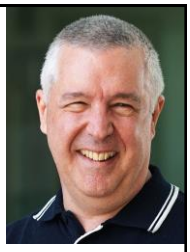
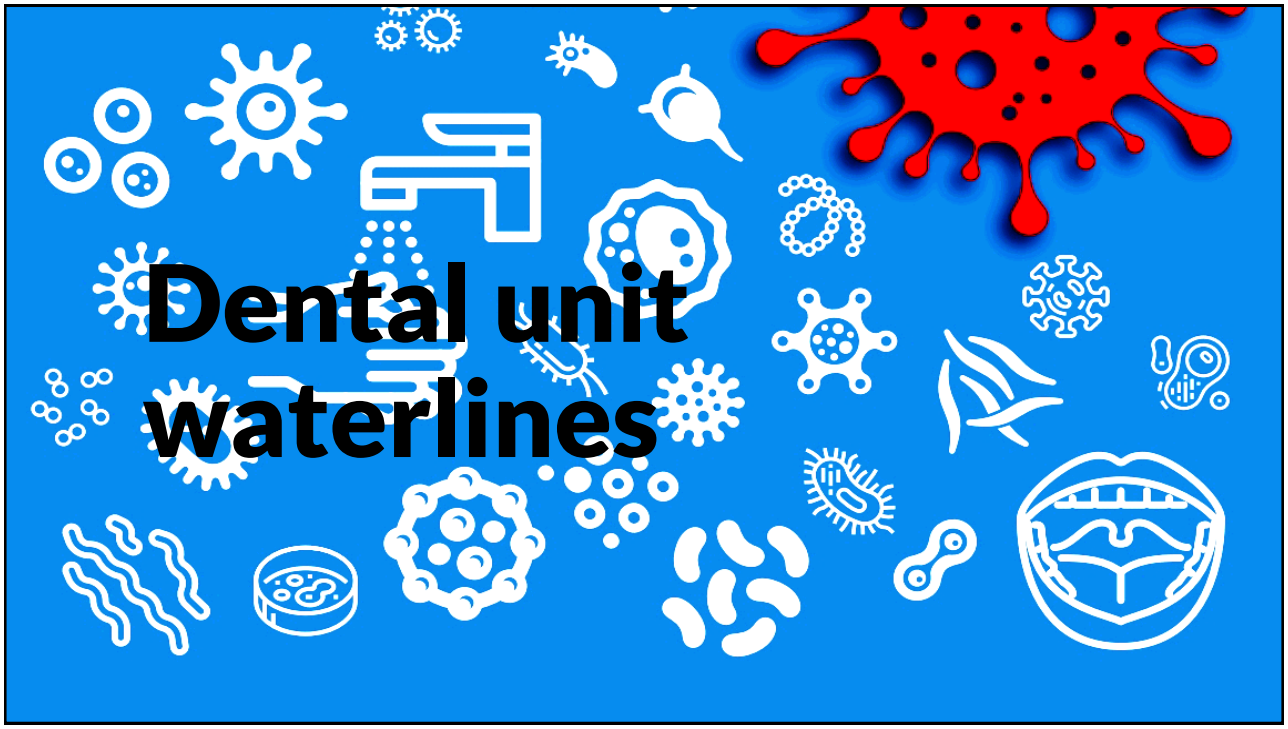
Contemporary Infection Control

2022



Emeritus Professor Laurence J. Walsh AO

BDSc(Hons), PhD, DDSc, GCEd, FRACDS, FFOP(RCPA), FFDT RCS Edin



Managing dental unit waterlines

Focus on dental unit waterlines

- Australia – August 2021 ADA ICG 4th edition
- Mandatory UK HTM 01-05 / EU water requirements
- USA: CDC 2003, AmDA 2012, and OSAP

- Growing data on NTMs
- Field study data prior to the pandemic
- Limitations of existing protocols

OSAP

Dental unit waterlines have many factors that make them more susceptible to biofilm. They are reviewed in the chart below.

Dental unit	Tap
Small diameter (1/16 in. to 1/8 in.)	Larger diameter (½ in.)
Very slow rate (drips/ second)	5L/min. flow rate (>1000x dental lines)
Plastic tubing makes biofilm attachment easy	Copper, as a metal and as a dissociated ion, is antimicrobial/bacte-riostatic
Large surface area to volume ratio	Small surface area to water volume ration
Biofilms form in small bore tubing of dental units	Smooth interior
Left stagnant for long periods of time	Fresh every time turned on
Chlorine rapidly dissipates over 24 hours and can even be absorbed by tubing	Chlorinated and replen-ished with every use

Water requirements in dental practice

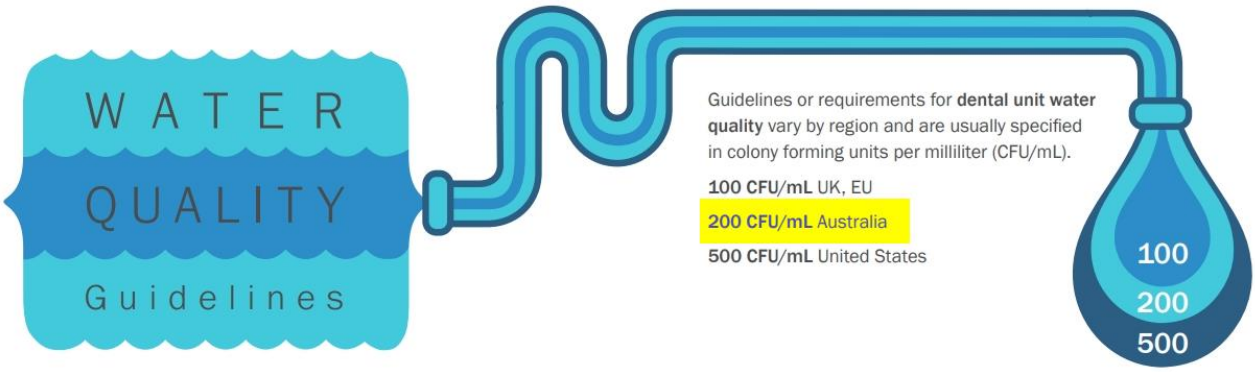
- Drinking water for staff
- Washer disinfector, instrument rinsing: softened (if local supply water is hard)
- DUWL supply: zero or low microbial count; chemical additives
- DUWL exit water including cup fill: potable, < 200 CFU/mL for immune compromised patients
- Oral surgery: sterile irrigant (saline rather than water)
- Steam sterilizer: ion free (distilled, deionized, reverse osmosis treated)

Dental unit waterlines

- *An independent (**bottled**) water supply can help to reduce the accumulation of biofilm*, **as it makes adding chemical agents to the water easy to undertake**. *The manufacturer's directions should be followed for appropriate methods to maintain the recommended quality of dental water and for monitoring water quality*. **This may include purging water from the lines or using sanitizing solutions when the chair is not being used for extended periods**.
- *Biofilm levels in dental equipment can be minimised by using a range of measures, including ozonation, electrochemical activation, and chemical dosing of water (e.g. with hydrogen peroxide, peroxygen compounds, hypochlorites, chloramines, iodine, silver ions, or nanoparticle silver).*

DUWL testing 1

- Same CFU targets as before (200 CFU/mL for medically compromised; otherwise potability)
- **Can use dipslide or dipstick tests.**
- Included reference to Australian Drinking Water Guidelines
  - Last updated in August 2018).
  - https://nhmrc.gov.au/about-us/publications/australian-drinking-water-guidelines



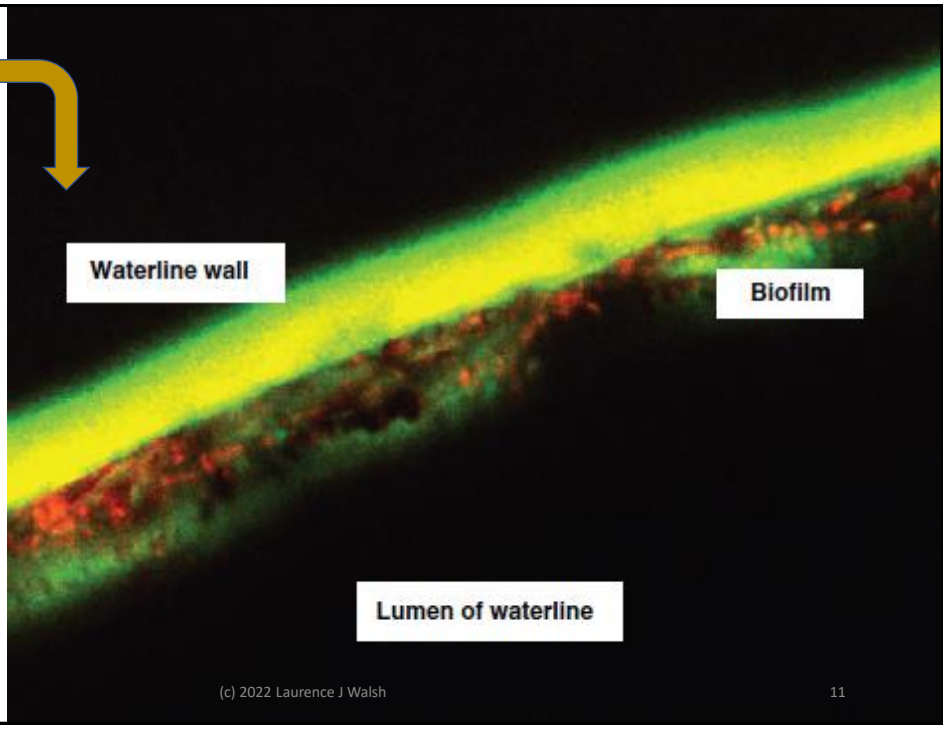
DUWL testing 2 (*taken from ADA NB article*)

- It is **good practice** to test microbial levels in water from dental unit waterlines on a regular basis, **for example**, six monthly or annually when tested levels are found to be **below** the target level of 200 CFU/mL.
- When **high counts** are found, the waterlines will need to undergo additional sanitising treatments to remove biofilm, and bring the bacterial levels back to within an **acceptable** range.
- Follow the instructions from the supplier of the dental chair. Ensure that any sanitising agent is flushed completely from the waterlines, before using the chair. **After** sanitizing, test water levels more frequently to ensure that the biofilm control measures being used are adequate.

Key compliance points for dental unit waterlines

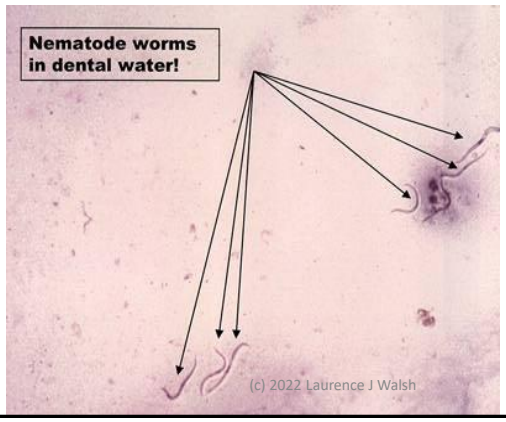
- Each practice has developed a protocol for testing water lines and water quality.
- Follow the dental chair manufacturer's instructions for appropriate chemical treatments for waterlines, as these vary between brands.
- If water samples reveal high loads of bacteria, undertake a sanitizing treatment, then re-test.

Cross-section



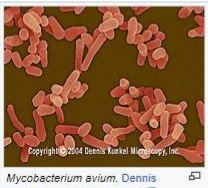
Bacteria, fungi, biofilm, slime worms, nematodes, amoebae, etc….

