



ISCO3 documents are recommendations which may become source of guiding and reference to all those who practice ozone therapy. However it is up to each ozone therapy to follow or not the recommendations issued by ISCO3.

Non-recommended routes of application in ozone therapy

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1. General Background

1.1 Scope

The scope of the present document is in line with two of the main important objectives of the ISCO3:

- 1) **INTERNATIONAL SCIENTIFIC AUTHORITY:** ISCO3 has been created with the clear intention that it has to turn into an international scientific authority due to the composition of its members; and that its recommendations may become **source of reference** to all those who practice this medical therapy.
- 2) **INTERNATIONAL GUIDING AND REFERENCE DOCUMENT:** To determine the scientific and medical merits of each particular use of ozone, as well as a code of good practice, in order to overcome more efficiently the **possibility of malpractice**.

The ISCO3 objectives are in line with two following important requirements of the modern ozone therapy:

“It is absolutely necessary to work with specific objectives and in a unified way to assure a practice **with great precision and safety**.¹

“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**.^{”1}

In addition, this document is in line with two general principles of the medicine:

- 1) **Primum non nocere:** Before anything else, not to do any harm.¹
- 2) **The ethical principles** in medicine.² These general principles should be taken into consideration in the clinical practice of the ozone therapy. As a consequence, any recommended application of ozone should be documented by pre-clinical and clinical trials.

The Madrid Declaration on Ozone Therapy, and this document are not legally binding instruments. The Declaration draws its authority from the degree to which it has been codified in, or influenced, national or regional legislation and regulations. Its role can be summarize in these words *"Even though the Madrid Declaration on Ozone Therapy is the responsibility of the ISCO3, the document should be considered the property of all patients"*



1.2. Acronyms, abbreviations and definitions

CAT	Catalase
DIV	Direct intra venous application
HBO3	Hyperbaric ozone application
HDO	High Dose Ozone therapy
HNE	4-hydroxy-2,3-trans-nonenal
HO1	Heme oxygenase 1
IPO3	Intraperitoneal Ozone
MAHT	Major autohemotherapy
O3SS	Ozonized Saline Solution
PUFA	Polyunsaturated fatty acids
RIO3	Rectal insufflation
RMDIV SM	Robins Method of Direct Intravenous Ozone Therapy SM
SOD	Superoxide dismutase

1.3 Why is it necessary a critical evaluation of inappropriate procedures of ozone therapy?

Ozone used within the determined therapeutic windows is absolutely safe and more effective than golden standard medications in numerous pathologies. However, there are practitioners who for the interest of increasing cost effectiveness and increasing speed in treatments, pretend to cure chronic diseases applying alternative administration ways, using high ozone doses, which are neither standardized, nor supported by pre-clinical / clinical data, nor evaluated toxicologically.⁴

Hence, the aim of this document is to emphasize the rules for a secure practice of ozone therapy in the hope that ozone therapy will be used under scientific evidence base to avoid irreversible damage to the patients.

1.4 Scientific requirements of a secure ozone therapy practice

1.4.1 Practitioner

As defined by the Madrid Declaration on Ozone Therapy¹ to carry out any procedure is required technically qualified personnel. Professionals should attend post-graduate formation courses which include at least the basic contents defined by ISCO3,⁵ or similar contents under the supervision of a local university or a scientific association of ozone therapy.

Practitioners should limit their practice to the field of their basic professional formation. This means: physicians will be in charge of human medical treatment or clinical trials; veterinarians should treat diseases, disorders and injuries in non-human animals; dentists should treat diseases and conditions of the oral cavity. Biochemists, pharmacists, biologists will participate in the molecular, preclinical and clinical research (in case of clinical research the direct interaction with patients will be responsibility of a physician). Nurses and technicians will act following the instruction of the corresponding doctor.



1.4.2 Devices / disposables

Generators used should be in line with the recommendations of ISCO3.⁶ and the oxygen-ozone gas mixture must pass an antimicrobial sterile filter ($< 20 \mu\text{m}$) before injection.

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.¹

1.4.3 Protocols

Clinical protocols should be based in evidences (pre-clinical / clinical) that must be conformed to generally accepted scientific / ethic principles; hinged on thorough knowledge of the scientific literature and other relevant sources of information, adequate laboratories and, as appropriate, animal experimentation.

