

International Scientific  
Committee of Ozone Therapy



Madrid  
Declaration on  
**OZONE  
THERAPY**

*(2nd. ed.)*

*June 12th 2015*

*“Towards a united approach to the  
practice of ozone therapy worldwide”*

**MADRID DECLARATION ON OZONE THERAPY**

*(2nd. edition)*

Official document of ISCO<sub>3</sub>

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ISCO<sub>3</sub> (International Scientific Committee of Ozone Therapy)

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# Madrid Declaration on OZONE THERAPY

*(2nd. ed., 2015)*

Official document of ISCO<sub>3</sub>

**1st edition:** Approved at the “International Meeting of Ozone Therapy Schools” held at the Royal Academy of Medicine in Madrid on **June 4th, 2010**, under the auspices of AEPROMO (Spanish Association of Medical Professionals in Ozone Therapy).

**2nd edition:** Approved by ISCO<sub>3</sub> on May 10, 2015 and officially presented at the “International Meeting of the Madrid Declaration on Ozone Therapy (2nd. ed.)” held at the Royal Academy of Medicine in Madrid on **June 12th, 2015**, under the auspices of ISCO<sub>3</sub> (International Scientific Committee of Ozone Therapy) and the administrative and logistical support of AEPROMO (Spanish Association of Medical Professionals in Ozone Therapy).





## MADRID DECLARATION ON OZONE THERAPY 2nd. ed., 2015 Official document of ISCO<sub>3</sub>

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The updating process of the Madrid Declaration took nearly one year since ISCO<sub>3</sub> in June 2014 decided to update the document which had been approved in June 2010. The principal authors wrote five drafts until agreeing on the text. Then ISCO<sub>3</sub> issued two international calls asking ozone therapists to send their proposals and suggestions for the updating of the Madrid Declaration. A high and important number of proposals from different parts of the world were received by ISCO<sub>3</sub> until January 31, 2015.

Based on the proposals received and the inputs presented by ISCO<sub>3</sub> members, several new drafts were written by the principal authors, who received additional proposals from ISCO<sub>3</sub> members. Finally the 21 members of ISCO<sub>3</sub> unanimously approved the 2nd. ed. of the Declaration on May 10, 2015 and became an official document of the committee.

ISCO<sub>3</sub> wants to thank all those who participated and/or supported the committee in the challenging efforts of updating the Declaration.



## MADRID DECLARATION ON OZONE THERAPY 2nd. EDITION, 2015 Official document of ISCO<sub>3</sub>

**Taking into account** since the discovery of ozone by the German chemist Christian Friedrich Schönbein in 1840, its medical use has increased in different parts of the world and health professionals are showing more interest in ozone's benefits and how it works. Accordingly, with the increase in the number of ozonetherapists all over the world, the number of patients reaping benefits from ozone has risen. Although great efforts and advances have been made since the approval of the 1st edition of the Declaration in 2010, the consolidation of ozone therapy has not been easy. Resistance is still found within the medical community and ozone's recognition in the legal field will require more coordinated efforts.

**Recalling** that pre-clinical research and clinical trials on the use of ozone therapy have been carried out in Cuba, Germany, Italy, Russia, Spain and other countries, with considerable scientific rigor, obtaining results that support its practice using different medical protocols.

**Bearing in mind** that the preclinical studies, genotoxic, toxicology and clinical studies carried out, endorse the application and the innocuous character of this medical therapy using a fairly wide range of doses. More detail in ISCO<sub>3</sub> official document: *Ozone Therapy and its Scientific Foundations*<sup>1</sup>.

**Emphasizing** that research and clinical experience with medical ozone are making progress despite varied obstacles. However, the main and permanent challenge for researchers and for ozone therapy associations is the lack and inaccessibility of financial resources that are essential to conduct the required scientific research.

**Stating** that, it is absolutely necessary to work with specific objectives, and in a unified way to assure a practice with great precision and safety.

**Recognizing** that there is variance that the medical community wishes to standardize, and that progress already has been made, that it should be taken into account; it is necessary to continue with the development of medical definitions of procedures and protocols determining the best applications where it is necessary, as well as a code of good practice, in order to overcome more efficiently the possibility of malpractice.

**Welcoming** with great satisfaction that ozone therapy practice has been regularized in the following countries: Ukraine (2001 and 2014); Italy in the Regions of Lombardy (2003), Emilia-Romagna (2007) and Marche (2009), and favorable court decisions have been taken by the Administrative Court of Lazio (1996 and 2003); China (2005); Russia (2005 and 2007); Spain, in 15 Autonomous Communities out of 17 (between 2007 and 2012), and ozone therapy is implemented in 22 Pain Treatment Units of the public health sector;<sup>2</sup> Cuba (2009); the Sultanate of Oman (2010); Emirate of Dubai of the United Arab Emirates (2011); Portugal (2013 and 2014); and Turkey (2014). Important efforts are being deployed in other countries towards regularization. So it is likely that other countries may follow. More details can be found in ISCO<sub>3</sub> official document: *Ozone Therapy and Legislation - Analysis for its Regularization*.<sup>3</sup>

**Taking into account** that the International Scientific Committee of Ozone therapy (ISCO<sub>3</sub>), as the depositary of the Madrid Declaration on Ozone Therapy and as the responsible body for its updating,



invited ozone therapists around the world to send their proposals to improve it. ISCO<sub>3</sub> received a high number of proposals and based on them and the internal inputs provided by the members of the committee, ISCO<sub>3</sub> approved it on May 10, 2015. Through this updating and working process the whole ozone therapist community had the opportunity to participate in a serious medical scientific exchange with the objective of having a global document applicable to patients by health professionals.

**Considering** that the updated version of the Declaration reflects the advances in the field of ozone therapy, provides tools for its right application to patients, and reflects a great amount of unanimity among the community of ozone therapists around the world.

### **The International Scientific Committee of Ozone Therapy (ISCO<sub>3</sub>) has adopted the following CONCLUSIONS**

**First.** To approve the “**Therapeutic Ranges for the Use of Ozone**” detailed within the “Recommendations” section of this Declaration.

**Second.** To increase the exchange of knowledge, research, and experiences, both positive and negative that occur in the field of ozone therapy, in furthering and increasing the knowledge of the huge benefits that this therapy has. To stimulate the publications of research results in specialized medicine journals.

**Third.** To encourage health researchers to increase their creative efforts, so that ozone therapy continues to demonstrate its therapeutic benefits with safety and effectiveness under the development of controlled clinical trials.

**Fourth.** To stimulate the creation of Standardized Operative Procedures, according to good clinical practices for each procedure, taking into account new developments, with the view of increasing the quality and making diverse homogeneous treatments.

**Fifth.** To make systematic efforts to ensure that each scientific congress/meeting to be organized adopts conclusions that reflect the progress made and set achievable and realistic targets, sharing the findings and aims to encourage and promote research to deepen the understanding of ozone therapy. To work towards the harmonization and unification of criteria at the international scientific level.

**Sixth.** To encourage the different associations to work in their own countries where the ozone therapy has not yet been regularized to get it properly regularized and therefore to enjoy a legal status.

**Seventh.** To encourage the edition of text books, the organization of theoretical and practical courses on ozone therapy in a systematic way, so that those who practice it do so based on sound knowledge; this will necessarily be reflected on a more efficient medical health care which will benefit the patients and the therapy.

**Eighth.** To encourage that training courses may follow the same guidelines and structure as per guidelines issued by international competent organizations.



***The International Scientific Committee of Ozone Therapy (ISCO<sub>3</sub>)  
has adopted the following  
RECOMMENDATION***

That the ***Therapeutic Ranges for the Use of Ozone*** as detailed in the annex to this “Madrid Declaration on Ozone Therapy (2nd. ed.)” and an integral part thereof, serve as a reference to ozone therapists in order for them to implement them carefully and systematically. These ***Therapeutic Ranges for the Use of Ozone*** are the summary of scientific research in different countries and are the result of many years of experiential and clinical practice.

**ACKNOWLEDGEMENTS**

**The International Scientific Committee of Ozone Therapy (ISCO<sub>3</sub>)**

**Express** its most sincere recognition to ***Dr. Velio Bocci***, Emeritus Professor of Physiology at the University of Siena, for the significant and important contributions he has made in favor of ozone therapy in the fields of research, teaching, information and patient care, to the point that in the history of ozone therapy he must be considered as one of its most important pioneers.

**Express** its most sincere gratitude and recognition to ***Dr. Adriana Schwartz*** and the **Spanish Association of Medical Professionals in Ozone Therapy (AEPROMO)** for their great vision in detecting the need to draft a document that unifies criteria and for the initiative to write under the international consensus the first Madrid Declaration, which was launched at the “International Meeting of Ozone Therapy Schools”, organized by AEPRMO and warmly housed in the centenarian walls of the Royal National Academy of Medicine in Madrid on 4th June 2010.

**Express** its most sincere gratitude to the Spanish Association of Medical Professionals in Ozone Therapy (AEPROMO) for providing its administrative and logistical support to the “International Meeting of the Declaration of Madrid on Ozone Therapy (2nd. ed.)” warmly housed for the second time in the centenarian walls of the Royal National Academy of Medicine in Madrid.



Madrid  
Declaration on  
**OZONE  
THERAPY**

*(2nd. ed., 2015)*





**ANNEX TO THE MADRID DECLARATION ON OZONE THERAPY  
(2nd. ed.)**

**WHICH IS INTEGRAL PART THEREOF**

Approved by

The International Scientific Committee of Ozone Therapy (ISCO<sub>3</sub>)

on May 10, 2015

**Index**

**THERAPEUTIC RANGES FOR THE USE OF OZONE**

<b>1. THERAPEUTIC BASIS</b> .....	11
<b>2. OZONE THERAPY (O<sub>3</sub>X) BASIC PRINCIPLES</b> .....	15
<b>2.1 Contraindications</b> .....	15
<b>2.2 Interactions with ozone</b> .....	15
<b>2.3 Adverse effects</b> .....	16
2.3.1 Grade 1 Mild.....	16
2.3.2 Grade 2 Moderate .....	16
2.3.3 Grade 3 Severe .....	16
2.3.4 Grade 4 .....	16
2.3.5 Grade 5 .....	17
<b>2.4 Toxicity</b> .....	17
<b>2.5 Pediatrics dosages through rectal insufflation</b> .....	17
<b>3. MAIN ROUTES OF APPLICATION</b> .....	18
<b>3.1 Recommended routes of application</b> .....	18
3.1.1 Major Autohemotherapy (AHTmajor).....	18
3.1.2 Minor Autohemotherapy (AHTminor) .....	19
3.1.3 Intramuscular, paravertebral and intrarticular injection .....	19
3.1.4 Paravertebral intramuscular injection .....	19
3.1.5 Hernias .....	19
3.1.6 Intradiscal Treatment .....	20
3.1.7 Sacral Hiatus/transluminal peridural infiltration .....	20
3.1.8 Intraforaminal infiltration .....	20
3.1.9 Intra-articular Treatment .....	21
3.1.10 The gloves technique (Emphysema subcutaneous technique).....	21
3.1.11 Gasification in plastic bag.....	21