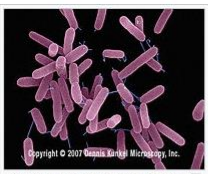
Legionella species



BACTERIA	FUNGI	PROTOZOA
· *Acinetobacter*	· *Penicillium*	· *Acanthamoeba*
· *Mycobacterium avium*	-*Cladosporium*	· *Cryptosporidium*
· *Actinomyces*	· *Alternaria*	· *Microsporidium*
· *Nocardia spp.*	· *Scopulariopsis*	· *Giardia*
· *Flavobacterium*		
· *Alcaligenes*		
· *Pasteurella spp.*		
· *Bacteroides*		
· *Peptostreptococcus*		
· *Caulobacter*		
· *Pseudomonas aeruginosa*		
· *Moraxella spp.*		
· *Klebsiella pneumoniae*		
· *Pseudomonas cepacia*		
· *Proteus vulgaris*		
· *Lactobacillus*		
· *Salmonella typhimurium*		
· *Legionella*		
· *Streptococcus spp.*		

Williams et al. 1996

Legionella pneumophila	 *Legionella pneumophila*. Dennis Kunkel Microscopy, Inc.<4>	L. pneumophila is a gram-negative bacterium which spreads via the air-conditioning system. They are called chemo-organotrophs because they are capable of using certain amino acids as primary carbon and energy sources. Their cell walls are unique in a way that they contain a huge amount of branched fatty acids. Once they enter the cells, they reside and multiply in numbers within a membrane-bound compartment. A respiratory trace and the lungs are the main sites of infections. Legionnaire's disease is an atypical lung infection caused by Legionella pneumophila [19].
Mycobacterium spp.	 *Mycobacterium avium*. Dennis Kunkel Microscopy, Inc.<5>	It is a bacterial genus which contains a vast number of species. The best known species are M. leprae and M. Tuberculosis which cause leprosy and tuberculosis respectively. In their cell wall, it consists of special fatty molecules called mycolic acids, and these complexes make the cell walls less permeable. They are aerobic, non-motile rods [20].

Pseudomonas aeruginosa	 *Pseudomonas aeruginosa*. Dennis Kunkel Microscopy, Inc.<6>	It is a heterogeneous genus comprising gram-negative, aerobic, rod-shaped bacteria. They exhibit motility since they have one or more polar flagella. They also contain fimbriae as an attachment to surfaces of other organisms. They grow fastest at the room temperature [21]. Pseudomonas aeruginosa is the best known and the most virulent pathogenic pseudomonad. It is known as one of the most abundant life forms on the planet. Despite their widespread distribution, P. Aeruginosa infections are relatively rare [22].
Staphylococcus spp.	 *Staphylococcus aureus*. Dennis Kunkel Microscopy, Inc.<7>	They are small cocci that exist in irregular clumps or grape-like clusters. There are three main species of Staphylococcus that share morphological and biochemical features. The pathogenic organisms produce many extracellular products known to play as a factor that leaves effects on humans [23].

***"Pseudomonas aeruginosa* was transferred from the (dental) unit to the patient in all cases."**

Martin, M.V., The significance of the bacterial contamination of dental unit water *Systems Dental Journal*. pp: 152-153. September 1987

"*Pseudomonas aeruginosa* strains were found both in water from the dental equipment and in the CF (cystic fibrosis) patients' sputum."

Jensen, E.T., Giwercman, B., Ojeniyi, B., Bangsborgl, Hansen, A.§, Koch, C.§, Fiehn, N.-E. I, and Hoiby. Epidemiology of *pseudomonas aeruginosa* in cystic fibrosis and the possible role of contamination by dental equipment. *Journal of Hospital Infection*. 36. pp: 117-121. 1997.

International Journal of
Environmental Research and Public Health

MDPI

Article

Dental Unit Waterlines in Quito and Caracas Contaminated with Nontuberculous Mycobacteria: A Potential Health Risk in Dental Practice

Orlando J. Castellano Realpe [1,†], Johanna C. Gutiérrez [2,3,†], Deisy A. Sierra [2,3,†], Lourdes A. Pazmiño Martínez [1], Yrneh Y. Prado Palacios [3], Gustavo Echeverría [4] and Jacobus H. de Waard [3,5,*]

1 Facultad de Ciencias Químicas, Universidad Central del Ecuador, 170521 Quito, Ecuador
2 Facultad de Medicina. Escuela de Bioanálisis, Universidad Central de Venezuela, 1053 Caracas, Venezuela
3 Instituto de Biomedicina "Dr. Jacinto Convit", Universidad Central de Venezuela, 1010 Caracas, Venezuela
4 Instituto de Investigación en Salud Pública y Zoonosis CIZ, Universidad Central del Ecuador, 170521 Quito, Ecuador
5 One Health Research Group, Facultad de Ciencias de la Salud, Universidad De Las Américas, 170504 Quito, Ecuador
* Correspondence: jacobusdeward@gmail.com
† The three authors contributed equally to this work.

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Abstract: Three cases of severe odontogenic infections due to nontuberculous mycobacteria (NTM) in Venezuela that were directly associated with dental procedures and the finding of dental unit waterlines (DUWLs) in dental offices that were colonized with mycobacteria species was the reason for assessing the water quality of DUWLs in dental offices in two capital cities in South America, namely, Quito and Caracas. The main water supplies and the water from 143 DUWLs in both cities were sampled and especially checked for contamination with NTM. To measure the overall bacteriological quality of the water also the presence of heterotrophic bacteria, coliform bacteria, and *Pseudomonas* was determined. Results showed that respectively 3% and 56% of the DUWLs in Quito and Caracas yielded NTM species (up to 1000 colony-forming units (CFU)/mL). Furthermore, high and unacceptable total viable counts of heterotrophic bacteria and/or coliform bacteria and *Pseudomonas* were detected in 73% of the samples. We conclude that, in both cities, the water in the majority of DUWLs was contaminated with NTM and other potential pathogens, presenting a risk to human health. The detection of NTM in DUWL water with acceptable heterotrophic bacteria counts shows the need to include NTM in water quality testing. Mycobacteria are more resistant to disinfection procedures than other types of vegetative bacteria, and most testing protocols for DUWLs do not assess mycobacteria and thus do not guarantee risk-free water.

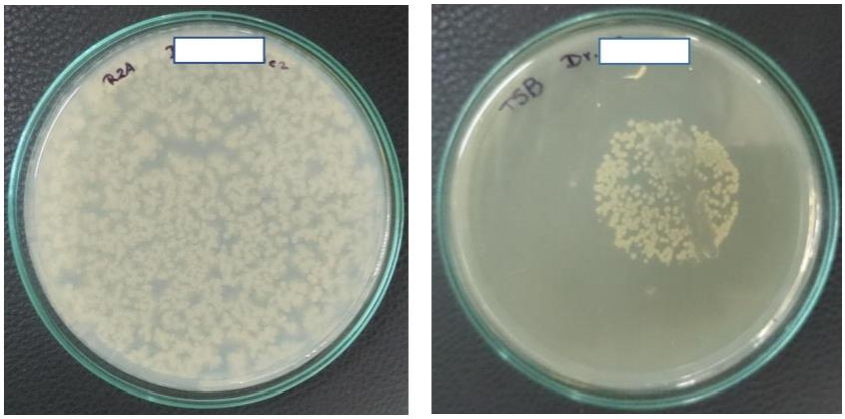


Figure 1. Examples of positive water cultures. On the left, 100 µL of water was plated on Reasoner's 2A (R2A) agar, and the Petri dish was incubated at 36–37 °C for 72 h. On the right is the result of a water sample that was positive for mycobacteria after 7 days of growth at 37 °C. An uncountable number of mycobacteria was isolated from 200 mL of DUWL water. The mycobacteria were isolated using the membrane filtration technique with a 0.45 µm nitrocellulose filter with a diameter of 47 mm, that was placed upside down on TSB agar with glycerol and an antibiotic mix, and again removed after two days of preincubation (see Materials and Methods). The visible streak through the colonies on this plate is where a loop full of bacteria had been taken for confirmation of the presence of acid-fast bacilli using the Ziehl–Neelsen staining technique. The mycobacteria on this plate were identified with the PCR-restriction enzyme analysis (PRA) technique as *M. abscessus*. For the purpose of anonymity, the name of the dentist on the Petri dish has been hidden.

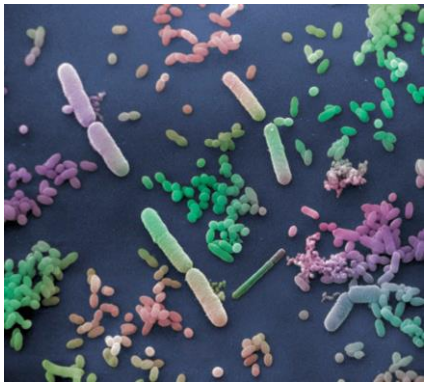
Organism	Associated with	Reference
Pseudomonas and *Proteus* spp.	Rhinitis	Clark, 1974
P. aeruginosa	Oral abscesses	Martin, 1987
L. pneumophila	Humoral responses initiated	Reinthaler *et al.*, 1988
Legionella dumoffi	Legionnaires' disease	Atlas *et al.*, 1995
Mycobacterium gordonae	Endocarditis	Pankhurst *et al.*, 1998
Non tuberculosis *Mycobacterium*	Cervical lymphadenitis	Lohr *et al.*, 1978; Wallace *et al.*, 1983
Bacterial endotoxins	Asthma, inflammation due to acute phase cytokine release, hypersensitivity pneumonitis	Putnins *et al.*, 2001; Pankhurst *et al.*, 2005; Pankhurst and Coulter, 2007
Acanthamoeba	Ocular keratitis	Barbeau, 2007
Aspergillus spp.	Oral aspergillosis	Iatta *et al.*, 2009
P. aeruginosa	Acute purulent maxillary sinusitis	D'Ovidio *et al.*, 2011
L. pneumophila	Legionnaires' disease	Ricci *et al.*, 2012

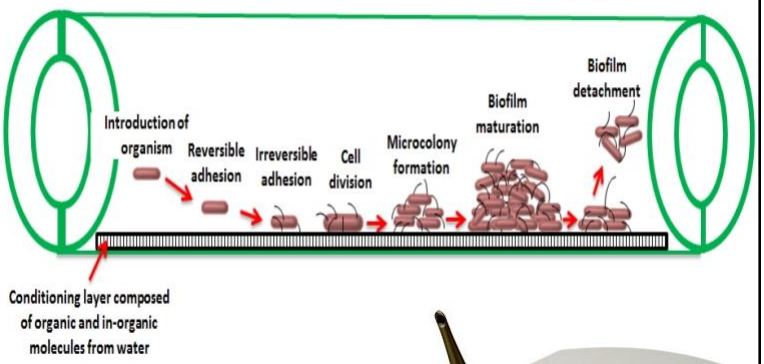
Disease manifestations linked to contaminated DUWL water

Legionnaires' disease	Meningitis	Diarrhea	Gastrointestinal infection
Caries progression	Chronic lung disease	Endocarditis	Septicemia
Bronchitis	Severe dehydration	Urinary tract infections	Respiratory infections
Chronic pneumonia	Acute pneumonia	Wound infections	Bacteremia
Abscesses	Conjunctivitis	Allergic respiratory reactions	

Williams et al. 1996

DUWL stagnation during pandemic shut-downs





Bite magazine, April 2021

02/09/2021 Managing dental unit waterlines - Bite Magazine

Managing dental unit waterlines

By **Editor** - April 12, 2021

Estimated reading time: 5 minutes

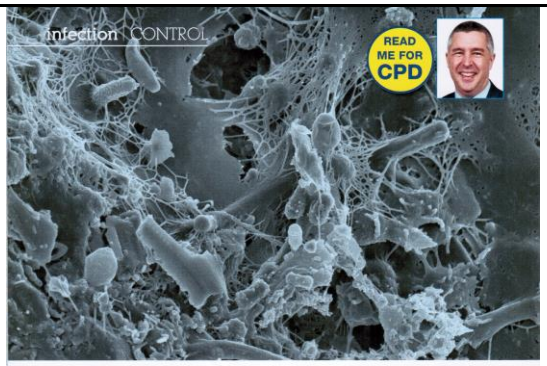
Photo: Olga David Tadevosian 123RF

Smarter management of dental unit waterlines is essential for the safety of dental patients and staff—and it could just save your practice.

To say that clean dental unit waterlines are crucial to the health of a practice—for patients, staff and the bottom line—is an understatement.

As dentists operate in an environment of heightened infection-control risks because of COVID-19, the spotlight is on the dangers of waterline contamination from biofilm—a layer of microorganisms such as bacteria and fungi that forms in wet places. The upshot of poor processes can be the transmission of diseases such as legionella, Pontiac fever and the coronavirus.

