To be always safe

Health = Security

SONICA®

ULTRASONIC CLEANERS

THE SPECIALISTS IN ULTRASONIC CLEANING

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SONICA® DECONTAMINATION TANKS

Hygiene = Safety

- Completely made of **stainless steel**
- The decontamination tank contains the **electropolished stainless steel** basket that can be used in ultrasonic tanks as well, avoiding the contact with infected instruments.

Our decontamination tanks:

3 Litres	6 Litres	14 Litres	21/28 Litres
			

SONICA® ULTRASONIC CLEANERS

All with SWEEP SYSTEM!

- Ultrasonic cleaners to reach high cleaning standards in a short time.
- **SONICA®** models have different dimensions and capacities and they are available from **3 litres to 130 litres**.

SONICA® TRENDMATIC

- **Pre-washing** function with pressurised spray water jets.
- Ultrasonic cleaning with **automatic** detergent dosing.
- Multiple rinses with pressurised clean water spray jets.
- Drying function.
- Possibility of **thermal** disinfection cycle at **93° for 3 minutes** for model **9 TD**.
- Traceability system on **USB** memory of each cycle data.
- Wide graphic **LCD** display **with resistive touchscreen** (it is possible to key in also using surgical gloves).

All in one

All essential operating protocols in one multifunction ultrasonic device.
More available space in the sterilization room, more time saving and traceability of each cycle since it is completely automatic.

Available models

TRENDMATIC	**9**	capacity 9 litres
TRENDMATIC	**9 TD**	capacity 9 litres
TRENDMATIC	**18**	capacity 18 litres

Double overlapping basket with the possibility of half load washing cycle.

SONICA® CL 4%

How does it work and when you need it?

SONICA CL4% is a Concentrated Disinfectant Aqueous Solution with decontaminating and cleansing action for invasive Medical Devices.

100 g of SONICA CL4% solution contain the following ingredients:

INGREDIENTS

ACTIVE INGREDIENTS	Chlorexidine gluconate	1,50 g 15,00 g
EXCIPIENTS	Co-formulants, Essence, Colouring and Purified water	q.b. to 100,00 g

Product presentation (Chemical and physical characteristics and incompatibility)
SONICA CL4%® is a concentrated aqueous solution with decontaminating and cleansing action. This Chlorexidine and Cetrimide-based solution has a near-neutral pH value, which allows cationic bis-biguanides to exert maximum antimicrobial action. Combination with Cetrimide creates a high-level synergetic disinfectant action as well as a cleansing effect. Solutions that contain Cetrimide from 0.1% to 1% are commonly used for the sterile preservation of medical devices. Cetrimide is a quaternary ammonium compound and, as such, behaves as a cationic surfactant. Chlorexidine in gluconate form also has a positive charge. It is evident that this product is incompatible with anionic detergents, soaps, and emulsifiers. Chlorexidine salts are incompatible with borates, bicarbonates, carbonates, chlorides, citrates, nitrates, phosphates, and sulphates, since with these elements it forms low-solubility salts. Chlorexidine salts are rendered inactive by cork.
The isopropyl alcohol contained in SONICA CL4%® guarantees improved product preservation, lower risk of product contamination, and also increases the antibacterial properties of the active ingredients.

Application fields and procedures
1. Decontamination and simultaneous detergency of surgical instruments and medical devices as per the Decree of 28th September 1990: Regulations for protection against professional contagion from HIV in public and private health and care structures;
"After use, reusable devices shall be immediately immersed in a chemical disinfectant with recognised effectiveness upon HIV, prior to any disassembly or cleaning operations to be carried out in preparation for sterilisation."
2. Temporary sterile conservation of surgical instruments.
When removing instruments from the solution, extract them aseptically and rinse them thoroughly in sterile water. SONICA CL4% is a concentrated solution for use only when diluted with tap water.

