The Multiple Inert Gas Elimination Technique: A User's Manual

US Army Institute of Surgical Research Technical Report Number 2016-x

Alexander T. Dixon, BS, MLT(ASCP)[CM]

Brendan M. Beely, AAS, RRT

Bryan S. Jordan, MS, RN

Leopoldo C. Cancio, MD

Andriy I. Batchinsky, MD

Editor:

Daniel S. Wendorff, BS

USAISR, San Antonio, Texas

11 February 2016

Contents

Acknowledgements (2002)

This work would not have been possible without the ongoing support, advice, expertise, and encouragement of Dr. Peter D. Wagner, Professor of Medicine and Bioengineering, School of Medicine, University of California, San Diego. In addition, Dr. Wagner kindly provided the software programs P50.exe, SHORT.exe, and VQBOHR.exe mentioned in the text, lent us an expired gas-mixing box, and critically reviewed the original manuscript.

We wish to thank Mr. Bryan S. Jordan, RN, and Dr. William Weiss for performance of much of the animal work; the technical support team at Hewlett-Packard, SPC Alejandro Gerena-Alvalle, and Ms. Janice Grassel, for assistance with the gas chromatography equipment; and SSG Luis Cardenas, Mr. Raul "James" Jimenez, Mr. Doug Cortez, Dr. Ron Walton, and the entire Veterinary Support Branch for animal care and surgical support. Mr. Guy Drew designed the new ISR 4.5-liter mixing box, and SSG Eric Smeed designed the new 1-liter mixing box and coordinated the construction of the boxes. Mr. Glen Gueller provided audiovisual support.

Finally, we are grateful for the guidance and leadership of the Commander and Director at the time these studies were performed, COL Cleon W. Goodwin, and of the Senior Scientist, Dr. Albert T. McManus.

Acknowledgements (2016)

Mr. Alexander Dixon wishes to thank Bill Baker, Mark Espinoza, Logan Leatherman, Mike Lucas, Belinda Meyers, Dr. Corina Necsoiu, Johnny Nelson, Jr., and Pete Walker for animal care and Austin Frye, Isaiah Land, and David Trapolsi for assistance outside of the ICU setting.

Disclaimer

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. The mention of specific products does not constitute endorsement of any kind. In conducting the research described in this report, investigators adhered to the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and with *The Guide for the Care and Use of Laboratory Animals*, National Institutes of Health Publication 85-23. The Animal Care and Use Committee of our Institute approved all experimental protocols and supervised all animal care.

Abstract

The Multiple Inert Gas Elimination Technique (MIGET) is a method of assessing ventilation/perfusion (V_A/Q) inequalities, shunt, and diffusion limitation. It was initially developed as a means of quantifying V_A/Q mismatch as an intrapulmonary cause of hypoxemia.[1] The lung is viewed as a set of 50 respiratory units, each with a distinctive V_A/Q ratio. Six inert gases of varying solubility in blood are infused, and the arterial and expired gas concentrations of the gases are measured under steady-state conditions. Then, distribution of blood flow and ventilation to each of the 50 compartments is calculated. Although technically challenging, this method can be applied in a wide range of basic and clinical settings, and enables the evaluation

of V_A/Q mismatch in a relatively low-risk, non-invasive fashion. This report describes the MIGET methodology from a technical standpoint, as currently performed at the U.S. Army Institute of Surgical Research (USAISR). General set up, assessment of gas chromatograph (GC) linearity, preparation and infusion of the 6-gas solution, specimen collection and processing, and data analysis are covered in detail. Creation of a novel, complex, multi-ramped GC program is described.

A Brief History

In the late 1970s, Dr. Peter D. Wagner, of the Division of Physiology at the University of California – San Diego, developed the Multiple Inert Gas Elimination Technique (MIGET).[2-11] Using MIGET, distribution of blood flow (Q) and ventilation (V_A) to each V_A/Q compartment is calculated and graphically depicted, thereby providing insight into the intrapulmonary causes of hypoxemia. Shimazu *et. al.* introduced the MIGET to the U.S. Army Institute of Surgical Research (USAISR) in a study focusing on smoke inhalation in an ovine model.[12] Shimazu was assisted in 1987 by Wagner, who visited to consult with technique development. Shimazu, through utilization of the MIGET, helped define the primary role of V_A/Q mismatch in the pathophysiology of hypoxemia following inhalation injury.[13] In subsequent work, Ogura and Tasaki used the MIGET in an exploration of the pathophysiology of sepsis-induced lung injury; these investigators also utilized the MIGET to assess various palliative treatments for smoke- and sepsis-induced lung injury in ovine and porcine models.[14-20]

In 2001, Batchinsky *et. al.* reintroduced the MIGET to the USAISR in their studies of pulmonary contusion in a porcine model[21] and chlorine inhalation in an ovine model[22]. In the presence of new analyzers and equipment, methods needed to be relearned and refined. This process was again expedited by a visit from Wagner, who consulted during animal experiments. Batchinsky also visited Wagner's laboratory to view an application of the MIGET in a human model; each of these visits were instrumental to the success of the current methodology at the USAISR.

In 2015, after a 4-year hiatus, Batchinsky returned to the MIGET to assess the efficacy of various palliative measures against acute respiratory distress syndrome (ARDS). This project also marked the handing over of the MIGET to Alexander Dixon. The transition from Batchinsky to Dixon began with a study investigating the effects of nebulized racemic epinephrine on ARDS brought about by smoke inhalation and thermal burns in a porcine model. The Multi-Organ Support Technology task area (MOST) of the USAISR hopes to extend the usage and utility of this technique to all appropriate studies within the group and with outside collaborators.

Purpose

The intent of this report is to describe the current MIGET methodology at the USAISR. Furthermore, it is the desire of the authors to empower a MIGET newcomer to a reach a level of practical proficiency. To this end, this manual is accompanied by photographs and schematics. Nevertheless, successful implementation of this technique requires dedication, practice, and determination on the part of at least one, full-time investigator to produce reliable results.

Basic Considerations

The Principle of Mass Balance

One of the foundations of the MIGET is the mass-balance principle.[8] "Mass balance" means that there is conservation of mass. The entire mass of a particular inert gas entering the lung from mixed venous blood exits the lung by arterial blood and exhaled, alveolar air. There can be no MIGET if the gases utilized react with the surrounding tissues into which they are infused. This model is represented by the following equation:

$$(1) \quad \lambda P_v Q_T = \lambda P_a Q_T + P_E V_E$$

where P_V is the partial pressure of the inert gas in mixed venous blood in mmHg, P_a is the partial pressure of the inert gas in arterial blood in mmHg, P_E is the partial pressure of the inert gas in the expired gas in mmHg, V_E is the volume of gas expired in L/min, Q_T is the cardiac output in L/min, and λ is the partition coefficient (i.e. the ratio of the concentration of the gas in the liquid phase to the concentration in the gaseous phase) where:

$$(2) \quad \lambda = \beta \cdot \frac{(P_B - P_{H_2O})}{100}$$

where β is the solubility of the inert gas in mL per 100mL of blood per mmHg, P_B is the barometric pressure in mmHg, and P_{H2O} is the water vapor pressure in mmHg (obtainable from standard reference tables).

For Equation (1) to hold true, Henry's law must apply. The gas is biologically inert and has a very rapid, virtually unlimited diffusion capacity across the alveolar-capillary membrane. In addition, a linear relationship exists between partial pressure of the gas and the mass of gas dissolved in blood.

Applying Equation (1) to an individual lung unit that is characterized by a specific V_A/Q ratio:

$$(3) \quad P_C = P_a = P_v = \frac{\lambda}{\lambda + \left(V_A/Q\right)}$$

where P_C is the end-capillary partial pressure for the inert gas in mmHg. Thus,

$$(4) \quad \frac{P_c}{P_v} = \frac{P_a}{P_v} = \frac{\lambda}{\lambda + \left(V_A/Q\right)}$$

where P_C/P_V defines the retention of the gas in the blood (R) and P_A/P_V defines the excretion (E) of the gas into the ambient air. Knowing this, we can substitute into Equation (4) to obtain:

$$(5) \quad R = E = \frac{\lambda}{\lambda + \left(V_A/Q\right)}$$

This equation represents an important principle; the retention and excretion of the inert gases depend only on the solubility of the gas and the V_A/Q ratio of the lung unit. To characterize the various units within the lung, the gases chosen must cover a wide range of partition coefficients. To this end, the MIGET analysis suite uses a multi-compartmental approach with enforced smoothing.[2,4,8] Based on the retentions of the gases, the continuous distribution of the pulmonary blood flow as a function of V_A/Q ratios is estimated. Similarly, the continuous distribution of the alveolar ventilation as a function of the V_A/Q ratios is assessed via the excretion levels of the gases.

The Lung

The MIGET does not appreciably alter the physiology of the lung being studied. The lung is assumed to consist of a number of compartments that are homogeneous and arranged in parallel. The ventilation and perfusion in every compartment is assumed to be constant over a time scale of minutes. The distribution of blood flow and ventilation are modeled as smooth, without sudden irregular changes. The concentration of the inert gases in the inspired air is assumed to be zero.[2]

Steady-State Conditions and Diffusion Equilibrium

For the mass-balance equations of the V_A/Q units to remain valid, a steady-state condition must exist. This ensures that during sample collection, retentions and excretions are constant.[4,8,23] A steady ventilation status can be documented by way of repeated minute-ventilation and respiratory-frequency measurements. In our practice, a Wright's respirometer was installed after the exit port of the mixing box, and expired volumes were measured manually before each sampling.

Ideally, the condition of the subject should be maintained before and during sampling. In our practice, diligent attention was given to the steadiness of blood pressure and heart rate. Fluctuations greater than ±10mmHg in blood pressure or ±5 beats/min in heart rate may point to inconsistencies in the steady-state condition. Similar values from duplicate arterial blood samples can also point to a well-maintained steady-state condition. In fact, the MIGET data itself can provide the greatest insight into the condition via a retrospective recapitulation. The absence of systematic differences between distributions obtained from the first and second MIGET samples support steady gas exchange; smaller values of the residual sum of squares (RSS) also correspond to a steady-state condition.

Of comparable importance is the maintenance of temperature. The temperature of the subject need not be maintained at a specific point but rather within a reasonable range, as much as possible given physiologic state, prior to sampling. The temperature of the equilibration water bath should also be maintained at a consistent temperature that is within the range of normal body temperatures for the subject under consideration; it need not be matched, degree for degree, to the subject. The rate of infusion of the 6-gas mixture must also be steadily maintained; a deviation of as little as 10cc/hr can disrupt the steady-state condition. Infusion rates, expressed in mL/min, equivalent to one quarter the minute ventilation in L/min yield concentrations in the range of ppm or lower.[1]

Cardiac Output

Accurate measurement of cardiac output is crucial in determining V_A/Q inequality. Cardiac output (CO) describes the volume of blood being pumped by the heart over a specified

period of time, reflects the quantity of blood delivered to all parts of the body, and indicates how efficiently the heart responds to metabolic demands of the body.[24]

The most widely-employed method is thermodilution (TD) via flow-directed pulmonary catheter (see **Figure 1**[25]). The pulmonary artery catheter enables convenient indirect measurement of cardiac output, the major clinical determinant of oxygen transport.[26-28] In our practice, we have relied upon the Swan-Ganz CCO/EDV Thermodilution Catheter (model 117HF75) used in conjunction with a Vigilance II Monitor™ (Edwards Lifesciences, Irvine, CA).