3.1.12 Subcutaneous application.....	21
3.1.13 Ozone suction cup.....	21
3.1.14 Insufflation in fistulas.....	21
3.1.15 Ophthalmologic .....	21
3.1.16 Vaginal insufflation .....	22
3.1.17 Insufflation vesico-urethral .....	22
3.1.18 Otic route.....	22
3.1.19 Intratonsillar infiltration route .....	22
3.1.20 Ozone micro doses in trigger and acupuncture points.....	22
3.1.21 Subcutaneous application.....	23
3.1.22 Ozonized Saline Solution .....	25
3.1.23 Extracorporeal blood oxygenation-ozonation (EBOO).....	26
3.1.24 Rectal Insufflation.....	27
<b>3.2 Application routes not recommended for not being safe .....</b>	<b>27</b>
3.2.1 Direct intravenous infection of ozone .....	27
3.2.2 Intra-arterial injection .....	28
<b>3.3 Application route prohibited .....</b>	<b>28</b>
<b>3.4 Application routes that have not received total consensus .....</b>	<b>28</b>
3.4.1 Intra joint injection of ozonated water.....	28
3.4.2 Intra peritoneal.....	28
<b>3.5 Essential requirements .....</b>	<b>28</b>
<b>4. PATHOLOGIES MORE APPROPRIATE TO BE TREATED WITH OZONE THERAPY .....</b>	<b>29</b>
4.1 Diseases in the level A .....	29
4.2 Diseases in the level B .....	29
4.3 Diseases in the level C .....	30
<b>5. GENERAL BASICS FOR TREATMENT.....</b>	<b>32</b>
<b>6. BASIC RULES TO PERFORM TRAINING IN OZONE-THERAPY.....</b>	<b>33</b>
<b>ADDENDUM A. Clinical application of ozone in dentistry. ....</b>	<b>35</b>
<b>ADDENDUM B. Mutagenicity, toxicity and adverse reactions in ozone therapy. ....</b>	<b>43</b>
<b>REFERENCES .....</b>	<b>45</b>



## 1. THERAPEUTIC BASIS

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**Ozone therapy (O<sub>3</sub>)** is a medical treatment that uses an oxygen-ozone mixture (95% - 99.95% of oxygen and 0.05% - 5% of ozone) as a therapeutic agent to treat a wide range of diseases. Since ozone has no receptors, its pharmacological mechanism of action is indirect, through their mediators. The response is dependent on activation of nuclear transduction mechanisms' signals (Nrf2: Nuclear factor (erythroid-derived 2)-like 2) and protein synthesis, e.g. SOD (superoxide dismutase), CAT (catalase), HO1 (heme oxygenase 1), etc.<sup>4,5</sup>

**Ozone Therapist:** The doctor who practices this therapy is called an ozone therapist. The word therapist comes from the Greek (*therapeutes*) composed by the verb *therapeuein* and meaning: care for, attend, alleviating, hence the word therapy refers to the person who is dedicated to curing diseases, in this case with oxygen-ozone mixture.

**Ozone generators:** The ozone must be produced by a medically reliable and certified generator, complying as any other medical device with the standards set up by, for example, CE,<sup>1</sup> CSA, UL. The generator must allow the measurements of precise ozone concentrations (1 µgN/mL - 80 µgN/mL). Within the European Union the generators are obliged to comply with the RoHS directives.<sup>6</sup> The generator must produce ozone exclusively from medicinal grade, at least 99.5% pure oxygen, coming from a medical quality certified container.<sup>7</sup>

Medical grade oxygen should fit the quality standard of the local Pharmacopoeia. If local Pharmacopoeia is not available, the reference Pharmacopoeia should be: European Pharmacopoeia,<sup>8</sup> United States Pharmacopoeia<sup>9</sup> or Japanese Pharmacopoeia.<sup>10</sup>

According to these Pharmacopoeias, the quality criteria are mentioned.<sup>11</sup> The machine must be able to generate the therapeutic, i.e. homogeneous oxygen-ozone mixture with a range of ozone concentration between 1 µg/NmL and 80 µg/NmL. No other substances besides O<sub>2</sub> and O<sub>3</sub> may be present in the produced gas mixture. To insure the accuracy of the ozone concentration, the calibration of the ozone generators should be done regularly, once a year. The medical ozone generators must have a seal of approval or Certification of Medical Device, given by a CE medical device Notified Body or by an equivalent institution.

**Concentrations:** The concentration's unit of measurement is µg/NmL. The µg/NmL units take into account the pressure and room temperature. The "N" of the µg/NmL means normalized milliliter therefore Standard Conditions of Temperature (0°C) and Pressure (1 bar). This is the only unit recognized by the International Ozone Association - IO3A.<sup>12</sup> The concentration expressed in µg/NmL must have a margin of error equal or better than ±10%.

### Dose and volume of blood to be extracted

Ozone therapeutic indications are based on the knowledge that low physiological doses of ozone may play important roles within the cell.<sup>13,14</sup> At the molecular level, different mechanisms of action have been shown to support the clinical evidence for this therapy.<sup>15</sup> Data summarized in this document are

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<sup>1</sup> *CE Marking* means "European Conformity". CE Marking on a product is a manufacturer's declaration that the product complies with the essential requirements of the relevant European health, safety and environmental protection legislation. <http://www.ce-marking.org/what-is-ce-marking.html>



based on more than 2000 scientific books and papers listed in the ISCO<sub>3</sub> *Ozone Therapy International Library*.<sup>16</sup>

There are therapeutic, non-effective and toxic concentrations of ozone. It has been proved that concentrations of 10  $\mu\text{g}/\text{NmL}$  or 5  $\mu\text{g}/\text{NmL}$  and even smaller, have therapeutic effects with a wide security margin, so it is now accepted that the therapeutic ozone dosage for **systemic treatment** (AHTmajor, rectal insufflation, intramuscular, etc.) ranging between 500  $\mu\text{g}$  and maximum 4000  $\mu\text{g}$  per treatment and concentrations ranging from 10  $\mu\text{g}/\text{NmL}$  to 40  $\mu\text{g}/\text{NmL}$  are safe and effective.<sup>17</sup>

It is necessary to define the volume of blood to be extracted. This is done based on the weight of the patient being treated. Hemodynamic / hypovolemia disorders with a loss of 15 % of total circulating blood volume (CBV) are not considered. In case of AHT major, a withdrawal of 2 % or more than 1.5 % is conservative. A person of 85 kg has CBV of 65 mL / kg x 85kg = 5,525 mL. The 2 % corresponds to 110 mL blood withdrawal. Ranges of a safe blood collection are: 1.2 mL / kg to 1.3mL / kg with the limit of 150 mL in individuals of 150 kg.

**For example:** a person of 85 kg;  $1.2 \cdot 85 = 102$  mL blood to be extracted. These dosages have been shown to be safe and effective. They activate cellular metabolism and have immunomodulatory and anti-oxidant effects. It should be emphasized that each route of application has a minimum and a maximum dosage as well as concentration and volume to manage.

The total ozone dose is equivalent to the gas volume (mL) multiplied by the ozone concentration ( $\mu\text{g}/\text{NmL}$ ) (Dosage= Volume x Concentration). The Dose is not given by kg body weight but, by dose dependent response and the concentration can be expressed as well in  $\mu\text{g}/\text{NmL}$  or as mg/NL of ozone.<sup>18</sup> We strongly advise applying the *up-dosing system*, as Dr. Bocci stated, *start low, go low*.<sup>19</sup>

Studies, involving the calculation of the ozone dose based on body weight, are ongoing. All therapeutic dosages are divided into three types, according to their mechanism of action (Table 1):

- a) **Low doses:** These doses have an immunomodulatory effect and are used in diseases where there is suspicion that the immune system is very much compromised. For example, in cancer, for the elderly and for debilitated patients, etc.
- b) **Medium doses:** They are immunomodulatory and stimulate the antioxidant enzyme Defense System. They are most useful in chronic degenerative diseases such as diabetes, atherosclerosis, COPD, Parkinson syndrome, Alzheimer, and senile dementia.
- c) **High doses:** They have an inhibitory effect on the mechanisms which occur in autoimmune diseases such as rheumatoid arthritis and lupus. They are especially employed in ulcers or infected injuries and are, also, used to prepare ozonized oil and water.

**Materials to be used:** All materials used must be disposable and ozone resistant: glass, silicone probes, catheters and silicone tubes, connections of Kynar or stainless steel 316, and siliconized syringes.

**Table 1.** Guidelines for ozone concentration / volume, according to the most common routes of administration (Tab. 1A, Local Routes; Tab. 1B, Systemic Routes)

**Table 1 A.** Local Routes

Most common routes of application: LOCAL					
Method	O <sub>3</sub>	Levels			Remarks
		High	Medium	Low	
Auricular	C. (µg/NmL)	20	10	4	In dynamic application: Flushing manually, very slowly, using a siliconated syringe of 50 mL for 5 min.
	V. (mL)				
	Dose (mg)				
Vesicouretral	C. (µg/NmL)	20-25	15	10	Dynamic flow, flushing flow 0.1-0.2 L/ min.
	V. (L)	1-2			
	Dose (mg)	20-25 40-50	15-30	10-20	
	V. (mL)	200	150	100	
	Dose (mg)	4.0-5.0	2.25	1.0	
Bags	C. (µg/NmL)	80-60	50-40	30-20	20-30 min. The area must be moistened before treating the area.
	V. (L)	Depending on the bag dimension			
	Dose (mg)	Depending on the bag volume			
Paravertebral	C. (µg/NmL)	20	15	10	See Reference: ISCO <sub>3</sub> (2014) <sup>20</sup> <i>Ozone in Non-Rheumatic Locomotor System Pathologies</i> , for details.
	V. (mL)	5-20			
	Dose (µg)	100-400	75-300	50-200	
Subcutaneous	C. (µg/NmL)	10	8	5	Maximum 100 mL/session
	V. (mL)	1-2			
	Dose (µg)	10-20	8-16	5-10	
Intra-articular	C. (µg/NmL)	15-20	10	5	See Reference: ISCO <sub>3</sub> (2014) <sup>20</sup> <i>Ozone in Non-Rheumatic Locomotor System Pathologies</i> , for details.
	V. (ml)	1-2 mL (finger)/ 5-20 others			
	Dose (µg)	15-20	15-20/ 50-200	5-10/ 25-100	
		30-40 75-100 300-400			

Note: Not recommended route: inhalation (high risk of toxicity, see text for details).

Legend: C, concentration; V, volume.

**Table. 1 B.** Systemic Routes

Most common routes of application: SYSTEMIC					
Method	O <sub>3</sub>	Levels			Remarks
		High	Medium	Low	
Major Autohemotherapy	C. (μg/NmL)	30-40	20-30	10-20	In some cases it could be assessed that up to 60 μg/NmL has proved to be safe and with a greater capacity of induction of cytokines. Venous blood volume 50-100 mL.
	V. (mL)	50 - 100			
	Dose (mg)	1.5-2.0 3.0-4.0	1.0-1.5 2.0-3.0	0.5-1.0 1.0-2.0	
Minor Autohemotherapy	C. (μg/NmL)	30-40	15-20	5-10	5 mL blood is removed intravenously and drawn into a 20 mL disposable syringe (containing already the same amount of ozone-oxygen mixture).
	V. (mL)	5			
	Dose (μg)	150-200	75-100	25-50	
Vaginal	C. (μg/NmL)	30-35	20-25	10-15	Dynamic flow, flushing flow 0.1-0.2 L/min for 10 min.
	V. (L)	1-2 Lt			
	Dose (mg)	30-35 60-70	20-25 40-50	10-15 20-30	
Sauna*	C. (μg/NmL)	10	8	5	20-30 min, 10-15 with ozone, followed by 10-15 min with water vapor / Temp. 40-45 °C
	V. (mL)	Depending on the design and type of sauna			
Acupuncture / reflexology	C. (μg/NmL)	30	20	10	
	V. (mL)	0.1 - 0.3			
	Dose (μg)	3-9	2-6	1-3	
Rectal Insufflation	C. (μg/NmL)	30-35	20-25	10-15	Major concentrations of 40 μg/mL can hurt the enterocyte. The only exception is, in case of acute bleeding, in ulcerous colitis, beginning with a high concentration of 60-70 μg/mL / and 50 mL Vol. Once the bleeding diminishes, reduce concentration.
	V. (mL)	200	150	100	
	Dose (mg)	6.0-7.0	3.0-3.75	1.0-1.5	

Note: \* Can be also used for systemic effect (e.g. to facilitate heavy metal elimination).

Not recommended routes: direct intra-venous (no clinical evidence available, high risk of embolism).

Legend: C, concentration; V, volume.



