



**International Scientific Committee
of Ozone Therapy**

Tel/Fax (+34) 913515175. Cell Phone (+34) 669685429
Avenida Juan Andrés 60. Local 1 – Bajo Izquierdo 28035,
Madrid (Spain) info@isco3.org www.isco3.org

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Version: 1
Date: 11/06/2023
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International Scientific Committee of Ozone Therapy ISCO3

ISCO3/MVE/00/03 Rectal Insufflation in Small Animals

Approved by ISCO3 on 8th July, 2023

Original drafter of the paper: Roberto Siqueira. Members ISCO3 (International Scientific Committee of Ozone Therapy), Veterinary group.

Suggestion on how to cite this paper:

ISCO3 Rectal insufflation in Small Animals. Madrid, 2023, International Scientific Committee of Ozone Therapy: www.isco3.org

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Contact for correspondence: ISCO3 Secretariat: E.mail: info@isco3.org



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Title

ISCO3/MVE/00/03 Rectal Insufflation in Small Animals

1.1. Brief background

The rectal administration of ozone is one of the oldest systemic and local forms of application. The biological effects of the Rectal Insufflations of Ozone (RIO₃) has been demonstrated extensively either experimentally or clinically. Furthermore, preclinical studies demonstrated its low toxicity. RIO₃ has been now extended to treat many diseases and is increasingly being used as a systemic therapeutic form. RIO₃ is already being viewed as an alternative to Major autohemotherapy (MAH). Using standardized clinical protocols, a therapeutic success can be reached with RIO₃. Handling the advantage and disadvantage of RIO₃, not as alternative to MAH but used properly (e.g. paediatric, geriatric, when MAH cannot be performed because i.v. is difficult due to unfavourable vein conditions, etc.), this method is a valid route of O₃/O₂ administration.¹ The RIO₃ is a systemic route. The gas is quickly reacted 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.¹ This non-invasive technique can be used without risk in pediatric and elderly patients, and on patients with difficult venous access for MAH. Generally, this is well tolerated and allows scaling doses similar to those used by MAH.

1.2. Purpose

The purpose of this SOP is to describe the procedure for Rectal Insufflations of Ozone (RIO₃), in small animals.

1.3. Scope

This procedure specifies the technique, doses, volume of gas and frequency of application of ozone by rectal way.

1.4. Acronyms, abbreviations and definitions

MAH	Major autohemotherapy
RIO ₃	Rectal Insufflations of Ozone
ROS	Reactive Oxygen Species
SOP	Standard Operational Procedure
Total doses	Total dosage amount, in micrograms, of ozone given per session, calculated as volume in mL multiplied by concentration in µg/mL



2. Responsibility

The responsibility for this medical act will fall mainly on the veterinarian and also on the staff under his charge (VTA). We will differentiate three aspects:

2.1 Responsibility of the veterinarian towards the owner

Veterinary

Description of the protocol (purpose, desired effects, forms of application, number of sessions, possible side effects, etc.)
Explanation of the purpose of the treatment
Request the informed consent (ISCO3/QAU/00/21)

2.2 Responsibility of the veterinarian towards the patient

Veterinarian

Clinical records registration
Applications and monitoring of the therapy by duly accredited professionals and in the asepsis, measures required so that the procedure is carried out in the best conditions.
Ensure a relaxed environment to minimize risks
Patient follow-up
Recording all data on medical records
Evaluation of the results
Reporting any late complications

VTA

Accommodate the patients
Preparation of the material to perform the procedure
Detect and alert the doctor to anomalies due to possible reactions
Notification of possible complications

A RIO₃ session should be done by a veterinary, adequately trained in ozone therapy. It is the veterinarian's responsibility to see that all steps of the procedure are done in the correct manner, in order to always avoid errors, accidents, and to prevent incidents.



3. Procedure

3.1 Indications

As a complementary systemic use, these generally comprise chronic inflammatory diseases or inflammatory symptoms,² asthma.³ Angiopathy,⁴⁻⁶ arterial circulatory disturbances,^{7,8} retinitis pigmentosa,⁹⁻¹¹ chronic inflammatory rheumatic disease,¹² virus-conditioned diseases,^{13,14} immune deficiency,¹⁵ vestibular syndrome,¹⁶ complementary oncology.¹⁷ In addition, local diseases, such as: colitis,¹⁸ proctitis, wounds¹⁹ or anal fissures.

