The *125L* Ozone Sterilizer

The Sterilization Technology for the 21st Century

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125L OZONE

STERILIZER

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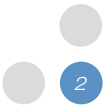
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1.0 PREFACE

The *125L* Ozone Sterilizer uses a new and unique ozone sterilization process developed by TSO_3 Inc. This sterilizer produces its own sterilant from USP grade oxygen, water and electricity.

The duration of the sterilization cycle is about 4 hours and 30 minutes, and occurs at temperatures between 30.8 and 36°C (87.4 to 97°F), just slightly above room temperature. The sterilization process is compatible with the requirements of delicate, heat-sensitive surgical and diagnostic instruments. Additionally, a variety of steam-sterilizable instruments can also be processed in the *125L* Ozone Sterilizer.

Microbial efficacy has been demonstrated by achieving a minimum SAL of 10^{-6} with a variety of microorganisms, including the most resistant microorganism: *Geobacillus stearothermophilus*, formerly known as *Bacillus stearothermophilus*. Furthermore, the official test to determine sporicidal activity of liquid and gaseous chemical disinfectants recommended by the Association of Official Analytical Chemists was completed successfully. The ability of the process to sterilize hinges and lumen devices was also demonstrated.

TSO_3's *125L* ozone technology uses an effective sterilization agent, is safe for employees and patients, and does not harm the environment.

2.0 INTRODUCTION

TSO_3 has developed a new technology (ozone sterilization) which is a viable alternative to current technologies. The process was developed to sterilize the new generation of surgical and diagnostic instruments, made of heat-sensitive materials, especially polymers. The Ozone Sterilizer operates at near ambient temperature, thus allowing it to overcome the thermal limitations of polymeric instruments.

Ozone has been recognized as a safe disinfectant for water and food. It may be safely used in gaseous and aqueous phases as an antimicrobial agent for the treatment, storage, and processing of foods, including meat and poultry.

Ozone sterilization does not require the handling of dangerous gas cylinders, and poses no threat to the environment or the user's health. The *125L* Ozone Sterilizer generates its sterilant on-site using USP grade oxygen and water. The sterilizer is connected to the hospital's existing oxygen network or, if mobility is required, to portable oxygen cylinders. Because ozone gas is created in an enclosed ozone generator within the unit, no sterilant handling is required.

The *125L* ozone cycle is composed of two identical half cycles. After the instruments have been loaded into the chamber, the door is closed and the cycle is started. A vacuum is drawn followed by a humidification phase. Ozone is then injected into the chamber and the sterilization process begins. When the half cycle has been reached, the steps, from the vacuum to the ozone injection phases, are again repeated and followed by a final ventilation phase to remove ozone from the chamber and packaging.

At the end of the sterilization cycle, the ozone is transformed into oxygen. There are no toxic or hazardous residues or waste products associated with the process, only oxygen and purified water.

The ozone process is compatible with most reusable medical items currently sterilized by other oxidative processes, ethylene oxide, peracetic acid or steam. Sterilizing sealed ampoules, liquids, natural rubber, latex, and fabrics are not recommended. Sterilization of implants and flexible endoscopes has not been validated.

Preparation of instruments for sterilization should follow the Canadian Standard Association (CSA) and Association of periOperative Registered Nurses (AORN) recommendations or guidelines from other regulatory or professional organizations. The instruments to be sterilized must be cleaned according to the instrument manufacturer's recommendations before being placed in the sterilizer. Follow infection control practices and hospital central processing department procedures for washing and rinsing the instruments before sterilization.

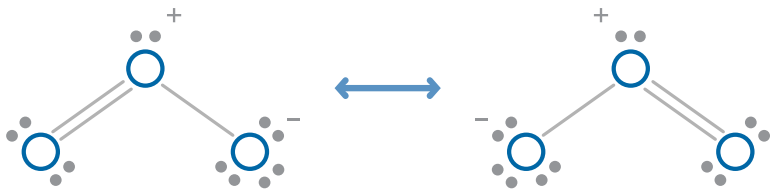
The ozone-based process is compatible with current packaging such as nonwoven material/polyethylene pouches and commercially available anodized aluminum containers using disposable filters.

2.1 MODE OF ACTION

Ozone, an allotropic form of oxygen, is a molecule comprised of three oxygen atoms, whose chemical symbol is O_3 (Figure 1). In normal conditions of use, ozone is in a gaseous state and soluble in water. It is a powerful oxidant (the reduction potential for the half-cell reaction $O_3 + 2H^+ + 2e^- \rightarrow O_2 + H_2O$ is 2.07 V) and is chemically unstable in gas and liquid mixtures. Fluorine (F_2/F^-, 2.87 V), fluorine monoxide (F_2O/F^-, 2.15 V), and atomic oxygen (O/H_2O, 2.42 V) are examples of chemical molecules that have a reduction potential higher than ozone. However, they are either extremely toxic or nearly impossible to use or generate. Oxidoreduction potential of hypochlorite ($HClO / Cl^-$) is 1.49 V and the potential of hydrogen peroxide (H_2O_2/H_2O) is 1.78 V.

In its pure state, ozone is a pale blue gas. At room temperature, ozone is a colorless gas that can easily be detected because of its pungent smell when present at levels of 0.003 to 0.01 ppm, well below the acceptable level of 0.1 ppm. Ozone is 1.66 times heavier than air and will tend to stay near the ground. The UV spectrum shows a single broad absorption at 254 nm and is frequently used as a means to measure the production of ozone in generators.