The catheter is introduced through a large vessel and advanced through the right atrium into the right ventricle and eventually into the pulmonary artery. Careful observation of the insertion distance plus concurrent analysis of pulsatile waveforms provides visual cues to successful placement.[29-30]

The left internal jugular vein is cannulated via surgical incision. After incision, the catheter is advanced, with balloon inflated, from the superior vena cava (SVC) into the right atrium. Insertion beyond this point directs the catheter into the right ventricle and, ultimately, the pulmonary artery until occlusion occurs. When the catheter is in position the lumen used for TD CO determination resides approximately in the SVC near to the right atrium.

TD CO measurement is made in our lab by injecting 10cc 5% dextrose in water, held at 0-5ºC, into the proximal port, which resides in the SVC and detecting the change in blood temperature at the distal tip of the catheter. The degree of cooling of the blood caused by the injection is measured by a flow-through temperature probe at the point of injection on the downstream at the thermistor. Three measurements of TD CO are taken, averaged, and recorded as CO in L/min.

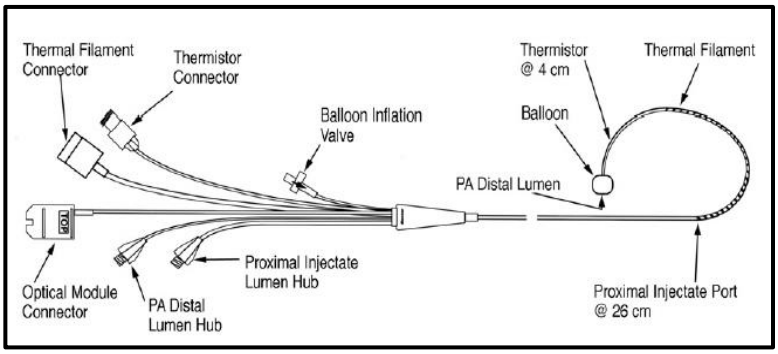


Figure 1: Flow-directed catheter
Used with Permission: Edwards Lifesciences, LLC

Selection of Subjects

A cursory literature search reveals that the MIGET has been performed on a wide variety of subjects: healthy and diseased human, canine, equine, porcine, ovine, leporine, reptile, and avian models. The MIGET has been proven safe and widely applicable in clinical and research settings. Moreover, the presence of the gases usually goes largely unnoticed by the subject.

The Ventilator

As long as the expired gas is directly transferred to the mixing box without passing through the ventilator, the composition of the gases will not be affected. However, accurate measurement of the minute ventilation requires the use of a reliable instrument, such as a

respirometer or calibrated pneumotachometer. For minute ventilation or expired volume quantities, the ventilator should not be used as a definitive source. Fraction of inspired oxygen (F_IO_2) is routinely measured using the same analyzer cells as standalone oxygen analyzers (± 2-3%), and thus the values displayed on the ventilator can be utilized.

Water Bath Maintenance

Well before samples are collected, ensure water level and temperature are within desired range. In our practice, the water bath is warmed to a consistent 37ºC. Depending on the subject species, the temperature may need to be adjusted; however, be certain the set point is always within the range of body temperatures appropriate for the subject and study. Ideally, body temperature during infusion and the water bath temperature should match, but there is no need to precisely match these temperatures since the MIGET software suite can correct for differences between body temperature and water bath equilibration temperature.

P50

P50 is defined as the partial pressure at which the saturation of a particular enzyme is fifty percent. In brief, the oxygen P50 of hemoglobin can give insight into the affinity of the subject's hemoglobin for oxygen at sampling. While there are chemistry and blood gas analyzers that are capable of deriving a value for P50, some use an approach that is derived purely mathematically[31], with little regard to physiology. This approach is also flawed inasmuch as the model breaks down with oxygen saturations greater than 90%, which are common in arterial samples.

Since the P50 of hemoglobin is requisite information for our calculations, a separate program has been developed for this specific instance. This program, which requires only simple blood gas information, generates reliable, reproducible values for P50.

Pre-Heparinized Syringes

As a general rule, the use of pre-heparinized syringes with dry lithium heparin should be avoided. In our experience, we have found that some manufacturers of pre-heparinized syringes use acetone as a drying agent for the anticoagulant. The effects of acetone-contaminated heparin have been investigated[32]; the proximity of blood-gas sampling syringes to the MIGET sampling syringe in the blood-sampling manifold[*] requires these syringes to be heparinized in house to minimize contamination.

Heparin Testing

As we have utilized several varieties of heparin derived from different species and organs, we continually sample and test our heparin for contamination. In our studies, each lot of heparin is tested utilizing a methodology similar to that described by Powell, *et. al.* for detecting acetone contamination.[31] From each new case of heparin opened in our lab, one third of the vials, determined by systematic sampling, are tested for the presence of contaminating substances that may interfere with peaks of interest in our chromatograms. It is highly advisable that this sort of testing be performed regularly and with greater frequency upon arrival of heparin from new lots or new suppliers.

[*] For further insight, see **Blood-Sampling Manifold**, p. 17.

Modalities

Originally, the technique was described using inert-gas levels obtained using arterial and mixed venous blood alongside expired gas samples. The technique can be performed, with comparable accuracy and less invasiveness with the omission of mixed venous sampling. When utilizing arterial blood samples only, cardiac output should be measured directly via a pulmonary artery catheter.[7]

In our practice, sampling mixed venous blood via a pulmonary artery catheter proved challenging. Due to low hydrostatic pressures at the sampling site, blood cannot be withdrawn from this point as safely and efficiently as through an arterial line. This obstacle can be surmounted by applying greater suction to the syringe and dropping the catheter as low as possible.

Depending on the force applied, air may be aspirated into the sampling syringe around the plunger. This can be avoided by the use of a disposable waste syringe as a "collection vessel". Once the blood has been collected, it can be injected into the glass syringe in such a manner that will avoid any extraneous air. Alternatively, this situation may be avoided by using a narrower-bore syringe.

As a final note on mixed venous sampling, this procedure increases overall sample processing time, number of samples, and the probability that handling errors will be made. In our estimation, the sampling of expired gas and arterial blood only is best suited for operations in which rapid, frequent sampling is preferred or in which many samples must be analyzed.

A third approach to the MIGET exists in which only expired gas and peripheral venous blood samples are obtained. Using this approach, the inert gas mixture is infused into a peripheral vein of one arm, and samples are taken from a peripheral vein in the opposite arm. This modality is based on the assumption that the arterial and venous levels of inert gases will approximate one another after a significant period of time has elapsed (i.e. 60-90+ min) in a resting patient whose tissues are not being experimentally warmed. The measured values of the inert gases can therefore be substituted for arterial peak levels in the software.[33] This approach is well suited for operations in which studies will take place over several days or weeks or when cannulation of a central artery is not possible.

Materials

Syringes

In our practice, matched plunger and barrel glass syringes produced by Becton, Dickinson & Co. Yale (B-D) in 10, 30, and 50cc capacity were utilized for sampling arterial and mixed gas samples. These syringes must have smooth plunger movement, must be air tight when closed, and must be able to withstand applied pressure and suction without noticeable leaking. In preparing the MIGET infusion or in determining blood densities, B-D plastic syringes with Luer-Lok™ in 10 and 20cc capacity were utilized. In the MIGET blood-sampling manifold, 20cc B-D plastic syringes with Luer-Lok™ and 1cc tuberculin slip tips were utilized. To determine partition coefficients, Perfektum®-produced glass tuberculin slip tip 1cc syringes were utilized.

The use of plastic syringes for sampling should be eschewed as they tend to leak the inert gases. The plastics of the plunger and barrel also tend to become saturated by the gases, which leads to falsely decreased readings, especially in blood samples.

Stopcocks

For the preparation of the MIGET infusion and infusion into the subject, B-D 4-way metal stopcocks were used. Metal stopcocks are a necessity in preparing the infusion as plastic pieces can be destroyed by high concentrations of the liquid phases added to the infusion. However, in the sampling of blood and expired samples, plastic stopcocks can be used, and they confer an added advantage of being easily locked into one another allowing for air-tight transfers. In our practice, we used Discofix 4-way stopcocks for sampling.

Needles

In our practice, B-D PrecisionGlide® needles in the size of 18G were utilized for initial heparinization of sampling syringes, as guide needles for the infusion bags, and to prepare aliquots of the liquid phases for the infusion bags. To deliver the infusion into the subject, in preparation of the infusion bag, and to perform solubility calculations Pierce Reacti-Vap™, four-inch, Teflon-coated, blunt-edged needles were used.

Heparin

In our most recent studies, we used heparin derived from porcine intestinal mucosa (Heparin Sodium Injection USP, 1000 units/mL, Fresenius Kabi USA, LLC). The volume of heparin used on each glass sampling syringe ranged from 0.5 to 0.7cc, depending on the brand of the syringe. In previous studies, porcine-intestine-derived heparin (Heparin Sodium Injection 1000IU/mL, Elkins Sinn, Inc.) and bovine-lung-derived heparin (Heparin Sodium Injection USP, 1000IU/mL, Pharmacia & Upjohn) have been used.[23]

Dextrose Infusion

The vehicle for the 6-gas mixture that was most commonly used in our studies is a 5% Dextrose Injection USP, 1L (Baxter Healthcare Corporation, Deerfield, IL, 60015 USA).

Inert Gases

In our practice, sulfur hexafluoride (SF_6), ethane and cyclopropane were ordered as a mixture from Matheson Tri-Gas Inc. The specifics of the tank are as follows:

- Cylinder pressure: 1227kPa
- Cylinder volume: 1.416m^3
- SF_6: 20%
- Cyclopropane: 20%
- Ethane: Balance

The remaining gases are furnished in the liquid phase.

- Acetone (Certified ACS Reagent Grade)
    - Fisher Scientific, 500mL, CAS: 67-64-1
- Ethyl Ether Anhydrous (Certified ACS Reagent Grade)
    - Fisher Scientific, 1L aluminum bottle, CAS: 60-29-7
- Halothane* (IUPAC: 2-bromo-2-chloro-1,1,1-trifluoroethane)
    - Sigma-Aldrich, 100mL, purity > 99.0%, CAS: 151-67-7

* Enflurane can be used in the place of halothane.

Water Bath

A water bath is used to allow the blood sample to equilibrate and release the gaseous phase of the inert gases, in preparation for sampling by gas chromatography. The water bath used in our practice is a Lindberg Blue M water bath (model # RSWB 3222A). The water bath was equipped with a metal tray and a high-density foam system used for holding in place the syringes during agitation.

Balance

The balance should be tared at the beginning of each study and as infrequently as possible during the study. The balance used in our practice is a Sartorius GMBH digital scale, type 1212MP.