3.2 Contraindications

Absolute contraindication: Severe anemia.²⁰

Relative contraindications / special situations:

- ✓ Acute myocardial infarction
- ✓ Pregnancy (first stage)
- ✓ Uncompensated toxic hyperthyroidism
- ✓ Thrombocytopenia less than 50.000 and serious coagulation disorders
- ✓ Severe Cardiovascular instability
- ✓ Massive and acute hemorrhage
- ✓ During convulsive states
- ✓ Hemochromatosis
- ✓ Uncompensated diabetes
- ✓ Anemia (Hematocrit level less than 20%)
- ✓ Patients receiving treatment with copper or iron.

Concentrations higher than 40 $\mu\text{g}/\text{NmL}$ can hurt the enterocyte.²¹

3.3 Recommended dosage intervals

In chronic illnesses, the proper dosage of medical ozone produces temporary oxidative stress tolerance so patients require repeated cycles of ozone therapy (15-32 sessions, 2-5 per week, 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.²¹

The range of dose is (10 – 35) $\mu\text{g}/\text{mL}$ (Table 1). See also Indication of recommended dosage of ozone for rectal insufflation (Table 2).²⁰

The volume is (3 mL/kg).

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



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Table 1. General dosages by rectal insufflations.

O ₃	High	Medium	Low	Remarks
C. ($\mu\text{g}/\text{mL}$)	30-35	20-25	10-15	Major concentrations of 40 $\mu\text{g}/\text{mL}$ can hurt the enterocyte.
V x B.w. (mL/kg)	3	3	3	

Legend: C, concentration; V, volume; b.w., body weight.

Table 2. Indication of recommended doses of ozone for rectal insufflation.²⁰

Recommendation	Concentration of O ₃ ($\mu\text{g}/\text{mL}$)	Volume of (mL/kg)	Frequency of treatment / number of treatments
Acute gastroenteritis, canine parvovirus, parasitic diseases, gastrointestinal immune- mediated diseases, pancreatitis chronic gastroenteritis	10-30	3	12
Leishmaniasis	20-35	3	12-15
Immune-mediated anemia and thrombocytopenia	10-35	3	9-12
Acute and chronic hepatic diseases	10-35	3	10-20
Acute and chronic kidney disease	10-35	3	10-20
Idiopathic feline cystitis	10-35	3	10-20
Oncology	10-35	3	10-20
Periodontal disease	20-35	3	8-15
feline gingivostomatitis	20-35	3	8-15
Hypothyroidism, Hypoadrenocortism Diabetes Mellitus	10-35	3	10-20
Herpesvirus, Calicivirus Papilloma virus Corneal ulcers	10-25	3	10-15
Feline Asthma, Calicivirus Herpes Virus	20-30	3	12-15
Pulmonary fibrosis	20-30	3	12-15
cardiorespiratory failure	15-35	3	12-15
Prostatitis, BPH cysts for and intra-prostatic Orchitis	15-35	3	12-15
Vaginitis Pyometra Endometritis	15-35	3	12-15
Dermatitis: bacterial, fungal, viral and parasitic	15-30	3	12-15
Immune-mediated Dermatitis Vasculitis Hyperkeratosis Anal fistulas	20-35 20-35	3 3	12-15 12-15



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Recommendation	Concentration of O ₃ (µg/mL)	Volume of (mL/kg)	Frequency of treatment / number of treatments
Herniated disc, discopondylitis	10-35	3	9-12
Immune mediated encephalitis	10-35	3	9-12
Ischemic vascular changes	10-35	3	9-12
Cognitive dysfunction	10-35	3	9-12
Degenerative myelopathy	10-35	3	9-12
Degenerative myelopathy	10-35	3	9-12
Osteoarthritis	10-35	3	12-15
Septic arthritis	10-35	3	9-12
Osteomyelitis	10-35	3	12-15

The frequency of applications can vary from 1, 2, 3 or 5 times a week, depending on the pathology, general clinical status and oxidative stress of the patient.

3.4 Clinical evaluation

A clinical and/or laboratory evaluation is necessary to establish a precise diagnosis and to permit comparisons between the patient's status before, during and after ozone therapy.

3.5 Preliminary operations

The patient's owner must be fully informed in advance about the method itself, about all the steps of the procedure, about the desired effect(s) and also about the possible unwanted side effects. In addition, the written Informed Consent Form must be read, understood and signed by the person responsible for the patient.

Device: Must fit the standard requirement ISCO3/DEV/00/01

Syringes: Plastic-based devices, must meet the ISO 15747 standard: 2005 (This is the European Union regulation). All containers and devices used in O₃ therapy must be ozone-resistant and must not release phthalates.