New routes of application of ozone therapy imply a clinical research. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol. Any clinical research in the area of ozone therapy must fit the same criteria set up for a regular drug. The proposed new routes of application must demonstrate some advantage over available treatment, such as:

- Showing superior effectiveness
- Avoiding serious side effects of an available treatment
- Improving the diagnosis of a serious disease where early diagnosis result in an improved outcome
- Decreasing a clinically significant toxicity of an available treatment
- Addressing an expected public health need

2. Basic pharmacology / toxicology mechanism of the ozone

2.1. Dose effect relationship

Higher ozone concentrations are not necessarily better, in the same way that it occurs with all the medicines. Ozone therapy has in general two main action mechanisms 1) *Direct oxidation*, with immediate effect of O_3 (e.g. inactivation of microorganism or pain mediator) 2) *Surrogate effects*, that involve the activation of a nuclear effectors (Nrf2 or NFkB) to induce a pharmacological response. In both, there is a therapeutic window. The knowledge of the therapeutic windows for each application route is described in the Madrid Declaration of Ozone Therapy¹ and it represents a summary extracted from the clinical experience of the main schools of ozone therapy, derived from the clinical practice, or from the experimental research.

The hormetic response of ozone is not an hypothesis, is a fact demonstrated clinically and experimentally.^{7,8} The interaction of ozone mediators (mainly H_2O_2 and 4-hydroxy-2,3-transnonenal (HNE) with nuclear factor induces a therapeutic respond is now well established by scientific data.⁹⁻¹¹ Low doses of ozone is capable of utilizing known molecular redox master switches such as Nrf2/Keap1 or NF-kB/IkB to effect adaptive resistance. In that way, low doses



stimulate cell protective pathways and nuclear transcription without altering cell viability;¹² on the contrary high doses can be genotoxic.¹³⁻¹⁶

The use of non-appropriate range doses of ozone in clinic may originate serious side effects, from tissue necrosis^{17,18} to a potential cancer that may develop during chronic exposition or during high doses exposition.¹⁹

2.2. Modalities of ozone administration

Ozone can be administered with great flexibility by different routes (e.g. extravascular blood oxygenation-ozonation, subcutaneous, intramuscular, intradiscal, intravaginal, intrauretral, vesical, etc.); but it **should never be used**:

- 1) **By inhalation.** Ozone oxidizes available antioxidants and reacts instantaneously with surfactant's polyunsaturated fatty acids (PUFA) present at the air – epithelial lining fluid interface to form reactive oxygen species that damages the respiratory system.²⁰ Immediately after the exposition the first symptoms (headache, cough, dry throat, heavy chest, shortness of breath) became evident, and urgent aid measures should be taken.²¹

There are two exceptions of administration of ozone or derivates by inhalation.

- a) Small volume of ozone gas (O_2/O_3) at low concentration ($6 \mu\text{g/mL}$), only in apnea and conducted by a well-trained medical doctor, to treat sinus diseases.^{22,23}
 - b) Volatile organic compounds generated by ozone. There are different methods, such as the bubbling of essential oils (e.g. essential oil of pine, thymus, eucalyptus, tea tree) or fixed oils (sunflower oil or olive oil), which generate derivatives that can potentially be used from the therapeutic point of view.^{24,25} In this case, the inhaled vapor is not O_2/O_3 , but terpenes or other organic compounds.
- 2) **Directly injected** (intra-arterial injection or intra venous injection) as a gas mixture in the circulatory vessels because of the risk of provoking oxygen embolism, given the fact that the gas mixture never contains less than 95% oxygen.²⁰ 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 ranges 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 to 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.²⁶⁻²⁹ 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.¹

Additionally, ozone is a very unstable gas, as the minutes pass, the concentration is lost. If it starts with $20 \mu\text{g/mL}$, at the end of the 5 min, the concentration will end up in $10-14 \mu\text{g/mL}$ or even less. Taking into account the current scientific knowledge, the use of DIV (direct intra venous application) involves an unnecessary risk, which should not be done outside of a clinical trial, nor in a center with no capacity to solve potential complications.



On the other hand, scientific societies should officially make clear their position against it (outside of its registered studies), to safeguard the image of ozone therapy in the event of accidents.

It would be enough that only one iatrogenic result produced for using DIV would originate a chaos in the ozone therapy professional community. So, it is of utmost importance to avoid in daily medical practice interventions that put the patient life in risk, especially if the procedure has not been scientifically proved. Any bad result would be detrimental for the ozone therapy.