FIGURE 1. DIAGRAM OF THE RESONANCE MOLECULAR STRUCTURE OF OZONE



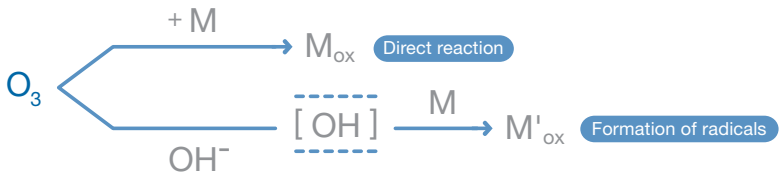
In an aqueous solution, ozone reacts in two different ways (Figure 2):

1. Directly, the ozone molecule is highly selective.
2. Indirectly, due to the action of secondary species reacting like free radicals and formed by the decomposition of ozone on contact with water. These free radicals are not selective. The term "free radical" is used to explain a physical reality of the sequence of reactions. During certain chemical reactions involving the breaking of bonds, there is a transitory formation of short-lived highly unstable neutral entities that carry an unpaired electron called a "free radical".

In the presence of water vapor, the oxygen atom produced by the decomposition of ozone will react with a molecule of water to form hydroxyl radicals as in the following equation:

$$O + H_2O \rightarrow 2HO$$

FIGURE 2. REACTIVITY OF OZONE IN AQUEOUS MEDIUM (M: UNDEFINED COMPOUND)



2.2 KINETICS OF THE STERILIZATION MECHANISM

In the early 1900s, Dr. Harriet Chick described a method for estimating the destruction of microorganisms by chemical disinfectants. She postulated that the microbial mortality would follow first-order kinetics. If the reaction conforms to Chick's law, the data will give a straight line on a semi-logarithmic graph. This law works for all liquid disinfectants and for many sterilization processes. Chick's law has evolved into what is now referred to as D-value.

The TSO_3 ozone sterilization process follows this law. The mortality of microorganisms is linear on a semi-logarithmic graph. However, the critical parameter for ozone is not time, as in steam or ethylene oxide sterilization processes, but rather the ozone dose (expressed as a concentration in mg/L) injected into the chamber. This process can be compared to the radiation process, where a minimal dose is needed to achieve the sterility assurance level.

2.3 OZONE PRODUCTION

Due to its thermodynamic properties, ozone is a metastable product: it decomposes slowly (in minutes) at ambient temperatures and rapidly (in seconds) at higher temperatures. Ozone is produced naturally from oxygen in the upper atmosphere by the absorption of ultraviolet radiation. Passing air or pure oxygen across an electrical field can artificially generate ozone as it occurs in nature when lightning discharges pass through the air.

Ozone is metastable, and is therefore generated as needed within the sterilizer. For an industrial applications scale, ozone is produced by electrical discharges in tubular generators which are basically comprised of two conducting electrodes. Dry-compressed air or oxygen is passed between these two electrodes where it is subjected to a high-voltage alternating current field. Some of the oxygen is transformed into ozone according to the following equation:

$$3\ O_2 \rightarrow 2\ O_3$$

3.0 MICROBIAL EFFECTIVENESS

The efficacy of the *125L* Ozone Sterilizer is established by demonstrating that the process can:

1. Achieve an overkill sterilization as demonstrated by testing a large number of challenge microorganisms recognized as being highly resistant to a variety of sterilants (i.e., to achieve a minimum SAL of 10^{-6} equivalent to a 12 logs total reduction at the end of the cycle).
2. Pass the AOAC sporicidal test, as defined by the American Association of Official Analytical Chemists.
3. Sterilize medical devices (non-metallic and metallic materials) in simulated use tests.
4. Sterilize long, narrow lumens (section 3.5).
5. Sterilize actual medical devices in hospital loads (referred to as “in-use tests”).
6. Achieve and maintain the required physical/chemical process lethality conditions within specifications as demonstrated in repeated runs and in different units.

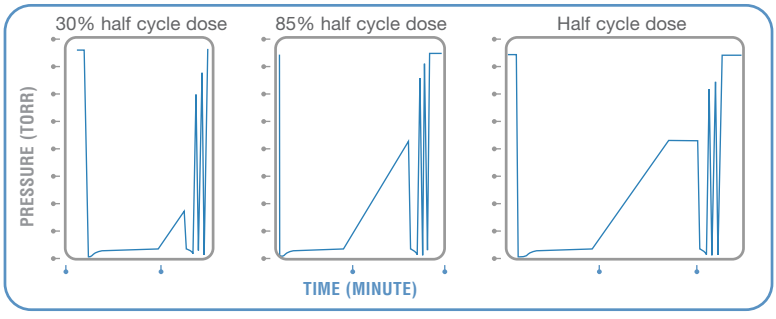
3.1 DEMONSTRATION OF 10^{-6} STERILITY ASSURANCE LEVEL (SAL)

An overkill approach was used for the determination of the highly resistant organism, based upon an initial concentration of at least 10^6 CFU (colony-forming units) of the recommended test organisms.

For the TSO_3 ozone sterilization process, the concentration of ozone injected into the chamber (called “dose”) is the parameter that is controlled to obtain different levels of microorganism survival. It represents the “power” of the process, or the lethal stress of the process.

In an experimental setup, the process is carried out so that the total concentration of ozone injected into the chamber (“dose”) in order to obtain several lethal stress levels. By varying the concentration of ozone injected into the chamber, a partial to complete lethality of microorganisms is achieved (Figure 3). The dose is expressed as a concentration of ozone in milligrams per net-chamber volume (mg/L). Consequently, the D-value for the Biological Indicator will be expressed in mg/L and not in minutes.