The patient must be placed on the treatment table or on the owner's lap, in cases of large animals the procedure can be performed on the floor as long as it is a clean and disinfected environment. Have a relaxed atmosphere to avoid sticky situations. The environment has to be airy. After applying ozone therapy, you should try to hold it for a few minutes after instillation, for better absorption of ozone gas.

3.6 Main procedure

Veterinarians should wash their hands and wear gloves. The foil wrap should be removed from the rectal catheter. External lotions, ointments or creams can be applied directly, using a gloved finger. Prior to administering the tip of the catheter, or applicator should be lubricated with a



water-soluble lubricant. To insert a rectal catheter, the lubricated, tapered end of the catheter should be placed at the rectal opening and gently pushed into the rectum. The catheter should be pushed continually forward for about 3 to 5 cm (it will depend a lot on the size and age of the patient). Gently and slowly administer the ozone.

3.7 Side effects

When ozone was administered by rectal insufflation, cases of bloating and constipation were reported.^{17,22} Is also reported slight irritation and transitory flatulence²³ and mild, short-term irritation.¹⁵

If the concentration used causes irritation or discomfort, consider lowering the concentration used or discontinuing treatment until irritation subsides.

Abdominal discomfort, hypotension, vagal syndrome can also be observed in the case of very fast applications.

According to clinical trials more than 46 984 rectal applications have been done in 716 human patients, number of applications varies between 1 and 40 treatments per year, only in two cases, was there described slight transient flatulence immediately after rectal ozone insufflation.²⁴

3.8 Patients Follow-up

Patients may be followed and re-evaluated from time to time, from the clinical / subjective point of view and/or with laboratory and/or image examinations.

3.9 Effect Mechanism

At low doses, systemically applied ozone in the form of RIO_3 acts as a bioregulator, ozone intermediary (H_2O_2 , 4-hydroxynonenal, etc.)²⁵ induces a signal transduction via the oxidation of glutathione or cysteine residues and the corresponding nuclear factors, resulting in a regulation of the antioxidants via Nrf2 information,^{25,26} or an immunomodulation via NFkB.²⁴

In adequate and controlled amounts, these derivatives of the ozone reaction exert biological and therapeutic functions, acting as second messengers, activating enzymes as chemical mediators and immune responses.²⁷

The systemic application of ozone leads to the delivery of super-enriched oxygen at the cellular level and optimizes function. Many biological effects have been attributed to ozone, such as increased glycolysis, effects on red blood cells, effects on rheology, bactericidal, fungicidal and virustactic, immunomodulatory, analgesic and anti-inflammatory. The application of low doses of ozone inhibits synthesis of prostaglandins, release of bradykinin and algogenic substances and proteinase secretion from macrophages and polymorphonuclear leukocytes.²⁸

It is indicated to start treatments with low doses and increase with time and according to the patient's response. Starting with larger dosage does not necessarily have a better effect. If the



patient has oxidative stress, a high dose of ozone can damage antioxidant mechanisms and worsen the clinical picture.²⁹

For rectal administration, there were 6 controlled studies, 2 randomized,²⁴ with 227 ozone human patients, 6 studies without a control group comprising 484 ozone patients. The indications agree with the classic indications of ozone therapy, as a rule associated with a chronic inflammatory process; the type of application also corresponds to the classic and standardized application forms.

For RIO₃ all studies show statistically significant clinical and/or pharmacological improvements, without adverse effects or adverse reactions.²⁴

4. Contingencies; Corrective Actions

In case of side effects follow the instructions of ISCO3/CLI/00/01 "First Aid in Ozone Therapy (Inhalation exposition and accidental over dose)" and report the side effect using ISCO3/REC/00/03 "The ISCO3 Safety Information and Adverse Event Reporting Program Form".

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6. Documentation and Attachments

6.1 List of recommended medical disposables

Silicone Luer lock syringe or 5, 10, 20, 50 mL or 60 mL glass syringe
Gloves and disinfectant solution
The female vaginal catheter, syringes with care or in syringes with rectal catheter
4,6,8,10,12,
Water soluble lubricant

7. Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.
MVE/00/03	28/04/2023	Draft under revision	First version
	11/06/2023	English grammatical correction by Dr. Wayne McCarthy	

8. Document Records

	Name	Title	Signature	Date
Author	Roberto Siqueira, V.D. E.mail. robertosiqueira.vet@gmail.com	Member ISCO3		11/06/2023
Authoriser / Approved	ISCO3 Board and veterinary members 2020-2024	All members		07/07/2023