DOSES, METHODS, AND TIMES

Use	Dilution in water (%)	Dilution Example	Contact Time
Decontamination and simultaneous detergency of medical and surgical devices in ultrasonic baths	2%	20 ml of SONICA CL4% per 1 litre of tap water	15 minutes
Decontamination and simultaneous detergency of surfaces, sheling and equipment in operating theatres (surfaces, furnishings, beds, strechers, etc.)	4%	40 ml of SONICA CL4% per 1 litre of tap water	20 minutes
Temporary sterile conservation of surgical instruments	4%	40 ml of SONICA CL4% per 1 litre of tap water	----------

Note: for the complete and correct use of the product refer always to the Technical and Safety Datasheet

All information in this brochure is reserved for professional health sector workers.

SONICA®

Unique and effective!

Specific detergent for decontamination and ultrasonic cleaning of surgical instruments

A single product that ensures quality and cleaning in order to be always safe.

SONICA® CL 4%

Medical Device **CE 0476**

It is a concentrated solution with cleaning and disinfecting properties in 1 litre bottles.

The product can be diluted in tap water from a minimum of **1%** to a maximum of **4%**.

COD. 090.005.0017

OPERATING PROTOCOLS

Procedures for the correct sterilization of surgical instruments

MONITORING TEST

Periodic check of the cleaning quality of the surgical instruments.

The adoption of a correct sterilization procedure also implies a periodic check of the cleaning quality.
This not only secures the use of the instruments, but it also ensures a correct autoclave sterilization process, preventing any risk for patients and the medical staff.

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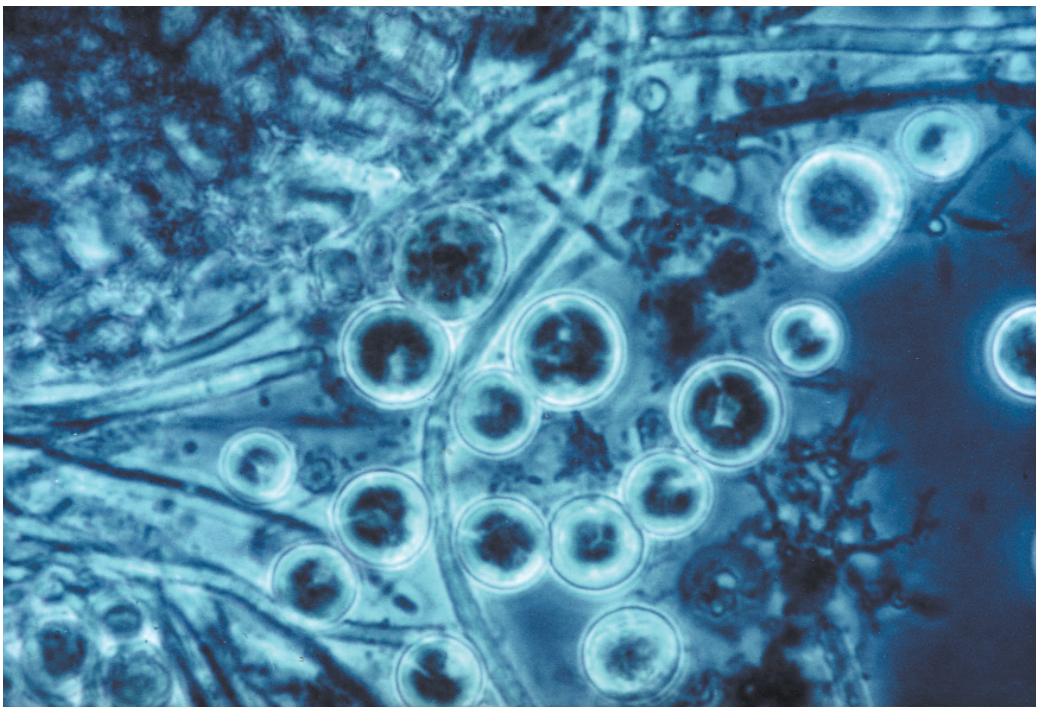

