International Scientific Committee of Ozone Therapy ISCO3

ISCO3/MVE/00/01 Major Autohemotherapy in Small Animals

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Suggestion on how to cite this paper:

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Major Autohemotherapy in small animals
ISCO3/MVE/00/01

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1.1. Brief background

Major Autohemotherapy (MAH) was developed by Dr. Hans Wolff in Frankfurt, (Germany), at the end of the 1960s. It constitutes one of the most frequent forms of application in ozone therapy. It is safe, effective and virtually free of side effects when performed by properly qualified professionals who comply with the principles of good practice.

Its idiosyncrasy allows applying a wide range of ozone concentrations, which is crucial depending on the oxidative stress (OS) of the patient. It can be considered a perfect adjuvant in most diseases, or used as the only therapy.

In Veterinary Medicine, due to the characteristics of the patient, the way of applying MAH is slightly different from Human Medicine. This form of application will be described later.

1.2. Purpose

The purpose of this SOP is to describe the procedure for a Major Autohemotherapy (MAH) session with ozone in small animals.

1.3. Scope

This procedure specifies the blood collecting technique, doses, volume of gas and frequency of application of ozone in small animals.

1.4. Acronyms, abbreviations and definitions

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>4-HNE</td>
<td>4-hydroxy nonenal</td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase</td>
</tr>
<tr>
<td>G6PD</td>
<td>Glucose 6 phosphate dehydrogenase</td>
</tr>
<tr>
<td>GSH</td>
<td>Reduced Glutathione</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen Peroxide</td>
</tr>
<tr>
<td>MAH</td>
<td>Major Autohemotherapy</td>
</tr>
<tr>
<td>Nfr2</td>
<td>Nuclear factor erythroid 2-related factor 2</td>
</tr>
<tr>
<td>NF-κβ</td>
<td>Transcription factor-kappa B</td>
</tr>
<tr>
<td>OS</td>
<td>Oxidative Stress</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operational Procedure</td>
</tr>
<tr>
<td>VTA</td>
<td>Veterinary Technical Assistant</td>
</tr>
</tbody>
</table>
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 MAH 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

In Veterinary Medicine, as in Human Medicine, MAH is considered a systemic route, therefore, it can be applied in all those pathologies that present OS: Immune-mediated, vascular, neuromuscular, tumor diseases, etc. Its use as oxidative preconditioning is becoming more and more frequent.

The evaluation of the OS is carried out by estimating certain parameters such as SOD, CAT, GSH, etc. In veterinary these tests are still far from our reach. Currently, the OS of the animal is assessed by evaluating its health status, which includes a complete physical examination, blood tests, X-rays, ultrasounds and other complementary tests to assess the current status and the level of associated OS. After this exhaustive examination, it is subjectively determined what dose of ozone the patient can receive, taking into account that the weaker the patient is (more OS), the lower the dose must be. The following tables will detail how to apply these doses.

3.2 Contraindications

- Massive and acute hemorrhage
- Thrombocytopenia (< 50,000) and coagulation disorders
- Unregulated hyperthyroidism
- Uncompensated diabetes
- Convulsive state
- Gestation (first stage).
- Severe cardiovascular instability
- Hematocrit level of less than 20%
- Concomitant use of parenteral iron or copper

3.3 Action Mechanism

**Action mechanism:** Chronic inflammatory processes are always accompanied by: high OS, reactive oxygen species, such as radical and nonradical oxidants, a suppressed antioxidant capacity and immunologic disbalance, each of which in turn promotes and maintains the inflammatory process. At low doses, systemically applied ozone in the form of MAH acts as a bioregulator, ozone intermediary (H₂O₂, 4-hydroynonenal, etc.) induce 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, or an immunomodulation via NFkB.
3.4 Application form

To assess the appropriate protocol in each case, several points will be taken into account:
- Pathology to be treated
- Age and condition of the patient (OS)
- Character of the animal (aggressive, nervous)
- Availability of the owner

3.5 Dose

The ozone dose is obtained by multiplying the ozone concentration expressed in µg by its total volume in NmL, with units expressed in µg/NmL. The volume of ozone will be identical to the volume of blood. The ozone concentration will be low in weakened animals. So, we will talk later about high, medium and low doses.