FIGURE 3. PRESSURE-TIME GRAPHS (PARTIAL CYCLES) FOR THREE D-VALUE POINTS OBTAINED BY VARYING THE OZONE DOSE ADMITTED INSIDE CHAMBER



3.1.1 SELECTION OF VALIDATION ORGANISM

The biological lethality profile has been exhaustively evaluated for the test organisms identified in Table 1.

TABLE 1. TEST ORGANISMS FOR NON TRADITIONAL STERILIZERS

A. BACTERIAL SPORES	B. VEGETATIVE BACTERIA
Bacillus atrophaeus var. *niger* ATCC 9372 formely known as *B. subtilis var. niger* *Geobacillus stearothermophilus* ATCC 7953 formerly known as *Bacillus stearothermophilus* *Clostridium sporogenes* ATCC 3584	*Staphylococcus aureus* ATCC 6538 *Salmonella choleraesuis* ATCC 10708 *Pseudomonas aeruginosa* ATCC 15442
C. MYCOBACTERIA	**D. FUNGI**
Mycobacterium terrae ATCC 15755	*Trichophyton mentagrophytes* (with conidia) ATCC 9533
E. NONLIPID VIRUS	**F. LIPID VIRUS**
Poliovirus Type II VR-301	Herpes simplex VR-260
G. ADDITIONAL TEST ORGANISM SUGGESTED IN LITERATURE	
Bacillus pumilus ATCC 27142	

3.1.2 EVALUATING THE D-VALUE

The D-value was evaluated for each microorganism of Table 1, except viruses. Paper strip carriers inoculated with the microorganism only, or with a mixture of the microorganism and hard water (400 ppm) or 10% serum were used. Contaminated carriers were placed in a 10-centimeter glass tube then distributed in a validation load.

The validation load used was a full, mixed load composed of medical instruments made of metal and polymers, some in pouches, some in anodized sterilization aluminum containers (Figure 4).

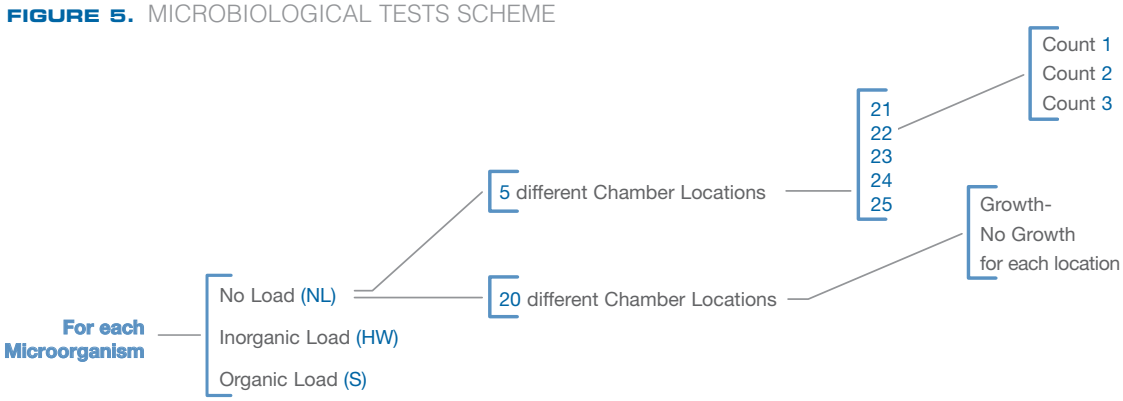
FIGURE 4. VALIDATION LOAD

Note: Some medical devices are not compatible with the process.

Microbial loads were placed in five different chamber locations for the estimation of the survivor curve by direct count (between 10^6 and 10^2 survivors) and in 20 different chamber locations for the estimation of the fraction negative (between 10^2 and 10^{-2} survivors).

For the survivor curve, the number of survivors was enumerated by plate count technique. Analyses were performed to estimate a survivor curve of the microorganisms and a D-value as a function of the dose in mg/L. For the fraction negative analysis, inoculated carriers were directly transferred to growth medium. D-values were calculated using the Stumbo-Murphy-Cochran and the limited Holcomb-Spearman-Karber methods (Pflug 1999) (Figure 5).

FIGURE 5. MICROBIOLOGICAL TESTS SCHEME



3.2 DETERMINATION OF MOST RESISTANT ORGANISM, BASED ON THE D-VALUE

The vegetative bacteria *S. aureus*, *Sal. choleraesuis* and *P. aeruginosa* are all killed (8 logs) at a dose less than 20% of the ozone dose injected in a half cycle even in the presence of organic matter.

The fungus, *T. mentagrophytes*, is slightly more resistant, especially when exposed in the presence of serum. *T. mentagrophytes* populations are reduced by approximately 8 logs in less than a quarter of the sterilization cycle (around 35% of a half cycle dose).

The mycobacteria are considered the most resistant microorganisms among the non-spore forming group. *M. terrae* has been shown to have a resistance approximately the same as *M. bovis* and *M. tuberculosis* in other sterilization processes. With ozone, the addition of serum does not increase the resistance of *M. terrae*. An ozone dose of less than 60% of a half cycle dose is sufficient to kill 8 logs of cells.

Among the spore-forming bacteria, spores of the obligate anaerobe *C. sporogenes* are less resistant to ozone than are the spores of aerobic species tested. This is most likely due to their sensitivity to oxygen and other oxidative products found during ozone exposure.

G. stearothermophilus has the highest D-values, when calculated using the survivor curve and fraction negative analysis, regardless of the type of load used.