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A new methodology for decontamination of dental instruments by an ultrasonic cleaner based on Sweep System Technology

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Abstract - The efficiency of a decontamination procedure by sonication for different dental instruments after experimental microbial and viral contamination was tested. Both germicidal and virucidal activity of sonication in the presence or absence of a cationic biobiguanid disinfectant was assessed following three different disinfection/sterilisation protocols. Dental instruments were contaminated with a mixed culture of *Enterococcus faecium*, *Staphylococcus* sp., *Pseudomonas aeruginosa*, *Mycobacterium* sp., *Escherichia coli* and *Bacillus subtilis,* or with Poliovirus type 1 and Herpesvirus simplex type 1 (HHV-1), exposed to ultrasonic treatment in an ultrasonic bath and the surviving microorganisms titered. The results showed that an effective disinfection of dental instruments, expressed by an equal or higher than 4 logs microbial and viral reduction, can be obtained after 15 min or 10 min sonication in an ultrasonic cleaner equipped with a Sweep System Technology. Conversely, by the combined action of chemical disinfection and ultrasonic treatment in the same device, a sterilising effect was obtained after only 5 min for microbial and 10-15 min for virally contaminated instruments. The synergistic effect of chemical and physical means, as already accepted as an effective cleaning procedure of medical instruments, can therefore be applied to obtain a safe and effective sterilisation of dental instruments potentially contaminated by organic fluids and dental material harbouring pathogenic microbes and viruses.

Key words: disinfection, sterilisation, ultrasounds, dental instruments, ultrasonic cleaner.

INTRODUCTION

The control of the transmission of infectious agents in practical medicine as well as in the practice of dentistry has become a very critical issue since the early 1980s, when Human Immunodeficiency Virus (HIV), Creutzfeldt-Jacob disease-variant (vCJD) and Human Hepatitis C Virus (HCV) were found to be

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transmitted by medical devices contaminated with human material (Will and Matthews, 1982; Smith *et al*., 2002). Since all patients can represent a potential source of infection (Ingrosso *et al*., 1999), it is necessary to adopt an appropriate protocol for the decontamination, cleaning and sterilisation of instruments used for each patient (Burkhart and Crawford, 1997). In particular, the treatment of dental instruments to be reused must follow a standardised protocol that provides subsequent steps of decontamination, cleaning and sterilisation. The decontamination procedure is mandatory when instruments are used in patients who have a case history of serious infectious diseases such as viral hepatitis, AIDS (Lewis and Arens, 1995) and tuberculosis. After decontamination, the cleaning of the instruments is also an obligatory step, because, if not performed correctly, it may compromise the final process of sterilisation (Stach *et al*., 1995). Indeed, the removal of organic material before sterilisation is essential (Burkhart and Crawford, 1997), since the presence of organic debris can protect the microorganisms from inactivation. The last step is the sterilisation or disinfection that depends on the nature and utilisation of the instruments. It is therefore important to consider that the incorrect execution of even a single phase of the process can influence the final result (Sanchez and MacDonald, 1995).

Depending on their utilisation, it is important to evaluate the risk of transmission of infectious material by non-disposable recycled dental devices that have been used on patients and managed by auxiliary personnel but not adequately pre-decontaminated, disinfected or sterilised.

The cleaning of instruments is therefore essential to make disinfection and sterilisation procedures effective, and to protect auxiliary personnel from potential cross-infections. A wide variety of chemicals were used, alone or in combination (Angelillo *et al*., 1998; Jatzwauk *et al*., 2001) with other physical methods like ultrasounds (Weller *et al*., 1980, Watmough, 1994; Cafruny *et al*., 1995; Bettner *et al*., 1998; Walmsley, 1998; Filho *et al*., 2001) to clean instruments and equipment, but their appropriate use was never defined (Miller *et al*., 1993, 2000). Recently, a new ultrasonic apparatus (SONICA® ultrasonic cleaner), equipped with a system of modulation frequency ranging between 43 and 45 kHz delivered by two separate transducers, was developed and commercialised by Soltec®, with the aim to improve the process of decontamination/sterilisation of medical and dental devices.