Ventilator

The ventilator used in our practice is a DrägerEvita XL. It is a full, intensive-care mechanical ventilator capable of various modalities of artificial ventilation for the subject. During MIGET sampling, continuous mandatory ventilation (CMV) is utilized owing to its ability to limit variability by maintaining the integrity of breath size and frequency. For MIGET sampling, the F_IO_2 is set to the lowest level possible to allow for stable oxygenation of the subject. The normal breathing circuit is removed, and a specialized MIGET circuit is attached to the endotracheal tube.[*]

Indirect Calorimetry

Crucial to the calculations performed by the software that analyzes the MIGET data are reading of $\dot{V}CO_2$ (i.e. the volume of carbon dioxide produced by the subject per minute) and $\dot{V}O_2$ (i.e. the volume of oxygen consumed by the tissues per minute). These quantities are measured for inspired and expired oxygen and carbon dioxide, as well as minute ventilation.

Expired Gas Sampling Platform

While there is some concern as to the loss of soluble gases to the materials that compose the ventilator circuit, we have been able to avoid this possible source of error via a specific connection between the ventilator and sampling circuit. For a detailed review of this set-up, see "Schematic of Ventilator/MIGET Sampling Circuit" (pp 28-30). By this mode, the ventilator is effectively, completely excluded from the sampling apparatus.

In past studies, we have utilized varying configurations of mixing boxes:

- A 1L mixing box with internal baffles, heated by means of a heating tape that is wrapped around the box.[†] This box is similar to that described by Ferrer, *et. al.*[34]
- A 10L mixing box with a copper coil (internal diameter (ID): 5.08cm, length: 4.877m, fitted into the box). The coil inside the box is heated while wrapped in heating tape. This was also the mixing box utilized in our work on pulmonary contusion.

[*] Please refer to the MIGET circuit schematics to view the details of the circuit (pp 28-30).
[†] This is the mixing box we utilized in the 2015-era smoke/burn studies.

- A 4.5L mixing box in which a stainless steel coil (ID: 3.175cm, length: 5.791m) was heated inside a digital oven. This device was designed by Mr. Guy Drew. The coil dimensions were copied from the previous USAISR model.[13-20]

Because of its small volume, the 1L mixing box is especially convenient for rapid, repeated sampling. The internal baffles allow for complete mixture of the expired gas inside the chamber. This has been confirmed by demonstrating the absence of respiratory variation in carbon dioxide levels measured at the exit port of the box. The same has been done for the other boxes as well. In the event of a study in which excessive minute ventilation occurs, a bigger volume box should be used.

As far as the tubing that connects the subject to the mixing box and the exhaust from the mixing box to the ventilator, temperature control is of the highest priority. The tubing and mixing box are wrapped with heating elements and covered in heat-insulating tape. In the case of the tubing, there is a layer of heat-insulating foam between the heating elements and tape. The heating elements are controlled by temperature controllers (Cole-Parmer Digi-Sense® 89000-00, Barnant Co., Barrington, Il MODEL 89000 00), programmed to levels that ensure a step-wise increase in temperature from subject to the tubing and again from the tubing to the mixing box. This is requisite as unheated elements can serve as a surface upon which condensation will occur, which in turn will provide a substrate into which water-soluble gases can escape. Also, any elements of the tubing that are not fully insulated in the previously-stated manner are heated by lamps that also preclude condensation.

Ventilator Tubing

In our practice, we utilize AirLife brand, 22mm corrugated tubing for construction of the ventilator circuit, as well as the MIGET sampling circuit.

Heat Lamps

In our practice, we utilize two Emerson brand ThermaLamps (Model 96-DL) to provide further protection from condensation in the tubing.

Thermometer

A thermometer is placed at the wye junction of the subject's endotracheal tube to measure ambient temperature. In our practice, we utilize a self-indicating liquid-in-glass thermometer (H-B Inst. Co., Philadelphia, PA).

Mylar Covering

A reflective, Mylar blanket is utilized in our practice to isolate the subject's endotracheal tube, which allows for more efficient heating of the exposed areas of the ventilator tubing. It is also useful to drape the blanket across the back of the subject to protect from the heat of the lamps.

Testing the GC for Linearity

Testing the GC for linearity is integral to the success of the MIGET before studies can commence.[9] To perform this procedure you will need 4, nitrogen-washed and filled 30cc, glass syringes. Obtain initial samples via the 3-gas mixture tank or headspace sampling from bottles. The following is the procedure to perform a linearity test:

1. Mark one syringe with an identifier corresponding to the gas sampled (e.g. "3-Gas", "ether", etc.)
2. Sample 30cc of gas from the 3-gas mixture tank
3. Regulating outflow with your finger, expel all but 3cc of the mixture from the syringe
4. Draw in 27cc of nitrogen into the syringe. This produces a 1:10 dilution
5. Repeat Steps (3) and (4) until you have obtained a 1:10,000 dilution*
6. Transfer 5cc of the mixture to a washed, 10cc syringe
7. Analyze the aliquot via GC
8. At this point, perform serial dilutions of 1:2 with the remaining 3-gas mixture, analyzing 5cc aliquots via GC, until the desired dilution is reached†
9. After all linearity GC runs have been completed, a calibration table should be built comparing concentrations and peak heights

Linearity tests for the gaseous phases of any remaining gases can be established using the preceding procedure. While linearity can be reestablished whenever desired, there is generally no need for this during a study if all samples and runs are repeatable and reasonable with no changes in operating conditions or hardware of the GC.

Gas Chromatography

What follows is the basic setup of our GC. Any functional GC with an electron-capture detector (ECD) and flame-ionization detector (FID) will suffice, and this technique can be used with a variety of columns under various conditions that can and should be optimized for each system. It is important to note that the customized nature of our GC column has led to increased efficiency and greater ease of use. By adding a glass wye to the end of our column, we have been able to split one sample between two detectors. If this is not feasible, two runs through one chromatograph or independent runs through two chromatographs may be necessary to expose the samples to both the ECD and FID.

Chromatograph

The GC used in our practice is a Hewlett-Packard (model #6890) equipped with a capillary column. The capillary column used is a J&W GS-Gas pro (SN 0752927) with a nominal length 30.0m and nominal diameter 320.0µm. At the end of the column, a glass wye is installed to split the sample between the two detectors. Our GC is equipped with a constant volume inlet loop, with a volume of 0.5cc, to which a metal stopcock has been attached.

Software Package

For our most recent studies, Agilent MSD Chemstation (Revision E.02.01) was used to obtain and interpret all chromatograms. The GC method is detailed below:

Front Inlet

In our studies, the front inlet was turned off, and all of our samples were introduced through the back inlet.

* Dilution is necessary to avoid overwhelming the detectors of the gas chromatograph.
† Six to eight serial dilutions are generally adequate.

Back Inlet

- Mode: Split
- Gas: N_2
- Heater: 250ºC
- Pressure: 36.54 psi
- Total Flow: 110 mL/min
- Split Ratio: 7.0:1
- Split Flow: 94.7 mL/min
- GasSaver: Off

Column

- Mode: Const Flow
- Inlet: Back
- Detector: Back
- Outlet psi: Ambient
- N_2 Pressure: 36.56 psi
- N_2 Flow: 14
- N_2 Average Velocity: 131 cm/s

Oven

- Oven: On
- Setpoint: 60ºC
- Oven Config Max: 450ºC
- Oven Config Eq: 0.50

Oven Ramp

	ºC/min	Next ºC	Hold time (min)	Run Time (min)
Initial		60	0.00	0.00
Ramp 1	45.00	175	0.00	2.56
Ramp 2	40.00	200	0.00	3.18
Ramp 3	30.00	220	1.2	5.05

Front Detector (ECD)

- Heater: 300ºC
- Makeup Flow Gas: N_2
- Const Col + Makeup: 45.0mL/min
- Electrometer: On

Back Detector (FID)

- Heater: 250ºC
- H2 Flow: 40.0mL/min
- Air Flow: 400mL/min
- Makeup Flow (He): 30
- Flame: On
- Electrometer: On

Signal 1

- Source: front det – col comp 1
- Data Rate: 20Hz
- Min Peak Width: 0.01min
- Save Data: On, All

Signal 2

- Source: back det – col comp 2
- Data Rate: 20Hz
- Min Peak Width: 0.01min
- Save Data: On, All

Data Integration

Data integration parameters are saved into the method files with data analysis available via an offline menu. Specific integration sequences can be constructed on demand by following the instructions from the Agilent operational manuals.

Preparation of Sampling Syringes

Blood-Sampling Manifold

This procedure outlines the process by which manifolds for blood sampling should be obtained. The sampling manifold allows for anaerobic acquisition of blood samples and can be used for arterial, peripheral venous, or mixed venous blood samples.

1. Attach a plastic stopcock to a clean and dried 30cc glass syringe
    a. Label the syringe according to its draw position and blood content (e.g. A1)
2. Record the mass of the syringe as "M_{dry}"
    a. All masses should be recorded to at least two decimal places
3. Draw a sufficient volume of heparin to lubricate the interior surface of each syringe
    a. We have found that a volume of ~4cc of heparin per glass syringe should suffice
4. While holding the syringe vertically, withdraw and advance the plunger, making full rotations, until the heparin has coated all interior surfaces
    a. The plunger should be withdrawn to the 30cc mark consistently to ensure reproducibility. A minimum of three passes of the plunger are recommended
5. While advancing the plunger on the final pass, move slowly until the heparin pools in the upper portion. Keep pressure on the plunger until heparin fills the stopcock
    a. This step may require tapping to ensure the system is gas free
6. Attach the second similarly prepared, labeled syringe via the stopcocks on the side port of the first
7. Holding both syringes in your non-dominant hand, close the stopcocks' free ports with your fingers
8. Open the stopcocks and transfer the heparin from the first to the second syringe under some pressure
9. Close the stopcock of the first syringe immediately to ensure no gas enters
10. Withdraw the plunger of the second syringe to remove any residual heparin from the dead space of the interconnected stopcocks
11. Close the stopcock of the second syringe
12. Dry the stopcock of the first syringe using compressed air
13. Record the mass of the heparinized syringe as "M_{+H}"
14. Subtract "M_{dry}" from "M_{+H}" to obtain the mass of heparin in the syringe ("M_{Hep}")
15. Divide "M_{Hep}" by the density of heparin ("ρ_{Hep}") to obtain the volume of heparin ("V_{Hep}")
    a. In our practice, we have experimentally derived a "ρ_{Hep}" value of ~1.02g/mL
16. Repeat the procedure listed above for all subsequent syringes, refilling your syringes with heparin as needed
17. After the final blood-sampling syringe has been heparinized, the remaining heparin is used for the 1cc blood gas syringes
    a. 1cc syringes should be equipped with plastic stopcocks and heparinized using the same principle as the glass syringes

By tracking the serial numbers of each syringe, masses for dry and heparinized syringes can be tracked to ensure consistency of technique. Comparing these quantities to historical runs will give insight into the accuracy and reproducibility of handling skills. As a technical aside, heparinized syringes should be used within twelve hours. Heparinized 1cc syringes should be

marked correspondingly to the MIGET syringes (e.g. A1, ABG1; V1, VBG1; etc.). The two heparinized syringes and a waste syringe are assembled into a manifold used for the actual sampling (Figure 2).