Moreover, it is observed that the dynamics of *G. stearothermophilus* kill follows first order kinetics against the dose with an r^2 value (coefficient of determination) of more than 0.9 (Figure 6). The presence of organic matter significantly increases the challenge of *G. stearothermophilus* spores to the sterilization process. Under these conditions *G. stearothermophilus* spores are the most resistant of all species tested, including *M. terrae.* Indeed, all the spore forming bacteria are considerably more resistant to ozone sterilization than non-spore forming microorganism.

Results of the microbial efficacy tests of the ozone sterilizer on microorganisms are summarized in Table 2.

FIGURE 6. KILL CURVE BY PLATE COUNT AND FRACTION NEGATIVE ANALYSIS

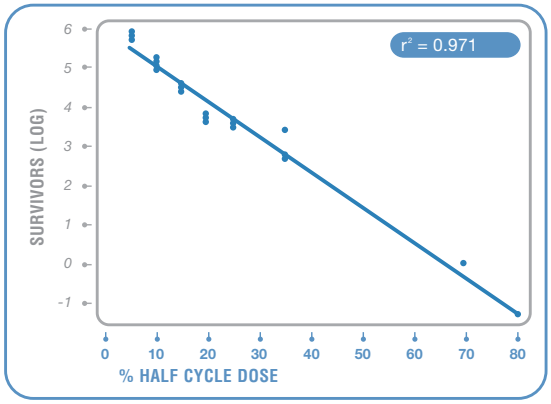


TABLE 2. MICROBIAL EFFICACY OF THE OZONE STERILIZER ON MICROORGANISMS EXPOSED TO THEIR KILLING DOSE (LESS THAN THE HALF CYCLE)

MICROORGANISMS	CONTROL CFU*	RESULTS (# POSITIVE / # SAMPLE) NO LOAD	HARD WATER	SERUM
VEGETATIVE BACTERIA				
Pseudomonas aeruginosa	$1.34x10^6$	0/20	0/20	0/20
Staphylococcus aureus	$2.23x10^6$	0/20	0/20	0/20
Salmonella choleraesuis	$1.07x10^6$	0/20	0/20	0/20
FUNGI WITH CONIDIA				
Trichophyton mentagrophytes	$1.11x10^6$	0/20	0/20	0/20
MYCOBACTERIA				
Mycobacterium terrae	$1.12x10^6$	0/20	0/20	0/20
SPORE-FORMING BACTERIA				
Clostridium sporogenes	$0.79x10^6$	0/20	0/20	0/20
Bacillus atrophaeus	$1.18x10^6$	0/20	0/20	0/20
Bacillus pumilus	$1.97x10^6$	0/20	0/20	0/20
Geobacillus stearothermophilus	$1.38x10^6$	0/20	0/20	0/20

** CFU: colony-forming unit*

Geobacillus stearothermophilus was chosen to be the reference microorganism and Biological Indicator for the sterilization process as it represents the greatest challenge over the other microorganisms tested, and as it demonstrates a linear lethality relation to the critical process parameter "dose".

3.2.1 VIRUCIDAL EFFICACY

The effects of sterilization on $TCID_{50}$ (tissue culture infectious $dose_{50}$) values for both viruses tested are shown in Table 3. The reduction titer in log was calculated to be ≥ 3.8 for Poliovirus type 2 and ≥ 2.8 for Herpes simplex type 1.

TABLE 3. RESULTS OF OZONE STERILIZATION PROCESS *125L* AGAINST TEST VIRUSES : EFFECT ON $TCID_{50}$

TEST VIRUS	TEST	INFECTIVITY
Poliovirus type 2	#1	Not detected
	#2	Not detected
Herpes simplex type 1	#1	Not detected
	#2	Not detected

3.3 AOAC SPORICIDAL TEST

The AOAC sporicidal screening test, as defined by the American Association of Official Analytical Chemists, was conducted to confirm the sporicidal effectiveness of the chemical sterilant to sterilize different types of porous carriers contaminated with resistant aerobic and anaerobic spores, in the presence of organic soil and inorganic salts. The test stipulates that no failures can be tolerated for sterilizing claims.

The results of this study, in Table 4, show that no growth was obtained for any AOAC inoculated carriers when exposed to a complete cycle in the *125L* Ozone Sterilizer for more than three sterilant "lots". Since ozone gas is created in an enclosed ozone generator within the unit as needed, each cycle can be considered as a new sterilant "lot".

TABLE 4. AOAC SPORICIDAL SCREENING TEST RESULTS WITH THE *125L* OZONE STERILIZER

TEST NUMBER	CARRIER	# POSITIVE / # TESTED *B. SUBTILIS*	# POSITIVE / # TESTED *C. SPOROGENES*
1	Porcelain penicylinder	0/60	0/60
	Silk suture loops	0/60	0/60
2	Porcelain penicylinder	0/60	0/60
	Silk suture loops	0/60	0/60
3	Porcelain penicylinder	0/60	0/60
	Silk suture loops	0/60	0/60

3.4 SIMULATED USE TESTS

Once the process parameters are established to achieve a SAL of 10^{-6}, the effectiveness of the process must be confirmed by simulated use tests.

3.4.1 MATERIALS STUDY

The goal of this study was to verify that materials used in medical devices could be sterilized in the *125L* Ozone Sterilizer. To do so, material samples inoculated with more than $1x10^6$ *Geobacillus stearothermophilus* spores mixed with organic or inorganic load were placed in a validation load condition.

Simulated use tests performed on inoculated material samples have demonstrated the ability of the ozone sterilization process to sterilize a wide range of material (Table 5).

