural and may vary from one batch to another with no impact on the performance of the product.

Precautions

pH:

- Use of gloves is recommended. .
- Store in the original container, closed, . between +4°C and +25°C.
- End of life: 24 months after production

Main Components*

Non-ionic surface agents: <5% Phosphonates: <5% Enzymes: <5%

* Does not contain carcinogens, mutagens, reproductive toxicants or endocrine disruptors.



Delivery Units

Ref OL20304 6 x 750 ml [EN, NL, FR, DE] Ref OL20304A 6 x 750 ml

Enzymes for patients' safety



onelife-bf.com



Babyscope 2.0

- Ergonomic: New compact & easy to use design (enhanced light, vision, & magnification)
- Modular: Interchangeable flexible inspection scope attachments available for small diameters devices such as: pediatric bronchoscopes & cystoscopes (diameters 1.06mm & 1.9mm).
- Smart: Offers the option to document & share what they see, enabling immediate corrective action.
- Cost efficient: Helps keeping repair cost & infection risks down



Endoscopes & instruments with lumens. Among them: Pediatric bronchoscopes & cystoscopes





Bayscope : Operating procedure

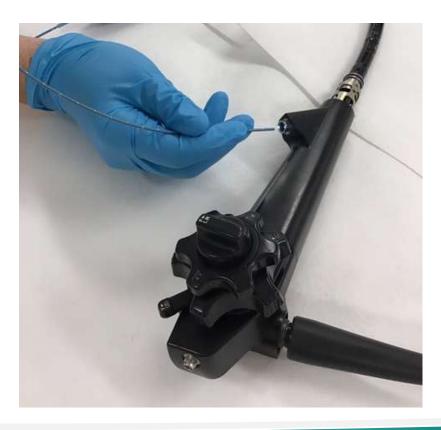
- Standard operating procedure for installation and use of the Babyscope's 2.0 Software
- <u>https://www.hmark.com/wp-content/uploads/2022/09/FIS-</u> 007U_Installation.mp4

 https://www.hmark.com/product-so3ware/ Choose version FIS005 (if purchased prior to 5/5/2020)

Babyscope's use : Inspect endoscope chanels & lumens



Prevent costly repairs







Babyscope's manipulation :



Insert in cavity





Insert in distal end

Handle with caution :

- No pushing if the passage is blocked
- Clean and disinfect according to instructions



Insert behind the elevator riser



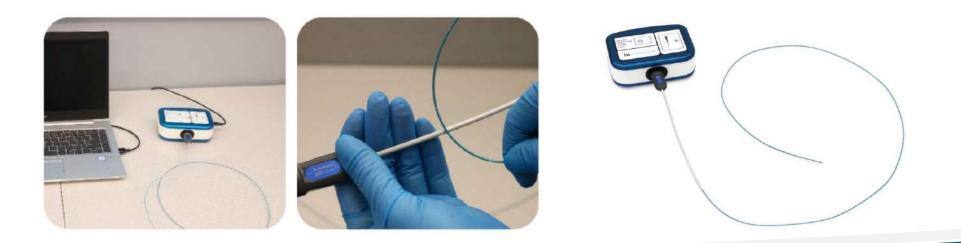
Insert through biopsy or aspiration port



Babyscope 2.0

Inspection camera for medical devices with lumen and hollow instruments

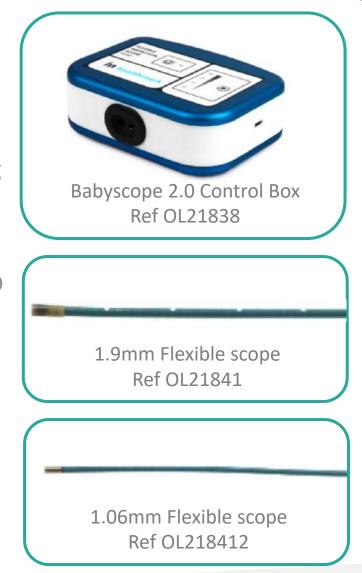
- ✓ New compact design easy to use
- ✓ Flexible, interchangeable inspection scopes of 1,06 mm and 1,9 mm
- $\checkmark~$ Photo or video recordings on PC
- ✓ Helps to reduce repair costs and infection risk





Babyscope 2.0

- Flexible scope 1,9 mm or 1,06mm
- Length 110 cm
- Box with camera processor and LED lighting
- Resolution :
 - CT-102 1.06 mm: 40.000 pixels (of 200 x 200 pixels)
 - CT-101 1.90 mm: 160.000 pixels (or 400 x 400 pixels)
- 120° vision
- Light control knob
- Accessories :
 - Connecting cable USB-C
 - USB key (software and instructions for use)





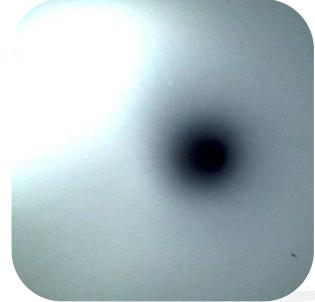
 Seeing inner mechanism and springs is normal







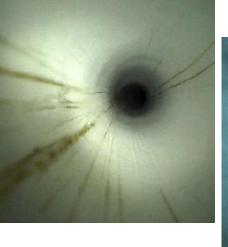




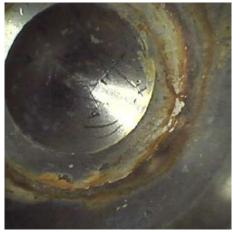


Learn to identify examples of deviations









Can you name these 4 problems?

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