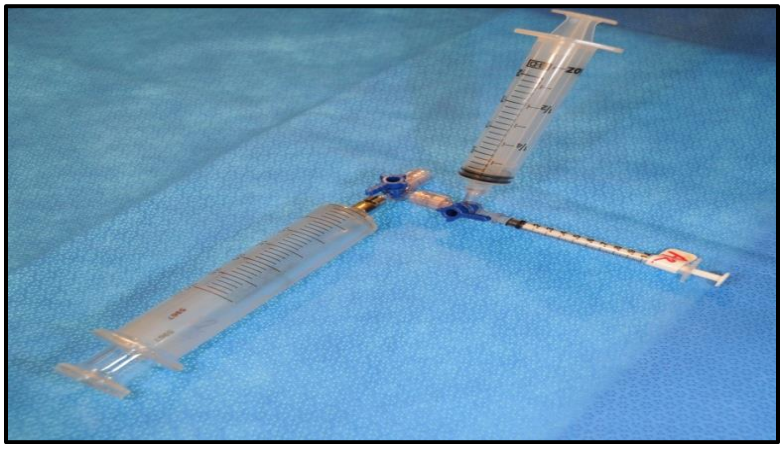


Figure 2. Fully-assembled manifold used for arterial or venous sampling.

We recommend preparing syringes in duplicate for each time point. Until good technique is established, triplicate or quadruplicate sampling may be advisable to account for possible loss of samples.

At this point in the process, the following data should have been obtained: serial numbers, dry masses, heparinized masses, masses of heparin, and heparin volumes for all sampling syringes.

Expired Gas-Sampling Syringes

In our practice, 30cc glass syringes are used for expired gas sampling, marked correspondingly to the blood sampling syringes mentioned above (e.g. A1/E1, A9/E9). After each expired gas sample was run through the GC, any remaining expired gas was expelled from the syringe. The syringes should be washed with nitrogen before being stored in an airtight configuration via a closed stopcock. These syringes should be stored far away from stocks of the inert gases to avoid contamination. These syringes do not need to be cleaned or stored in any particular or peculiar way beyond the methods mentioned above. Syringes should be replaced after any of the following deficiencies are noted:

- Obvious moisture in the barrel or upon the plunger
- Stickiness during plunger movements
- A high degree of variability unexplained by any other means (e.g. different results from duplicate samples)
- Other situations evaluated by common sense

GC-Delivery Syringes

GC-delivery syringes are used for sampling headspace from arterial and venous blood samples. For this purpose, 10cc glass syringes are washed and filled with ultra-high purity (UHP) nitrogen to capacity for easy differentiation of syringes that are in use from those awaiting analysis. The serial numbers for these syringes should be annotated along with the contents to disallow possible mix-ups. The nitrogen in these syringes serves to flush the dead

space of the stopcocks before headspace sample collection. This simple convention is useful in situations wherein many samples from multiple subjects are being processed and mistakes may occur.

Nitrogen Reservoir Syringes

Three to four 50cc glass syringes are washed and filled with nitrogen after being correspondingly marked. These syringes are kept on hand to add a nitrogen headspace to the blood sample-containing syringes as necessary.

Nitrogen Washing

All syringes used for gas collection should be washed to a full volume at least 30 times to full volume to ensure that any contaminating gases in the syringe are fully evacuated. Syringes that are being cleaned of mixed-gas samples should be washed a minimum of 40 times to full volume.

Solubility Calculations

Solubility of the inert gases in the subject's blood is dependent on temperature and hematocrit values as well as lipid, cholesterol, phospholipids, and protein content.[3] Therefore, these solubilities are ever changing. For these reasons, before any inert gas infusion begins, partition coefficients should be calculated via the following methodology to provide reasonable insight into the subject's baseline condition:

Preparation of 6-Gas Addition

A 6-gas addition should be prepared prior to the collection of solubility samples:

1. Into a clean, dry 30cc glass syringe labeled "3-Gas," introduce 30cc of the 3-gas mixture directly from the tank
2. Dilute this mixture via four successive 1:10 dilutions utilizing UHP nitrogen
3. Set aside
4. Label a second, clean, dry 30cc glass syringe "Halothane" (or "Enflurane" as appropriate) and fill with 30cc from the headspace of the bottle
5. Dilute this by two successive 1:10 dilutions utilizing UHP nitrogen
6. Set aside
7. Label a third, clean, dry 30cc glass syringe "Ether" and fill with 30cc from the headspace of the bottle
8. Dilute this by one 1:5 dilution utilizing UHP nitrogen
9. Set aside
10. Label a fourth, clean, dry 30cc glass syringe "Acetone" and fill with 30cc from the headspace of the bottle
11. Set aside (this syringe does not require a dilution)
12. Label a fifth, clean, dry 30cc glass syringe "6-Gas" and combine into it:
    a. 3-Gas dilution 15cc
    b. Halothane dilution 10cc
    c. Ether dilution 5cc
    d. Acetone 10cc

Blood Sampling

1. Prepare two, clean, dry 30cc glass syringes as described previously*
2. Label them "A_{con}" and "V_{con}"
3. Dry the stopcock of each syringe with compressed air and weigh to obtain "$M_{+Heparin}$"
4. Collect 7cc of arterial and venous blood into the correspondingly marked syringes
5. Weigh each stopcock to obtain "M_{+Blood}"
6. Utilizing a 50cc glass syringe as a nitrogen reservoir, transfer 15cc of UHP nitrogen to each blood sample
7. Place the syringes into the prepared water bath for 40 minutes to equilibrate
8. After equilibration, transfer headspace from each syringe into a correspondingly-labeled sampling syringe
    a. Before a transfer is made after equilibration, the stopcock of the wet syringe should be thoroughly dried with compressed air
    b. During transfer from one syringe to the next, the dead space of the stopcocks should be voided by the nitrogen from the "gaining" syringe
9. Introduce these samples individually to the GC for analysis
    a. This step is initially performed to ensure there are no contaminating sources in the blood that may interfere with analysis
10. All residual headspace should be expressed from the syringe
11. The syringe and stopcock should be carefully dried before the next step
12. Add 15cc of the "6-Gas Addition" to each syringe
13. Equilibrate the syringes in the water bath for 40 minutes
14. After equilibration, transfer headspace from "A_{con}" syringe into a 30cc glass syringe labeled "S1"
15. Set aside "S1"
16. Express all residual gas from "A_{con}"
    a. This step ensures full first extraction of gases and avoids re-equilibration of the gases with the blood
17. Repeat Steps (14) – (16) for "V_{con}"
    a. The sampling syringe for "V_{con}" should be labeled "S2"
18. Analyze via GC "S1" and "S2"
19. Utilizing a 50cc glass syringe as a nitrogen reservoir, transfer 15cc of UHP nitrogen to each syringe
20. Equilibrate the syringes in the water bath for 40 minutes
21. Remove the syringes and read the total volumes of blood and gas. Annotate this volume as "VG_1." Replace the syringes into the water bath for the final 10 minutes of equilibration time
22. Remove "A_{con}" from the water bath, dry the stopcock, and transfer the headspace to a 30cc glass syringe labeled "S1R"
23. Set aside and express all residual gas from the syringe
24. Repeat Steps (22) – (23) for "Vcon"
    a. The sampling syringe for "Vcon" should be labeled "S2R"
25. Analyze "S1R" and "S2R" via GC

* **Blood Sampling Manifold**, Steps (1) – (12) pp. 17-18.

Note: The preceding procedure provides all necessary information for the calculation of partition coefficients for SF_6, ethane, cyclopropane, and halothane. What follows is the continuation of the procedure for the determination of partition coefficients for ether and acetone.

26. Wash and fill two clean, dry 50cc glass syringes with 40cc of UHP nitrogen and mark as follows: for "A_{con}" label "S1R 1:50", and for "V_{con}" label "S2R 1:50"
27. Connect a glass 1cc tuberculin syringe to the side port of the stopcock on "A_{con}" and a Reacti-Vap™ needle to the end port
28. Open the stopcock to the tuberculin syringe and aspirate 1cc of blood
29. Close the stopcock to the blood-containing syringe and express all of the gas through the needle
    a. Attention to detail must be paid to this procedure to ensure that all bubbles are removed
    b. This procedure may need to be repeated to ensure a gas-free tuberculin syringe
30. After the tuberculin syringe is filled, express all but 0.25cc of blood through the needle
31. Turn the handle of the stopcock such that it forms a 45-degree angle between the "A_{con}" syringe and the tuberculin syringe to ensure that flow in all directions is stopped
32. Insert the needle, while still attached to the manifold, into the stopcock of the "S1R 1:50" syringe
33. Slowly express the entirety of the blood volume from the tuberculin syringe into "S1R 1:50"
    a. It is imperative that this is performed slowly such that no blood is allowed to contact the plunger, which will lead to sticking and preclude smooth motion of the plunger inside the barrel
34. Remove the needle and quickly close the stopcock to "S1R 1:50"
35. While maintaining the syringe at an angle that provides separation between blood and plunger, allow the syringe to equilibrate in the water bath for 5 minutes
    a. In our practice, the syringe is held in hand during this phase of equilibration
36. Read and record the total volume of the syringe and dry the stopcock with compressed air
    a. Record the volume of the gas as "VG1*"
37. Attach a UHP nitrogen-filled 30cc glass syringe labeled "S1 1:50" to the stopcock of "S1R 1:50" and transfer as much sample as possible
38. Record the volume of blood, in this case 0.25cc, as "VB1*"
39. Analyze "S1 1:50" via GC
40. Repeat Steps (27) – (39) for "Vcon"
    a. For "Vcon" the labels are as follows: "S2R 1:50," "S2 1:50," and "VB2*"

Calculation of Partition Coefficients

To calculate the partition coefficients for any of the gases, first we must elaborate on some terminology. "Sol_x" refers to the solubility of gas *x*. "$P1_x$" refers to the peak height of gas *x* from sample "S1". "$P1R_x$" refers to the peak height of gas *x* from sample "S1R". "$P1R^*_x$" refers to the peak height of gas *x* from sample "S1 1:50".

The equation for the determination of the partition coefficient for SF_6, ethane, cyclopropane, halothane, or enflurane is as follows:

$$(6) \quad Sol_x = \left(\frac{VG1}{VB1}\right) \Big/ \left(\frac{P1_x}{P1R_x - 1}\right)$$

The equation for the partition coefficient for ether or acetone is as follows:

$$(7) \quad Sol_x = \left(\frac{VG1*}{VB1*}\right) \Big/ \left(\frac{P1_x}{P1R*_x - 1}\right)$$

Very similar, if not nearly identical calculations can be obtained using the data collected from "V_{con}," substituting "VG1" for "VG2", "VB1" for "VB2", etc.

Advisements

The timing of all steps is adjusted to allow minimal delay between obtaining a GC sample and analysis. The rationale is that gaseous analytes can leak from syringes via the plastic stopcock connection point. In our practice, a delay of no more than ten minutes allows for the most accurate results.