## 2. OZONE THERAPY (O<sub>3</sub>X) BASIC PRINCIPLES

---

The three basic principles that must be taken into account before any ozone treatment process is implemented are the following:

a) ***Primum non nocere***: Before anything else, not to do any harm.

b) **Stagger the dose**: Always start with low doses, and increase them gradually.<sup>14,19</sup>

The exception will be in infected ulcers or injuries, where the reverse will be applied. In this case, start with a high concentration, and diminish it according to the improvement in the patient's condition. See table 1 for details. Higher ozone concentrations are not necessarily better, in the same way that it occurs with all the medicines.

### 2.1 Contraindications

Administration of ozone is contraindicated in:

1. Glucose-6-phosphate dehydrogenase deficiency (favism, acute hemolytic anemia)\*
2. Toxic Hyperthyroidism - Basedow Graves status
3. Thrombocytopenia less than 50.000 and serious coagulation disorders
4. Severe Cardiovascular instability
5. Acute alcohol intoxication
6. Acute Infarct of myocardium
7. Massive and Acute Hemorrhage
8. During convulsive states
9. Hemochromatosis
10. Patients receiving treatment with copper or iron

\* The prevalence of Glucose 6 phosphate dehydrogenase (G6PD) deficiency varies among ethnic groups with overall lower frequency in the Americas (3.4%), Europe (3.9%), and the Pacific (2.9%) as compared to sub-Saharan Africa (7.5%), the Middle East (6.0%), and Asia (4.7%).<sup>21</sup> Test of G6PD is recommended prior to O<sub>3</sub> therapy to avoid complications.

### 2.2 Interactions with ozone

During the treatment with ozone, antioxidant supplements may be used (e.g. vitamin C and vitamin E). However, the presence of these compounds in high concentrations in the blood interferes with the ozone's action as an oxidant agent and in the good course of the therapy. Consequently, oral vitamins or antioxidants, should never be given during treatment, but only before or after the ozone therapy. The time of suppression depends on the bioavailability of each specific antioxidant. It is recommended that intravenous antioxidant therapy, such as vitamin C or glutathione, neither be administered, before nor during, but only after ozone therapy.

Ozone increases the effects of ACE Inhibitors. Treatment with ozone in patients under anticoagulation therapy such as Coumadin / heparin/ must be done under control of INR. Patients receiving treatment with copper or iron cannot receive ozone treatment.

Synergic effect with other oxidative therapy (U.V., H<sub>2</sub>O<sub>2</sub> etc.) should be expected. Complementary effects can be expected in association with laser therapy, magnetic therapy, acupuncture, diathermia and physiotherapy.

### 2.3 Adverse effects

Most of the side effects reported could be related to *mala praxis*: administration technique, administration route, concentration of ozone administered, etc.

Grade of reported adverse effects (AE) according to NIH (2010) criterion.<sup>22</sup>

#### 2.3.1 Grade 1 Mild

(Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated).

- Some patients reported a brief and transient feeling of local heat and slight pain during the ozone injection.<sup>23</sup>
- Hematoma at the ozone infiltration site in one patient.<sup>24</sup>
- Four patients reported the sensation of itching on lips and tongue at the end of the session, 3 patients described nausea and a bad taste in the mouth during re-infusion of ozonated blood and one patient suffered dyspnea during the administration of therapy.<sup>25,26</sup>
- Onset of euphoria after the application of ozone using the oxygenation and extracorporeal ozonation of blood in 15 patients treated for skin lesions secondary to arterial ischaemia.<sup>27</sup>

#### 2.3.2 Grade 2 Moderate

(Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)).

- Onset of reduction of sensitivity in the legs of two patients in the group treated with ozone and corticoids that remitted in two hours.<sup>28</sup>
- Five patients reported lumbar and leg pain after the ozone injection that resolved spontaneously; and eight patients showed mild corneal irritation and reversible dyspnea after the administration of ozone.<sup>29</sup>
- When ozone was administered by rectal insufflation, cases of bloating and constipation were reported.<sup>27</sup>

#### 2.3.3 Grade 3 Severe

(Or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL).

- Vertebrobasilar stroke.<sup>30</sup>
- One acute bilateral vitreoretinal haemorrhage.<sup>31</sup>
- One case of meningeal irritation.<sup>32</sup>
- Three cases of viral hepatitis.<sup>33</sup>

#### 2.3.4 Grade 4

(Life-threatening consequences; urgent intervention indicated).

- One case of gas embolism was reported in the peri-ganglionic venous plexus involving the vertebrobasilar artery which manifested clinically as local pain for several minutes and which cleared in a few days.<sup>34</sup>



### 2.3.5 Grade 5

(Death related to AE)

- Four cases of death by gas embolism after the administration of ozone by direct intravenous injection.<sup>35-37</sup>
- One case death following ozone application by autohemotherapy to treat psoriasis.<sup>38</sup>
- One case death by fulminating septicemia following ozone therapy for lumbar disc herniation.<sup>39</sup>

## 2.4 Toxicity

Ozone should never be inhaled. Ozone is not toxic when used in the adequate range dose (Table 1), and by trained professionals with the right clinical protocols. Fatal cases are the result of *mala praxis*. (see Addendum B for references on ozone toxicity).

## 2.5 Pediatrics dosages through rectal insufflation

Systemic application via, only by via rectal.

- The concentrations to be used depend on the grade of the oxidative stress of the patient and the pathology to be treated (Table 2A).
- The volume to be administered depends on the age of the patient (Table 2B).
- To perform the rectal insufflation a catheter is introduced (1-2) cm inside the anal sphincter.

**Table 2.** Pediatric dosages by rectal insufflations

**Tab. 2 A.** According to the oxidative stress

Weeks of treatment	Concentration O <sub>3</sub> (µg/NmL)		
	Oxidative stress		
	Low	Moderate	Severe
First	20	15	10
Second	25	20	15
Third	30	25	20
Fourth	35	30	25

**Tab. 2 B.** Volumes to be administered according to patient's age

Age of the patient	Volumes to be administered (mL)
28 days-11 months	15-20
1 -3 years	20-35
3-10 years	40-75
11-15 years	75-120

The dosage changes every five sessions. Cycles of 15-20 sessions are indicated every four-five months during the first year. Later the patient will be evaluated to determine the frequency of the cycles for the second year.



### 3. MAIN ROUTES OF APPLICATION

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Medical ozone can be applied locally or parentally. In order to attain a synergistic effect, the various routes of application of ozone can be used combined or alone.

#### 3.1 Recommended routes of application

The routes of application described below have been proven and safe. They are the result of many years of research and experience, with more than 2,000 documented papers. Consult the *ISCO<sub>3</sub> Ozone Therapy International Library*.<sup>16</sup>

We welcome the therapeutic range indicated by the Guidelines of the Russian Ozone Therapy Association, published in its *Handbook of Ozone Therapy* (2008);<sup>40</sup> the *Guidelines for the Use of Medical Ozone* published by the German Medical Society for the Use of Ozone in Prevention (2009);<sup>41</sup> the guidelines published by the Ozone Research Centre, scientific unit of the Cuban National Centre for Scientific Research, in its book *Ozone Basics Aspects and Clinical Applications* (2008);<sup>17</sup> the significant contribution from Dr. Velio Bocci in the book *Ozone: A new medical drug* (2010)<sup>42</sup> and *The Guide for the medical use of ozone. Therapeutic Basis and indications* (2011) published by AEPROMO.<sup>43</sup>

##### 3.1.1 Major Autohemotherapy (AHTmajor)

The volume of blood to use varies between 50 mL and 100 mL. However, blood volumes greater than 200 mL must be avoided to prevent any risk of hemodynamic disturbances, especially in elderly or unbalanced patients.

**Ranges of a safe blood collection are:** 1.2 mL / kg to 1.3 mL / kg. Example: a person of 85 kg;  $1.2 \cdot 85 = 102$  mL blood to be extracted.

**Perfusion set:** Plastic-based devices, intended to contain blood, must meet the UNI EN ISO 15747:2005 (This is the European Union regulation). All containers and devices used in O<sub>3</sub>x must be ozone-resistant and must not release phthalates because these particles are toxic to the organism. For that reason, it is preferable to use glass for AHTmajor. The plastic bags for AHTmajor must be ozone-resistant and certified for blood collection by the EU or FDA. No other modification to perform ozonated blood transfusion is admitted.

**Ozone concentrations for systemic uses** range from 10 µg/NmL to 40 µg/NmL, concentrations of 70 µg/NmL - 80 µg/NmL and above should be avoided because of the increased risk of hemolysis, reduction of 2, 3 DPG and anti-oxidant and a consequent inability in activating immune-competent cells.

**Anticoagulant:** it is most advisable to use ACD-A *Anticoagulant Citrate Dextrose* Solution A, USP (2.13% free citrate ion), or Citrate Sodium 3.8% 10 mL per 100 mL of blood. Heparin is not advisable because it can induce thrombocytopenia<sup>44</sup> and Platelet aggregation<sup>45</sup>, and Citrate Sodium chelates Calcium. The quantity of ACD-A ranges from 7 mL -10 mL per 100 mL of blood.

**Frequency of treatment:** The number of treatment sessions and the ozone dosage administered will depend on the general condition of the patient, age and main disease. As a general rule, every five sessions the dose of ozone is increased and it is given in cycles that vary between 15 and 20 sessions. From the clinical point of view, a patient's improvement occurs between the fifth and tenth session, and it is considered that after the twelfth session the antioxidant defense mechanism has already been activated. The treatment is given daily, from Monday to

Friday. It could also be administered two to three times a week. Cycles can be repeated every 5-6 months.

### 3.1.2 *Minor Autohemotherapy (AHTminor)*

AHTminor is an immune stimulant therapy, comparable to «auto-vaccination».

**Indications:** As an auto vaccine in psoriasis, dermatitis, eczema, acne vulgaris, allergies and furunculosis, as an adjuvant in cancer or in chronic debilitating pathologies.

**Method:** 5 mL of blood is removed intravenously and collected into a 20 mL disposable syringe prefilled with the same amount of ozone-oxygen mixture (5 mL). Intensively shake for 30 seconds and slowly inject intramuscularly.

Cycles: of 5-10 treatments once a week.

### 3.1.3 *Intramuscular, paravertebral and intrarticular injection*

For details see: ISCO<sub>3</sub> (2014). *Ozone in non-rheumatic locomotor system pathologies*.<sup>20</sup>

### 3.1.4 *Paravertebral intramuscular injection*

The **classical paravertebral** infiltration is performed by locating the upper part of the spinous process and injecting the cervical and dorsal column with 5 mL of ozone at (10-20)  $\mu\text{g}/\text{NmL}$ , 1.5 cm laterally from the spine/column, with a (0.8 x 40) mm needle.

The infiltration for lumbar spine is made 2.0 cm from the spinous process, and 10 mL at the same concentration. The distribution of the needles is always bilateral, lateral or 2 cm above and 2 cm below the hernia. A depth from 2 to 4 cm should be considered when taking into account the patient's constitution and/or the area to be treated (smaller in thin patients and in the dorsal region, and greater in obese patients and in the lumbar region).

Local anesthesia (1 mL procaine or 1 mL lidocaine) in the muscle is optional. This may reduce the pain caused by ozone.

The treatment is done twice a week for the first two weeks. Once clinical improvement is achieved, the treatments should be spaced to once a week, for four to six weeks. And then, one session every 15 days until one cycle of 20 sessions is completed; these can be shortened once the symptoms have disappeared.

The recommended needle sizes for this procedure are (0.4 x 40) mm to 30 G (0.3 mm) x 1½" (40 mm). In some cases and with expert hands, longer needles may be used.

It is important that the physician adequately examines the muscles within the lumbo sacra region and the sacroiliac articulations to detect inflammation at this level or *trigger points* in that zone, above all in patients with discartrosis that do not respond adequately to the paravertebral infiltrations. If these points are detected they must be infiltrated. Concentration: (10-20)  $\mu\text{g}/\text{NmL}$ ; Volume: (5-10) mL; Dose: (50-400) mg.