3. Modalities of ozone administration non-supported by pre-clinical / clinical data

3.1. Emerging therapies

With the term “emerging therapies” in ozone therapy, can be summarized a series of methods non-supported by scientific evidences which do not take into consideration any of the issues described in the point 1.3 or 2 of this document. Nor even a “case report” can be found nor a pre-clinical data that support those aberrant variants. The only channels for the diffusion of the “success” of the therapy are Facebook, YouTube, personal web sites, testimonials from patients, etc.

Therefore, in most cases, they constitute serious cases of human experimentation that skip the elementary medical ethical standards.² In most cases, the methods are advertised as "curative" or "resolving" chronic or terminal diseases (e.g. cancer or HIV). However, the list of diseases that “cure” these methods is endless.

In contrast to appear as more effective methods than the traditional ones, there are reports of deaths and severe adverse events among patients who have been treated with emerging therapies.

The most representative of the emerging therapies are: direct intra venous application (DIV), Robins method of direct intravenous ozone therapySM (*RMDIV*SM), Hyperbaric (HBO3) multi passes method , (3-10, or more passes) with 200 mL blood + 200 mL O₃ at 70 µg/mL at 1 bar pressure, and (2000- 25 000) IU heparin per pass, high dose ozone therapy (HDO), and Intraperitoneal Ozone (IPO3).

3.1.1 Direct intra venous application (DIV)

DIV has not any scientific evidence. A simulation of the effect of DIV in a preclinical study using mouse and rabbit models, get this conclusion: “The preclinical results obtained provide evidence that the implementation of direct intravenous ozone is highly risky, because of the severe adverse effects and the mortality that can lead, so its use is not justified in humans.³⁰ In clinic, there are not even a case report supported the benefit of this methods. Due to the lack of homogeneity in the terminology used in ozone therapy, a bibliographic search using the keyword "intravenous ozone" may lead to the appearance 15 papers.³¹⁻⁴⁵ However, the reading of the "materials and methods" section, evidences that the authors have called "intravenous ozone" to the classic major autohemotherapy (MAHT) or to the administration of ozonated saline solution (O3SS). In any case they do not use the ozone gas directly into the vein.



Gaseous embolism symptoms are evident in patients undergoing DIV. Despite the theoretical discussion, on whether oxygen (the main component of O_2/O_3) may be embolic gas or not, the fact is that there are reports of deaths by the application of this method.²⁶⁻²⁸

3.1.2 Hyperbaric (HBO3) multi passes method

Hyperbaric multi passes method uses extra-doses of ozone and extra-doses of heparin. As DIV, HBO3 has not any pre-clinical or scientific clinical evidence. According to anecdotal evidences from patients or practitioners the main side effects are: loss of vision, lung disturbances, colored urine (red, brown). Is well know, that the association of heparin with ozone increases the activations of platelets.^{46,47} This is the reason why the MAHT uses a citrate based anticoagulant. In a typical 10 passes (200 mL blood + 200 mL O_3 at 70 $\mu\text{g/mL}$ + 2000 U of heparin) the patient receives a total dose of 140 mg of ozone (In MAHT 100 mL of O_3 at 40 $\mu\text{g/mL}$, the patients receive 4 mg) and 20 000 U of heparin. The dose of heparin is too high for a patient without coagulation disorder, and can exacerbate the main side effect of heparin: thrombocytopenia, mild pain, hematoma, hemorrhage, local irritation, erythema, increased liver aminotransferase, anaphylaxis and immune hypersensitivity reaction.⁴⁸ The observed side effects during de HBO3 multi pass are indicative of the toxicity of high ozone.

3.1.3 Intraperitoneal (i.p.)Ozone

It is been said that in case of mesothelioma, peritoneal carcinomatosis or peritonitis, endoperitoneal or endopleural injection of up to 2 500 mL of gaseous mixture with an ozone concentration of 10-20 $\mu\text{g/mL}$ can be performed.⁴ This modality is rarely used and must be performed by a specialist.⁴ There is not any clinical trial documented its benefits. However, the use of this therapy in cancer is supported by a pre-clinical study.⁴⁹⁻⁵¹ The administration of drug in pre-clinical study by intraperitoneal (i.p.) way is usual, because the difficult approach to the animal's veins. I.p. is mainly considered an experimental way. The experimental model of cancer in rabbits, is done by the implantation of the tumor in the rabbit ear, as consequence the marginal ear vein cannot be used for drug administration. It is mean that result observed in preclinical trial probably does not depend of the administration way.