The average blood volume will be 1 mL per kg of weight. There are numerous pathologies where MAH can be used, always taking into account the patient's state/OS at the start of therapy (Table 1 and 2).

3.6 Materials

- Butterfly needle 21G; 3-way stopcock; Syringes 3 bodies of 20, 60 and 100 mL; 23G needles; Physiological saline solution NaCl 0.9%; ACD-A anticoagulant or 3.8% Citrate; Ozone generator; Electric Razor; Antiseptic; Compressor; Nitrile gloves (Fig. 1).

- Optional: Hemo-Nate® filter 18 microns (Is a disposable filter with stainless steel filter media for absolute retention of harmful microaggregates of 18 microns).

Figure 1. Main materials needed to perform the MAH.
**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. Generally, heparin is not advisable because it can induce thrombocytopenia\(^4\) and Platelet aggregation,\(^5\) but it could be acceptable or even preferred in some pathologies and Citrate Sodium chelates Calcium. The quantity of ACD-A ranges from 7 mL -10 mL per 100 mL of blood.

**Device**: must fit the standard requirement ISCO3/DEV/00/01.

### 3.7 Method

- Have a relaxed environment to avoid complicated situations.
- Place the animal on a suitable and disinfected table/surface.
- Shave and disinfect the area where the venipuncture is to be performed.
- Join the wing nut to the 3-way stopcock.
- Insert the needle in the vein and fix it properly.
- In one of the ends of the 3-way stopcock, connect the pertinent syringe with the anticoagulant (Fig. 2).
- Aspirate the necessary amount of blood (Tab. 1 and 2).
- At the other end of the 3-way stopcock, connect the syringe previously loaded with ozone. Move the 3-way stopcock in order to connect the syringe with blood with the syringe with ozone. Pass the O\(_3\) it to the syringe containing the blood. Put the syringe in a horizontal position and rotate it gently for about 2 min so that the ozone comes into contact with the largest possible surface of blood (Fig. 3).
- Aspirate the gas with the syringe that previously contained it. Move the 3-way stopcock in order to connect the syringe with ozonized blood with the vein line and slowly infuse the ozonated blood (Fig. 4). Hemo-Nate® filter 18 microns would be inserted in the line.
- Wash with saline solution (Fig. 5).
- Caution, do not introduce remaining gas into the vein!!
- Remove the track.
- Keep at rest and monitor the animal for a few minutes in case there are adverse reactions.

### Table 1. Range of doses for Major Autohemotherapy according to Madrid Declaration.

<table>
<thead>
<tr>
<th>Method</th>
<th>O(_3) (µg/NmL)</th>
<th>Dosage</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>MAH</td>
<td>30-35</td>
<td>20-30</td>
<td>10-20</td>
</tr>
<tr>
<td></td>
<td>45-52</td>
<td>30-45</td>
<td>15-30</td>
</tr>
</tbody>
</table>

Legend: C, concentration; MAH: Major Autohemotherapy; V, volume.
Table 2. Indicative recommended ozone doses for Major Autohemotherapy.\textsuperscript{6}