### 3.1.5 *Hernias*

#### **Paravertebral deep injection**

For this injection it is necessary to use a longer needle, 0.4 mm or 0.5 mm x 90 mm spinal needle to inject over the *laminae*, close to the foramen, or around the *facet* joint. Cervical / dorsal hernias:

concentration (10–20)  $\mu\text{g}/\text{NmL}$ , volume (3–5) mL is given and for Lumbar hernias: the concentration is of (10–20)  $\mu\text{g}/\text{NmL}$ , and a volume of (7–10) mL.

### 3.1.6 Intradiscal Treatment

In general, only one intradiscal infiltration should be performed under mobile radiologic arch or fluoroscopic control or CT. The patient has to be under sedation (not general anesthesia) and with an antibiotic prophylactic therapy on the same day of the procedure. In some cases, the intradiscal infiltration can be repeated within 2–4 weeks.

For **lumbar discolysis**, a (5–10) mL mixture of oxygen – ozone at a concentration of (25–35)  $\mu\text{g}/\text{NmL}$  is used.<sup>46,47</sup> All animal models have shown annulus disruption secondary to concentrations of 50  $\mu\text{g}/\text{NmL}$  or more, so it is advisable not to use concentrations over 40  $\mu\text{g}/\text{mL}$ .<sup>48</sup> The needle used is Chiba 22 G (0.7 x 203) mm.

For **cervical discolysis**, (2–3) mL with ozone at a concentration of (25–35)  $\mu\text{g}/\text{NmL}$  is used.<sup>46,47</sup> The needle used is Chiba 25 G x 3 1/2 (0.5 x 90) mm.

The discolysis with ozone, although effective after only one treatment, requires specific infrastructure (for radiological control), an anesthetist and experienced personnel in the execution of the technique. Despite the fact that the paravertebral technique requires more sessions, it is equally effective and has a minimum level of risk.

### 3.1.7 Sacral Hiatus/transluminal peridural infiltration

An infiltration is performed in the peridural space twice weekly, with previous identification of the peridural space by echography guide. A mixture of oxygen–ozone in a volume of (10–20) mL at a concentration of (10–20)  $\mu\text{g}/\text{NmL}$  is used.

The translaminar peridural method or through the sacral hiatus route is an alternative to consider in the treatment of herniated discs with ozone therapy despite being an indirect method compared to the intradiscal because:

- With this method, neither the patient nor the operator is exposed to the risk of radiation.
- Ozone acts over both the disk and the damaged root upon deposit of the gas in the peridural space at the level of the conflict zone disco–radicular.
- It is easy to perform, causing no neurological damage, and a patient would resume normal life soon enough.
- It requires few material resources and equipment, making it a less expensive and effective method.
- Compared to the paravertebral, this indirect method requires fewer sessions and is very useful in the presence of multiple disc hernias.
- The success rate frequency is above 70 %.
- It requires minimum time to recover.
- It can be performed on patients with major associated diseases.

In all cases, the three commented techniques require strict asepsis, sterility measures and an informed written consent.

### 3.1.8 Intraforaminal infiltration

**Concentration range:** 10–20  $\mu\text{g}/\text{mL}$

Intraforaminal approach for cervical injection: requires 5 mL volume and a 25G x 3 1/2 (0.5 x 90 mm) cervical needle can be used.

Intraforaminal for lumbar injection: 7-10 mL and a Chiba 22G x 11 (0.7 x 203 mm) lumbar needle can be used.

### ***3.1.9 Intra-articular Treatment***

Concentration: (2-10-20)  $\mu\text{g}/\text{NmL}$

Volume in function of the articulation size: Fingers: (1-2) mL, Rest: (5- 20) mL.

### ***3.1.10 The gloves technique (Emphysema subcutaneous technique)***

Subcutaneous Infiltration of hands: (10 - 40) mL of oxygen-ozone mixture at (5-20)  $\mu\text{g}/\text{NmL}$  of concentration, with a 30 G (0.3 mm) needle. This infiltration is efficient in the treatment of neuropathic pain and osteoarthritis.

### ***3.1.11 Gasification in plastic bag***

Concentrations of (80,70, 60, 40, 30, 20)  $\mu\text{g}/\text{NmL}$  are used for periods of (5, 10, 20) min, depending on the stage and evolution of the lesion. A (60-80)  $\mu\text{g}/\text{NmL}$  is used only in purulent infections and for a very short time and for no more than 5 min. Once the infection is controlled and the healthy granulation tissue appears, the frequency and the concentration of the procedure have to be reduced to accelerate and induce the healing process.

**Note:** It is necessary to moisten the area and to remove all the air from the bag by vacuum before insufflating the gas into the bag. At the end of the procedure, the remaining ozone gas must be suctioned before removing the bag.

### ***3.1.12 Subcutaneous application***

This application is used for cosmetic purposes in cellulite. In this particular case, never use a volume larger than 200 mL per session, one injection every (5-10) cm in skin fold and in a volume of (2-3) mL per point. Concentration of 15  $\mu\text{g}/\text{NmL}$  to 20  $\mu\text{g}/\text{NmL}$  with a 27 G (0.3 mm) needle. Cycles of 15 - 20 sessions, twice a week.

These results are better if they are associated with ozone rectal insufflation or AHTmajor applied twice a week.

### ***3.1.13 Ozone suction cup***

Using concentrations ranging from 15  $\mu\text{g}/\text{NmL}$  to 60  $\mu\text{g}/\text{NmL}$ , with a variation in the duration of the treatment between 5 to 20 min. Using ventosa, vacuuming is necessary to remove air and ozone from the bell. Vacuum increases the blood flow and ozone can react better.

### ***3.1.14 Insufflation in fistulas***

The practitioner must always be sure, first that no communication exists with the respiratory tract. It is important to keep in mind the possible gas build-up in a closed cavity, blocked or cystic, to avoid dangerous or painful increases in pressure, for example, in cutaneous, perianal and surgical fistulas. A fistula wash with ozonated water must be carried out previously to insufflate the gas. Within the duration of 5 min to 20 min, the concentration of the oxygen-ozone mixture is (10 - 80)  $\mu\text{g}/\text{NmL}$ .

### ***3.1.15 Ophthalmologic***

In ophthalmological cases (keratitis, corneal ulcers, conjunctivitis and ocular burns), a special

glass attachment adapted to the contour of the eye is used. Due to the burning sensation of the topical application of ozone in gas or in form of ozonated oil, it is recommended to use anesthetic eye drops before the application of the ozone. The concentration of ozone is between (20 – 30)  $\mu\text{g}/\text{NmL}$ , application time 5 min, two to three sessions per week, combined with sub-conjunctival application at a concentration of 35  $\mu\text{g}/\text{NmL}$  with a volume of (1– 2) mL and ozonated oil at (200 – 400) IP. Ozonated oil due its bactericidal and virucidal properties is advisable to apply in the form of eye drops four or five times a day after topical anesthesia for the ocular burning that occurs as when the ozone gas is applied.

### **3.1.16 Vaginal Insufflation**

Ozone concentrations of (10–30)  $\mu\text{g}/\text{NmL}$  and a volume between (1 – 2) L at a continuous flow rate of 0.1 L/min to 0.2 L/min for 10 min are used. A vaginal wash with ozonated water must be carried out previously. For this application an ozone destructor device and special vaginal device are needed to acquire the equal, proper and safe distribution of the gas to the folds of the vaginal mucosa.

### **3.1.17 Insufflation vesico-urethral**

According to the case treated, insufflate between 50 and 100 mL of ozone into the bladder or urethra. The recommended concentration is (10 to 25)  $\mu\text{g}/\text{NmL}$  (increasing them progressively in steps of 5  $\mu\text{g}/\text{NmL}$ ). This treatment could be combined with ozonated water as a pre-irrigation procedure.

### **3.1.18 Otic route**

Check that the eardrum is intact. Due to the dryness properties of ozone, it is recommended to moisten the ear canal and the eardrum membrane before applying the ozone.

For insufflation, a syringe or a special headset with an ozone destructor device can be used or performing otic insufflation with a modified stethoscope with silicone tubes, connected between them with a “Y” and female luer lock connector of Kynar to assemble the syringe filled with ozone at the concentrations described, to be manually and slowly administered, so that the ozone can be absorbed in the ear canal and on the tympanic membrane. If there is minimal leakage of ozone, the administration should be done much more slowly. It will not be necessary to connect this device directly to the ozone machine.

**Concentration:** (10 – 25)  $\mu\text{g}/\text{NmL}$ ; application time: 5 min.

**Indications:** otitis, dermatitis of the ear canal, sinusitis and circulatory problems of head and neck.

### **3.1.19 Intratonsillar infiltration route**

Concentrations of (10 – 20)  $\mu\text{gN}/\text{mL}$  with a volume of 2.5 mL per point to infiltrate at the anterior and rear pillar of both tonsils are used. Four to five sessions are required. In case of nasal polyps, infiltrate directly into the polyp tissue a volume of 2.0 mL at a concentration of 50  $\mu\text{gN}/\text{mL}$ <sup>49</sup>. Inhalation<sup>50</sup> is performed only in a state of hydrosol of ozonized oil using an ultrasonic generator of hydrosol of ozonides forming particles of about 5 $\mu\text{m}$  (Terpenes). Never inhale ozone.

### **3.1.20 Ozone micro doses in trigger and acupuncture points**

As a general rule the trigger points are located in the muscles and are often deep, so the application has to be intramuscular and the volume can be between (5–10) mL depending on the anatomical place, and if the concentration is between (10 – 20)  $\mu\text{g}/\text{NmL}$ .

For acupuncture points or reflexology areas, the application is intradermal and fluctuates between 0.1 to 0.3 mL and up to 1 mL (maximum) of the gas mixture of O<sub>2</sub>/O<sub>3</sub> with concentrations below 20 µg/NmL.

### 3.1.21 Topical application of water, oil and ozonized creams

Ozone in water and ozonized oil are applied on ulcers, dirty traumatic lesions, chronic torpid ulcers, bed sores, burns, herpetic lesions, fungal infections, insect stings, in dental infections, as surgical cavity cleaner and in several infected lesions at different concentrations: high, medium, and low, depending on what it is intended to achieve (to disinfect, to regenerate) and on the type of tissue (Table 3).

The preparation of ozone in water is carried out by using a glass cylinder, filled about ¾ with bi-distilled water through which the gas mixture has to be bubbled continuously for at least 5 - 10 min. to achieve saturation. The unused ozone flows out via silicone tubing into a destructor and is converted to oxygen. (Table 3A).

The study of the physicochemical properties of ozonized vegetable oils has great importance for their characterization and identification. To determine the quality of ozonized products, analytical methods of peroxide, acidity and iodine values, relative density and viscosity are usually carried out. The peroxide value represents the quantity of peroxide expressed in mill equivalents of active oxygen contained in a 1,000 g sample (mEq/kg). This index will be used for dosages criterion (Table 3 B).