The use of i.p. in humans is not frequent, and as it involves a very invasive method needs quirophan condition. Therefore, the benefits of ozone as adjuvant in cancer should be reached using other way as the MAHT,⁵² with low side effects, low cost and low invasiveness compared with the i.p. (Intraperitoneal hemorrhage, pain, etc.). Any intervention on cancer should be approved by the patient and consulted with an oncologist. The only fact available today about the role of ozone in cancer, is it role as adjuvant,⁵³ not as a cure. Promising or creating expectations of healing a patient with cancer is a serious lack of medical ethics.

3.2 Comparative analysis of various methods

A comparative table shows the differences between well established methods of application of ozone and “the emergent methods” (Table 1).



Table1. Evidence basis comparison of some regular and “emergent” methods of applications of ozone therapy.

Method	Support by preclinic research	Support by clinical trials	Side effects
MAHT	Biochemical, Molecular and preclinical ⁵⁴	>11 000 treatments. ⁵⁵ > 577 patients ⁵⁵	* Hepatitis C virus infections. ⁵⁶ Release of plasticizers when use PVC bag. ⁵⁷ Death ⁵⁸
RIO3	Biochemical, Molecular and preclinical. ⁵⁹	>47 000 treatments. ⁵⁵ > 716 patients ⁵⁵	** Minor side effects. ⁵⁹
O3SS	Biochemical, Molecular and pre-clinical. ⁶⁰⁻⁶²	>500 Russian doctoral theses. ⁶³	
DIV	1 Toxicological report, result in fatal death. ³⁰	0 Clinical trials. >140 000 treatments.*** > 2000 patients***	5 fatal deaths documented. ^{26,27,64} Symptoms of gas embolism: axillary bubbling sensation, cough, a feeling of retrosternal weight, dizziness, changes in vision (ambliopia), hypotensive crisis, signs of cerebral ischemia (paresis of the members), and death.
HBO3	-	0 Clinical trials. Number of treatment and patients unknown	Loss of vision, lung disturbances, colored urine (red, brown).
IPO3	3 Pharmacology in Cancer. ⁴⁹⁻⁵¹	0 Clinical trials. Number of treatment and patients unknown	Intraperitoneal hemorrhage, pain.

Legend: MAHT, Major autohemotherapy; RIO3, Rectal insufflation; O3SS, Ozonized saline solution; DIV, Direct intra venous application; HBO3, Hyperbaric multi passes; IPO3, intraperitoneal Ozone.

*Described MAHT side effects are very discrete taste of metal at start of reinfusion, tiredness on next day, need to adjust the antidiabetic medication to lower doses, need to adjust the anti-hyperthyroidism medication to lower doses, need to adjust the Digitalis heart medication to lower doses, need to adjust anti-hypertensive medication.⁵⁴ Side effects describe in Table 1 for MAHT were consequence of *mala praxis*.

**When ozone was administered by rectal insufflation, cases of bloating and constipation were reported.^{65,66} Is also reported slight irritation and transitory flatulence⁶⁷ and mild, short-term irritation.⁶⁸ In two case was described slight transient flatulence immediately after rectal ozone insufflation.⁵⁵

*** Number of treatment and patients was referred by Howard F. Robins, D.P.M. in a letter to ISCO3 on 2014, entitle: The Safety and Benefits of Direct Intravenous Ozone Therapy (DIV). But not supported by any bibliography or study.



4. Conclusion Remarks

The new (emergent) methods in ozone therapy should be validated according to the scientific methods and following the ethical principles promoted by the World Medical Association.² A rational clinical protocol fits to the good clinical practice and supported by and ethic committee is needed. The current emergent methods in ozone therapy are characterized by lack of scientific support and represent a serious risk for the human health. The ISCO3 does not recommend the clinical application of any of the emergent methods (except patients enrolled in a clinical trial) until new scientific evidence of the benefit/risk ratio is provided.



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6. Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.
ISCO3/LEG/00/10	13/07/2017	Draft	First version
	5/09/2017	Version 1. Minor spelling and wording changes. Argument about SSO3 was separated to be included in a dedicated document on that subject. In “conclusion” was specified that new method will be accepted if patients are enrolled in a clinical trial.	First version
	21/09/2017	Spelling and wording changes.	First version

7. Document Records

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