The aim of this study was to evaluate the efficacy of a new method based on the combined effect of chemical and ultrasound treatment for disinfection of dental instruments experimentally contaminated by human pathogenic bacteria and viruses.

MATERIALS AND METHODS

The ultrasonic apparatus. The ultrasonic bath cleaner was the SONICA 2200EP Sweep System, an ultrasonic device developed and commercialised by Soltec® company (Milano, Italy) to clean and disinfect/sterilise medical and dental instruments by the combined use of a chemical disinfectant and ultrasounds. The ultrasonic waves are delivered from the bottom of the unit with a frequency of 43-45 kHz by two separate transducers delivering a total of 130

Watts. To improve the disinfection's procedure the apparatus can also warm up the cleaning solution by a resistance of 305 Watts fitted under the bottom panel.

The SONICA® CL 4% is a cleaning/disinfectant solution with a pH of 6.5-7.5 composed of 15% cetrimide, 1.5% chlorexidine gluconate, 6% isopropyl alcohol, and 0.1% E110 in water. As specified by the manufacturer, the solution was utilised at 2% in sterile distilled water warmed up to 40 °C in the SONICA 2200EP Sweep System ultrasonic device.

Bacterial strains and growth conditions. *Enterococcus faecium*, *Staphylococcus* sp., *Pseudomonas aeruginosa*, *Mycobacterium* sp., *Escherichia coli* and *Bacillus subtilis* strains were utilised to perform the bacterial contamination of the instruments. These strains were grown overnight at 30 °C in Luria-Bertani (LB) medium. As *Mycobacterium* sp. growth was slow, this strain was grown in LB medium added with glucose 0.2% and incubated until the cultural optical density reached the same value as the cultures of other bacteria. *Bacillus subtilis* was used to provide cultures with a high percentage of spores.

Viral strains and cell cultures. The attenuated Poliovirus type 1 (Sabin vaccine strain) and the clinical isolate of Human Herpes simplex virus type 1 (HHV-1) we used are a RNA non-enveloped and a DNA-enveloped human viruses respectively. They are characterised by a high replicative activity in a broad range of human and animal cells. These viruses are the prototype viruses generally used in antiviral tests for their relative resistance to common disinfectants. Both viruses were grown on confluent mycoplasma-free green monkey kidney cells (VERO), plaque purified and titred on the same cells.

Dental instruments contamination. Sets of dental instruments (dental pincers and tongs) were contaminated with: 1) a mixed bacterial culture containing at the same ratio *Enterococcus faecium*, *Staphylococcus* sp., *Pseudomonas aeruginosa*, *Mycobacterium* sp., and *Escherichia coli*; 2) the endospore-forming *Bacillus subtilis*; 3) the RNA-virus Polio1; 4) the DNA-virus HHV-1.

The dental instruments were immersed at room temperature in the bacterial suspensions, 10^9 Colony Forming Units (CFU)/ml in LB medium, for 1 h or in the viral suspensions only for 5 min to minimise the spontaneous viral inactivation.

For viral contamination, 10 ml of Dulbecco Modified Essential Medium (DMEM), supplemented with 10% newborn calf serum (CS) to mimic the protein content of biological material, was inoculated with 10^9 Plaque Forming Units (10^8 PFU/ml) of Poliovirus1 or 10^{10} PFU (10^9 PFU/ml) of HHV-1.

The dental instruments were then drained away for 15 s and then utilised for three different cleaning/disinfection procedures called Protocol A, B, and C.

Protocol A - Chemical decontamination without ultrasonic treatment. The contaminated instruments were placed into the decontamination tank containing 1000 ml of 2%-diluted SONICA® CL 4% disinfectant, with an initial number of 10^6 CFU/ml mixed bacterial cells or *Bacillus subtilis*, or 4.25 x 10^5 PFU/ml Poliovirus1, or 2.2 x 10^6 PFU/ml HHV-1. The treatment was maintained for 30 min at 40 °C without sonication. Samples were collected at 0, 5, 15, 30 min and their residual bacterial or viral population determined.