TABLE 5. SUMMARY OF SIMULATED USE TEST RESULTS

MATERIALS	# POSITIVE / # TESTED 10% SERUM	HARD WATER
Polyamide	0/3	0/3
Polyetherimide	0/3	0/3
Polyethylene high density	0/3	0/3
Polyethylene low density	0/3	0/3
Polymethyl methacrylate	0/3	0/3
Polypropylene	0/3	0/3
Polystyrene	0/3	0/3
Polyvinyl chloride	0/3	0/3
Polytetrafluoroethylene	0/3	0/3
Silicone	0/3	0/3
Stainless steel 316L polished	0/3	0/3
Stainless steel 316L unpolished	0/3	0/3

3.4.2 INSTRUMENT STUDY

The potential of the *125L* Ozone Sterilizer to sterilize complex medical instruments was also evaluated. Medical instruments were chosen due to their design rather than their materials content and were inoculated with 10^6 spores of *G. stearothermophilus* mixed with 5% serum. More than one site was selected on each instrument tested. After inoculation, the instrument were packaged and sterilized. Quantitative recovery was

performed on each inoculation site at least three times. The inoculum was 1.3 x 10^6 CFU for the first and second trial, and 2.2 x 10^6 CFU for the third. Results are shown in Table 6.

TABLE 6. SIMULATED-USE ON MEDICAL INSTRUMENTS USING *G. STEAROTHERMOPHILUS* SPORES MIXED WITH 5% SERUM

INSTRUMENTS	INOCULATED PART	RESULTS (CFU RECOVERED)
FLEXIBLE BIOPSY FORCEPS	Jaws	0, 0, 0
	Handle hinge	0, 0, 0
	Axe hinge	0, 0, 0
	Forceps mechanism	0, 0, 0
BRONCHOSCOPE TUBE UNIVERSAL	Inside lumens	0, 0, 0
DEFLECTING DEVICE	Articulated end	0, 0, 0
	Luer lock	0, 0, 0
	Pivot mechanism	0, 0, 0
OPTICAL ALLIGATOR FORCEPS	Inside lumen	0, 0, 0
	Jaws mechanism	0, 0, 0
	Luer lock	0, 0, 0
	Handle	0, 0, 0
	Lock bridge	0, 0, 0
CYSTOSCOPE STOPCOCK	Inside lumen	0, 0, 0
	Handle	0, 0, 0
HARDY SELLA PUNCH	Gliding mechanism	0, 0, 0
	Hinge	0, 0, 0
REUTER TIP DEFLECTING HANDLE	Open screw thread	0, 0, 0
	Close screw thread	0, 0, 0
ELECTROCAUTERY PEN	On/Off press button cavity	0, 0, 0
	Side slot	0, 0, 0
NASAL BIVALVE SPECULUM	Hinge	0, 0, 0
	Screw	0, 0, 0
RESECTOSCOPE SHEET	Lock bridge	0, 0, 0
BISTOURI BEAVER HANDLE	Screw cut	0, 0, 0
	Screw thread	0, 0, 0
BULL DOG CLAMP	Hinge	0, 0, 0
	Spring	0, 0, 0
	Jaws	0, 0, 0
FORCEPS MICRO CUP	Gliding mechanism and jaws	0, 0, 0
	Hinge	0, 0, 0
ANGULAR SUCTION TUBE	Inside lumen (angle)	0, 0, 0
HAEMORRHOIDAL LIGATOR	Hinge	0, 0, 0
	Circular gliding end	0, 0, 0
	Handle screw	0, 0, 0
	Handle	0, 0, 0
	Flexion mechanism	0, 0, 0
SYRINGE	Gliding system	0, 0, 0
	Screw	0, 0, 0

3.5 RIGID LUMEN DEVICES

Tests on lumen devices were done using inoculated stainless steel wires placed directly inside (in the middle of) the lumens without a fitting device, or by direct inoculation of the lumen. The carriers were inoculated with a minimum of 10^6 spores of *Geobacillus stearothermophilus*.

Rigid lumen devices packaged in sterilization pouches or rigid sterilization containers were exposed to the half cycle (Table 7A and Table 7B).

TABLE 7A. SUMMARY OF RIGID LUMEN STERILIZATION TESTING AT THE HALF-CYCLE

INTERNAL DIAMETER (mm)	LENGTH (mm)	LOG REDUCTION VALUE (LRV)
0.5*	450	> 7.27 logs
0.9	485	> 6.54 logs
1	500	> 7.24 logs
2	575	> 6.98 logs
3	650	> 6.98 logs
4	700	> 6.98 logs

* Note: This lumen claim has not been reviewed by the US Food and Drug Administration

It is possible to sterilize medical devices having a single stainless steel lumen within the following lengths and internal diameters:

TABLE 7B. SUMMARY OF RIGID LUMEN LENGTHS SUCCESSFULLY STERILIZED IN THE *125L* OZONE STERILIZER

INTERNAL DIAMETER (mm)	LENGTH (mm)
0.5*	450
0.9	485
1	500
2	575
3	650
4	700

* Note: This lumen claim has not been reviewed by the US Food and Drug Administration

For example, a uretero-renoscope with 2.5 x 3 French (0.83 x 1.0 mm) stainless steel lumen 485 mm long can be effectively processed.

3.6 IN-USE TESTS (HOSPITAL STUDY)

Instrument sterilization plays a key role in the reduction of transmission of nosocomial infection. A way to prevent infection is to understand all the factors that can interfere with the sterilization process and device reprocessing. Consequently, in-use tests were performed on previously used medical instruments, then cleaned and lubricated by hospital personnel in a routine manner. Instruments were selected on the basis of the challenge they offer to the sterilization process, packaged and sterilized in the *125L* Ozone Sterilizer.