Since oil steam diffusion in the high-voltage pipes is unavoidable, the ozonization of oils must never be executed with a medical generator. Otherwise, the result would be the production of several toxic substances and the danger of explosion. The recommended method to assay Peroxide values is as described in the European Pharmacopoeia,<sup>8</sup> modified by Zanardi *et al.* (2008).<sup>51</sup>

**Table 3.** Dose ranges of ozone in water and ozonized oils

**Table 3 A.** General specifications to preparer ozone in water

O <sub>3</sub> in bidistilled water					
Method	Specifications	Levels			Remarks
		High	Medium	Low	
Ozone / water 1. Local treatment, essentially use high O <sub>3</sub> concentrations 2. For ingestion, low O <sub>3</sub> concentrations are used.	O <sub>3</sub> C. (µg/NmL)	100-80	60-40	20-10	Final concentration of ozone in water (bi distilled water) usually corresponds to 1/4 (25 %) of the O <sub>3</sub> bubble concentration at 20 °C. Estimate bubble time is (5-10) min at flow 3L/h. Those parameters are variable, depending on the O <sub>3</sub> flow and type of bubble device.
	V. H <sub>2</sub> O (mL)	V. of water depends on the area to be treated (see practical examples below).			
	Final O <sub>3</sub> C. (µg/NmL)	25-20	15-10	5-2,5	
Ozone / water (Practical examples)  1) For external use  2) Ingestion	O <sub>3</sub> Concentration (µg/NmL)	100			
	Volume of bi-distilled H <sub>2</sub> O (mL)	500			
	Bubbling time (min)	10			
	Final O <sub>3</sub> C. (µg/NmL)	25			
	E.g. of applications	Ulcer, bed sores			
	O <sub>3</sub> Concentration (µg/NmL)	10			
	Volume of bi-distilled H <sub>2</sub> O (mL)	250			
	Bubbling time (min)	5			
Final O <sub>3</sub> C. (µg/NmL)	2,5				
E.g. of applications	Gastric ulcer				

**Note:** The ozone in water must be maintained in a tightly closed glass bottle with a silicone or teflon cap, possibly in the refrigerator. If it is kept at 5 °C, the ozone concentration is halved in some 110 h, but at 20 °C the ozone half-life is only 9 h.<sup>42</sup>

**Legend:** C<sub>g</sub>, ozone gas concentration; C<sub>w</sub>, ozone concentration in water; V, volume.

**Table 3 B.** Example of adjust the concentration to 60 µg/NmL and flow rate of 3 L/h.

Time of ozonization (min)	Concentration of ozone in water (mg/L). At 20 °C, 500 mL of bi-distilled water
2	13
5	32
8	61
10	54
15	97
20	129
30	194

**Table. 3 C.** Specifications of ozonized oil.

Ozonized Oils					
Method	Specifications	Levels			Remarks
		High	Medium	Low	
Ozonized oil (local treatment)	PV (mEq/kg)	800-1200	600-400	400-200	Recommended method to assay Peroxide values in the method describes in the European Pharmacopoeia, <sup>8</sup> modified by Zanardi <i>et al.</i> (2008). <sup>51</sup>

### Indications

- 400 IP: for oral administration in post-surgery<sup>52</sup> and diseases of the intestinal tract like *Helicobacter pylori*. In facial revitalization, rosacea, acne and stimulation of granulation.
- 600 IP: in vaginal mucosa (vulvo-vaginitis), rectal (hemorrhoids), nasal, trophic ulcers in epithelialization phase, care of the scalp and skin.
- 400-600 IP: in wounds, trophic ulcers and burns under clear and frank granulation.
- 800-1200 IP: in severely infected wounds and ulcers, gingivitis, alveolitis, herpes simplex, herpes zoster.

### Notes:

Some commercial formulations include enhancing skin penetrating agent and are appropriated for intact, uninjured skin: In psoriasis, viral diseases and fungal infestation of the skin, onychomycosis, furunculosis, abscess.

The oils must be kept in dark glass bottled under refrigeration of 4°C.

Ranges of dose based on peroxide index are indicative and based on a summary of current data available.<sup>53</sup> The lack of quality control of peroxide values induces bias in the current available studies, e.g.:

- Sunflower ozonized oil (Peroxide value 75 mEq/kg – 100 mEq/kg) reduces symptoms related to skin burns, and is effective in preventing the post-lesional hyperpigmentation.<sup>54</sup>
- Topically applied ozonized sesame oil for acute cutaneous wound healing in mice indicate that both low (<1,000 mEq/kg) and high doses (>3,000 mEq/kg), as expressed in terms of peroxide





value, delay cutaneous wound healing. “Middle” concentration (about 1,500 mEq/kg) has the most beneficial effect in accelerating the wound closure ratio.<sup>55</sup>

Legend: PV, peroxide values.

### 3.1.22 *Ozonized Saline Solution*

The Russian and Ukrainian schools utilize ozonized saline solution (OSS) as another form of systemic application of ozone, and its practice is well extended mainly in these two countries.<sup>40,56</sup> Its efficiency is testified by the results of a high number of scientific research studies submitted at the eight Practical Scientific Conferences which took place in Russia from 1992 to 2014.<sup>57</sup>

A team of researchers led by Prof. S. Razumovsky, a major world expert in the chemistry of ozone, found out, through an investigation of the processes of the decomposition of ozone in aqueous media, that the decomposition of ozone in the aqueous solution of NaCl is not accompanied by the formation of products different from the oxygen, and no noticeable amounts of hypochlorites and chlorates were observed in particular. This is significant for the medical applications of ozonized isotonic solution.<sup>58</sup>

At the Scientific Research Center of the Nizhny Novgorod Medicine Academy, Russian scientists, under the leadership of the academician A. Korolev, successfully developed the method of ozonated saline solution in October 1977. In April 1979, for the first time in the world, a cardioplegic ozonated solution in the coronary system of a patient with congenital cardiac injury was administered. In November 1986, the first extracorporeal blood ozonation during placement of a prosthetic mitral valve was conducted.

#### **Ozonated saline solution may be prepared by three methods:**

- First method: The three needles: Requires constant bubbling of ozone to ensure the solution is constantly saturated with ozone gas.
- Second method: The two needles: The solution is saturated for 10 min and requires rapid transfusion due to the decrease of the concentration over time.
- Third method is a combination of methods using two and three needles. In this case, the ozonation saline method takes two needles and intravenous infusion followed by periodic bubbling ozone from a special tank. The ozone concentration in saline solution is stable. This requires special equipment.

#### **Recommended dose of ozone:**

The ozonization is carried out with very low ozone concentrations which are calculated according to the weight of the patient.

**Low ozone dose:** 1  $\mu\text{g}/\text{Kg}$ .

**Medium ozone dose:** 2  $\mu\text{g}/\text{Kg}$ .

**High ozone dose:** 5  $\mu\text{g}/\text{Kg}$ .

#### **Calculation of the ozone gas concentration to prepare ozonated saline:**

(Please note that the dissolved ozone concentration is 25% of the ozone gas concentration)

#### **Dose Formula:**

Dose (mg) = dissolved ozone concentration ( $\mu\text{g}/\text{mL}$ ) \* Volume (mL) saline solution.

**Example:** Patient's weight = 80 Kg; Saline solution volume = 200 mL

**Low ozone dose:**  $1 \mu\text{g}/\text{Kg} * 80 \text{ Kg} = 80 \mu\text{g}$

80 = ozone gas concentration \* 25% \* 200

Dissolved ozone concentration in saline =  $0.4 \mu\text{g}/\text{mL}$

Ozone concentration to mark from the generator =  $1.6 \mu\text{g}/\text{NmL}$

**Medium ozone dose:**  $2 \mu\text{g}/\text{Kg} * 80 \text{ Kg} = 160 \mu\text{g}$

160 = ozone gas concentration \* 25% \* 200

Dissolved ozone concentration in saline =  $0.8 \mu\text{g}/\text{mL}$

Ozone concentration to mark from the generator =  $3.2 \mu\text{g}/\text{NmL}$

**High ozone dose:**  $5 \mu\text{g}/\text{Kg} * 80 \text{ Kg} = 400 \mu\text{g}$

400 = ozone gas concentration \* 25% \* 200

Dissolved ozone concentration in saline =  $2 \mu\text{g}/\text{mL}$

Ozone concentration to mark from the generator =  $8 \mu\text{g}/\text{NmL}$

The upper limit of the concentration of ozone in the ozonized saline solution is  $2 \mu\text{g}/\text{L}$ ; exceeding this limit is dangerous and can cause phlebitis. The exceptional cases are severe sepsis and severe viral infections. In such cases, the concentrations may be increased up to  $8 \mu\text{g}/\text{L}$ .

**Note:** The volume of saline solution used for one procedure is (200-400) mL. The number of procedures for one course of treatment is 6 to 10. Procedures are conducted daily or every two days.

**Low doses ( $0.4 \mu\text{g}/\text{mL}$ )** are used to stimulate the immune system for diseases of the cardiovascular system, and for obstetrics, to prevent toxicity in the first trimester of pregnancy and fetal hypoxia in the third trimester.

**Medium doses ( $0.8 \mu\text{g}/\text{mL}$ )** are used for detoxification in endo-toxemia and chronic inflammatory diseases of different etiologies.

**High doses ( $2 \mu\text{g}/\text{mL}$ )** are used in the treatment of infectious diseases, as well as in skin and burn diseases.

### 3.1.23 Extracorporeal blood oxygenation-ozonation (EBOO)

This method is used in Italy, Russia, Ukraine, and in some Latin American countries, mainly to treat severe peripheral arterial disease, coronary disease, cholesterol embolism, severe dyslipidemia, Madelung disease, deafness of vascular origin, necrotizing fasciitis, septicemia infection resistant to antibiotics, ischemic stroke, chronic heart failure and viral hepatitis C. The method EBOO is an advanced variant of the Autohemotherapy (AHTmajor). The EBOO amplifies the therapeutic benefits reported of AHTmajor by treating a greater volume of blood (4 L/h) at a lower ozone concentration ( $<1 \mu\text{g}/\text{NmL}$ ). The procedure EBOO represents a simultaneous oxygenation and ozonation of blood which is transferred from one vein system of the patient to a gas exchange device (GED), and then from GED into another venous system. Upper and lower veins can be used for this procedure. There are two basic procedures of the EBOO.

*The first method* is based on GED of microporous, ozone-resistant, polypropylene hollow fibers with an external diameter of  $200 \mu\text{m}$ , a thickness of  $50 \mu\text{m}$ , and a membrane surface area of 0.22 m. Concentration of the ozone-oxygen mixture is around 99 % and 1%, respectively. During this procedure the patient's blood is transferred inside the hollow channels, and the ozone-oxygen mixture surrounds the channels from the outside.

*The second method* is based on the use of rotor and film GED (consisting of a glass bottle revolving horizontally and an immovable cork where are three nipples made of ozone-resisting polypropylene).



If the procedure lasts more than an hour, it is necessary to introduce to the patient an extra dose of heparin (1 mL, 5,000 IU) in one hour. The procedure is completed by blood displacement from the lines and GED, using saline solution and removal of intravenous cannulas.

**Note:** Modern dialyzers used for hemodialysis are made of polysulfone, cuprophane and other non-ozone-resistant materials. The use of such devices for EBOO is provoking a risk of undesirable products of ozone-dialysis in the blood.<sup>59-62</sup>

### **3.1.24 Rectal Insufflation**

The Rectal insufflation of ozone is a systemic route. The gas is quickly dissolved in the luminal contents of the bowel, where mucoproteins and other secretory products with antioxidant activity readily react with ozone to produce reactive oxygen species (ROS) and lipid peroxidation products. These compounds penetrate the muscular mucosa and enter the circulation of venous and lymphatic capillaries.<sup>63</sup> This non-invasive technique can be used without risk in pediatric and elderly patients, and on patients with difficult veins' access for Major Autohemotherapy. Generally, this is well tolerated and allows scaling doses similar to those used by Major Autohemotherapy.

In chronic illnesses, the proper dosage of medical ozone produces temporary oxidative stress tolerance so patients require repeated cycles of ozone therapy (20 sessions daily, constituting one cycle). It is recommended to increase the dose in each consecutive cycle, repeated at a 3 to 4 month interval in the first year. If there is more than six months between each cycle, doses must be the same as in the first cycle. Beneficial results are reported following rectal dosing (low, middle and upper middle doses). High doses will only be used after two cycles of ozone therapy with an interval of three months each.

The range of dose is 10 - 30  $\mu\text{g}/\text{NmL}$

The range of volume is 100 - 200 mL

Concentrations higher than 40  $\mu\text{g}/\text{NmL}$  can hurt the enterocyte.