Emeritus Professor Laurence Walsh, of the UQ Oral Health Centre at The University of Queensland School of Dentistry, is a renowned researcher on waterlines.



Waterline management: The problem of stasis

By Professor Laurence J. Walsh AO

Maintaining a low level of biofilm in dental unit waterlines remains an important consideration in everyday infection control. The issue has come to prominence with dental clinics closing down and then reopening because of various restrictions to practice. Stagnation of water inside the tubing of a dental chair which has been shut down is a major problem because waterborne organisms such as Legionella overgrow readily whenever water sources become stagnant. In addition to Legionella, many other bacteria and protozoa that may be found in reticulated water will also overgrow, forming a thick biofilm which can completely occlude the lumen of the tubing and prevent the subsequent flow of water.

Closing down a dental chair

This problem of stagnation is the reason why when closing down the dental chair, it is critical to remove water from the waterlines, for example, by displacing it using compressed air, or to render the water that remains incapable of supporting the growth of microorganisms. This can be done using a hibernation solution which contains a long acting antimicrobial agent, designed to prevent the overgrowth of microorganisms for several months. Such a solution needs to be flushed thoroughly from the waterlines when the chair is brought back into its normal operation. An example of a hibernation solution which is used extensively around the world is Bilpron. This is a mixture of propylene glycol, parabens, biguanides and complexing agents in aqueous solution.

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A key consideration for any hibernation solution is that it must not cause chemical problems (such as corrosion) by reacting with the components of control blocks, nor must it cause degradation of the waterline tubing.

As an example, leaving sodium hypochlorite solutions in the waterlines for an extended period is likely to cause significant corrosion in the control blocks of many types of dental chair that are manufactured in Europe.

> 'The first consideration when restarting a dental chair will be what has grown in the waterlines during the period of inactivity. Prolonged flushing of the waterlines will often reveal surprisingly large deposits of biofilm, held together with a dense matrix of extracellular polymeric substances (EPS), with the appearance of a 'slime worm'...'

Restarting a dental chair

The first consideration when restarting a dental chair will be what has grown in the waterlines during the period of inactivity. Prolonged flushing of the waterlines will often reveal surprisingly large deposits of biofilm, held together with a dense matrix of extracellular polymeric substances (EPS), with the appearance of a "slime worm" (Figure 1).

Dislodged portions of biofilm that have accumulated during the period of inactivity can block the fine tubing inside dental handpieces and triplex syringes and can also occlude the irrigated spray nozzles of dental handpieces.

When bringing a dental chair back into service, it is important to consider the nature of the water which is now being fed into that dental chair. Ideally, this should have as low a level of microorganisms as possible - ideally, the water should be sterile. If this is not possible and tap water is being used as the source of water, then some chemical treatment of that water will be required to reduce the levels of normal waterborne bacteria and to prevent the growth within the waterlines. Common chemical treatments for tapwater include the addition of an oxidant (such as hydrogen peroxide), the inclusion of a long acting antimicrobial agent such as ionic silver or iodine, or various combinations of these measures.

Generating sterile water "on demand" is possible when the water has been appropriately treated, for example, using ultrafiltration or ozonation. Both approaches have different running costs. For ultrafiltration, the final filter (usually with pore sizes of 0.2 µm or less) requires periodic replacement and there are normally several (up to 4) pre-filters that also require replacement. If these filters are not replaced, blockage of the more expensive 0.2 µm filter will occur rapidly. For an ozonation system, the most common approach is to use a platinum electrode for electrolytic decomposition of water and such electrodes normally last for several years. Ozonation systems that use corona discharge require periodic replacement of the corona discharge chamber because of progressive damage from nitrogen compounds that are formed when normal air is broken down using high electrical voltages. Using pure oxygen as a source to supply a corona discharge chamber overcomes this issue and while this particular approach is used industrially for water treatment, it is uncommon in dental practice. It is much more common for clinics to use point-of-care water ozonation systems with platinum electrodes.

Figure 1. A typical example of a "slime worm" and several fragments of biofilm, that were produced from dental unit waterlines following a shock treatment in mid-2020. This particular slime worm emerged from a dental chair which had been shut down during the April-May 2020 period of dental practice restrictions because of the COVID-19 pandemic. The scale bar represents 10 mm.

Shock treatment

Electroconvulsive therapy is still used today in contemporary psychiatry for the management of patients with certain severe mental illnesses to effectively reboot neurotransmitters in the brain. The analogy to shock treatment for a dental chair is surprisingly strong, since a chemical shock treatment of the waterlines of the dental chair is designed to, ideally, reboot the situation back to when the dental chair was brand-new and the tubing was completely clear of any biofilms.

Agents used for shock treatment are designed to disrupt the EPS matrix of the biofilm, so that the biofilm fragments can be flushed from the waterline.

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This leaves the tubing clean. Chemically, they are somewhat different to agents used to reduce the tenancy of organisms to overgrow during the normal operation of the dental chair, since these may only impair or reduce microbial overgrowth, but may not necessarily damage the structure of the biofilm. In some cases, products that are designed for continuous dosing have special formulations which allow them to be used for shock treatment (e.g. they may be used at a much higher concentration and left in place for extended periods of time). Most commonly though, for a dental chair, a separate shock treatment agent will be used, which is compatible with the control blocks in the tubing of that dental chair. This is left in for a prolonged period of time, for example, overnight in the case of Bilpron, for dental chairs such as Belmont, Planmeca, Ritter or Sirona.

Only a small number of brands of dental chair on the market have control blocks that are compatible with sodium hypochlorite. The indiscriminate use of sodium hypochlorite can easily cause significant corrosion to occur, as this material is extremely reactive to most pure metals as well as to many metal alloys. The general principle is to only use sodium hypochlorite when the manufacturer has explicitly stated that this is their recommended protocol. In other cases, use a purpose-built shock treatment agent (such as Bilpron) that has broad compatibility with many brands of dental chairs.

Interpreting the results of microbial testing

While many dental practices will collect their water sample from the triplex syringe, of the available waterlines in the dental unit, this is the one which will have the most regular flow and thus is the least likely to show heavy accumulation of biofilm. It is best practice to sample a waterline which is not used heavily, since this will tend to have the most biofilm.

The Kona Ozone tap produces ozonated water that is perfect for use in dental unit water lines, pre-procedural mouth rinses and hand washing - www.konaozone.com.au.

> 'Generating sterile water on-demand is possible when the water has been appropriately treated, for example, using ultrafiltration or ozonation. For an ozonation system, the most common approach is to use a platinum electrode for electrolytic decomposition of water and such electrodes normally last for several years. Ozonation systems that use corona discharge require periodic replacement of the corona discharge chamber...'

The most commonly used method for assessing the microbial quality of water that is exiting from the tubing of dental chairs is to undertake microbial culture. Ideally, the method used should be one that allows for the recovery of slow-growing organisms, since the nutritional situation of a waterline environment is quite impoverished compared to that of a typical agar plate where numerous nutrients and growth factors may be present in the medium. Particular media have been developed for water testing, such as R2A, which match the nutritionally deprived environment of the waterline. Organisms are counted after a growth period at room temperature of 5 days.

The target level for microbial counts is a value which is below the threshold of 200 colony-forming units (CFU) per mL. This has been the recommended level in Australia and in North America for dental chairs for the past 20 years and it takes into account the fact that many dental patients may have some degree of frailty or medical compromise. The threshold level used in Europe is only 100 CFU/mL.

When a water sample is checked, a test result which yields a value above 200 CFU/mL should trigger some urgent action regarding the waterlines in that dental unit. There should be a shock treatment and once the treatment agent has been flushed out fully, a further sample should be taken from the same waterline. If this second sample yields a value which is still above the threshold, then the routine of shock treatment must be repeated, until the value falls below the threshold. Note that the process of shock treatment involves extensive flushing of all the waterlines of the dental chair for an extended period to remove the shock treatment agent.

Future perspectives

The COVID-19 pandemic has focused attention on the issue of what dental staff and dental patients inhale while in a dental clinical environment. Procedures that generate aerosol do so both from patient derived fluids, as well as from fluids from waterlines of the dental unit and the waterlines of any standalone benchtop equipment (such as a benchtop ultrasonic scaler unit). The occupational health and safety of staff who work in the dental clinical environment is an important responsibility for practice owners and this is why following the correct protocols for managing dental unit waterlines is important.

About the author

Professor Laurence J. Walsh is based at The University of Queensland School of Dentistry where he leads research in advanced technologies. He serves as the technology editor of Australasian Dental Practice magazine and is a longstanding member of the ADA Infection Control Committee. Prof. Walsh is a noted commentator on and user of new technologies.

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"Slime worm"

This particular slime worm emerged from a dental chair which had been shut down during the April-May 2020 period of dental practice restrictions because of the COVID-19 pandemic.

The scale bar represents 10 mm.

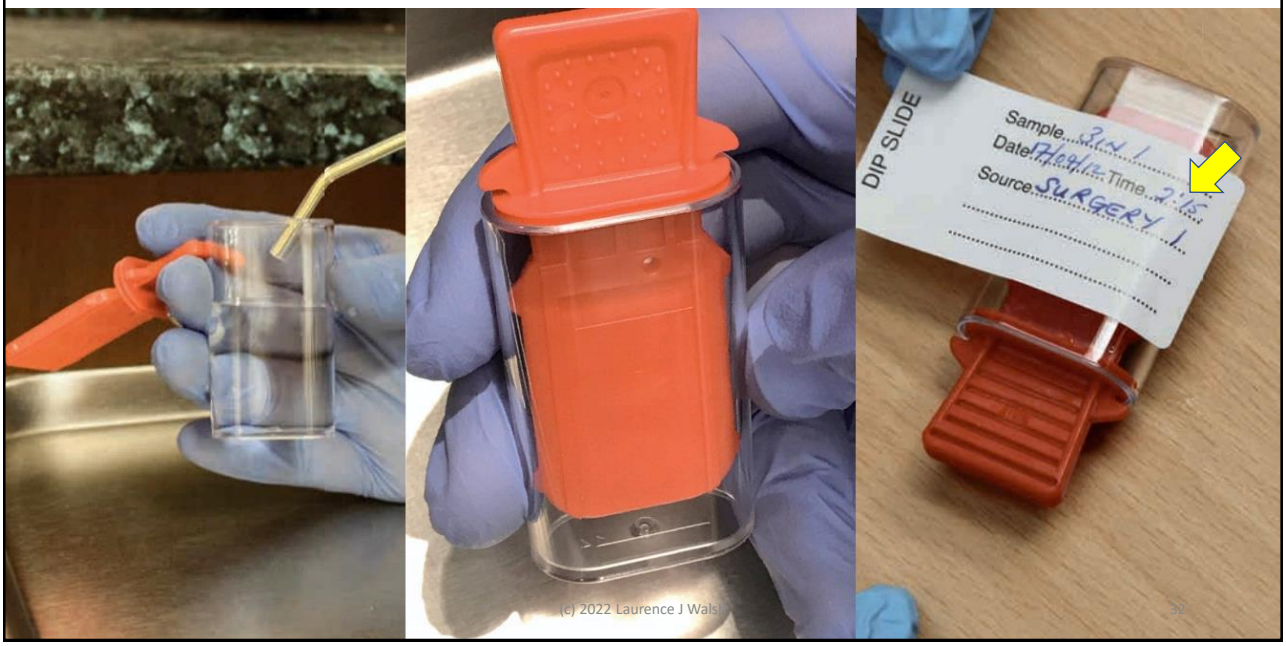
Are the spray jets clogging often?

Water quality thresholds

- When treating immune compromised patients, water from dental unit waterlines should contain **less than 200** colony forming units per mL.