<table>
<thead>
<tr>
<th>Indication</th>
<th>O\textsubscript{3} Concentration (μg) // Dose mL/kg</th>
<th>Blood volume (mL)</th>
<th>Treatment frequency / Number of treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GASTROINTESTINAL DISEASES\textsuperscript{7-11}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute gastroenteritis, Canine parvovirus, Parasitic diseases, Immune-mediated gastrointestinal diseases, Pancreatitis</td>
<td>15-35 // 1-1.5 mL/kg</td>
<td>8</td>
<td>Series of 10 treatments weekly</td>
</tr>
<tr>
<td>Chronic gastroenteritis</td>
<td>15-35 // 1-1.5 mL/kg</td>
<td>8 -10</td>
<td></td>
</tr>
<tr>
<td><strong>LEISHMANIASIS\textsuperscript{7,12}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>20-35 // 1-1.5 mL/kg</td>
<td>8-10</td>
<td></td>
</tr>
<tr>
<td><strong>HAEMATOLOGY</strong> (Note: Controversial use in MAH with haematocrit &lt; 20%)\textsuperscript{7}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemias &amp; Immune-mediated thrombocytopenia</td>
<td>10-35 // 1-1.5 mL/kg</td>
<td>4-8</td>
<td></td>
</tr>
<tr>
<td><strong>LIVER DISEASES\textsuperscript{7,13-15}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute and chronic liver diseases</td>
<td>10-35 // 1-1.5 mL/kg</td>
<td>8-16</td>
<td></td>
</tr>
<tr>
<td><strong>NEPHRO-UROLOGY\textsuperscript{7,16}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute and chronic kidney disease</td>
<td>10-35 // 1-1.5 mL/kg</td>
<td>8- undefined according to the chronicity of the process</td>
<td></td>
</tr>
<tr>
<td>Idiopathic feline cystitis</td>
<td>10-25 // 1 mL/kg</td>
<td>8-12</td>
<td></td>
</tr>
<tr>
<td><strong>ONCOLOGY\textsuperscript{7,9,17}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjuvant or treatment</td>
<td>10-35 // 1-1.5 mL/kg</td>
<td>8-undefined, according to the severity of the disease</td>
<td>Cycles every 3 months</td>
</tr>
<tr>
<td><strong>DENTISTRY\textsuperscript{7,9,18}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periodontal disease</td>
<td>15-30 // 1 mL/kg</td>
<td>8-15</td>
<td></td>
</tr>
<tr>
<td>Feline gingiva-stomatitis</td>
<td>15-30 // 1 mL/kg</td>
<td>8-15</td>
<td></td>
</tr>
<tr>
<td><strong>ENDOCRINOLOGY\textsuperscript{7,19}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothyroidism, Hypoadrenocortism Diabetes Mellitus</td>
<td>15-35 // 1.5 mL/kg</td>
<td>8-20</td>
<td></td>
</tr>
<tr>
<td><strong>OPHTHALMOLOGY\textsuperscript{7,9,20-22}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herpesvirus, Calicivirus Papilloma virus Corneal ulcers</td>
<td>10-25 // 1-1.5 mL/kg</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>CARDIORESPIRATORY DISEASES\textsuperscript{7,23}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feline Asthma, Herpes virus Calicivirus</td>
<td>20-30 // 1 mL/kg</td>
<td>4-8</td>
<td></td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>20-30 // 1 mL/kg</td>
<td>4-8</td>
<td></td>
</tr>
<tr>
<td>Cardiorespiratory insufficiency</td>
<td>15-35 // 1 mL/kg</td>
<td>4-8</td>
<td></td>
</tr>
<tr>
<td><strong>GENITOURINARY DISEASES\textsuperscript{7}</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostatitis, BPH Cysts for and intra-prostatic, Orchitis</td>
<td>15-35 // 1-1.5 mL/kg</td>
<td>4-8</td>
<td></td>
</tr>
<tr>
<td>Vaginitis Pyometra Endometritis</td>
<td>15-35 // 1-1.5 mL/kg</td>
<td>4-8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DERMATOLOGY*7, 9, 13, 15, 24-26</td>
<td>NEUROLOGY*7, 13, 15, 27-29</td>
<td>TRAUMATOLOGY9, 13, 15, 29-32</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------------</td>
<td>-----------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Dermatitis:</td>
<td>15-30 // 1-1.5 mL/kg</td>
<td>Hemiated disc,</td>
<td>Osteoarthrosis</td>
</tr>
<tr>
<td>bacterial,</td>
<td></td>
<td>discospondylitis</td>
<td>10-35 // 1-1.5 mL/kg</td>
</tr>
<tr>
<td>fungal,</td>
<td></td>
<td>Immune-mediated</td>
<td>Septic arthritis</td>
</tr>
<tr>
<td>viral and</td>
<td></td>
<td>encephalitis</td>
<td>10-35 // 1-1.5 mL/kg</td>
</tr>
<tr>
<td>parasitic</td>
<td></td>
<td>Ischemic vascular</td>
<td>Osteomyelitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>alterations</td>
<td>10-35 // 1-1.5 mL/kg</td>
</tr>
<tr>
<td>Immune-mediated</td>
<td>20-35 // 1–1.5 mL/kg</td>
<td>Cognitive dysfunction</td>
<td></td>
</tr>
<tr>
<td>dermatitis</td>
<td></td>
<td>Degenerative myelopathy</td>
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<tr>
<td>Vasculitis</td>
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<td>Neuromuscular disorders</td>
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<td>Hyperkeratosis</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Anal fistulas</td>
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</tbody>
</table>