## **3.2 Application routes not recommended for not being safe**

### **3.2.1 Direct intravenous injection of ozone**

Its application is strongly discouraged due to the risk of gas embolism which can occur even in the case of using a slow infusion pump and volumes of 20 mL. The complications of stroke range from a simple axillary bubbling sensation, then cough, a feeling of retrosternal weight, dizziness, to changes in vision (amblyopia), hypotensive crisis, with signs of cerebral ischemia (paresis of the members) and death. It is important to note that five patients died as a result of a gas embolism after administration of ozone by direct intravenous injection.<sup>35-37</sup>

It ought to be kept in mind that oxygen solubility at 37 °C is only about 0.23 mL per 100 mL of plasmatic water, and therefore, venous plasma cannot dissolve oxygen quickly enough, leading to the formation of a gas embolus.

Furthermore, there is no justification to put the patient and the therapy at risk when there are other methods which are safe, have been tested and are effective, such as, major autohemotherapy, minor autohemotherapy and rectal insufflation.

Some ozone therapists claim the effectiveness of direct intravenous ozone, but neither pre-clinical or clinical trials, nor peer-reviewed publications support such claims.

### **3.2.2 Intra-arterial injection**

Its application is strongly discouraged due to the risk of gas embolism.

## **3.3 Application route prohibited**

### **Inhalation route**

Being highly toxic, the inhalation route is absolutely prohibited. The anatomical and biochemical characteristics of the lung make it extremely sensitive to oxidative damage by ozone.

## **3.4 Application routes that have not received total consensus**

### **3.4.1 Intra joint injection of ozonated water**

This method (practiced essentially in China) involves the joint injections of ozonated water at 22  $\mu\text{g}/\text{mL}$ . Validity of the procedure needs to be demonstrated by clinical trials.

### **3.4.2 Intra peritoneal**

This route is still in the scientific experimental phase in animals – to which various tumor cell lines have been implanted – but results show that ozone is more cytotoxic to tumor cells than many of the cytostatics used and without causing the adverse effects of the chemotherapy. The research into this matter has been essentially undertaken at the Laboratory Animal Medicine of the Philipps-University of Marburg (Germany) by the Medical Veterinarian Professor Siegfried Schulz.<sup>64,65</sup>

It is exhorted that investigations in animals continue being carried out. Experimental studies for the treatment of cancer using this way of administration in human beings have not yielded convincing data so far.

However, the washing of the abdominal cavity intra-operatively in peritonitis with (5–10) L ozonated saline solution at a concentration of (4–6)  $\mu\text{g}/\text{mL}$  for 20 min and with the placement of a silicone tube as drainage for further washing, has been used in human beings.<sup>43</sup>

## **3.5 Essential requirements**

To carry out any procedure, the described routes of application require technically qualified personnel, as well as a written informed consent, followed by strict measures of asepsis and sterility.

As any another medical practice, all the material used in ozone therapy, be it in contact with patient's tissue or fluids, must be either disposable after only one use, or be sterilized (ex. surgical equipment), and the oxygen-ozone gas mixture must pass an antimicrobial sterile filter (< 20  $\mu\text{m}$ ) before administration.

Generator used should be in line with the recommendations of ISCO<sub>3</sub>.<sup>7</sup> Professionals should attend post-graduate formation courses which include basic contents defined by ISCO<sub>3</sub>.<sup>66</sup>



## 4. PATHOLOGIES MORE APPROPRIATE TO BE TREATED WITH OZONE THERAPY

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The diseases sensitive to the ozone treatment may be classified into three categories, according to Evidence-based medicine (EBM). Evidence quality was assessed based on the source type (from meta-analyses and systematic reviews of randomized clinical trials) as well as other factors including statistical validity, clinical relevance, currency, and peer-review acceptance.

Levels of evidence were adapted from the U.S. Preventive Services Task Force and the Centre for Evidence Based Medicine, Oxford. Selected levels of evidence in ozone therapy were classified as:<sup>67,68</sup>

**Level A:** Good scientific evidence suggests that the clinical benefits of ozone substantially outweigh the potential risks. Based on systematic reviews with randomized controlled trials, systematic reviews with homogeneity of cohort studies or systematic reviews with the homogeneity of case-control studies.

**Level B:** At least fair scientific evidence suggests that the clinical benefits of ozone outweigh the potential risks. Based on individual randomized controlled trials (with a narrow confidence interval), cohort studies or case-control studies.

**Level C.** At least fair scientific evidence suggests that there are clinical benefits provided by ozone, but the balance between benefits and risks are too close. Based on expert opinions without explicit critical appraisals, case reports, or based on physiology, bench research, or “first principles”, or descriptive epidemiology.

### 4.1 Diseases in the level A

Spinal diseases (disc herniation, spondylolysis, etc.).<sup>69-72</sup>

For details see: ISCO<sub>3</sub> (2014). *Ozone in non-rheumatic locomotor system pathologies*.<sup>20</sup>

### 4.2 Diseases in the level B

These include among others:

- a. Orthopedic diseases and localized osteoarthritis.
- b. Painful disorders of musculoskeletal soft tissue.
- c. Patellar chondromalacia, Gonarthrosis.
- d. Tendinopathies (tennis elbow, jumper’s knee, painful shoulder and Rotator Cuff Tendinopathy.)
- e. Quervain’s tenosynovitis.
- f. Carpal tunnel.
- g. Diabetes and diabetic foot.
- h. Chronic fatigue syndrome and fibromyalgia.
- i. Sudden sensorineural hearing loss.<sup>73</sup> Menière’s disease.
- j. Advanced ischemic diseases. Lower limb arterial ischemia.
- k. Age-related , macular degeneration (atrophic form).
- l. Dental caries lesions (see Addendum A for more details).
- m. Osteomyelitis, pleural emphysema, abscesses with fistula, infected wounds, bed sores, chronic ulcers, diabetic foot and burns.



- n. Acute and chronic infectious diseases, particularly those caused by bacteria resistant to antibiotics or to chemical treatments, viruses, fungi (hepatitis, HIV-AIDS, herpes and herpes zoster infection, papillomavirus infections, onychomycosis and candidiasis, giardiasis and cryptosporidiosis). Bartolinitis and vaginal candidiasis. Athlete's foot. Onychomycosis.

Although the ozone therapy represents a useful support for the treatment of these diseases, it is worthy to underline that neither the ozone nor its metabolites, among which the  $H_2O_2$ , reach a germicide tissue concentration, because the free pathogens are protected by plasma antioxidants and intracellular viruses which are unattainable.

Papers supporting these applications are available in ISCO<sub>3</sub> *Ozone Therapy International Library*.<sup>16</sup>

### 4.3 Diseases in the level C

For these pathologies the ozone therapy, either used only as an exclusive form or as support for a specific treatment, according to the cases, becomes a medicine/treatment with a high therapeutic success rate according to preliminary clinical reports.

These include:

- a. Cancer-related fatigue. The ozone therapy associated with orthodox treatments may accelerate and improve results. However, ozone therapy has so far not been able to show a therapeutic effect on cancer. For all these pathologies ozone treatment should be integrated with the conventional treatment, and there is evidence of its utility, but more precise studies are required.
- b. Asthma.

In the following cases the combination of orthodox treatments and ozone therapy, at least on theoretical grounds, shows that it may be useful but there is no real clinical evidence. The anecdotal evidence suggests the existence of therapeutic effectiveness but, in many cases the efficacy has been achieved by using various types of therapy, therefore the results are not reliable. In some studies the combination of ozone therapy with another treatment has been evaluated, concluding that ozone therapy acts as complement.

- a. Autoimmune diseases: Multiple sclerosis, rheumatoid arthritis, Cohn's disease, chronic inflammatory bowel disease.
- b. Lung diseases: Emphysema, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and acute respiratory distress syndrome.
- c. Skin diseases: Psoriasis, eczema and atopic dermatitis.
- d. Sepsis: Severe sepsis and multiple organ dysfunction, necrotizing fasciitis, peritonitis, burns, maxillary infection, suppurative otitis media, tonsillitis, frontal sinusitis, cystitis.
- e. Respiratory diseases: Tuberculosis, bronchitis, respiratory failure, rhinosinusitis, pleural emphysema.
- f. Gastro intestinal diseases: Cholelithiasis and peptic ulcer, gastrointestinal hemorrhage.
- g. Ophthalmology: Dry eye syndrome, diabetic retinopathy, endophthalmitis, choroid diseases, age related macular degeneration, retinitis pigmentosa, chronic glaucoma.
- h. Nervous system disorders: Ethanol withdrawal.
- i. Pain: Fibromyalgia, metatarsalgia, migraine.
- j. Pregnancy: Placental failure, preeclampsia, infertility caused by fallopian tube adhesion.
- k. Vascular diseases: Ischaemic heart disease.



- l. Cancer metastasis (as adjuvant or to reduce side effects of chemo or radiotherapy): Refractory hemorrhagic radiation proctitis. Prostatic hyperplasia.
- m. Raynaud's syndrome.
- n. Chronic kidney failure.
- o. Liver diseases: hepatitis A, B, C.
- p. Edematous fibrosclerotic panniculopathy.
- q. Thyroid-nodule.
- r. Senile dementia, alzheimer.

Papers supported this applications are available at the *ISCO<sub>3</sub> Ozone Therapy International Library*.<sup>16</sup>

#### **LOW RANGE**

- Biological regeneration.
- Gout.
- Fibromyalgia.

#### **LOW-MIDDLE RANGE**

- Chronic kidney failure.
- Cancer.
- Nephropathies.

#### **MIDDLE RANGE**

- Neurovegetative illnesses: Alzheimer, parkinson, dementia syndromes.
- Pulmonary illnesses: Emphysema, COPED, acute respiratory distress syndrome.
- Ophthalmological illnesses: Retinosis pigmentarias, cataract, glaucoma, macular degeneration related to age.
- Hematology illnesses: Thalassemia B, sicklemlia.
- Vascular illnesses: HTN, venous insufficiency, peripheral arterial illness, CVA, cardiac.

#### **MIDDLE-HIGH RANGE**

- Viral Illnesses: Herpes simplex, herpes zoster, HIV/AIDS, hepatitis A, B, C, papilloma human virus.
- Diabetes.
- Cerebral palsy.
- Dermatological illnesses.
- Orthopedic illnesses.
- Giardiasis.
- Candidiasis and cryptosporidiosis.
- Allergic illnesses.
- Chronic fatigue syndrome.
- Systemic lupus erythematosus.
- Rheumatoid arthritis.
- Crohn's illness.
- Intestine inflammatory illnesses.
- Multiple sclerosis.

## 5. GENERAL BASIS FOR TREATMENT

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Not all patients respond equally to the small, controlled oxidative stress that is produced by ozone therapy. Therefore, the ozone treatment should always be applied in a gradual and progressive manner, starting with low doses and increasing gradually to avoid unnecessary risks, until a clinic diagnostic method for the oxidative stress is available, which allows the dose to be adjusted.

It is possible to measure and classify the state of the oxidative stress of the patient. Only one variable of the antioxidant / pro-oxidant system (as indices of total antioxidant activities), is not advisable. Markers of bio-molecules damage (such as malondialdehyde, advanced product of protein oxidation, etc.), activities of enzymes (e.g. catalase, superoxide dismutase, glutathione peroxidase), antioxidants (e.g. glutathione) and indicators of the total antioxidant activity are recommended. Unfortunately, reliable methods or equipment for measuring oxidative stress are not available. Research in this direction is underway.

If the redox balance is not well known (antioxidants/pro-oxidants balance) and the patient is in an oxidative stress, an initial medium or high dose, may damage cellular antioxidant mechanisms and aggravate the clinical picture. *It is therefore preferable to start with low doses and to increase according to patient response. This is the general practice rule.*

However, it is very important that the physician takes into consideration the nutritional status of patients (by anamnesis and anthropometric index). Food is the source of exogenous antioxidants and is of paramount importance in the clinical response of ozone. According to the patients' initial clinical state, the decision of whether they are eligible to receive the treatment with ozone or not would be made. In some cases, it will be necessary to improve the nutritious state of patients first before proceeding with ozone.