- Bacterial levels can be tested using commercially available test strips (e.g. **Millipore** MHPC10025; **R2A agar** dipslides (better for stressed slow growing bacteria)

Be consistent with the time of day when samples are taken – e.g. lunchtime



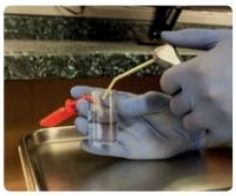
DUWL tests - MHPC

HENRY SCHEIN®

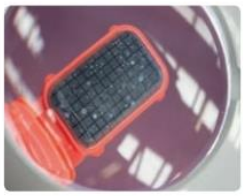
Dental Unit Waterline CFU and HPC water test

Product Code: ST-9MERHPCSAMP

Water test for Heterotrophic Plate Counts and Colony-Forming Units



Test



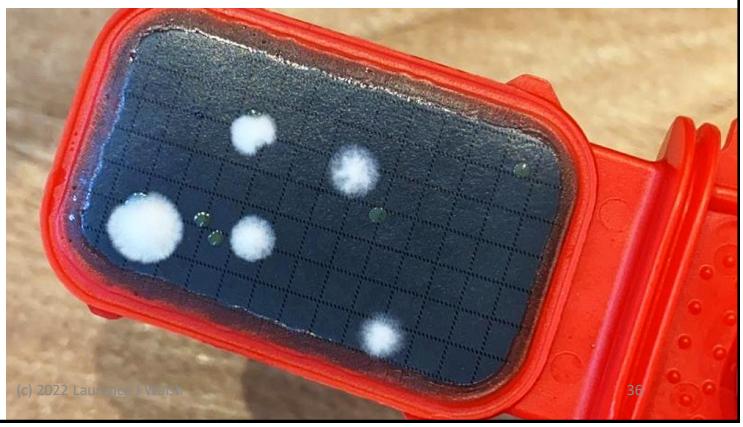
Inspect Under Mag Lamp

Record

Airborne Contamination

Proper sample collection

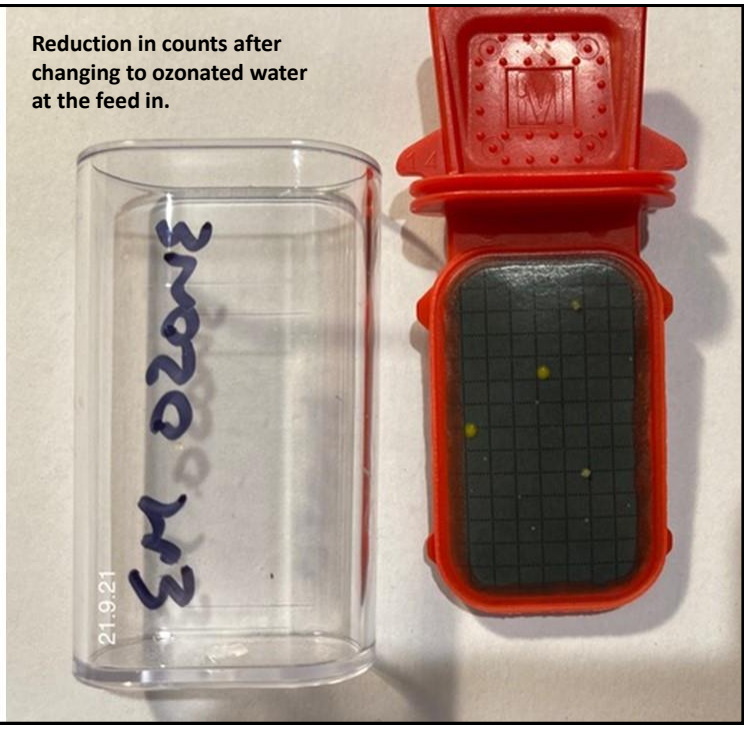
- Sufficient volume and contact time with medium
- Least used lines, e.g. u/sonic scaler during restrictions
- Decontaminate external instrument surfaces before sampling
- Prevent air-borne contamination of test device



Zero CFU/mL in sterile
or ozonated water

20 CFU/ml in tap
water

350 CFU/mL in handpiece
or triplex exit water



Reduction in counts after changing to ozonated water at the feed in.

Step 1 | Check Sampler After 7 Days

It is important to check after exactly 7 days for an accurate result. Use a mag lamp for accurate inspection. Remember you're looking for distinct colonies or a complete overgrowth where all colonies have formed together.

Step 2 | Check Sampler After 7 Days

Is the Sampler 100% Clear, with a matt black Sampler surface?

Yes | The Sampler is 100% Clear

Record 0 c.f.u on your audit sheet.

No | The Sampler is not Clear, Or has a shiny surface

Now you need to count the colonies (dots). Use a mag lamp.

Step 3 | How many Dots

Record the number as c.f.u (Not as Pass/Fail).

Example| 1 c.f.u

Example| 41 c.f.u

Example| 100 c.f.u

Pass | Under 99 Colonies

Record the Number.

Fail | Over 99 Colonies

Record the Number.

Fail | Total Overgrowth/Slimy

Record TMTC (Too many to count)

Total Overgrowth | Fail Condition

Total overgrowth means the slide has been completely overgrown with bacteria and is a fail result. Usually this presents itself as a "slime" across the paddle.

In some cases, such as example 1 to the right, the overgrowth and slime is obvious as it appears shiny and slimey under the light.

Othertimes the slime is less obvious in natural light, but becomes apparent under a magnifying lamp. As such we always recommend checking samplers under a magnifying lamp.

Another way to check for slime is to run a tongue depresser over part of the paddle. As seen in example 2 this will remove part of the slime and reveal total overgrowth.

In cases of total overgrowth this should be recorded as **too many to count (TMTC).**

Example 1| Total Overgrowth

Example 2| Slime being removed

Sampling frequency

- : 0-200 CFU/mL
- : 201 or Above CFU/mL

How often should you test?

- Test your waterlines every month.
- Once passing results are consistent, test quarterly.

When should you shock?

- Before first beginning a treatment protocol or when changing treatment products.
- Shock regularly (recommended every 90 days to start) and extend if testing reveals less frequent is possible.
- When your waterline test reveals a contamination level of 200 CFU/mL or more.

https://dip-slides.com/dental/68-dental-dipslides-box-of-10.html

OUR DIPSLIDES | DIPSLIDE INCUBATORS | KITS | EXTRAS | ABOUT OUR DIPSLIDES | BLOG | MO

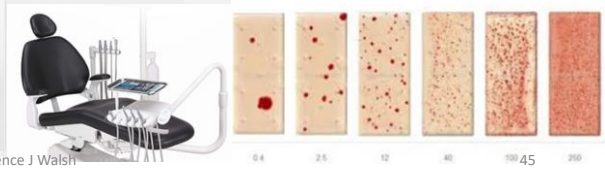
✓ Ready To Be Shipped

DENTAL DIPSLIDES (BOX OF 10)

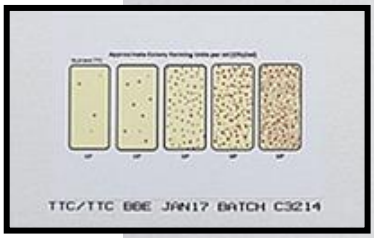
Dental practices often require the use of dipslides to
dental unit water lines. The dipslides that we recom
agar dipslides.

£11.96 Tax excluded

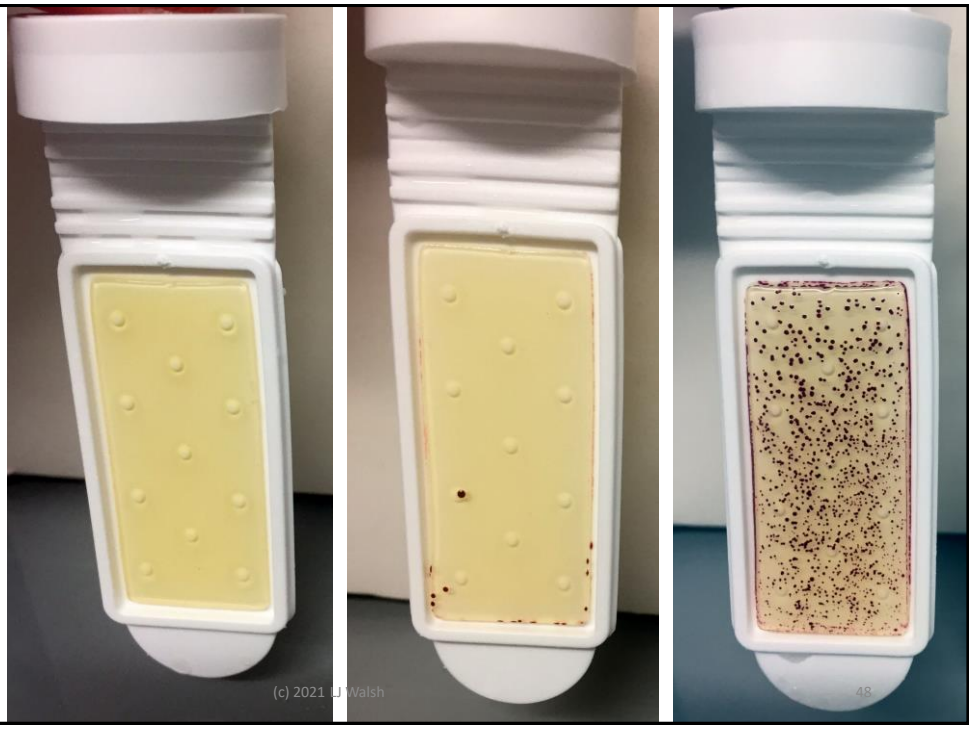
Front Agar Type	R2A
Front Agar Colour	Straw
Front Agar Growth	Heterotrophic Total Count
Rear Agar Type	R2A