Preparation of the 6-gas Mixture

This procedure should be performed in a designated area set aside specifically for mixture preparation. As a best practice, gloves should be changed before the addition of each gas. While this preparation does not need to take place in a sterile setting, the use of proper aseptic technique is vital. The concentration of gases in the mixture will decrease with time, so the mixture should be used within three to four hours of preparation. In our practice, a 5% dextrose infusion is used as the base for the mixture. In reality, the mixture can be prepared in Ringer's lactate or normal saline to accommodate variances in study design or product availability.

The volumes of the gases/liquids infused are standardized inasmuch as they ensure sufficient saturation of the infusion circuit, blood volume, and ventilation circuit plastics. Some room may exist for customization of these volumes; however, such customization will require experimental confirmation of the concentrations prior to use.

1. The Pierce needle/ B-D stopcock set should be assembled and set aside
2. Open the overwrapping of the infusion
    a. Be mindful to avoid touching the rubber aspiration port
    b. Ensure the fluid is not expired, and that the fluid bag is free from leaks and overt contamination

3. Carefully, introduce a sterile PrecisionGlide needle to create a port of entrance through which the Pierce needle can pass
4. Remove the guide needle and introduce the infusion needle into the hole created by the guide needle. This step may require patience.
5. Lay the bag on a table or counter and push all of the gas together into as few bubbles as possible in the middle under the upper cupola of the bag. Open the stopcock and, while applying constant pressure, remove as much gas as possible.
    a. If needed, tap on the bag to coerce any remaining bubble to coalesce when expressing the gas.
6. Hang the bag from an IV pole and connect the hose from the 3-gas tank to the stopcock
7. Open the regulator to allow the gas to flush the hose and stopcock for a few moments to avoid infusing air into the bag
8. Open the stopcock and add 400cc of 3-gas mixture
9. Close the stopcock and shake the bag vigorously for 15-20 seconds
    a. Exercise caution to avoid puncturing the bag with the needle at this step
10. Allow the bag to rest for 10-15 minutes. This allows the partial pressures in the gaseous and liquid phases to equilibrate
    a. During this stage, the small bubbles will generally coalesce into one large bubble
    b. Keep in mind that these gases may be driven out of the solution by excessive, unnecessary agitation
11. Remove as much gas as possible from the bag utilizing the method outlined in Step (5)
12. Repeat Steps (6) – (10) twice more
    a. While fewer saturations may suffice, repeated saturations serve to protect from technical missteps
13. Using a 10cc Luer-Lok™ syringe equipped with a PrecisionGlide needle to draw up 3cc of ether
14. Disconnect the needle and connect the syringe to the stopcock. Inject the ether into the bag
    a. Be sure to expel as much as air as possible from the syringe before any liquid additions.
15. Aspirate some fluid into the syringe and flush total volume into the bag
16. Following Steps (13) – (15), use a 20cc Luer-Lok™ syringe to inject 18cc of acetone
17. Following Steps (13) – (15), use a 10cc Luer-Lok™ syringe, inject 4cc of halothane/enflurane
18. Gently invert the bag two to three times before infusion
19. The bag should be marked with subject identification criteria and the time and date of preparation before use

Quality Assurance

While it is not necessary to confirm the concentrations of the constituents of the prepared mixture, it can be achieved via the following methodology:

1. Remove a 1cc aliquot of the mixture using a tuberculin slip tip syringe and PrecisionGlide needle
2. Inject approximately 0.25cc into a 1L container pre-filled with UHP nitrogen
3. Incubate the container for 10-15 minutes at room temperature

4. Utilizing a clean, washed 10cc glass syringe, remove a 5cc sample and run through the GC

This methodology can provide an "internal standard."

Delivery of 6-gas Mixture

Infusion of the 6-gas mixture should be administered via a peripheral vein. It is critical to separate the infusion and sampling sites from each other by as a great a physical difference as possible. In addition to this, the lines themselves must be separated as the inert gases can diffuse through the plastic by way of direct contact.

When we first began our studies, we infused the mixture via the right femoral vein and sampled via the right femoral artery. This approach jeopardized our results due to the diffusion of the highly concentrated mixture across the vessel walls and plastic catheters. This diffusion occurs because the concentrations of the gases in the mixture are orders of magnitude higher than those retrieved from biological sources. By infusing into a femoral vein on one side of the subject and sampling from the opposite femoral artery, we were able to resolve the "inexplicable" difficulties we had with the technique.

Continuing this thought process, with the smoke/burn application of the MIGET, we began infusing through the right femoral vein and sampling through the carotid artery, which, anatomically speaking, separated infusion/sampling by the greatest physical and anatomical distance feasible.

The minimum time required for the inert gases to come to equilibrium with the porcine model we have been using is approximately 45 minutes. As a rule, infusion should run between 45 and 60 minutes to ensure proper equilibrium while guarding against excessive fluid overload. These time scales may differ with the species of the subject, the body mass of the subject, and cardiovascular dynamics.

What follows is the procedure we use to ensure proper delivery of the 6-gas mixture:

1. Connect the metal stopcock to the infusion line via a male-to-male connector
2. Prime through the line to ensure the total volume is occupied by the infusion
3. Flush through the femoral cannula to ensure patency before infusion begins
4. Set the rate of infusion to the desired rate[*]
5. Start infusion and note the time

Quality Control of the Infusion Device

Steady delivery of the infusion into the subject is one of the most important conditions to achieving success with the MIGET. To avoid confusion, we suggest the following safety measures:

- Double-check the delivered amount of the infusion by weighing the bag prior to infusion and after infusion to verify that the desired amount is delivered. The weight difference should correspond to the delivered amount on the pump display
- During infusion, gently invert the bag at least once every 10-15 minutes to ensure halothane/enflurane does not pool in the lower portion of the bag
- Maintain the rate of infusion, in mL/min, at a minimum of half the minute ventilation in L/min

[*] In our practice, this rate ranges between 3-5cc/min.

- During the infusion, periodically check plastic connections. Repeat, prolonged exposure to the 6-gas mixture can cause any plastic piece to crack and leak. The male-to-male connector, if plastic, should be exchanged when deficiencies are noted or, at a minimum, every 24hrs

Sample Collection

Arterial Sampling[†]

Blood samples are obtained utilizing a pre-prepared sampling manifold, via the following procedure:

1. Prior to connecting the manifold to the arterial line, apply suction such that it fills with blood
2. After connection, pull 10cc of blood into the waste syringe
3. Open the sampling stopcock
4. Draw 7cc of blood into the sampling syringe
5. Immediately close the sampling stopcock
6. Draw an additional 10cc of blood into the waste syringe
7. Open the blood gas stopcock
8. Draw 1cc of blood into the heparinized tuberculin syringe
9. Close the blood gas stopcock
10. Return as much blood as possible from the waste syringe to the subject
11. Rinse stopcock and syringe to remove any residual blood

If followed closely, this maneuver will produce two, air-free samples for analysis while wasting as little blood volume as possible for the subject.

Mixed Venous Sampling

If venous sampling is performed, it should be taken via the distal port of a pulmonary artery catheter. A sampling manifold, constructed in the method of the arterial sampling manifolds, is utilized. This procedure of sampling is identical to the steps listed above.

Expired Gas Sampling

Expired sampling is performed at the exit port of the mixing box.(**Figures 3-5**). The sampling port should be located as close to the end of the heating coil as possible; this will ensure a collection of steady-state, homogeneously-mixed samples with respect to respiratory cycles. Expired gas collection is delayed with respect to arterial sampling via a precise methodology. The delay between arterial and expired gas samplings can be calculated via the following formula:

$$(8) \quad Delay = \frac{Volume_{mixing\ box} + Volume_{connecting\ tubing}}{Minute\ ventilation}$$

where volumes are measured in millimeters (mL) and minute ventilation is measured in millimeters per minute (mL/min). After approximately one half of the arterial sample has been

[†] The construction of the manifold is described in detail in "Preparation of Sampling Syringes", p 17.

drawn, a clock should be started for this delay. The expired gas sample should be taken smoothly, over approximately 60 seconds to ensure a representative sample carried over several respiratory cycles is obtained.

Sample Processing

General Comments

Depending on setup, it may be necessary to adjust the volume of sample injected. The amount of sample injected should not be less than three times the volume of the constant-volume inlet loop plus 1cc. In our case, there is a 0.5cc loop, so the minimum sample volume to inject would be 2.5cc. Any sample size that exceeds this convention can be injected into the loop, for its function is to introduce equal sample sizes with each injection. Allow the carrier gas flow to be left open, which ensures the entirety of the sample will be flushed through the column. If this is not possible, it may be the case that some portion of the sample is not flushed into the column. It is advisable to allow the carrier gas flow to remain open for the entirety of the run, if possible.

Expired Gas Samples

Expired gas samples should be introduced to the GC as soon as possible. It can be proven experimentally that syringes leak with time; the rate of sample loss can be tested via GC, if desired. Generally, samples should be run with minimal delay. Syringes should be tested for leaks if extended delays are expected; leak rates greater than 1-2%/hr are unacceptable. What follows is our procedure for the analysis of expired gas samples via GC; be certain to perform all of the following steps in a smooth yet deliberate manner and as quickly as possible:

1. Ensure GC software is prepared and ready to process a sample
2. Connect the plastic stopcock of the syringe to the metal stopcock of the injection port
3. Open the plastic stopcock
4. Inject sample
5. Turn the injection handle down and allow it to remain down until the run ends
6. Press “Start”
7. Close the plastic stopcock to lock transfer in all directions
8. Disconnect the syringe from the injection port

Blood Samples

Below we have outlined the procedure by which all blood samples can be processed regardless of their source (i.e. arterial, peripheral or mixed venous):

1. Thoroughly dry the syringe and stopcock using compressed air
2. Record mass as “M_{+Blood}”
3. Connect sampling syringe and nitrogen reservoir stopcocks
4. Holding the sampling syringe in your non-dominant hand, open the nitrogen syringe stopcock to flush the dead space of the stopcocks with at least 10cc of UHP nitrogen
5. While maintaining pressure over the Luer ports, place the reservoir syringe on a steady surface (e.g. a table)
6. Open the sampling syringe stopcock to draw in 8cc of UHP nitrogen; it is imperative that no blood enters the nitrogen reservoir and that the total volume of blood and nitrogen is precisely 15cc

7. Close the stopcocks by turning the handles toward the syringes
8. Place the syringe in the water bath and begin agitation
9. Annotate the time
10. After 30 minutes of equilibration time, remove each syringe from the water bath to read and record the total volume of fluid in the syringe
    a. During equilibration, gas will move from the blood into the headspace of the syringe, which causes an increase in total volume. Record the total volume as "V_{Total}"
11. Return the syringes to the water bath to complete equilibration for 10 minutes
12. Quickly remove each sampling syringe from the water bath and dry the stopcock with compressed air
13. Connect stopcocks of the blood-filled syringe and sampling syringe
14. Apply pressure over the Luer ports
15. Open the stopcock of the sampling syringe
16. Flush the dead space of the connected stopcocks with UHP nitrogen from the sampling syringe
17. Immediately close the ports with your fingers
18. Holding the blood-filled syringe vertically, open its stopcock and pull back on the plunger of the sampling syringe to extract the headspace sample
19. Close the stopcock of the sampling syringe
20. Introduce the sample for analysis via GC
21. Maintain the residual blood for density calculations

For the equilibration phase of processing, the rate of agitation that causes blood movement in the syringe but does not cause the blood to foam should be discerned. During the equilibration phase, be certain to gently rotate the barrel and plunger of each syringe to prevent sticking and ensure ease of movement. We generally perform two such rotations, approximately ten minutes apart.