After sterilization, sterility testing of three to five sites on each instrument was performed. Hinge, lock mechanism, handle, distal end (serrated surfaces), sliding systems, and lumens are some examples of site selection made. Survival of aerobic and anaerobic bacteria, yeast and fungi was evaluated. No surviving organisms were found on the instruments listed in Table 8.

TABLE 8. PARTIAL LIST OF INSTRUMENTS TESTED WITHIN IN-USE TESTING

INSTRUMENTS	NUMBER OF CONTAMINATED SITES / NUMBER OF SITES TESTED		
	AEROBIC BACTERIA	ANAEROBIC BACTERIA	YEAST AND FUNGI
Sponge classic holding forceps	0/4	0/4	0/4
Peers towel clamp	0/4	0/4	0/4
Gregory classic bulldog clamp	0/4	0/4	0/4
Satinsky forcep	0/4	0/4	0/4
Articulated Greenberg arm	0/4	0/4	0/4
Vaginal speculum	0/4	0/4	0/4
Biopsy forcep	0/4	0/4	0/4
Stopcock	0/4	0/4	0/4
Kerrisson	0/4	0/4	0/4
Rongeur	0/4	0/4	0/4
Lip retractor	0/4	0/4	0/4
Hardy nasal speculum	0/4	0/4	0/4
Raney clip applier	0/4	0/4	0/4
Anderson-Adson retractor	0/4	0/4	0/4
Detrich clamp	0/4	0/4	0/4
Needle holder	0/4	0/4	0/4

3.7 ENDOTOXINS - PYROGENS

Depyrogenation of instruments can also be accomplished with ozone. The pyrogenic substances (toxins), which are by-products of microbial growth, are not eliminated during sterilization in an autoclave or by heat. They adhere strongly to the container walls. Since these substances are comprised of unsaturated lipopolysaccharides, they are rapidly destroyed by ozone (Gurley 1985).

A study of ozone depyrogenation using the Limulus amebocyte lysate (LAL) end point method on low (0.5 µg ≈ 5000 endotoxin units or EU) and high (125 µg ≈ 1,250,000 EU) endotoxin control were performed (Table 9). Comparison with steam and EO sterilization was also done.

The ozone process is efficient when inactivating low amounts of endotoxins. Approximately 180,000 EU of residual endotoxin could still be detected in high (125 µg) endotoxin controls following ozone depyrogenation. Nevertheless, these high endotoxin controls contained approximately 1,250,000 EU to begin with, which indicated that more than one million endotoxin EU had been inactivated during ozone process. Ozone is a surface process, and since the high level endotoxin controls were on the bottom of a small bottle, the access for ozone was limited. Finally, the fact that modulation experiments were carried out successfully indicated that there were no inductors or inhibitors within these controls.

TABLE 9. COMPARISON OF VARIOUS DEPYROGENATION METHODS USING VARIOUS ENDOTOXIN CONTROLS

ENDOTOXIN LEVEL	RESIDUAL ENDOTOXIN (%)			
	STEAM	EO	DRY HEAT	OZONE
Low level 0.5 µg	0.5%	53.5%	0.3% (170°C)	0%
High level 125 µg	n.d.*	n.d.	n.d.	9.2%

**n.d. : not determined*

3.8 PRIONS

A preliminary study of prion destruction has shown a 3.5 logs reduction in a quarter cycle. This study was performed with hamster prions (263K). A 20 µL of 10% hamster brain extract was spread on a glass petri dish, dried and exposed to a quarter cycle in the *125L* Ozone Sterilizer. Prion proteins recovered were analyzed by electrophoresis and Western blot methods using 3f4 mouse monoclonal antibodies. A 3.5 logs of prion proteins were found on non-exposed control and none on the exposed sample. These tests demonstrate the potential of ozone to oxidize prion proteins even in the presence of rich lipid content (brain extract).

4.0 *125L* OZONE STERILIZER MONITORING SYSTEM

4.1 PROCESS CONTROL

The *125L* Ozone Sterilizer is totally controlled by a programmable logic controller (PLC). The software has been developed by TSO_3 to control the electromechanical components of the sterilizer and to control the touch screen and the printer.

All critical process parameters are monitored during the cycle. At the end of each cycle step, the process parameters are printed. At the end of the cycle, the screen will indicate “cycle completed” and a printout will be produced. During the sterilization cycle, if one of the critical process parameters is not reached, the cycle will abort and the reason of the interruption will be displayed on the screen and on the printout.

It was demonstrated that the SAL of 10^{-6} is achieved when the cycle operates inside the set parameters.

The sterilizer possesses a single standard cycle for all medical devices to reduce the risk of cycle selection error by the operator.

4.2 PROCESS CHALLENGE DEVICE (PCD)

A process challenge device (formerly known as a BI test pack) has been developed for routine process monitoring of an ozone sterilization process cycle. TSO_3 recommends using a process challenge device containing a Biological Indicator to monitor the performance of every sterilization cycle. This frequency of PCD usage is the standard recommendation for monitoring most commonly used low temperature gaseous sterilization methods.

The process challenge device is composed of commercially available items. For assembly of the test pack, a self-contained OZO-TEST® Biological Indicator containing a minimum 10^5 *Geobacillus stearothermophilus* spores is placed in a syringe with a 3.6 mm diameter catheter tip. As with the ethylene oxide test pack, the cap of the Biological Indicator should be inserted in the syringe first. The plunger diaphragm is then inserted into the barrel of the syringe, and then the syringe is placed in a TSO_3 sterilization pouch along with a TSO_3 Chemical Indicator before the pouch is sealed. The test pack configuration was established to obtain a successful “kill” of the BI at approximately 75% of the total sterilization cycle.