As with any medical treatment, patients may be divided into three types: Normo-responders, hyper-responders and hypo-responders.

There are factors which cannot be controlled and that depend on the patient's idiosyncrasy and the characteristics of how the disease manifests itself.

Ozone therapy is a *medical act* and should be practiced by medical doctors and implemented with scientific rigor. It can produce, with a low frequency, a minimum of adverse cases. For these reasons, we consider that the regularization of the ozone therapy carried out by the authorities should include the following requirements, and in cases where this has not been done the ozone therapists should apply them.

The medical centers where the ozone therapy is practiced should have mandatory sanitary authorization for its functioning and should abide by the following requirements:

1. To have a qualified doctor with training and recognized experience in ozone therapy. This will be the person responsible for the management of the treatment.
2. To use the appropriate equipment to generate and apply the ozone therapy. These should also have the required authorizations from the appropriate sanitary authorities. In the case of the European Community equipment should be marked with the CE. The equipment to generate ozone must be calibrated or revised periodically, according to the recommendation of the manufacturer, to avoid incorrect applications or concentrations.
3. To use medical oxygen provided by an authorized company.





4. To work with carbon mask, as a personal protection, during open applications of ozone (bags, dental, otic, vaginal applications, etc.)
5. To implement the various and appropriate protocols, according to the administration route chosen, in order to guarantee the quality of treatment. The protocols should be appropriately validated and recognized by the international scientific ozone therapy community.
6. To establish an informed written consent, which should be signed by the patient and the medical doctor responsible for the implementation of the ozone therapy, leaving a copy in the patient's clinical history.
7. To have an appropriate airing and ventilation system.
8. To have lifesaving drugs, ventilation support equipment or an Ambu balloon.
9. To take into account that the intra disk application of ozone should be done in a surgical room; in hospital or in an ambulatory unit for major surgery under fluoroscopic guidance.
10. The key to the therapeutic success depends on diverse controllable factors which include the scientific preparation and technique by the ozone therapist; the method that is employed, the quality of the ozone and the general application of the good clinical practices. The non-controllable factors depend on patient idiosyncrasy and on the state of the current illness.

## **6. BASIC RULES TO PERFORM TRAINING IN OZONE THERAPY**

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1. All ozone therapy trainers should have diplomas in ozone therapy issued by recognized bodies, preferentially by universities.
2. The training curriculum should be accredited and approved by a recognized body such as universities or an experienced prestigious international organization in ozone therapy.<sup>66</sup>
3. The practical training must necessarily be done in a controlled clinical environment that meets the current health legislation of each country.
4. All the disposable material used for training must comply with the rules listed in this Declaration.



Madrid  
Declaration on  
**OZONE  
THERAPY**

*(2nd. ed., 2015)*

## **ADDENDUM A. Clinical application of ozone in dentistry**

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### ***General Remarks***

This is the first attempt to write the general guidelines of ozone used in dentistry while taking into consideration the available academic research, published clinical studies, and the vast clinical experience of worldwide dental ozone users, the so called anecdotal clinical experience which is based on personal opinion and results. This task is indeed delicate and might be objectionable, but ISCO<sub>3</sub> took the challenge to start this process and to bring it to the level of ozone used in medicine where the general guidelines are better defined.

This dental addendum will be updated as new research and published articles become available, as well as the feedback of various dental ozone associations or groups and individuals. It is recommended to acquire a basic knowledge in ozone science in general, specifically the use of ozone in medicine and its biological effects, indications, contra-indications, precautions and safety of use.

These general guidelines are not intended to be a substitute for a thorough training in the use of ozone in dentistry. Please modify/add according to your standard of care and the clinical case.

### ***1. Safety and Precautions***

The use of ozone gas intra-orally is probably the most critical among all other ozone applications in industry and health care when it comes to inadvertent and accidental inhalation of ozone gas. Thus, it is paramount for the dentist and his/her staff to take all necessary precautions during the application of ozone gas intra-orally to avoid any accidental inhalation.

- Stop immediately the procedure if the particular odor of ozone is detected. Check for leaks and application modality.
- Vit.C (1 g) always available in the dental office.
- Whenever possible, use a silicone cup (i.e. a piece of 10, 8, 6 mm ø silicone tube adapted to the delivery handpiece) when applying ozone gas. If the handpiece is a single line type, puncture the silicone cup with an 18 G needle and suction excess gas. If the handpiece is a dual line type, turn ON the dedicated suction and then apply the ozone gas.
- At all times, use the dental unit high vacuum to suction any gas leaking outside the silicone cup or the treatment area even if the delivery handpiece has a dedicated suction pump.
- Custom thermoformed total arch trays must be well sealed with silicone impression material all around the edges. It is advisable to perform a hermetic test of the sealed trays (inside the mouth) by connecting the outlet port to the suction source and a 20 mL syringe filled with air to the inlet port of the tray. If the tray is properly sealed and hermetic, the suction could easily aspirate the air from the syringe. In case the syringe plunger is not efficiently and automatically pulled in by the suction source, this means the tray was not properly sealed and the potential for leakage is high. Recheck and reseal as needed.
- In the event of uncontrollable and severe adverse reactions due to accidental ozone gas inhalation, immediately implement the standard protocols for emergency and medical attention/referral.

### ***2. Oxygen source and water quality for ozone gas generation and ozonated water preparation***

Even though it is recommended to use a medical-grade oxygen to generate ozone gas, other oxygen sources i.e. desiccated and pre-treated ambient air, oxygen concentrator, might be used in some



dental procedures. For injections in soft tissues and in surgery applications, a medical-grade oxygen source is indicated.

It is recommended to use distilled water to prepare ozonated water. Reverse osmosis filtered city water can be used to prepare ozonated water for non-surgical procedures, and potable city water for disinfection of surfaces and countertops in the dental office.

### **3. Ozone-compatible materials**

Always use ozone-resistant materials which come into contact with ozone gas i.e. silicone, fluoropolymer plastics, PTFE polytetrafluoroethylene (Teflon®), PVDF polyvinylidene difluoride (Kynar®), Fluorocarbon (Viton®), laboratory-grade glass, 316 stainless steel, titanium.

### **4. The CT factor concept**

The CT factor, Concentration x Time of application, is commonly used in disinfection/sterilization procedures and is an indicator of the total reacted and residual concentration of the used disinfectant (i.e. ozone, chlorine, chlorine dioxide, etc.) needed to satisfy the ozone demand of various organic/inorganic substances and microorganisms present in the treated medium.

How this CT factor correlates in dental applications is that the dental therapist should assess the clinical case and take into consideration the organic/inorganic substances which might consume large amounts of the applied ozone gas and/or ozonated water, and to have enough residual or remaining ozone molecules to oxidize and kill microorganisms. The CT factor is also related to the ozone hardware configuration and ozone production.

Example: in a bleeding treatment area, the applied ozone dose should be higher than in non-bleeding situations due to the strong anti-oxidant capacity of blood constituents and ozone consumption, leaving little to no ozone residual for effective bacterial kill. The same applies in carious lesions and how much affected or partially infected dental tissues are left after excavation.

A low ozone concentration generator might need more contact time to achieve similar results than a higher ozone output generator. This doesn't mean that high ozone concentrations are always better, some users prefer longer contact times with lower concentrations, or using higher flow rates.

$$\text{Total Applied Ozone Dose (mg)} = \text{Oxygen flow rate (mL/min)} \cdot \text{Ozone concentration } (\mu\text{g/NmL}) \cdot \text{Time (min)} / 1000$$

The total amount of ozone application via total arch trays, ozonated water, ozone gas and ozonated oil, should be adapted to the severity of the clinical case and then reduced according to the progression of the healing process. The general rule is to start with high ozone doses and then to reduce according to the healing progress.

### **5. Range of ozone gas and ozonated water concentrations**

Concentration, contact time and flow rate or volume are all related and should be adapted to the clinical case. The right unit to express the concentration of ozone is  $\mu\text{g/NmL}$ .

It is still difficult to recommend the best or to narrow the range of ozone concentrations which are currently used in dentistry by various groups or individuals. Most of the initial dental ozone research and published clinical studies were done using the Healozone® unit which delivers around



4  $\mu\text{g}/\text{NmL}$  ozone gas at a flow rate of  $\sim 600$  mL/min, and contact times of 30 s up to 2 min. A newer version of this generator now delivers up to 20  $\mu\text{g}/\text{NmL}$ . Some users prefer even higher concentrations, up to (80–100)  $\mu\text{g}/\text{NmL}$  with lower flow rates.

Ozonated water concentrations and applied volumes also vary according to the clinical case. It is noteworthy to mention that ozonated water is considered to be more bio-compatible and less irritating to epithelial cells than the gas form (although no adverse events have been reported when ozone gas was applied for the duration commonly used in dentistry). The concentration range between 4  $\mu\text{g}/\text{mL}$  up to 20+  $\mu\text{g}/\text{mL}$  is used safely without any reported negative side effects. Ozonated water is used in all dental applications, with or without ozone gas, and for the patient's routine mouth rinse before, during, and after a dental procedure.

This said, and by extrapolation from the recommended general guidelines for topical applications in medicine, the following guidelines and ozone parameters are presented according to the application and the severity of the clinical case. Please note that ozone is considered to be an adjunct agent, not a substitute for other disinfectants and/or therapeutic agents commonly used in dentistry.

Some helpful adjuncts and materials:

Air Prophy (Sodium bicarbonate – Sylc); Fluid Air Abrasion (Aluminum oxide); Caphosol; GC MI paste; GIC (GC Fuji Triage – Fuji IX); tri-calcium silicate (Biodentine, Theracal); Novamin products; Probiotics; Xylitol; Caries detector dyes and equipment.

## General Procedures in Dentistry

### 1. Tooth Caries

#### Low severity clinical case

Flow rate:  $\pm 250$  mL/min ( $\text{O}_2$ ); Concentration ( $\text{O}_3$ ): 5–20  $\mu\text{g}/\text{NmL}$ ; Time: 30 s.  
 $\pm 500$  mL/min (air); Concentration ( $\text{O}_3$ ): 2–4  $\mu\text{g}/\text{NmL}$ ; Time: 1 min.

#### Moderate severity clinical case

Flow rate:  $\pm 250$  mL/min ( $\text{O}_2$ ); Concentration ( $\text{O}_3$ ): 15–30  $\mu\text{g}/\text{NmL}$ ; Time: 1 min.  
 $\pm 500$  mL/min (air); Concentration ( $\text{O}_3$ ): 2–4  $\mu\text{g}/\text{NmL}$ ; Time: 2–3 min.

#### High severity clinical case

Flow rate:  $\pm 250$  mL/min ( $\text{O}_2$ ); Concentration ( $\text{O}_3$ ): 30–60  $\mu\text{g}/\text{NmL}$ ; Time: 1–2 min.  
 $\pm 500$  mL/min (air); Concentration ( $\text{O}_3$ ): 2–4  $\mu\text{g}/\text{NmL}$ ; Time: 4+ min.

Repeat cycle if necessary; re-wet the treatment area frequently.

#### ***1.1 Low severity clinical case: development defect hypo-calcify fissures; caries in enamel only; partially erupted posterior teeth.***

##### Preventive treatment: <sup>74,75</sup>

- Ozonated water (8  $\mu\text{g}/\text{mL}$  – 10  $\mu\text{g}/\text{mL}$ ) mouth rinse.
- Fissures air prophy (Na bicarbonate / Sylc).
- Wash with ozonated water. Keep surface wet.
- Apply ozone gas.
- Rewet the treatment area each 30 seconds if longer application time is used.

- Apply mineralizing agent.
- Apply Fuji Triage.