After removing the syringes from the water bath, use your hand to shield the blood-containing portion of the syringe while drying. The compressed air will cool the blood otherwise, which may result in some loss of gases back to the blood. While sampling the headspace, make all efforts to avoid aspirating blood into the sampling syringe. In the event that some blood is drawn into the sampling syringe, attach a second sampling syringe and sample in the same methodology above described. In our practice, we maintain, at a minimum, two sampling syringes nearby when sampling headspace.

In the event that the number of blood samples to be analyzed exceeds the water bath's capacity, it is preferable to leave samples unprocessed rather than rush the equilibration phase. This delay, however, may cause some gas loss due to leakage. By far the most effective solution to this problem is proper planning and time management.

MIGET Analysis Suite

Be certain to always use uppercase letters in all aspects of the software suite. Check and double check entries before confirming as the data cannot be adjusted once confirmed. If, for some reason, a misstep is noticed after confirmation, delete all output files generated by the program, which are created in the same folder in which the programs are located. For the purpose of this manual, "extension" will refer to the last one to two characters of a file name, a period, and any digits following the period.

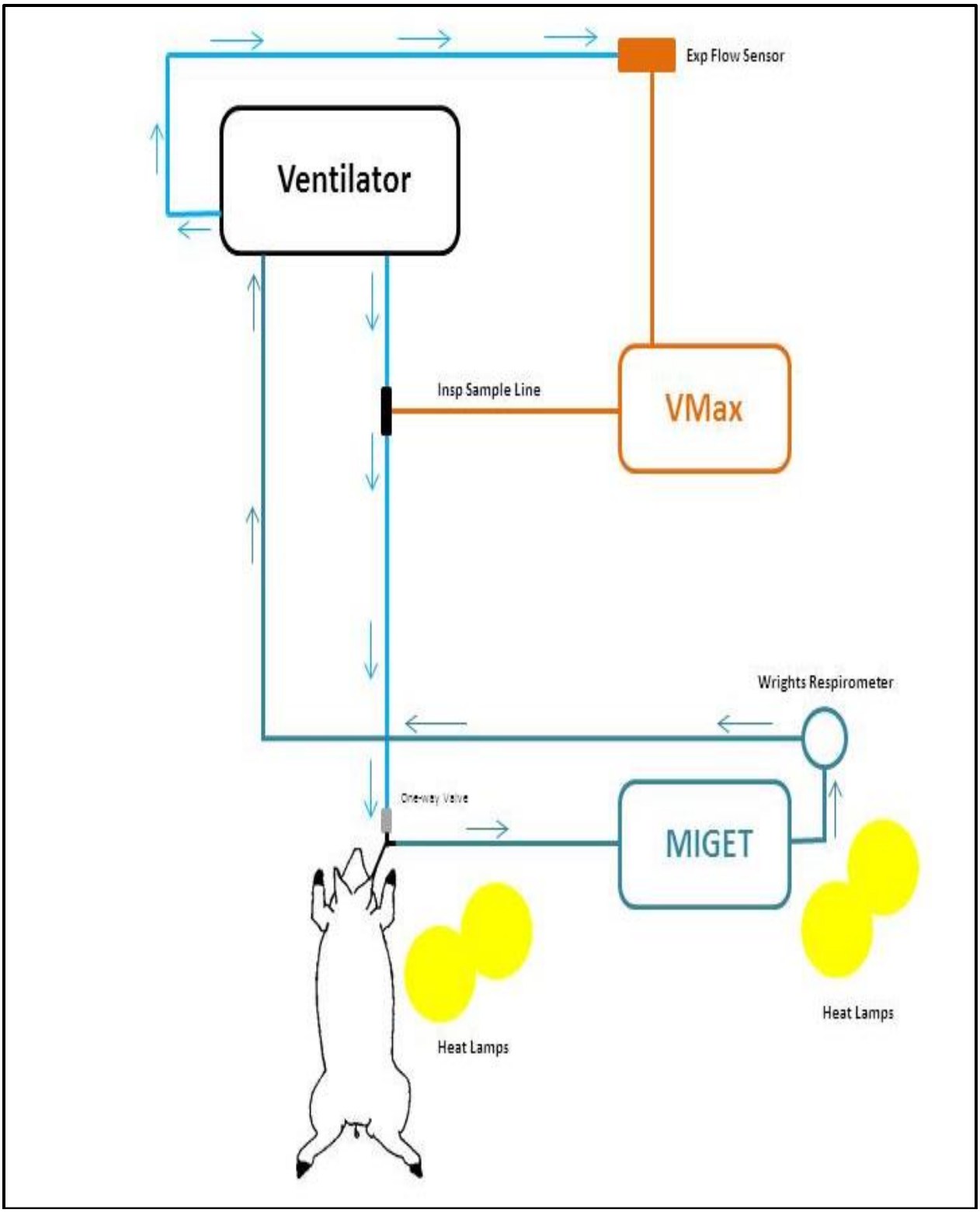


Figure 3. *MIGET Circuit overview*

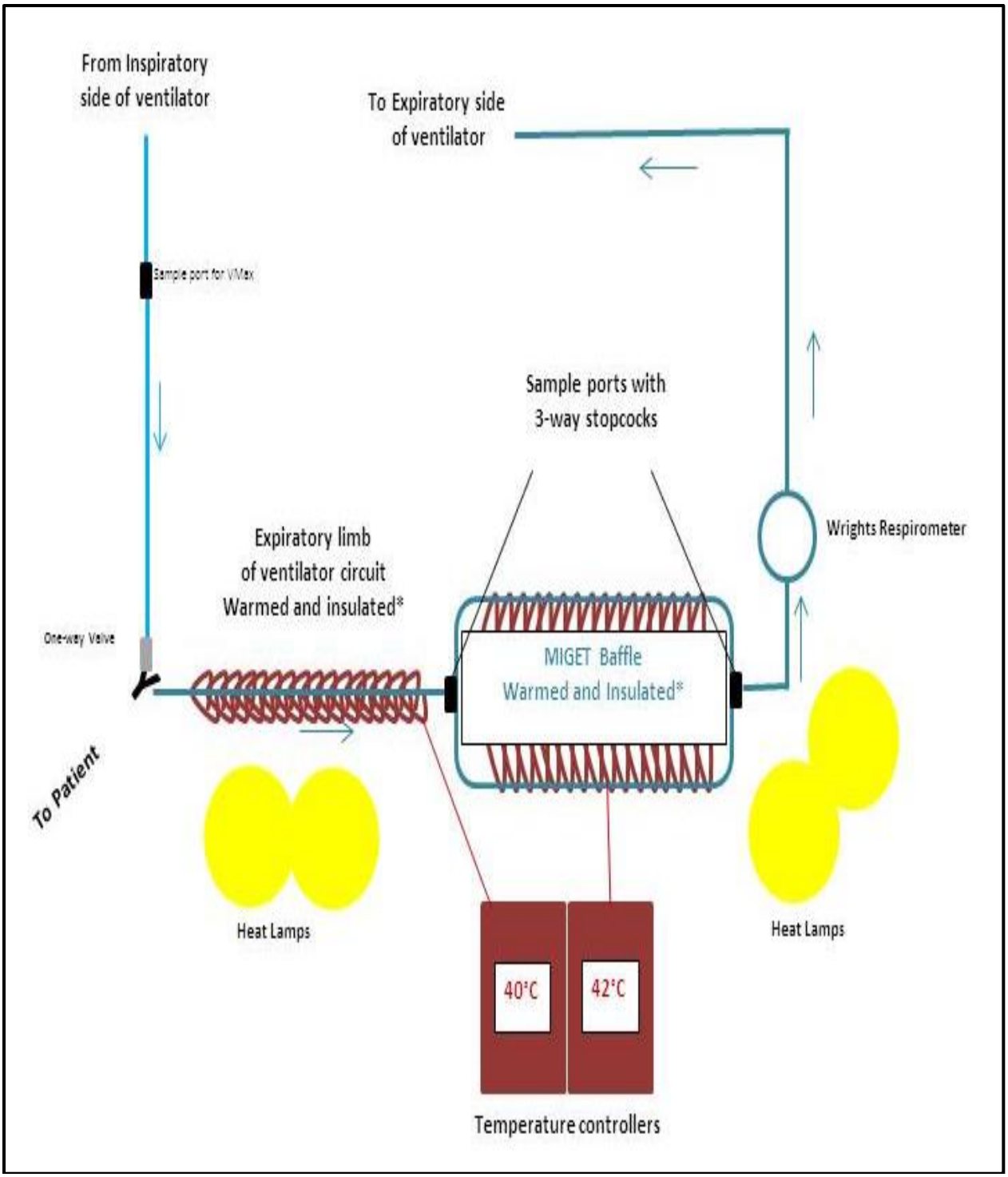


Figure 4. *MIGET Sampling Box Detail*: The circuit limb and baffled box are warmed, insulated, and wrapped with two turns of heat tape. Circuit limb is covered with foam insulation and held closed with duct tape. Baffled box is wrapped with Mylar blanket and covered with duct tape. Heat tape controllers are organized such that circuit limb (set to 40ºC) is warmer than the subject, and the baffle (set to 42ºC) is warmer than the circuit limb; this limits condensation. Heat lamps are positioned to further protect against condensation in the non-insulated parts of the circuit (e.g. ETT, wye, connection into the baffled box, sample port, respirometer).