This ozone process challenge device closely resembles the configuration of the Association for the Advancement of Medical Instrumentation (AAMI) and the Canadian Standards Association routine ethylene oxide test pack. The syringe acts as an ozone absorber, and also as a diffusion restrictor (a diffusion restrictor being a device or material that by its composition or geometry impedes the movement of the sterilant). The Biological Indicator represents a microbial challenge. The pouch offers an additional diffusion restrictor and represents the sterilization packaging that is routinely used to pack medical devices.

5.0 SAFETY OF STERILIZED INSTRUMENTS

5.1 SURFACE EFFECTS

The first phase for material functional compatibility covered 19 materials: 16 polymers, two types of stainless steel and one sample of glass.

Raw material samples were processed in one, 10 and 25 sterilization cycles in the *125L* Ozone Sterilizer and analyzed as described in Table 10.

The study results of the effects of the ozone sterilization process on physical and chemical properties will be used by TSO_3 as a basis for discussion with medical device manufacturers to provide them information on which materials have a good potential for compatibility with the ozone process. Latex, Kraton® (synthetic latex), and polyurethane (ether base) were assessed "not recommended" for the ozone process, and have not been analyzed for biocompatibility because of the significant physical changes during ozone processing.

TABLE 10. TECHNIQUES USED TO EVALUATE MATERIALS SAMPLES

TESTS AND ANALYSES	METHODOLOGY *	PURPOSES
Visual aspects	Optical microscope, UV-Vis	Deformation, color, transparence
Structural properties	DSC	Degradation, vitreous T° transition
Mechanical properties	MTS	Yield, flexibility, resistance
Surface properties	SEM, AFM, AES, XPS	Surface topography, contaminants, chemical surface composition

*DSC: Differential scanning calorimetry
SEM: Scanning electronic microscopy
AES: Auger electronic spectroscopy
MTS: Material tensile strength
AFM: Atomic force microscopy
XPS: X-ray photoelectronic spectroscopy

5.2 SAFETY FOR PATIENT USE (BIOCOMPATIBILITY)

The materials that are most commonly used in the manufacturing of medical instruments and devices have been subjected to a wide variety of toxicological tests to determine whether the ozone sterilization process affects biocompatibility.

All tests were carried out by an external GLP-compliant laboratory, in accordance with CDRH (Center for Devices and Radiological Health) Recognized Consensus Standards, ANSI/AAMI/ISO 10993 series, and other methods approved by the FDA.

Bulk materials have been processed in 25 complete sterilization cycles and evaluated using the following tests:

Blood compatibility: Hemolysis tests to establish the compatibility of materials processed by the TSO_3 ozone sterilization process with blood.

Systemic toxicity: Systemic injection tests on mice to establish that materials processed by the *125L* Ozone Sterilizer are not toxic.

Dermal allergenicity: Sensitization by the guinea pig maximization test (GPMT) to establish that the materials processed by the *125L* Ozone Sterilizer do not elicit contact dermal allergenicity.

Skin irritation: Primary skin irritation tests using the albino rabbit model to establish that the materials processed by the *125L* Ozone Sterilizer do not elicit dermal irritancy.

Cytotoxicity: The MEM elution cytotoxicity test to determine the cytotoxicity of materials after exposure to TSO_3 ozone sterilization.

5.3 COMPATIBLE MATERIALS

Many devices made of the following materials can be safely processed in the *125L* Ozone Sterilizer:

- Rigid polyvinyl chloride (PVC)
- Polyamide (nylon)
- Polypropylene
- Polyetherimide
- Polytetrafluoroethylene (PTFE) - Teflon™
- Silicone
- Polymethyl methacrylate (PMMA) Plexiglass™
- Stainless steel
- Low density polyethylene (LDPE)
- Pyrex™
- High density polyethylene (HDPE)
- Anodized aluminum

All medical devices should be processed in accordance with the medical device manufacturer's recommendations.

Medical device manufacturers and standards agencies recommend that medical devices be properly cleaned.

All instruments must be disassembled into their component parts before cleaning and processing.

6.0 USER AND HEALTH CARE FACILITY PERSONNEL SAFETY

Ozone is a bluish gas with a very pungent characteristic odor. The odor threshold for humans is from 0.003 to 0.01 ppm (Sittig, M. 1991). It is possible to detect ozone at a concentration lower than the exposure limit for an 8-hour period, which is 0.1 ppm, as established by OSHA.

Ozone is found naturally in the atmosphere as a result of the action of solar radiation and electrical storms. It is also formed around electrical sources. Ozone is used:

- As an oxidizing agent in the organic chemical industry;
- As a disinfectant for food in cold storage rooms and for water purification;
- For bleaching textiles, waxes, flour, mineral oils and their derivatives, paper pulp, starch and sugar;
- For aging liquor and wood;
- For processing certain perfumes, vanillin, and camphor;
- In treating industrial wastes;
- In the rapid drying of varnishes and printing inks;
- And for deodorizing feathers.

The permissible exposure limits in air have been determined by:

OSHA (Occupational Safety and Health Administration):

The legal permissible exposure limit (PEL) is 0.1 ppm (0.2 mg/m^3) average over an 8-hour period (TWA) and the short term exposure limit (STEL) is 0.3 ppm (0.6 mg/m^3) for a period of 15 minutes.

NIOSH (National Institute for Occupational Safety and Health):

The recommended airborne exposure limit is 0.1 ppm which should not be exceeded for any period of time.