Invasive treatment:

- Fluid (ozonated water (8  $\mu\text{g}/\text{mL}$  - 10  $\mu\text{g}/\text{mL}$ ) air abrasion (29  $\mu\text{m}$  aluminum oxide).
- Wash with ozonated water (8  $\mu\text{g}/\text{mL}$  - 10  $\mu\text{g}/\text{mL}$ ). Keep cavity wet.
- Apply ozone gas.
- Rewet the treatment area each 30 s if longer application time is used.
- Apply mineralizing agent.
- Fill with Fuji IX or your preferred material.

**1.2 Medium severity clinical case: caries in coronal third of dentin**

- Anesthesia most probably not indicated.
- Ozonated water mouth rinse.
- Fluid air abrasion (29  $\mu\text{m}$  aluminum oxide) / slow speed electrical handpiece (100  $\text{r}\cdot\text{m}^{-1}$ ) / hand instruments.
- Cutting assisted with caries detector dyes / DiagnoDent.
- Wash with ozonated water. Keep cavity wet..
- Apply ozone gas.
- Rewet the treatment area each 30 s if longer application time is used.
- Apply mineralizing agent.
- Fill with Fuji IX, EQUIA, or your preferred material.

**1.3 Medium-High severity clinical case: caries in middle third of dentin**

- Assessment if anesthesia is needed.
- Ozonated water mouth rinse.
- Fluid air abrasion (aluminum oxide) / slow speed electrical handpiece (100  $\text{r}\cdot\text{m}^{-1}$ ) / hand instruments.
- Cutting assisted with caries detector dyes / DiagnoDent.
- Recommended to leave the affected slightly leathery layer dentin (bottom 0.5 mm-1 mm)
- Wash with ozonated water at demand during caries removal. Keep cavity wet.
- Apply ozone gas.
- Rewet the treatment area each 30 s if longer application time is used.
- Apply mineralizing agent.
- Fill with Fuji IX, EQUIA, or your preferred material.

**1.4 High severity clinical case: caries in apical third of dentin. Assessment if anesthesia is needed.**

- Ozonated water mouth rinse.
- Fluid air abrasion (aluminum oxide) / slow speed electrical handpiece (100  $\text{r}\cdot\text{m}^{-1}$ ) / hand instruments.
- Cutting assisted with caries detector dyes / DiagnoDent.
- Remove totally necrotic dentin (not sensitive) and leave  $\pm 1$  mm of the affected slightly leathery dentin layer.
- Wash with ozonated water at demand during caries removal. Keep cavity wet.
- Apply ozone gas for 2 min or more. Rewet with ozonated water at (30-60) s interval.
- Apply mineralizing agent.



- In case a thick layer of affected leathery dentin is left, it is recommended to fill the cavity with GIC or Tri-calcium silicates, and reassess at 2-3 month interval.
- In case of a thin leathery dentin layer (0.5 mm -1 mm), a permanent filling might be provided
- Reassess at 3 month recall with XRay and clinical examination.

Please note that total arch ozone trays are recommended before and during the treatment, specifically in deep caries lesions.

## 2. Hypersensitivity: no caries involvement

Diagnosis – Risks factors assessment – Treatment planning according to clinical case –

- Ozonated water mouth rinse<sup>76</sup>.
- Air prophylaxis (Na bicarbonate – Sylc).
- Wash with ozonated water<sup>77,78</sup>.
- Apply ozone gas. Rewet with ozonated water if sensitivity is felt during ozone gas application.
- Apply mineralizing agent.

## 3. Root Canal Treatment<sup>79-82</sup>

- Ozonated water mouth rinse.
- Cavity access – Canal(s) ID<sup>83</sup>.
- Flush cavity with ozonated water and apply ozone gas (20-60  $\mu\text{g}/\text{NmL}$ ; 60 s)<sup>84</sup>.
- Proceed with your preferred chemical/mechanical shaping/cleaning technique.
- Final rinse with large amounts (100-200 mL) of ozonated water (8-12  $\mu\text{g}/\text{mL}$ ) using appropriate needles (Ultradent capillary tips).
- Irrigate with ozone gas (40-60  $\mu\text{gN}/\text{mL}$ ) for (1-2) min each canal. Keep the delivery tip freely moving inside the canal while suctioning the excess gas.
- In case a two session RCT is desired, fill the canal(s) with your preferred interim product.
- Inject (1-2) mL at 5-10  $\mu\text{g}/\text{NmL}$  in the peri-apical region. Repeat if needed.

## 4. Regular Hygiene / Scaling and Prophylaxis<sup>85,86</sup>

- Ozonated water mouth rinse.
- Fill the scaler fluid bottle with ozonated water (if applicable) and proceed with the scaling procedure.
- Irrigate at demand with ozonated water.
- Ozonated oils if needed<sup>87</sup>.

## 5. Mild Gingivitis<sup>88,89</sup>

- Ozonated water mouth rinse.
- Fill the scaler fluid bottle with ozonated water (if applicable) and proceed with the scaling procedure.
- Irrigate at demand with ozonated water.
- Total arch tray ozone application may be required before initiating the cleaning/scaling procedure.
- Apply ozonated oil and if needed slightly inside the sulcus at 400-600 IP.
- Provide the patient with ozonated oil for home use. Apply once or twice daily for few days.



## 6. Periodontitis<sup>90,91</sup>

Multi-session treatment guidelines

Session 1:

- Thorough irrigation with ozonated water.
- Total arch tray ozone application (250/500 mL/min; 20-45  $\mu\text{gN/mL}$ ; 5 min).
- Supra-gingival scaling. Ozonated oil application. Home use ozonated oil once or twice daily at 600 IP.

Session 2:

- Thorough irrigation with ozonated water.
- Total arch tray ozone application (250/500 mL/min; 20-45  $\mu\text{gN/mL}$ ; 5 min).
- Sub-gingival scaling/root planning. Ozonated water at demand inside pockets.
- Pockets irrigation with ozone gas using an appropriate applicator (Ultradent capillary tips; 27G – 25G blunt needle).
- Home use ozonated oil once each other day for one week.
- Reassessment – Decide if further treatment is indicated.

Please note that the total amount of ozone application via total arch trays, ozonated water, ozone gas, and ozonated oil should be adapted to the progression of the healing process. The general rule is to start with a high ozone dose and then reduce according to the healing progress.

## 7. Orthodontics

Irrigate thoroughly with ozonated water and apply ozone gas (20-30  $\mu\text{g/mL}$ ; 30-60 s) around each bracket. Repeat cycle each 3 months or as required. In presence of gingivitis, treat accordingly. Home use of ozonated oil (400-600 IP). It is easier to remove the orthodontic wires and elastics to apply ozone gas via a silicone cap and also to avoid any deterioration of non-ozone-resistant materials.

### Pre-Surgical Conditioning

In situations where the medical status of the patient (diabetes; low immunity; medicines side effects; elderly) might affect the healing process or contribute to post surgery complications, a pre-surgery conditioning might alleviate such events. The tooth or teeth to be extracted and surrounding soft tissues, or even the total mouth, are treated with ozonated water and ozone gas using any application modality most suitable for the case.

The frequency of ozone application is adapted to the clinical situation of the patient.

Parenteral ozone administration by medical physicians might also contribute in the pre-surgery conditioning.

## 8. Tooth Extraction

- Ozonated water mouth rinse.
- Remove any existing plaque and infiltrate the sulcus with ozone gas.
- Proceed with tooth removal.
- Flush the socket with ozonated water.
- Cover the site with a gauze, use an applicator tip to irrigate with ozone gas (45-70  $\mu\text{gN/mL}$ , 60 s) while suctioning excess gas. Fill the socket with few drops of ozonated oil. Home use ozonated oil once or twice daily and decrease application according to the healing phase.





## 9. Implants

### 9.1 *Implants placement*

- Ozonated water mouth rinse.
- Proceed with implant site preparation and irrigate with ozonated water at demand.
- Infiltrate the site with ozone gas using an appropriate applicator while suctioning excess gas.
- Place few drops of ozonated oil on sutures. Home use of ozonated oil and reduce as healing progresses.

### 9.2 *Peri-Implantitis*<sup>88</sup>

Diagnosis – Risks factors assessment – Treatment planning

- Non-invasive procedure if indicated.
- Ozonated water mouth rinse.
- Irrigate with ozonated water and ozone gas using an appropriate applicator.
- Use your preferred debridement technique and technology.
- Place a few drops of ozonated oil inside the affected area. Home use of ozonated oil of 400-600IP.
- Inject (1-2) mL ozone gas (10-15  $\mu\text{gN/mL}$ ) around the targeted site.
- Reassess at regular recalls and apply ozone as required.

### *Invasive procedure*

Same as above, plus your preferred technique for granulation tissue removal, implant surface decontamination (Sylc, aluminum oxide,...), grafting,...

## 10. Crowns & Bridges, Veneers

- Ozonated water mouth rinse.
- Pre-preparation: 30 s ozone gas using a handpiece/silicone cup or total arch tray (FMR).
- Post-preparation: ozonated water/gas 1 min; apply mineralizing agent.
- Pre-cementation: air prophylaxis (Na bicarbonate – Sylc) / fluid air abrasion, ozonated water/gas 1 min, mineralizing agent.
- Post-cementation: ozonated water and oil in case of bleeding gums due to finishing/polishing procedure.
- Prosthesis: rinse with ozonated water, ozone gas 1 min, ultrasonic bath with ozonated water.

## 11. Soft Tissue Lesions

### 11.1 *Herpes*

Topical application of ozone gas (500 mL/min; 30  $\mu\text{g/mL}$ ; 1-2 min). Inject 1 mL (10  $\mu\text{gN/mL}$ ) around the lesion. Place a few drops of ozonated oil at 800 IP and home use 1-2 times daily at 600 IP. Use topical anesthesia if patient feels any discomfort during ozone gas injection.

### 11.2 *Aphthous*

Flush with large amounts of ozonated water. Topical application of ozone gas as in herpes treatment. Cover lesions with a few drops of ozonated oil at 800IP and home use of ozonated oil at 600 IP.

### 11.3 *Cuts – Ulcers – Wounds*

Minor ulcers and wounds respond favorably with home use of ozonated oil. If needed, in office application of ozonated water and gas as required.



#### **11.4 Dentures Stomatitis**

Clean the dentures in an ultrasonic unit using ozonated water. Flush the affected areas with ozonated water and place ozonated oil 600 IP on the seating side of the dentures. Home use of ozonated oil 400 IP

#### **12. Ozone-assisted Whitening**

- Ozonated water mouth rinse.
- Air prophylaxis (Na bicarbonate – Sylc).
- Isolation with light-cured dam.
- Apply your favorite hydrogen peroxide gel.
- Using an appropriate applicator tip, infiltrate the gel with ozone gas (250 mL/min; 30-45  $\mu\text{gN}/\text{mL}$ ; 60 s) while suctioning excess gas. Repeat cycle for each tooth.

#### **13. Osteonecrosis of the Jaws ONJ/ BONJ**

In the event a surgical procedure is necessary in patients taking bisphosphonate medication, specifically by IV, or in patients at risk, a pre-surgical conditioning, as described above, is warranted to help minimize the risks of ONJ.

Recent research showed that a combination of antibiotic therapy and application of ozonated oil at 600 IP were successful in treating the lesions without any surgical intervention. Ozonated water application and localized cleaning are also suitable throughout the treatment phase. Systemic ozone administration (provided by an MD) might be recommended.

#### **14. DUWL – Instruments Cold Sterilization – Dental office countertop disinfection<sup>92,93</sup>**

##### ***Other Uses***

- Flush the dental unit water lines with ozonated water daily.
- Use ozonated water in an ultrasonic cleaner for cold disinfection of instruments.
- Disinfect office cabinets, countertops, etc, with ozonated water.
- Impressions, prosthesis, dentures, nightguards, bleaching trays, temporary crowns and bridges, implants abutments, etc, are all suited for disinfection with ozonated water/gas.

## ADDENDUM B. Mutagenicity, toxicity and adverse reactions in ozone therapy

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