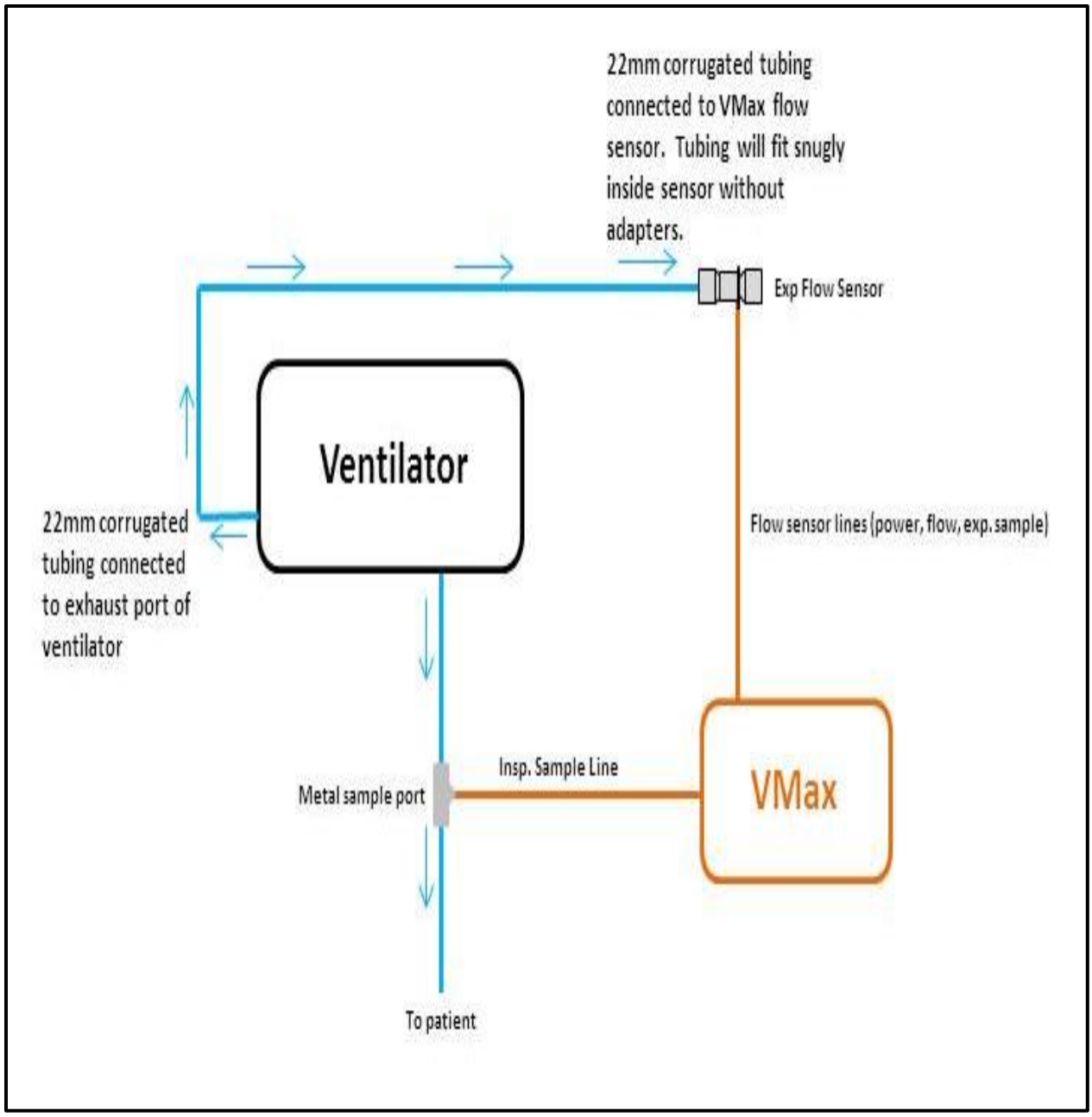


Figure 5. *VMax Connection Detail*. Ensure VMax is calibrated for both gas readings and flow prior to connection and sampling.

Be sure to keep file names alphanumeric and restricted to six characters, excluding extension (e.g. 3241BL3.3, 2019729.9, etc.). It is critical that all file names remain the same, with the exception of the extension. Raw data and output files can be viewed with Windows Notepad or Microsoft Word ®. Files with extensions "3.3" and "33.3" are output files, and files with extensions "7.7" and "9.9" are raw data files.

P50 Program

1. Double click icon to open the program
2. Press "0" to send output to disk, press Enter
3. Give the Unit 3 file a name with the extension "3.3" (e.g. subject number, time point, 3.3 - 2352BL3.3), press Enter
4. Enter a name or identifier for the subject (e.g. "Pig 2352"), press Enter
5. Enter the date of the study (e.g. 29 Jun 15), press Enter
6. Enter the number of blood gas sets that will be analyzed, press Enter
7. Press "1" to create a new file, press Enter
8. Give the Unit 7 file the same name given to the Unit 3 file with the extension "7.7" (e.g. 2352BL7.7), press Enter
9. Enter the following quantities and be sure to press Enter between each entry:
   a. F_IO_2 (fraction of inspired oxygen), as a decimal
   b. PO_2 (arterial partial pressure of oxygen), in mmHg
   c. PCO_2 (arterial partial pressure of carbon dioxide), in mmHg
   d. pH (arterial pH)
   e. SAT (arterial blood oxygen saturation), as a whole number
   f. Hb (arterial blood hemoglobin), in g/dL
   g. Hct (arterial blood hematocrit)
   h. Temp (subject's body temperature), in ºC
10. Confirm data are entered correctly with "Y", press Enter
11. Enter all subsequent blood gas sets following Steps (9) – (10)
12. Enter a lower bound for P50, in mmHg, press Enter
13. Enter an upper bound for P50, in mmHg, press Enter
14. Enter a lower and upper bound for arterial blood oxygen saturation, as a whole number in mmHg, press Enter
15. The window will disappear after all quantities have been entered

In our practice, the lower and upper bounds for P50 in swine are 20mmHg and 30mmHg, respectively; however, these values may vary based upon species of the subject and other parameters of the study.

SHORT Program

1. Double click icon to open program
2. Press "0" to write output to disk, press Enter
3. Give the file a name with the extension "3.3" (e.g. 3241BL3.3), press Enter
   a. "3.3" extension is required
4. Choose the appropriate number for the subject being studied, press Enter
   a. For swine, choose "1"
5. Choose "1" to correct for body/bath temperature difference, press Enter

6. Choose “1” to correct for expired acetone loss, press Enter
7. Choose “0” for the usual way, press Enter
8. Choose the appropriate number for the blood based on source, press Enter
9. Choose the appropriate number for the manufacturer of your GC, press Enter
10. If the raw data files already exist, which is unlikely, choose “Y” and continue to Step 13. If not, choose “N”, press Enter, and continue to Step 11
    a. If the raw data file is present, it will have a “7.7” extension
11. Type a short, descriptive label for data set, press Enter
12. Give the file a name with the extension “7.7” (e.g. 3241BL7.7), press Enter
13. Give the file a name with the extension “9.9” (e.g. 3241BL9.9), press Enter
    a. This will be the raw data file transmitted to the VQ Bohr Program
14. Enter the following quantities. Be sure to press Enter between each entry:
    a. Number of data sets (1, 2, 16, etc.)
    b. P_B (barometric pressure at sea level), in mmHg
    c. Blood gas-electrode temperature, in ºC, (37ºC in our case)
    d. Water bath equilibration temperature, in ºC
    e. Fractional correction value (CV) of gases other than SF_6: 0.03
    f. Oxygen solubility in blood: 0.003
15. Enter the following quantities and be sure to press Enter between each entry:
    a. Number of gases: 6, if all are used
    b. Number of compartments: 50
    c. Z-smoothing factor: 40
    d. VQ lowest: 0.005 compartments
    e. VQ highest: 100 compartments
16. Enter partition coefficients for the gases obtained from solubility calculations in the following order: SF_6, ethane, cyclopropane, halothane/enflurane, ether, acetone, press Enter after each value
17. Confirm partition coefficient values are entered correctly with “Y”, press Enter
18. Enter arterial peak heights or areas, pressing Enter after each value
19. Confirm peak data values are entered correctly with “Y,” press Enter
20. Enter expired peak heights or areas, pressing Enter after each value
21. Confirm peak data values are entered correctly with “Y”, press Enter
22. Enter mixed venous peak heights or areas, pressing Enter after each value
    a. If only arterial or peripheral venous blood is used, enter “0” for each value
23. Confirm peak data values are entered correctly with “Y”, press Enter
24. Enter the following quantities and be sure to press Enter between each entry:
    a. VE (volume expired), in L/min
    b. QT (cardiac output), in L/min
    c. P_B (barometric pressure of the room in which the study is taking place), in mmHg
    d. $Temp_B$ (body temperature), in ºC
    e. $Temp_R$ (room temperature), in ºC
    f. VGA (volume of arterial gas), in mL
    g. VBA (volume of arterial blood), in mL
    h. VHA (volume of heparin in arterial syringe), in mL
    i. VGV (volume of mixed venous gas), in mL
    j. VBV (volume of mixed venous blood), in mL

k. VHV (volume of heparin in mixed venous syringe), in mL
    i. If only arterial or peripheral venous blood is used, enter "0", "1", "0" for these values, respectively

25. Confirm data are entered correctly with "Y", press Enter
26. Enter the following quantities and be sure to press Enter after each entry:
    a. Hb (hemoglobin), in g/dL
    b. Hcrit (hematocrit)
    c. VO_2 (oxygen consumption), in mL/min
    d. VCO_2 (carbon dioxide production), in mL/min
    e. TOL (tolerance): 99000
    f. F_IO_2 (fraction of inspired oxygen), as a decimal
    g. F_ICO_2 (fraction of inspired carbon dioxide), as a decimal
    h. P_{50} (hemoglobin 50% saturation pressure), in mmHg
    i. PM_AO_2 (arterial partial pressure of oxygen), in mmHg
    j. PM_ACO_2 (arterial partial pressure of carbon dioxide), in mmHg
    k. pH_A (arterial pH)
    l. VO_2 (oxygen consumption), in mL/min
    m. VCO_2 (carbon dioxide production), in mL/min
27. Confirm data are entered correctly with "Y", press Enter
28. Summarized calculations of the program will now populate. Confirm data set is ok with "Y", press Enter
29. Repeat Steps 18 – 28 for any remaining number of data sets
30. When you confirm the last set of summarized calculations, the input screen will disappear

If you are using mixed venous and arterial blood samples in your practice, Step (26) should be amended as follows:

26. Enter the following quantities and be sure to press Enter after each entry:
    a. Hb (hemoglobin), in g/dL
    b. Hcrit (hematocrit)
    c. P_VO_2 (venous partial pressure of oxygen), in mmHg
    d. P_VCO_2 (venous partial pressure of carbon dioxide), in mmHg
    e. pH_V (venous pH)
    f. F_IO_2 (fraction of inspired oxygen), as a decimal
    g. F_ICO_2 (fraction of inspired carbon dioxide), as a decimal
    h. P_{50} (hemoglobin 50% saturation pressure), in mmHg
    i. PM_AO_2 (arterial partial pressure of oxygen), in mmHg
    j. PM_ACO_2 (arterial partial pressure of carbon dioxide), in mmHg
    k. pH_A (arterial pH)
    l. VO_2 (oxygen consumption), in mL/min
    m. VCO_2 (carbon dioxide production), in mL/min

VQ Bohr Program

1. Double click icon to open program
2. Enter the file name created by SHORT with extension "9.9", press Enter
3. Choose "0" to write output to disk, press Enter

4. Give the file a name with the extension "33.3" (e.g. 3241BL33.3), press Enter
5. Choose "0" to hardcopy plot the V/Q curves, press Enter
6. Choose "0" to hardcopy the R&E curves, press Enter
7. Choose "0" for the usual way, press Enter
8. Do not type anything, press Enter
9. Choose "Y" for O_2/CO_2 Calcs, press Enter
10. Choose "Y" for Bohr integration calculations, press Enter
11. Enter the desired number of integration steps within the provided range (100 in our case), press Enter
12. Choose "0" for no flag screen print, press Enter
13. The input screen will disappear

Measurement of Blood Density

What follows is the procedure we use to determine pooled blood density:

1. Weigh a dry, empty reservoir to obtain "M_R" in grams
    a. A disposable syringe or graduated cylinder can be used for this procedure
2. Pool together the residual blood remaining in the glass syringes used for blood sampling[*]
3. Ensure that as much blood on the surface of the reservoir is removed and that the surface is dried with compressed air
4. Weigh the reservoir again to obtain "M_{R+B}" in grams
5. Carefully observe the volume of the blood in the reservoir to obtain the quantity "V_B" in milliliters
6. Using the following equation, obtain "ρ_B" in g/mL:

$$(9) \quad \rho_B = \frac{M_{R+B} - M_R}{V_B}$$

As a side note, in our laboratory setting, we have created a library of blood density samples per species of subject to observe possible trends and shifts in this value. This quantity should be measured regularly in the beginning phases of experimentation, but it can be replaced by a representative mean after several iterations. If the latter is utilized as a strategy, the measured value should be obtained and compared to historical values before used to ensure quality control. The density calculated may not be exact or even approximate systems utilizing the MIGET on models of varying species.