Protocol B - Ultrasonic treatment. In order to evaluate the effect of sonication on the bacterial and viral population in the absence of a preliminary disinfection procedure, the contaminated instruments were placed into 1000 ml of 10 mM Phosphate Buffered Saline (PBS) in the ultrasonic bath set at 40 °C. The treatment was maintained for 30 min. Samples were collected and analysed at 0, 5, 15, 30 min.

Protocol C - Chemical decontamination combined with ultrasonic treatment. The contaminated instruments treated by chemical decontamination for 5 min in the decontamination tank (Protocol A) were therefore immersed in the ultrasonic bath containing the same disinfection solution at 40 °C. The treatment was maintained for 30 min. Samples were collected and analysed at 0, 5, 15, 30 min.

Bactericidal activity test. The number of living and dead bacteria, before and after the three treatments indicated in the A, B, C protocols, was determined either by plate counting or by fluorescence microscopy.

Plate counts. Aliquots (1 ml) of samples were diluted in M9 Mineral Medium and appropriate dilutions were placed on plate agar containing LB medium. The samples were incubated at 30 °C for 24 h. The number of viable bacteria (CFU/ml) were determined at the different times of treatment.

Fluorescence microscopy. The counts of total bacteria were performed in the presence of the fluorochromes SYBR Green I and propidium iodide, emitting light after excitation in the green and red fluorescence, respectively. SYBR Green is a molecule, which stains all the cells, either dead or alive. Conversely, propidium iodide can penetrate only cells with a damaged membrane. It is therefore possible to distinguish the living (green) cells from the dead (red) ones. For cell staining, 10 µl of each fluorochrome in 1 ml of culture sample were utilised. For each treatment, several samples were analysed by an epifluorescent microscope to identify and count the differently stained bacteria. The observation of the samples was performed with an Axiolab HB050 Zeiss, equipped with a high-pressure mercury bulb and a filter set type UV-2A EX 330-380 and G2AEX510-560.

Virucidal activity test. Virucidal activity was evaluated by the reduction of at least 99.99% infectivity (4 logs) of test viruses after treatments as in A, B, and C protocols.

At the end of each protocol the instruments were “washed” in 10 ml of DMEM 10% CS for 5 min and the residual virus in the medium titred on confluent VERO cells. As a control, the virus present on the instruments immediately after contamination was also titred.

RESULTS

Sets of dental instruments were contaminated with a mixed bacterial culture containing *Enterococcus faecium*, *Staphylococcus* sp., *Pseudomonas aeruginosa*, *Mycobacterium* sp., and *Escherichia coli*, or the endospore-forming *Bacillus subtilis*. Similarly, identical sets of dental devices were contaminated with

the RNA-virus Polio1 or the DNA-virus HHV-1. The dental instruments were then immersed in the bacterial suspension for 1 h or in the viral suspension for only 5 min to minimise the spontaneous viral inactivation. The contaminated instruments were then submitted to three different cleaning/disinfection procedures called Protocol A, B, and C as described in Methods.

Chemical decontamination without ultrasonic treatment (Protocol A)
Results of bacterial number after decontamination are reported in Table 1. Data show that the total bacterial number decreased of about 3 logs after 5 min of treatment and, after 15 min, the complete absence of living cells was observed.

The titration of Poliovirus1 and HHV-1 before and after treatment was done in triplicate on confluent VERO cells. The effect of chemical disinfection on the viruses present on contaminated instruments parallels the results obtained with bacteria. In particular, a uniform 3-logs decrease was observed after 5 min treatment and a complete virus inactivation after 15 min.