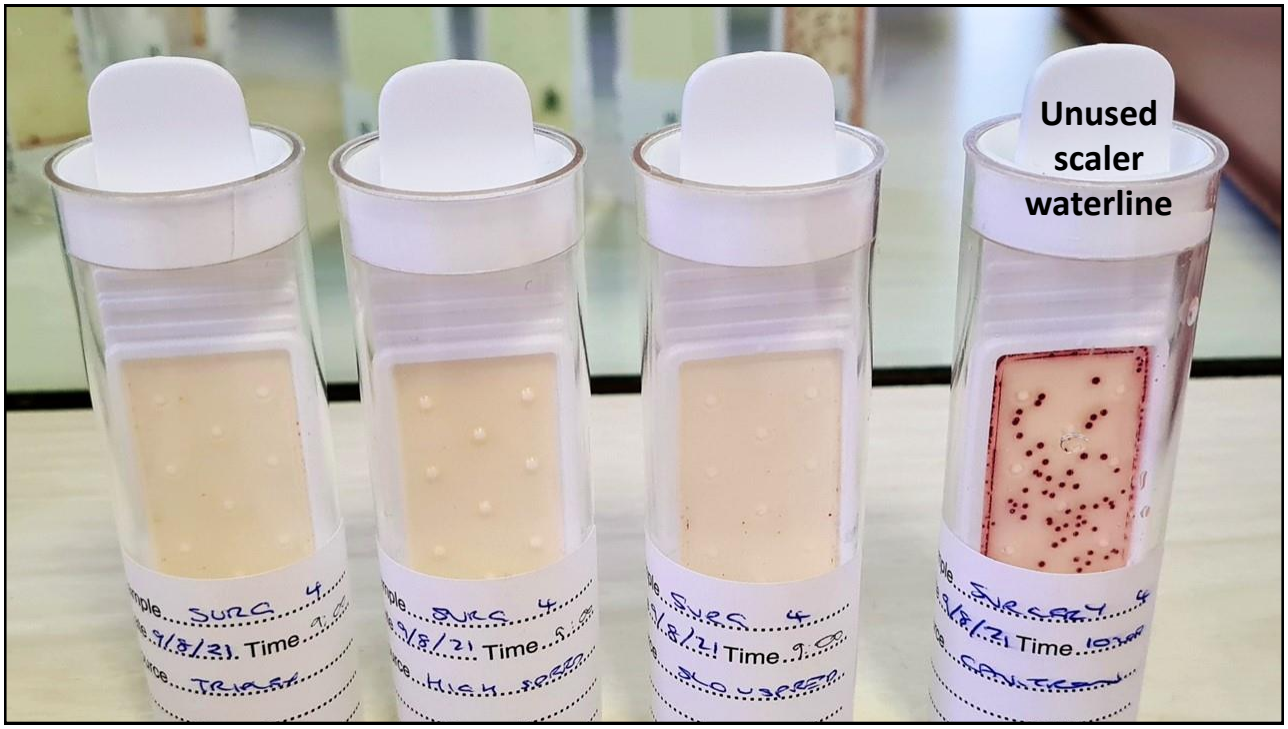


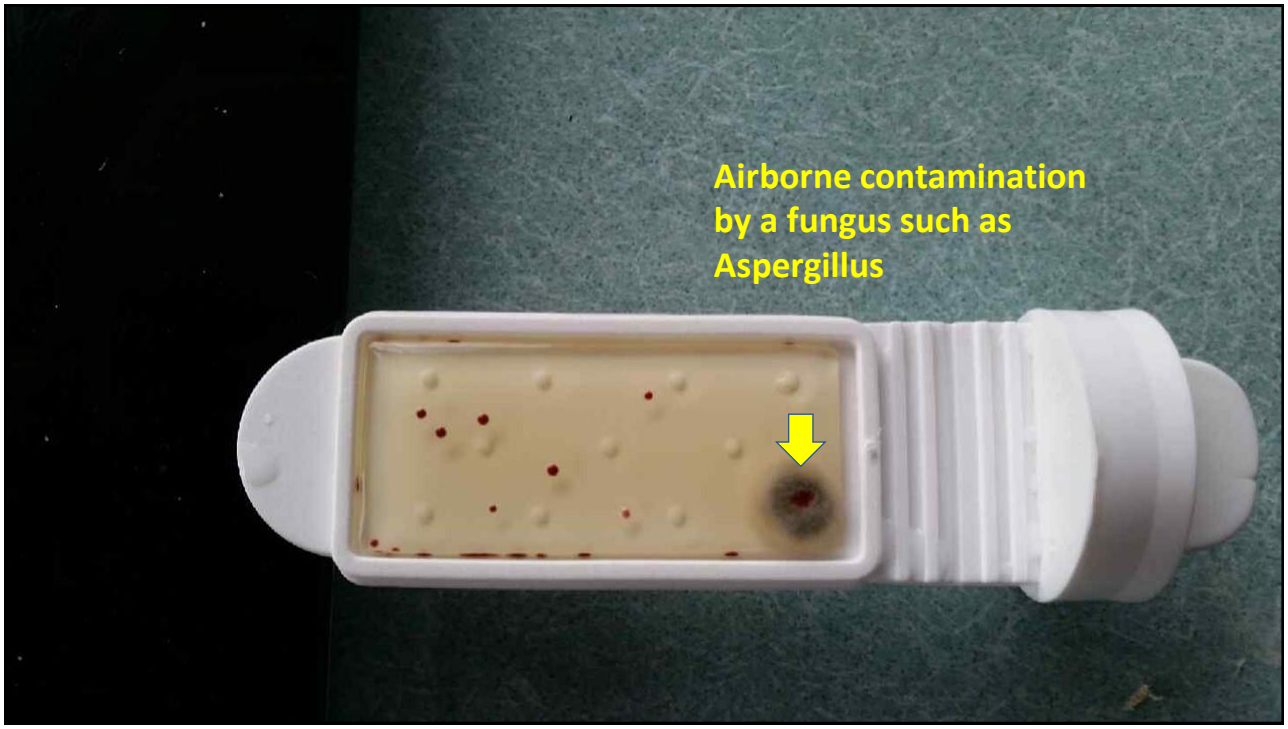
DUWL tests – R2A agar



Ordinary CBD tap water




Unused
scaler
waterline


Airborne contamination
by a fungus such as
Aspergillus

Lab based water testing

If a waterline is being treated with a continuous low-level antimicrobial like a tablet, straw, cartridge, or drops, when samples are taken, the antimicrobial agent left in the lines will also be present within the sample. During incubation, whether through a mail-in laboratory test or in-office water test, the antimicrobial continues to fight any bacteria present within the sample and can lead to false-negatives,

Porteous et al. 2003 JADA.

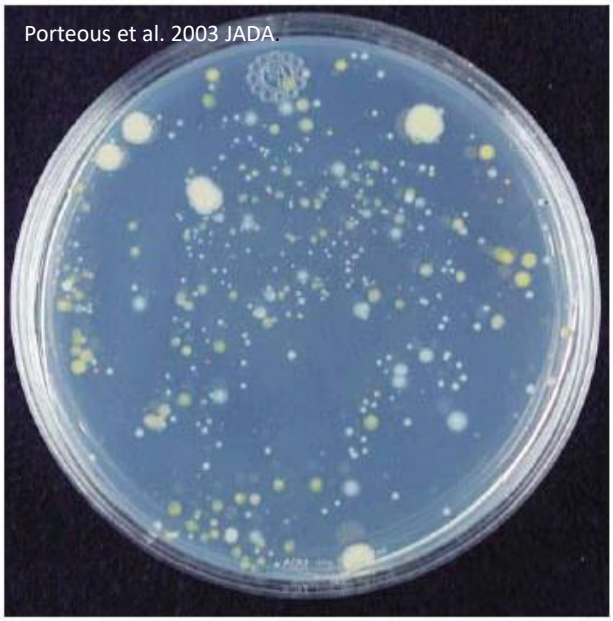


Figure 1. Typical appearance of colonies of heterotrophic, mesophilic bacteria isolated from dental units grown on low nutrient R2A agar plates.

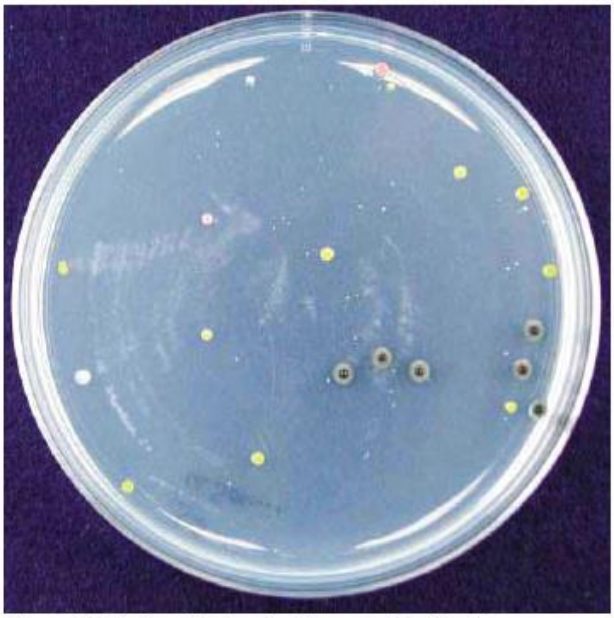
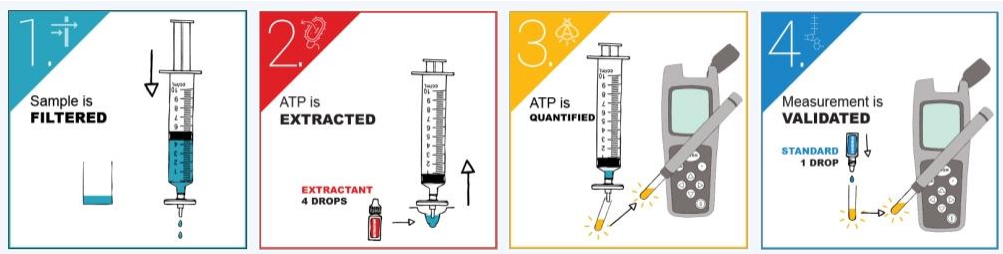
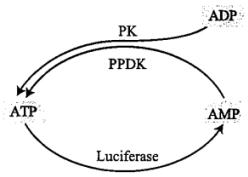


Figure 3. Similar black colonies were isolated from source tap water.

ATP analysis of bacteria: 2 mins



Bacterial detection: LuciPac™ A3 Water



PK：Enzyme for the conversion of ADP to ATP
PPDK：Enzyme for the conversion of AMP to ATP
Luciferase：Enzyme for producing light in the presence of ATP

- Uses the firefly luciferase enzyme reaction:
- Luciferin + luciferase + ATP (luciferin-luciferase-AMP) + pyrophosphate ➡ (luciferin-luciferase-AMP) oxyluciferin + luciferase + CO_2 + AMP+ **light**
- Due to the AMP recycling system integrated into the reaction by means of pyruvate-phosphate dikinase (PPDK), the considerably more stable AMP can also be detected on the surfaces in addition to ATP, which leads to an overall increase in sensitivity.
- https://food.r-biopharm.com/wp-content/uploads/2021/02/instruction-manual-lucipac-a3-water-3.pdf
- https://food.r-biopharm.com/products/lumitester-smart/

Table 1: Main components of each reagent

Reagent name	Main component
Luminescent reagent	Luciferin Luciferase Magnesium acetate Phosphoenolpyruvic acid Pyrophosphoric acid Pyruvate,orthophosphate dikinase Pyruvate kinase
Releasing reagent	Surfactant (Benzalkonium chloride)

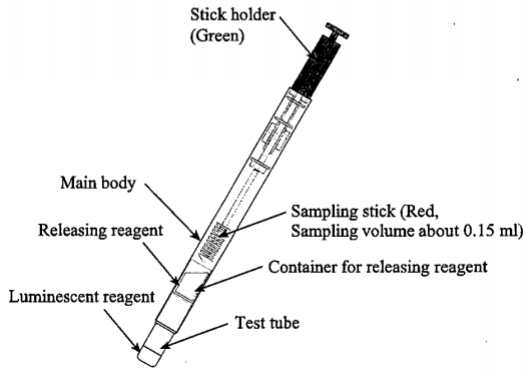


Fig. 2: Each part name of the LuciPac A3 Water

④ Open the measurement chamber cover and place "LuciPac A3/Pen" in the chamber.

⑤ Close the measurement chamber cover and press START.
The measurement starts. The measurement result is displayed after countdown.

⑥ Remove the "LuciPac A3/Pen" after measurement.
When the "LuciPac A3/Pen" tube is not removed from the measurement chamber, the error alarm continues to ring.

Follow local government regulations when disposing the "LuciPac A3/Pen".

Lumitester Smart from Kikkoman Biochemifa for Rapid bacterial measurement

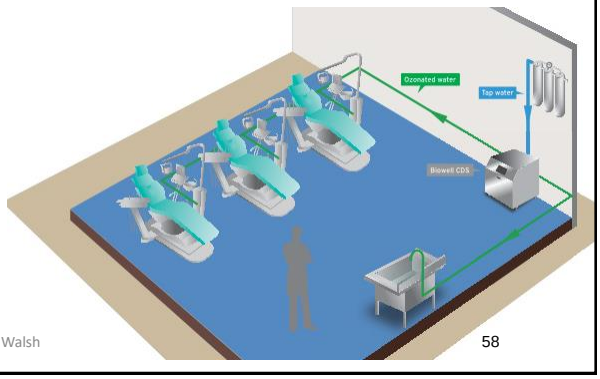
- Lumitester Smart has Bluetooth and can transfer data to a mobile or tablet or via a cable to a PC.
- The LuciPac pen has the advantage that it not only measures ATP, but AMP as well.
  - AMP is a degradation product of ATP. Enzymes and heat degrade ATP to ADP and AMP.
- LuciPac A3 measures ATP+ADP+AMP, while other luciferase-based systems measure only ATP. Measuring A3 rather than ATP gives some advantages:
  - More sensitive
  - Less issues with sample degradation after collection before measuring as the total of A3, i.e. ATP+ADP+AMP is stable.

1. **FROM INSTALLATION**
   - Sterile water supply
     - Ultrafiltration
     - Ozonation

2. **EXISTING CHAIRS**

- Chemical dosing of reticulated water
  - Ozonation
  - Peroxide and silver ions
- Independent (bottled) water systems
  - Tablets (Peroxide and silver)
  - Liquid dosing (Peroxide, Peroxide and silver, NaOCl)
  - Iodine (slow release resins)
  - Ozonated water
  - Chloramines

- **SHOCK TREATMENTS**
  - NaOCl (domestic bleach)
  - Dentisan BIOCLEAR
  - Alpro BILPRON



DUWL biofilm prevention and control

- **AGENT USED**
  - Oxidants
    - Hypochlorites
    - Chloramine T
    - Peroxides
    - Ozonation
- Silver
- Iodine

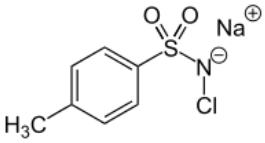
- **DELIVERY STRATEGY**
- DOSING SYSTEMS inside chair
- Impregnated TUBING for DUWL
- TABLETS for bottles
- LIQUID for bottles
- Collection "straws" on bottle

FLUSHING: ADA recommends rinsing all waterlines at the start of the work day for 2 minutes (with no handpieces attached) at all dispensing sites.