ACGIH (American Conference of Governmental Industrial Hygienists):

The recommended airborne exposure limit is 0.1 ppm averaged over an 8-hour workshift.

FDA (Food and Drug Administration):

Also regulates ozone as a toxic gas (21 CFR, part 801.415). According to this regulation, ozone concentration released by a device should not exceed 0.05 ppm by volume of air circulated through the device, or the device shall not cause an accumulation of ozone in excess of 0.05 ppm per volume (when measured under standard conditions at 25°C (77°F) and 760 mm of mercury) in the atmosphere of enclosed space intended to be occupied by people for an extended period of time.

6.1 EXPOSURE TO OZONE

The *125L* Ozone Sterilizer design limits the risk of exposure of hospital personnel. All ozone produced passes through a catalyst that reverts it back to oxygen before being exhausted into the room. Since the ozone gas is created in an enclosed ozone generator within the unit, there is no manipulation of the sterilant.

The *125L* Ozone Sterilizer possesses built-in safety features that protect the user from high ozone concentration exposure.

Measurements have been made to determine the maximum ozone concentration to the catalyst outlet. Measurements have been made on two different worst case conditions (when the quantity of ozone passing through the ozone destruction unit is at its maximum level). Measurement #1 took place at the end of the first half cycle, and Measurement #2 at the end of the full cycle. Results are presented in Table 11.

The maximum ozone concentration released by the device never exceeded 0.02 ppm in a worst case condition, hence demonstrating that the device is safe for users and health care facility personnel.

TABLE 11. MEASUREMENT OF THE MAXIMUM OZONE CONCENTRATION AT THE CATALYST OUTLET

# TEST	MEASUREMENT #1 (ppm)	MEASUREMENT #2 (ppm)
1	0.000	0.000
2	0.010	0.020
3	0.000	0.010
4	0.000	0.006
5	0.000	0.010
6	0.010	0.010

6.2 TOXICITY OF OZONE

The acute toxicological effects of ozone are due almost entirely to its extreme reactivity and to its being a powerful oxidizing agent. Ozone will oxidize several biochemical compounds, including fatty acids, amino acids, nucleotides, etc., (Carmichael 1982). Lungs are the primary, target of airborne ozone.

Signs and symptoms of acute exposure to ozone may be severe and include irritation and burning of the skin, eyes, and mucous membranes. Inhalation of ozone can cause sufficient irritation to the lungs to result in pulmonary edema. The onset of pulmonary edema may be delayed for some hours after exposure (21CFR, part 801.415).

In accordance with the Handbook of Toxic and Hazardous Chemicals and Carcinogens, an exposure level of 0.2 ppm for 3 hours may not produce symptoms. Levels of 0.3 ppm may cause tightness in chest and throat, dry throat, and irritation of throat and lungs within 30 minutes. Levels of 0.5 ppm and above may cause headache, drowsiness, loss of coordination, and accumulation of fluid in the lungs. Levels near 10 ppm may result in immediate, severe irritation of throat and lungs, excessive sweating, continual coughing, decreased blood pressure, weak and rapid pulse, and severe chemical pneumonia (Sittig, M. 1991).

Several studies among welders (industrial exposure, generally mixed exposure not limited to ozone) have shown that there are no effects after brief exposure to 0.2-0.25 ppm of ozone; throat irritation at 0.3-0.8 ppm of ozone; dryness of the mouth and throat, irritation of the nose and eyes at 0.8-1.7 ppm of ozone; severe headache and respiratory problems at 9.2 ppm (Beard 1982). An exposure as high as 11.2 ppm for 2 hours caused perspiration, coughing, and collapsing; oxygen inhalation relieved the symptoms and in 2 days all symptoms disappeared.

Although ozone is a powerful oxidant and may be dangerous, ozone has been used since the 18th Century. It is possible to use it safely, and many industries have proved it. Ozone was discovered by Schönbein in 1840, and as early as 1856 attempts at room disinfection using ozone were made in Paris hospitals. Water disinfection was one of its first uses and started more than 100 years ago. The pharmaceutical industry uses ozone to sterilize liquid medications, plastic, and glass containers, as well as the water used in drug manufacturing processes. Bottled water plants also use ozonated water to sterilize their containers. It is now approved to be used in food processing for the decontamination of poultry. The use of ozone is not new and no evidence suggests that ozone may be a potential carcinogen.

7.0 SUMMARY

The development of the *125L* Ozone Sterilizer is the beginning of a new era in low temperature sterilization. This unique process has the potential to improve sterilization processing throughout the world.

TSO_3 introduces this process that uses oxygen, water and electricity to generate on site its sterilant: ozone. Medical quality oxygen is released into the ozone-generating unit and then subjected to an electrical field, which converts the oxygen into ozone. The ozone is then fed into a humidified sterilization chamber where it sterilizes medical devices. The ozone is subsequently reverted into oxygen using an ozone converting catalyst. The only residues left at the end of the sterilization cycle are oxygen and clean water vapor. Instruments are ready for reuse immediately after sterilization, no aeration is required. The ozone process is a safe, efficient, fast and cost effective response to sterilization needs for heat sensitive materials.

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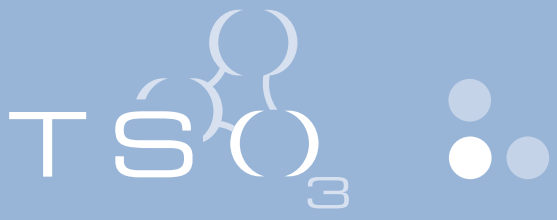

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