Ultrasonic treatment (Protocol B)
The results of the effect of sonication on the bacterial and viral population in the absence of a preliminary disinfection procedure are reported in Table 2. Sonication alone can only partially reduce the microbial population from 10^6 to 10^2 CFU/ml, thus suggesting the need of a chemical disinfectant to support and complement the physical action of sonication in killing of resistant microorganisms.

The ultrasound treatment alone resulted however very effective to inactivate both RNA (Polio 1) and DNA (HHV-1) containing viruses. In fact, the virus titer dropped by 3-logs after 5 min, and was reduced to zero after 15 min of sonication.

Chemical decontamination combined with ultrasonic treatment (Protocol C)
The results obtained by protocols A and B suggested a subsequent evaluation of the combined effect of chemical and ultrasound treatments. The result of the combined chemical/physical treatment on bacteria and viruses survival is reported in Table 3.

The enumeration of living microorganisms, after 5 min sonication of the contaminated instruments, revealed the complete inactivation of the bacterial mixture and *Bacillus subtilis*.

Moreover we could observe that when the experiments were performed with cultures of *Bacillus subtilis* in which most cells were spores, the contribution of ultrasound treatment to their inactivation was important. In this case, as shown in Table 2, a relevant part (8×10^3 CFU/ml) of bacteria remained viable in the solution still after 15 min, and only the effect of the ultrasonic bath determined their complete inactivation (Table 3, Fig. 1). A control experiment with cells incubated after this treatment was also performed to exclude that spores could become vegetative forms (data not shown).

The procedure outlined in Protocol C showed high efficacy on instruments contaminated by viruses. A 1-log reduction after 5 min and a complete sterilisation after 15 min were observed with dental instruments carrying the low but significant amount of virus left from the disinfection procedure.

TABLE 1 – Efficacy of chemical disinfection on bacterial and viral population (Protocol A)

Bacterial number (CFU/ml)												
Treatment time (min)	0			5			15			30		
	P. C.*	F. M.§		P. C.*	F. M.§		P. C.*	F. M.§		P. C.*	F. M.§	
		Green	Red		Green	Red		Green	Red		Green	Red
Bacterial mixture	1×10^6	1×10^6	0	2×10^3	1×10^3	9×10^5	0	0	1×10^6	0	0	1×10^6
Bacillus subtilis	1×10^6	1×10^6	0	1×10^6	1×10^6	0	0	0	1×10^6	0	0	1×10^6
Virus titer (PFU/ml)												
Treatment time (min)	0			5			15			30		
Poliovirus 1	4.25×10^5			1×10^2			0			0		
HHV-1	2.2×10^6			1×10^3			0			0		

*P.C. = Plate Counting; §F.M. = Fluorescence Microscopy.

TABLE 2 – Efficacy of ultrasound treatment on bacterial and viral population (Protocol B)

Bacterial number (CFU/ml)												
Treatment time (min)	0			5			15			30		
	P. C.*	F. M.§		P. C.*	F. M.§		P. C.*	F. M.§		P. C.*	F. M.§	
		Green	Red		Green	Red		Green	Red		Green	Red
Microbial mixture	9×10^6	9×10^6	0	9×10^3	8×10^3	8×10^6	9×10^2	8×10^2	9×10^6	8×10^2	7×10^2	9×10^6
Bacillus subtilis	1×10^6	1×10^6	0	1×10^3	1×10^3	9×10^5	8×10^3	1×10^2	9×10^5	1×10^2	1×10^2	9×10^5
Virus titer (PFU/ml)												
Treatment time (min)	0			5			15			30		
Poliovirus 1	4.25×10^5			2×10^2			0			0		
HHV-1	2.2×10^6			2.5×10^3			0			0		

*P.C. = Plate Counting; §F.M. = Fluorescence Microscopy.