Ozonators

Clean water system (CWS) using a bottle

- Bottles can be located internally or externally
- Check MFR instructions for correct water type and correct additives (e.g. take tap water and add in ***)



- **Alpron**:
- Water based solution with EDTA and **chloramine-T** disinfectant.
- Continuous treatment.
- Used at 1% concentration
- Made fresh weekly. Left in the waterlines of the chair continuously.

Silver

- Long acting disinfectant
- Used in chemical tablets, e.g. BluTab, Citrisilm and ICX
  - **BluTab and Citrisil: silver is ~ 0.8%**
  - **ICX: 0.5% silver nitrate**, 10% sodium percarbonate (becomes hydrogen peroxide), 45% citric acid, 10% EDTA, 1% surfactant
  - Silver and low conc HP does not affect bond strengths.
  - Wait two minutes for these tablets to fully dissolve before using the dental unit.
  - **With these tablets, they are not intended for use as biofilm removal agents. They are to be used in conjunction with regular dental unit water monitoring (e.g. 3 monthly) and shock treatment**.

a dec

Maintain. Monitor. Shock. **Three steps to clean.**

What can you do to deliver the highest quality water to your patients? Along with a daily waterline maintenance protocol, it's important to monitor water quality and periodically shock the lines to clear deposits and contamination. These three steps help maintain your dental unit waterlines.

a-dec ICX®

PRODUCT NAME: ICX

DIRECTIONS FOR USE:

1. Empty any remaining water left in the bottle.
2. Drop tablet into an empty dental unit water bottle (0.7L tablet in 0.7 liter bottle, 2L tablet in 2 liter). Avoid touching the tablet with unprotected skin.
3. Fill bottle with water and connect to the dental unit.
4. Wait 120 seconds for the tablet to fully dissolve.

NOTE: This product is intended for use with potable water and in conjunction with regular dental unit water testing, following flush (shock) treatment with an antimicrobial product registered with the U.S. EPA and recommended by the manufacturer.

ICX is intended for use in general dental applications by providing water to the dental syringe, various handpiece ancillary devices, and cuspidor cupfill. When used according to these instructions for every bottle refill, optimum efficacy for control of odor and foul taste bacteria can be achieved.

Peroxides

- 8% HP in KaVo Oxygenal 6, which is dosed at 0.25% (250 mg/L)
- 1.41% in Dentosept P solution (also contains 0.1% silver nitrate)
- HP is also released from ICX tablets, which contain 10% sodium percarbonate (becomes hydrogen peroxide)
- HP levels can be tested with commercial test strips.

Sirona chairs and Dentosept injectors

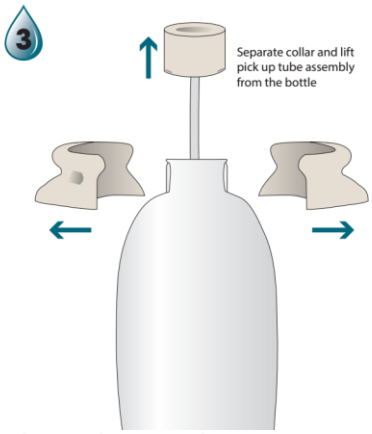
Iodine

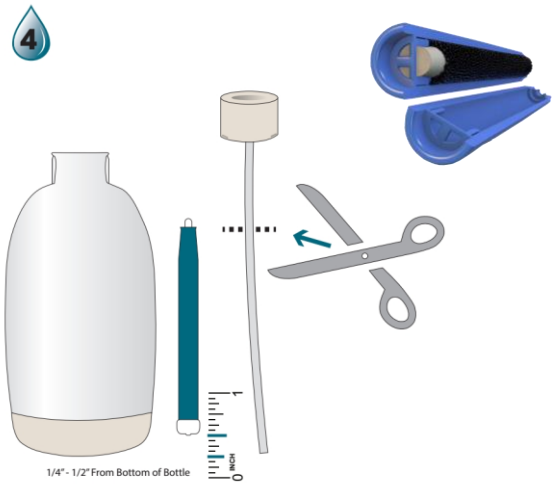
- Iodine is an essential trace element for humans and is used in the synthesis of thyroid hormones. The recommended dietary intake for adults ranges from 0.03 mg/day to 0.15 mg/day
- Resin-releasing straws dose water entering the dental unit with iodine **4-6 ppm over a 1 year period** (e.g. Dentapure)

- Low concentration avoids irritancy (threshold is around 10,000 ppm iodine); No allergy concerns as is not complexed to peptides
- Low maintenance approach with an engineering solution that does not involve daily compliance

Iodine releasing pickup straws

CROSSTEX A CANTEL MEDICAL COMPANY DentaPure

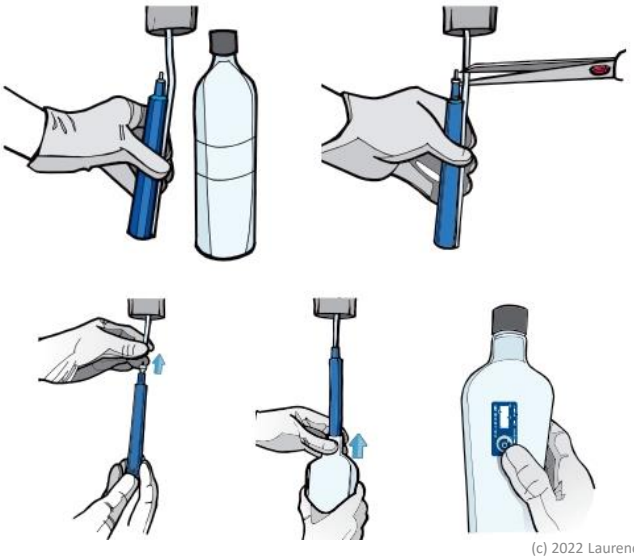


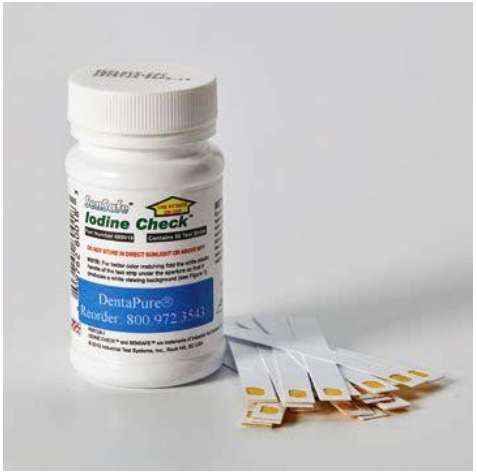


This advanced water treatment technology was originally developed for NASA and successfully used for over 20 years to ensure that water consumed in space was free of bacteria, viruses and other harmful organisms.

Dentapure (iodine dosing)

Iodine releasing pickup straws





Shock treatments

e.g. Alpro BILPRON; Dentisan BIOCLEAR; NaOCl (for A-dec chairs)

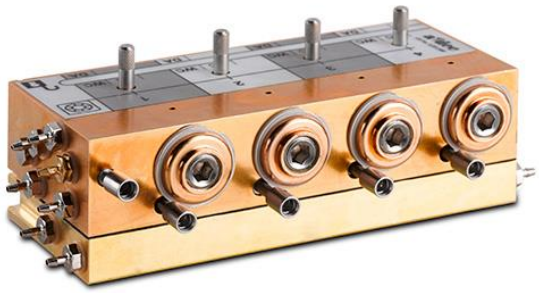
- Shock treatment clears deposits and bacterial contamination from dental unit waterlines.
- Whenever applying a shock treatment, be sure to adhere to the product instructions provided by the manufacturer.
- Check which products are compatible with the dental unit.
- Shock the waterlines whenever test results indicate a bacteria level greater than **200 CFU/mL.**
- **After** a shock treatment, **flush** the agent out and continue normal chemical treatments, then re-test levels of microorganisms.

Shock treatment (sanitizing)

NaOCl shock treatments (e.g. 1:10 dilution of 5% NaOCl for 10 mins)

- Stored sodium hypochlorite degrades over time; needs a cool environment away from sunlight
- Effectiveness is influenced by pH,, hence lower pH values around 6.5 or less are optimal
- Higher turbidity lowers the effectiveness of NaOCl

- Solution can be very corrosive to metal of control blocks (only some brands and designed to withstand it)



Problems using domestic bleach:

Alkaline pH, corrosion (of control blocks of non-US chairs), low hypochlorous acid concentration, low free available chlorine, FAC levels are unknown and reduce with temperature and storage time.

How to Shock Your Dental Unit Waterlines Using Diluted Bleach:

Step 1: Create Bleach-Water Solution

- Detach your chair's water bottle.
- Add bleach and cold water with measuring cup (or 5 oz. patient cup) (see table below).
- Re-attach your bottle to the unit.

	bleach		water
For 5.25-6.25% Sodium Hypochlorite	x1	+	x9
For 7-8.25% Sodium Hypochlorite	x1	+	x13

Step 2: Run Bleach Into Each Line

Air-Water Syringe
- Run each A/W syringe for 10-15 seconds or until you smell the bleach flow out the tip.

High & Slow Speed Lines
- Remove each handpiece from the end of the line.
- Place the handpiece connection into the collection cup, bucket, or sink.
- Switch and hold the "flush" toggle (or step on the rheostat) until you can smell the bleach at the handpiece connection.
- Repeat this procedure at every handpiece line, including slow speed handpieces.
- If no water flows through your slow speed lines, shocking these lines is not necessary.

Ultrasonic Scalers
- If you have an ultrasonic scaler in this operatory, run the scaler until you smell the bleach at the scaler tip, this may take up to 1 minute.

Pro Tip: If you are having a hard time recognizing if bleach is within the line, grab a neon sticky note and lightly spray it with the scaler, syringe, or handpiece. Wait 10 seconds. If bleach is within the line, the sticky note will be lighter in the area you sprayed.

Step 3: Wait and Flush

- Set your timer for 10 minutes
- Leave bleach in each line for 10 minutes and NEVER MORE than 15 minutes.
- After 10 minutes, disconnect the water bottle and dump the remaining solution into your collection cup, bucket, or sink. NEVER SEND BLEACH OR ANY DENTAL UNIT WATERLINE SHOCK PRODUCT THROUGH ANY EVACUATION/SUCTION LINES.
- Refill water bottle with warm water and flush lines for 2 minutes to help break up and remove biofilm.
- Lastly, flush lines with cold water for 1 minute to cool the lines

ProEdge DENTAL WATER LABS

Step-by-Step Video
ProEdgeDental.com/Shock

What You Will Need:
- Regular household bleach (check expiration and concentration)
- Dummy Straw (if you are using a straw)
- Water
- Standard PPE
- Collection bucket
- Timer
- Measuring cup (or 5oz. patient cup)

When Should I Shock?
- Before first beginning a treatment protocol or when changing treatment products.
- At least every quarter (90 days)
- When your waterline test reveals a contamination level of 200 CFU/mL or more.

Why Should I Shock?

Shocking is the key to dental waterline maintenance. Biofilm is stubborn, complex, and can be hard to remove, which is why it is critical to use a shock treatment such as diluted bleach to help clean your waterlines.

Is Bleach Safe for Equipment?

Several manufacturers (including Midmark, DCI International, DentalEZ, DENTSPLY Cavitron, Pelton & Crane, and Proma) have authorized weekly or daily water system treatment with a solution of bleach. If you want to shock with something other than bleach, Crosstex Liquid Ultra™ is an effective alternative.