Note: dose of ozone is expressed in a minimum maximum interval. Integral treatment of each pathology may involve the use of more than one way of administration (see Madrid Declaration 2020).

Ozone concentrations for systemic uses range from 10 μgN/mL to 35 μgN/mL, concentrations above 40 μgN/mL is not recommended; concentrations higher than 60 μgN/mL imply an increased risk of hemolysis, reduction of 2,3 DPG and anti-oxidant and a consequent inability to activate immune-competent cells.

Figure 2. Three-way connection with the syringe containing the anticoagulant. At that step syringe containing ozone is off.
Figure 3. Blood ozonation, ozone is transferred to the syringe with blood. Three-way connector links both syringes, venous access is off.

Figure 4. Remotion of the gas phase (A) and infusion (B) of ozonated blood. A. Three-way connector link both syringes, venous access is off. B. Three-way connector link venous access and ozonated blood contained syringe. Gas contained syringe is off.
Figure 5. Washing step, with physiological saline solution.

4. Side effects

The secondary effects, above all, derive from the handling, sometimes difficult, of the patient. The main adverse effects are bruising or phlebitis in the venipuncture area. Hypotension or vagal syndrome can also be observed in the case of rapid reinfusions.

5. Warning, Contingencies, Corrective Actions

Warning: in case of aggressive animals, use the rectal way.

In case of other side effects follow the instructions of ISCO3/CLI/00/01 "First Aid in ozone therapy (Inhalatory 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".

6. References

6.1 SOP References

ISCO3/DEV/00/01 Guidelines and Recommendations for Medical Professionals Planning to Acquire a Medical Ozone Generator.

ISCO3/CLI/00/01. First Aid in ozone therapy (Inhalatory exposition and accidental over dose)

ISCO3/REC/00/03 The ISCO3 Safety Information and Adverse Event Reporting Program Form.


6.2 Other References


### 7. Change History

<table>
<thead>
<tr>
<th>SOP no.</th>
<th>Effective Date</th>
<th>Significant Changes</th>
<th>Previous SOP no.</th>
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<td>ISCO3/MVE/00/01</td>
<td>13/12/2022</td>
<td>Grammatical and spelling errors was suggested by Dr. Wayne McCarthy, ND. Legend from figure 2 to 4 was modified. Peretyagin S.P. and A.A. Struchkov: Suggestion related with contraindication and technical precisions of the method.</td>
<td>First version</td>
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### 8. Document Records

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<th>Title</th>
<th>Signature</th>
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<td>Mercedez Hernández Avilés</td>
<td>Member ISCO3</td>
<td>28/12/2022</td>
</tr>
<tr>
<td>Author</td>
<td>Miriam Portero Fuentes</td>
<td>Member ISCO3</td>
<td>28/12/2022</td>
</tr>
<tr>
<td>Authoris / Approved</td>
<td>ISCO3 Board and members 2020-2024</td>
<td>All members</td>
<td>28/01/2023</td>
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