TABLE 3 – Efficacy of combined chemical and ultrasound treatment on bacterial and viral population (Protocol C)

Bacterial number (CFU/ml)															
Treatment time (min)	Initial bacterial number			0#			5#			15#			30#		
	P. C.*	F. M.§		P. C.*	F. M.§		P. C.*	F. M.§		P. C.*	F. M.§		P. C.*	F. M.§	
		Green	Red		Green	Red		Green	Red		Green	Red		Green	Red
Microbial mixture	1×10^6	1×10^6	0	2×10^3	1×10^3	9×10^5	0	0	1×10^6	0	0	1×10^6	0	0	1×10^6
Bacillus subtilis	1×10^6	1×10^6	0	1×10^6	1×10^6	0	0	0	8×10^5	0	0	7×10^5	0	0	7×10^5

Virus titer (PFU/ml)				
Treatment time (min)	Initial virus number	0	5	15	30
Poliovirus 1	4.25×10^5	1×10^2	1.7×10^1	0	0
HHV-1	2.2×10^6	1×10^3	1.1×10^1	0	0

*P.C. = Plate Counting; §F.M. = Fluorescence Microscopy; #Time (min) after 5 min chemical disinfection.

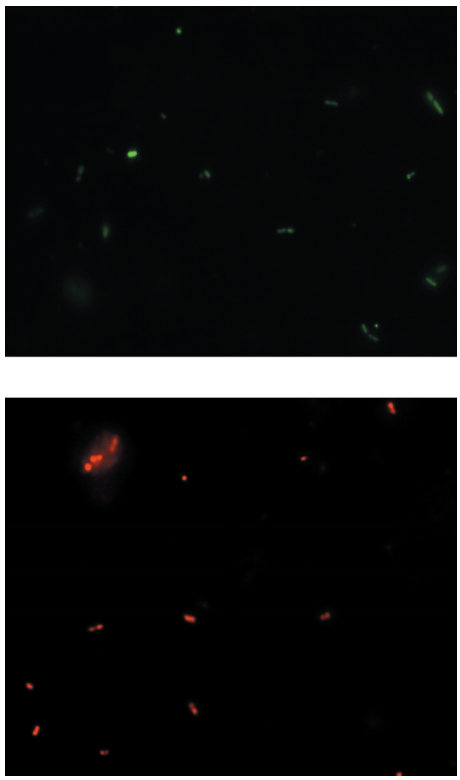

FIG. 1 – Fluorescence microscopy showing living (green) and dead (red) bacteria present in the solution of contaminated instruments before (A) and after (B) 15 min of treatment with Protocol C.

DISCUSSION

Ultrasounds have been utilised for long time as an effective means to clean surgical instruments (Weller *et al*., 1980) and, in particular, dental devices (Walmsley, 1998) before sterilisation. Although the virucidal effect of high frequency sound waves on tobacco mosaic virus was previously shown (Oster *et al*., 1947), our results demonstrated that the disinfectant solution we tested is able to inactivate different bacteria as well as non-enveloped RNA and enveloped DNA viruses after 15 min. Particularly resistant bacteria as *B. subtilis* need more time or a different treatment because Protocol A is unable to kill viruses or microbial cells in less than 15 min. Treatment with ultrasounds with or without the chemical disinfectant SONICA® CL 4% in the cleaning solution (Protocol B) was equally efficient with viruses although requesting more time (over 30 min) to completely inactivate bacteria. Conversely, the combined procedure (Protocol C) of a disinfectant and ultrasounds completely inactivates viruses and bacteria after 15 min of treatment.

On the basis of these results, we demonstrated that this methodology, based on the innovative Sweep System Technology delivering a homogeneous frequency of 43-45 kHz by two separate transducers, coupled to the use of a mild chemical disinfectant at 40 °C, was able to inactivate bacteria and viruses from experimentally contaminated instruments and confirm the general effectiveness of ultrasounds in cleaning dental instruments (Cafruny *et al*., 1995; Bettner *et al*., 1998).

Protocol C, which we found to be the only one able to sterilise in less than 15 min the contaminated dental devices, should therefore be utilised by all dental practitioners during their clinical interventions on patients of unknown case history.

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