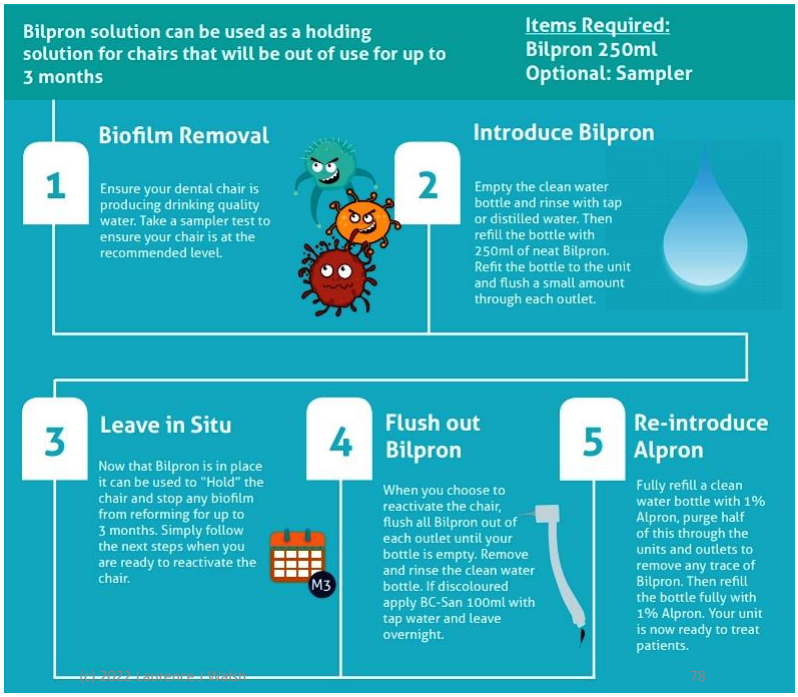
Questions?
Call our team at 888.843.3343 or visit ProEdgeDental.com

- **Bilpron: Waterline sanitizer**
- Water based solution with **bisguanides**, complexing agents, propylene glycol and preservatives
- **For SHOCK TREATMENT.**

- *Active for up to 3 months allowing long term "mothballing" of low use dental units, as well as during holidays and weekends.*

Bilpron

Holding solution for dental chair hibernation up to 3 months



Alpron for the continuous decontamination of treatment water in dental unit water lines of all treatment units.

Bilpron for the decontamination, cleaning and long-lasting inhibition of biofilm formation.

Biofilm-Removing-System (BRS)

Decontamination of treatment water and dental unit water lines

1 Weekend Closedown

An Intensive disinfection is ideally done **weekly**. The disinfection is best performed over the weekend so the Bilpron has a longer contact time and can perform a more intense disinfection.
As such we recommend you perform this on a friday afternoon.

2 Remove Alpron, Add Bilpron

Empty the clean water bottle of any Alpron. Rinse well with potable water, then refill with 250mls of NEAT Bilpron. Refit the bottle to the unit and flush a small amount through each outlet.

3 Contact Time

As mentioned in step one the intensive disinfection is ideally performed on the friday, so that the Bilpron is left in over the weekend. The minimum contact time is **12 hours**. Ensure Bilpron is left in for at least this amount of time, otherwise the disinfection will be ineffective.

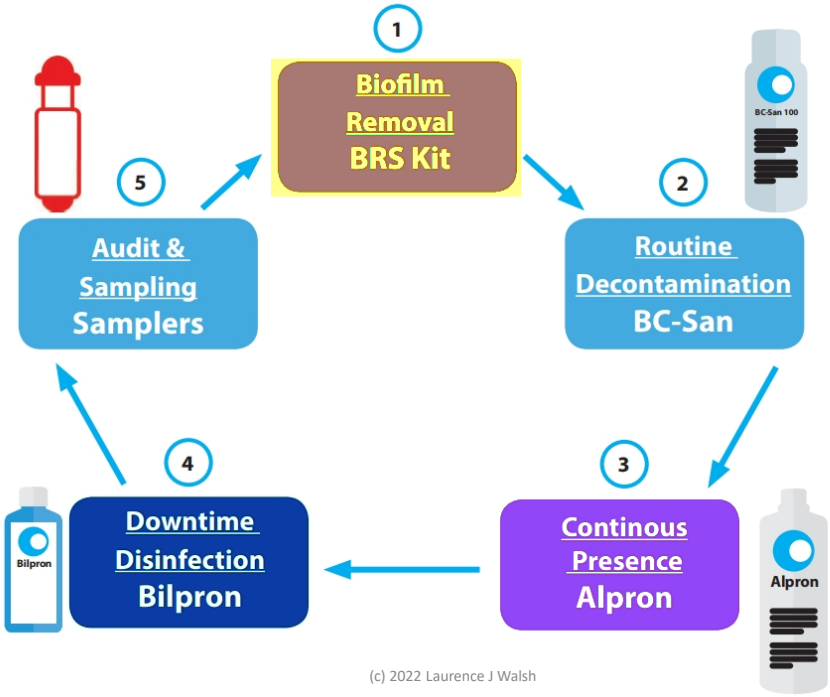
4 Flush out Bilpron

On the monday or after at least 12 hours, flush all Bilpron out of each outlet until your bottle is empty.

Remove and rinse the clean water bottle. If discoloured apply BC-San 100ml with tap water and leave overnight.

5 Re-introduce Alpron

Fully refill a clean water bottle with 1% Alpron, purge half of this through the units and outlets to remove any trace of Bilpron. Then refill the bottle fully with 1% Alpron. Your unit is now ready to treat patients.



ICXRenew™

- Part 1 solution = Hydrogen peroxide 1.5-2.5% @ pH 1.9-2.4
- Part 2 solution = Maleic acid 0.1-0.3% and sodium lauryl sulphate 1.5-2.5% @ pH 9.0-10.5.

a-dec

A-dec ICX Renew Solution 1 and Solution 2 is a specially formulated dental unit waterline shock treatment. It is intended to lower bacterial contamination in effluent and remove build-up of non-pathogenic microbial contamination from the waterlines. This ready to use two-part product cleans dental unit waterlines and removes the buildup of odor-causing, foul tasting bacteria. Always use ICX Renew Solution 1 and 2 together. Do not add water. This product is to be used in conjunction with regular testing of dental unit water.

ICX Renew™ Shock Treatment

Dental Unit Waterline Shock Treatment

Fast-acting liquid shock solution removes odor and foul-tasting bacteria in dental unit waterlines. Just mix the two solutions together into an empty water bottle, run through the lines, and soak overnight. The blue color improves visibility, so you know that the solution is in the waterlines. After completing the shock treatment, flush the lines with water and resume your regular use.

a-dec

The Benefits of ICX Renew

- Penetrates and removes the build-up of odor and foul-tasting microbial contamination bacteria.
- Blue color lets you know the solution is hard at work in the waterlines.
- Non-corrosive and gentle on equipment.
- Will not corrode or clog waterlines.
- Odorless formula.

Two-part solution.
Nine applications per box.

A-DEC RECOMMENDS

performing a shock treatment when dental unit waterline bacteria levels exceed the recommended action level.

If test results are < water quality standards = continue daily maintenance with ICX

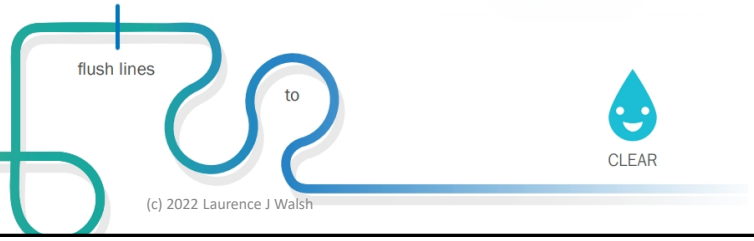
A-dec ICX tablets for daily treatments

If test results are > water quality standards = shock your dental waterlines with ICX Renew

A-dec ICX Renew for shock treatments

WHEN YOU SHOCK, LET ICX RENEW

sit overnight



Town water – shock Tx every 4 weeks

New as of: 02.2020

Dentsply Sirona

Teneo

Operating Instructions (not valid for USA)

English

Monthly or as required

Change the water and air filters [→ 269]

Microbiological water test [→ 220]

Microbiological water test

Perform the microbiological test of the water from the treatment center at regular intervals and after longer periods of disuse > 1 week; see "Media quality" [→ 16]. Start the checkups in intervals of no more than two weeks and adjust the time intervals depending on the results. In addition to running laboratory tests, you can also use the "Total Count Tester" as a simple means of performing this test.

Interactively guided sanitation of the treatment center

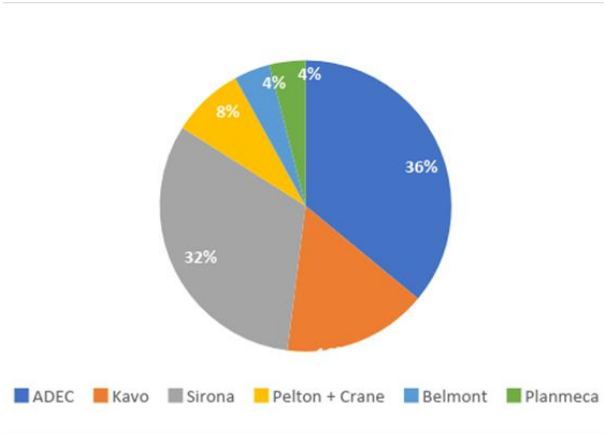
Sanitization, that is disinfecting the treatment water paths, can be performed by using the disinfection system. During sanitization, the treatment water is first emptied according to a pre-defined process, then the disinfectant for the water paths is filled undiluted into the treatment water paths and flushed out again at the end. Sanitization includes several phases and takes at least 24 hours but should not exceed 3 days.

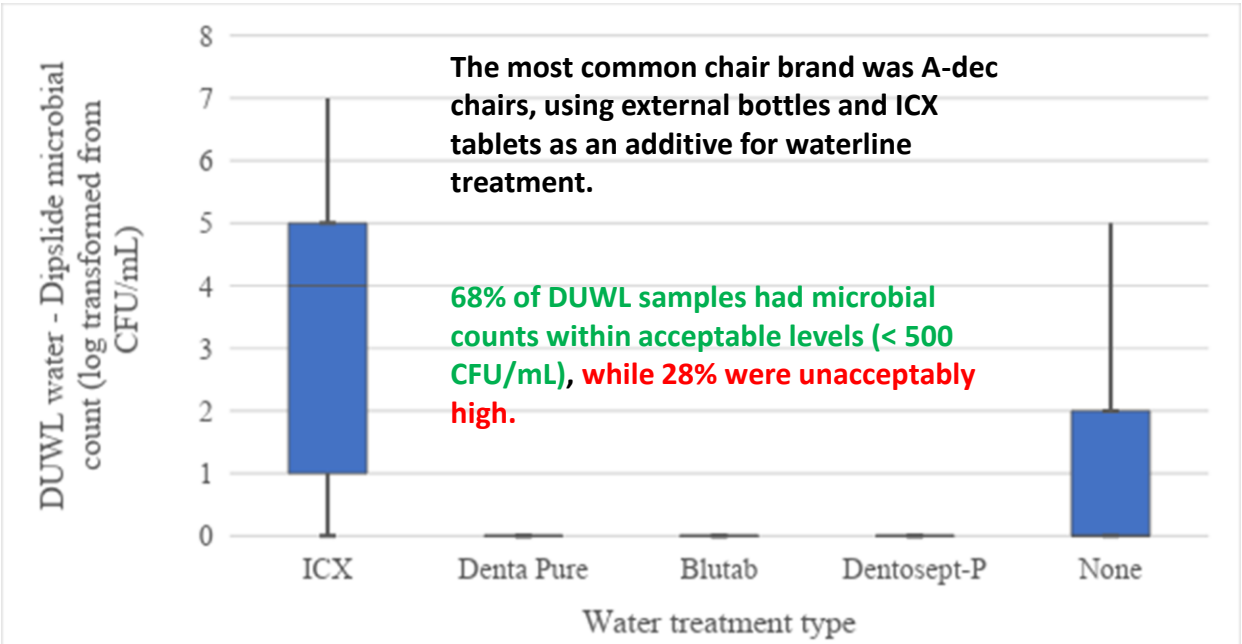
When operating with public drinking water supply (self-sufficient water supply switched off), interactive sanitization should be carried out:

- Regularly every 4 weeks
  The display *Days until next sanitization* appears in the status bar of the touchscreen. It first appears three days before the date of sanitization (3d = 3 days until sanitization).
- After longer periods of disuse (> one week)
- If the bacterial count exceeds 100 bacteria per milliliter; see "Microbiological water test" [→ 220]
- After changing from stand-alone water supply to public drinking water supply and the agent for disinfecting the water paths

UQ 2018 field study of DUWL

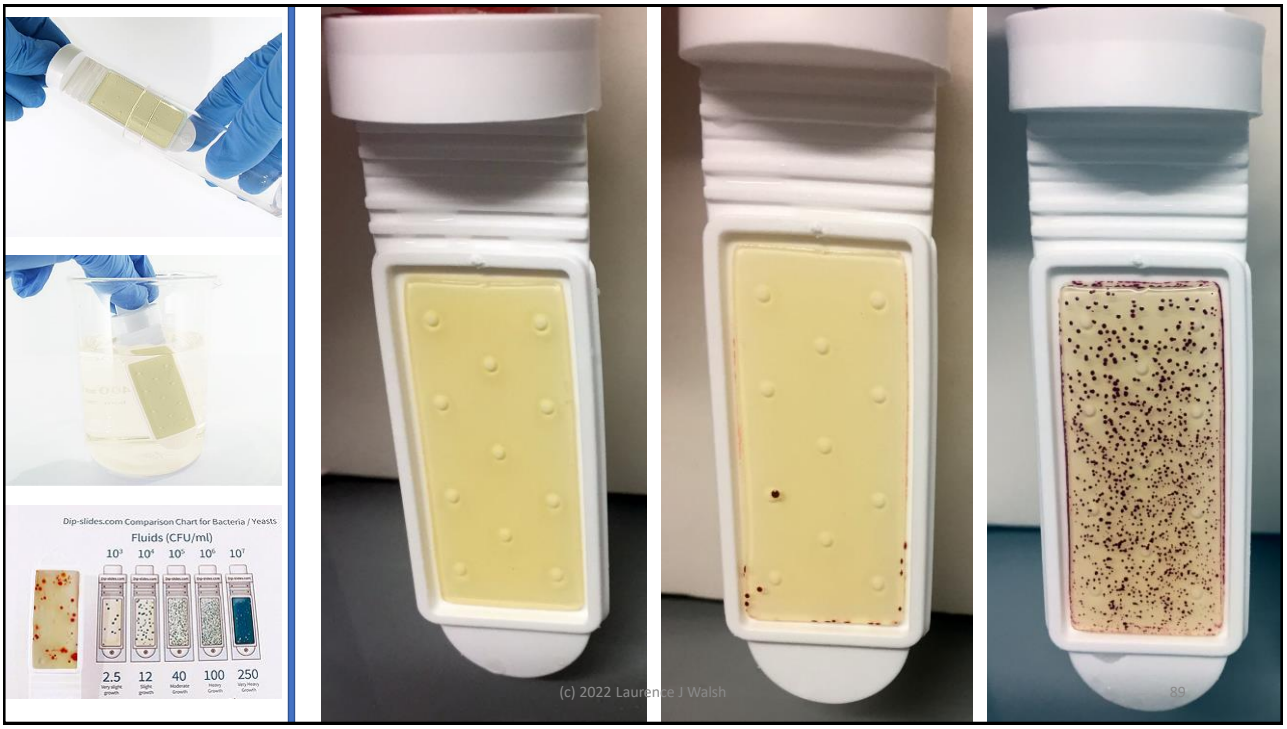
- The most common brand of dental chair units was A-dec.
- The dental chair age varied between 1 and 13 years, with the average age being 5.8 years.
- The most common water source was an external bottle. The most common water treatment was ICX tablets (sodium percarbonate and silver nitrate).





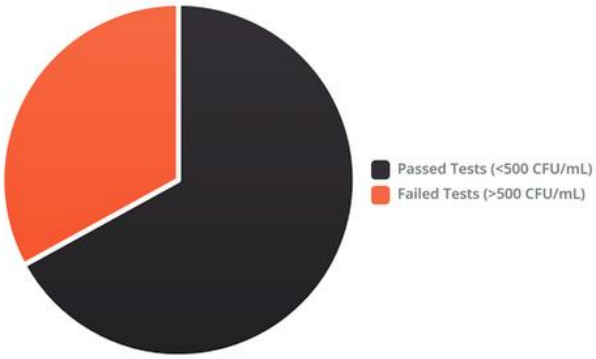
The most common chair brand was A-dec chairs, using external bottles and ICX tablets as an additive for waterline treatment.

68% of DUWL samples had microbial counts within acceptable levels (< 500 CFU/mL), while 28% were unacceptably high.



Dental unit waterline management issues

- **Common issues**
- Incorrect use of product
  - Failure to follow MFR IFU
  - Insufficient Tx time
- Infrequent shock Tx
- Lack of testing



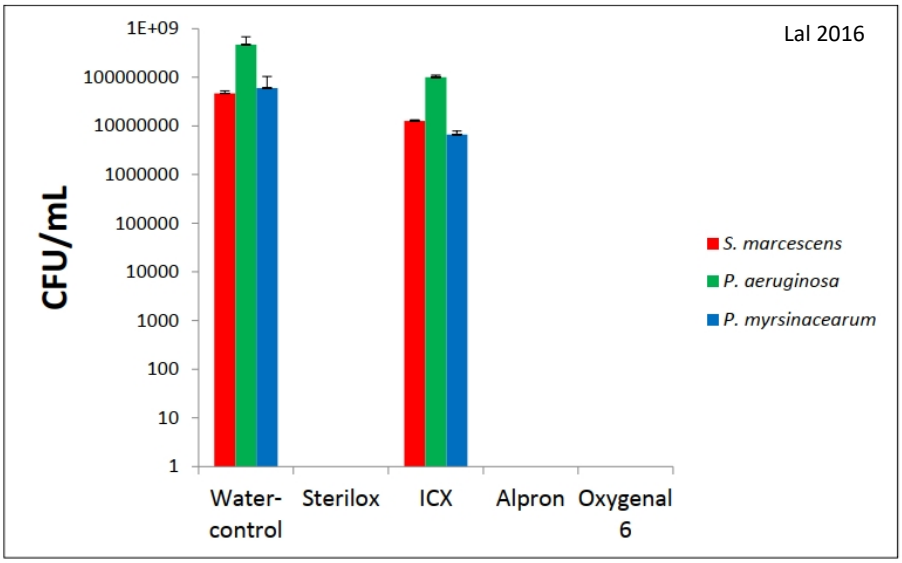


Figure 3.6: The effect of DUWL biocides on *S. marcescens*, *P. aeruginosa*, and *P. myrsinacearum* in 1×10^8 CFU/mL inoculum size.

ICX issues:
- HP degradation over 2 weeks
- Designed for biofilm growth reduction only, not biofilm elimination.
- Must be used with periodic monitoring and shock treatment (IFU).
- Not suitable for hibernation.

Hibernation agents:
Alpro BILPRON
Dentisan BIOCLEAR
- These remove biofilm.
- Also can be used for weekly shock treatments.

ICX issues:
- HP degradation over 2 weeks
- Designed for biofilm growth reduction only, not biofilm elimination.
- Must be used with periodic monitoring and shock treatment (IFU).
- Not suitable for hibernation.
- "White pill" set and forget issue
- Skin microflora contamination of water when ungloved hands are used to handle tablets

Hibernation agents:
Alpro BILPRON
Dentisan BIOCLEAR

- These remove biofilm.
- Can be used in the waterlines over extended periods of time (e.g. 3 months)
- Also can be used for weekly shock treatments.

How to Shock Your Waterlines

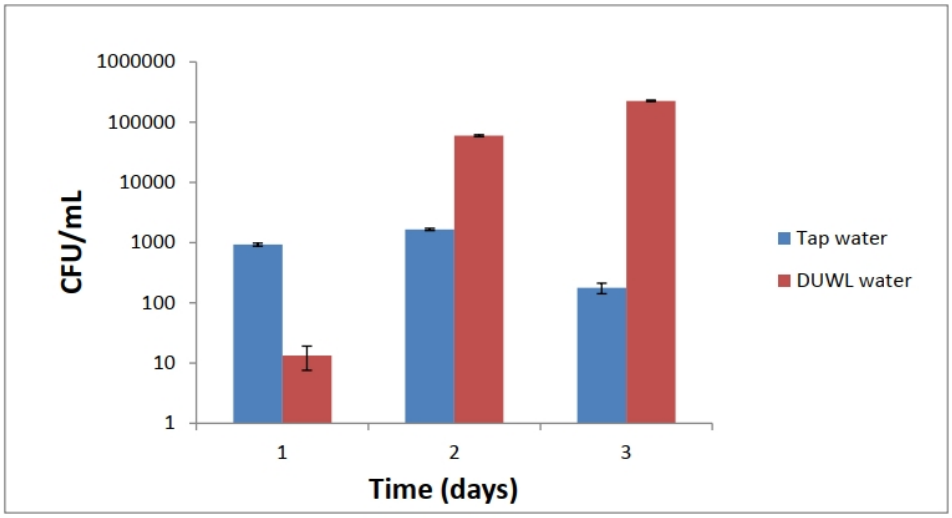


Figure 6.2: Planktonic bacterial counts from the sDUWL output water and tap water (source) during first three days. Difference in CFU/mL count was statistically significant ($p = 0.0001$).

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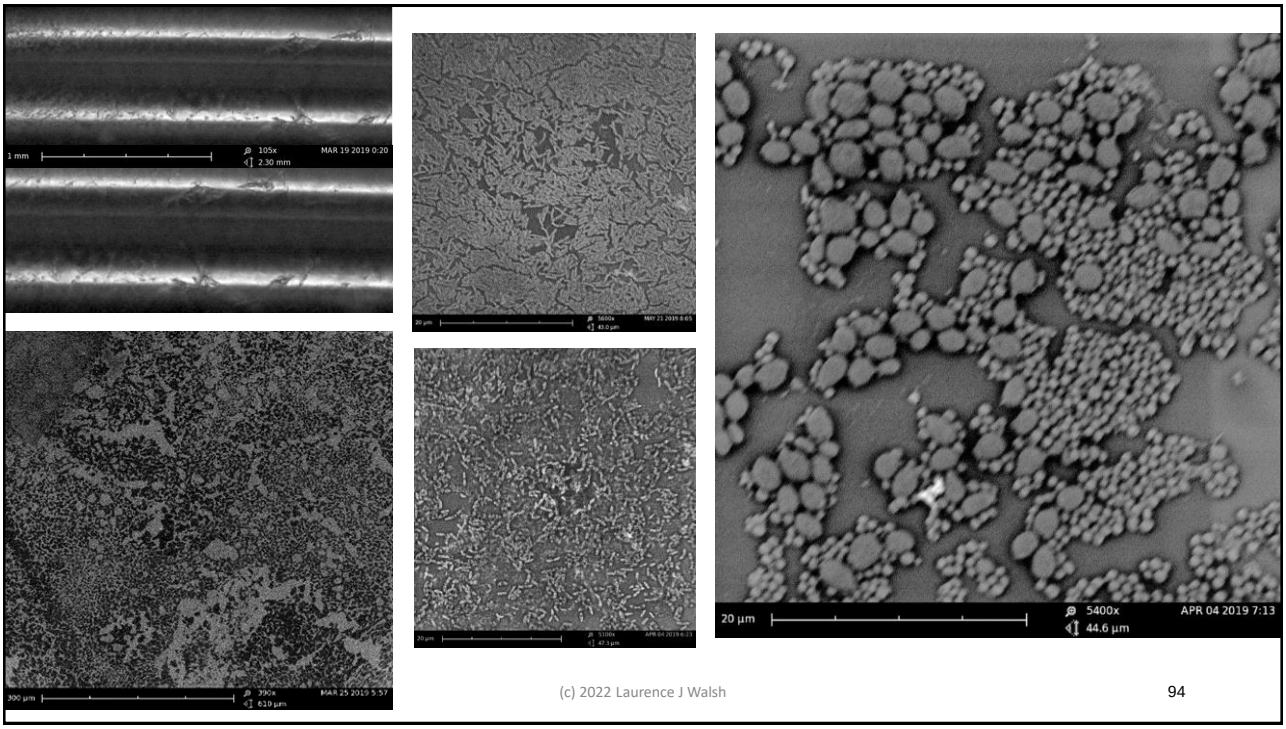

Biofilm removal example: crystal violet staining