Troubleshooting

Loss of Gases in Expired Samples

It is possible that the gases infused may not be recovered from the expired samples in the amounts expected in comparison to the excretion of other gases. The largest deficiency we have observed comes along with acetone excretion, for it is the most soluble of the gases we use. This may be observed, for example, when condensation accumulates in the expiratory limb of the

[*] If sampling arterial and venous blood, be sure to maintain integrity between the samples.

ventilator circuit. We combat this source of error by ensuring a step-wise increase in the temperature of the expiratory-sampling platform. The expiratory limb of the ventilator circuit is heated to a temperature greater than the subject's expected body temperature, and the sampling box is heated to a temperature that is yet higher than the expiratory limb. All surfaces of the platform that are not directly heated are exposed to high-grade heating lamps; all of these are efforts to reduce the probability of acetone loss. In the case of a subject who is not fully anesthetized, the problem of excessive salivation should also be taken into account. If saliva is allowed to pool in the sampling platform, it can serve as an acetone sink.

This should not, however, be taken to mean that other soluble gases cannot also be lost. If acetone excretion is normal, and the excretion of other gases is comparably low, it can point to an aspect of the sampling system for which a particular gas has an affinity. As a rule, all component pieces of the connection apparatus should be evaluated to discern which materials may be absorbing the gases in question such that they may be replaced. Be certain to refer heavily to the *Materials* section to ensure that the correct supplies are available.

The problem of acetone loss is also directly addressed via the MIGET software suite. The SHORT program corrects for acetone loss via an adjustment of its excretion value based upon the excretion of ether. However, SHORT correction cannot ameliorate deficiencies in technique or handling; this is to say that a high residual sum of squares cannot be corrected via this method.

Interpretation of VQ Bohr Output

The following is a series of images from a "33.3" output accompanied by descriptions:

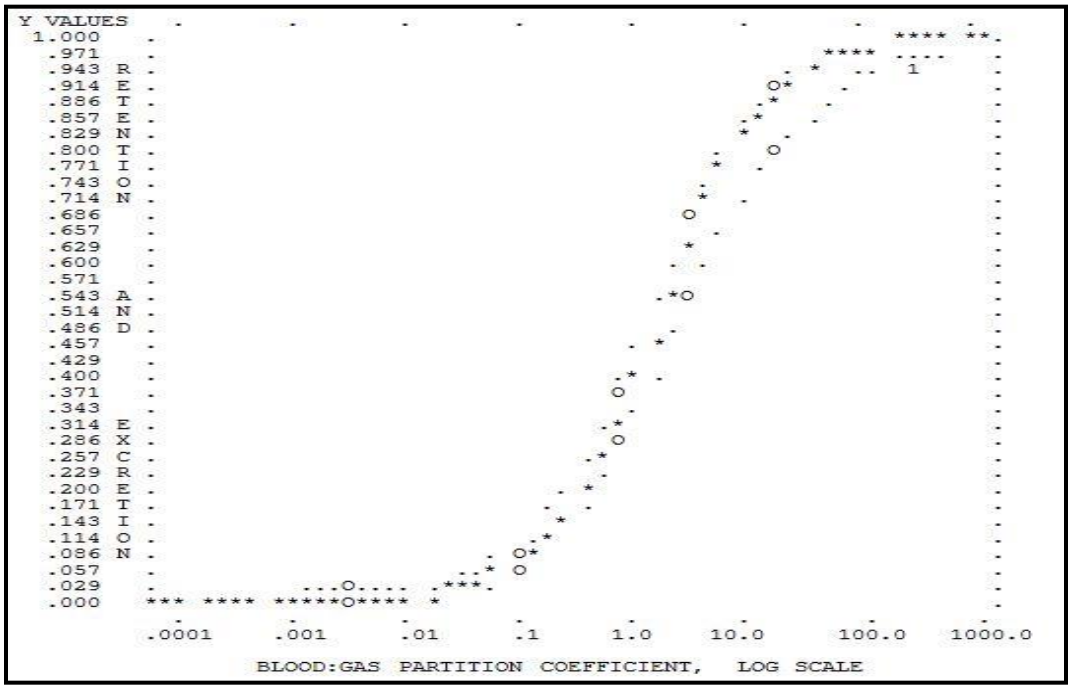


Figure 6. These curves represent retention and excretion as a function of solubility. These curves allow for estimation of how close the experimental data compares to expected values for a homogenous lung. In the graph pictured above, we observe good fit.

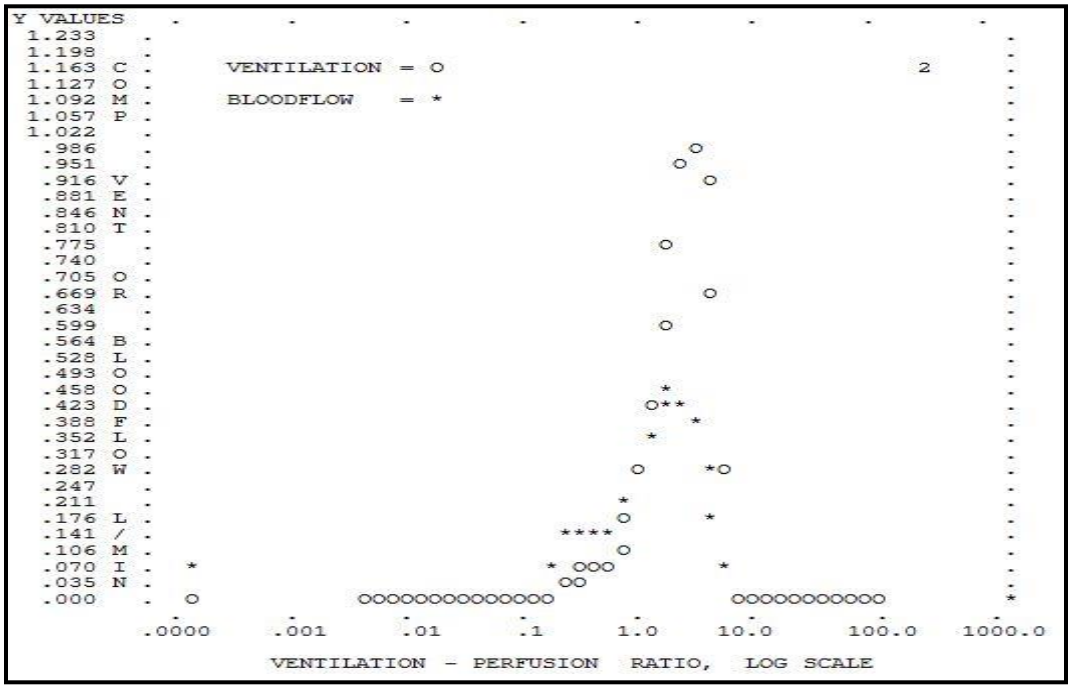
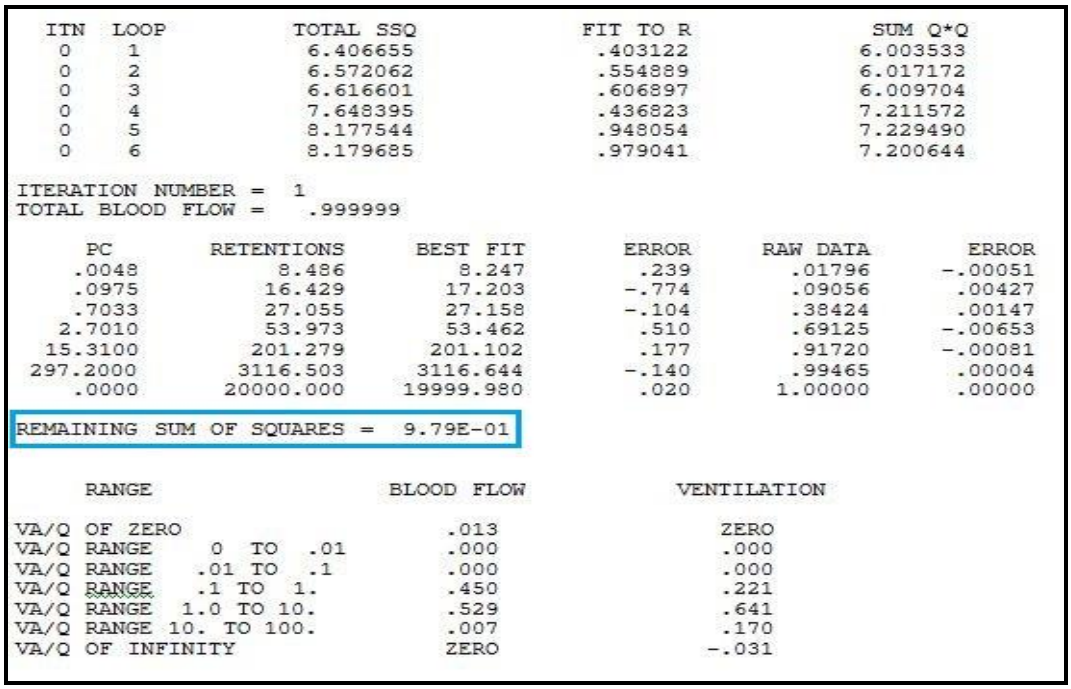


Figure 7. Inspection of these curves allows for a general estimation of the match between ventilation and blood flow. The *x*-axis represents the V_A/Q ratio, and the *y*-axis represents blood flow or ventilation (L/min). The form and shape of this curve can provide insight into a normal or pathologic case.



ITN	LOOP	TOTAL SSQ	FIT TO R	SUM Q*Q
0	1	6.406655	.403122	6.003533
0	2	6.572062	.554889	6.017172
0	3	6.616601	.606897	6.009704
0	4	7.648395	.436823	7.211572
0	5	8.177544	.948054	7.229490
0	6	8.179685	.979041	7.200644

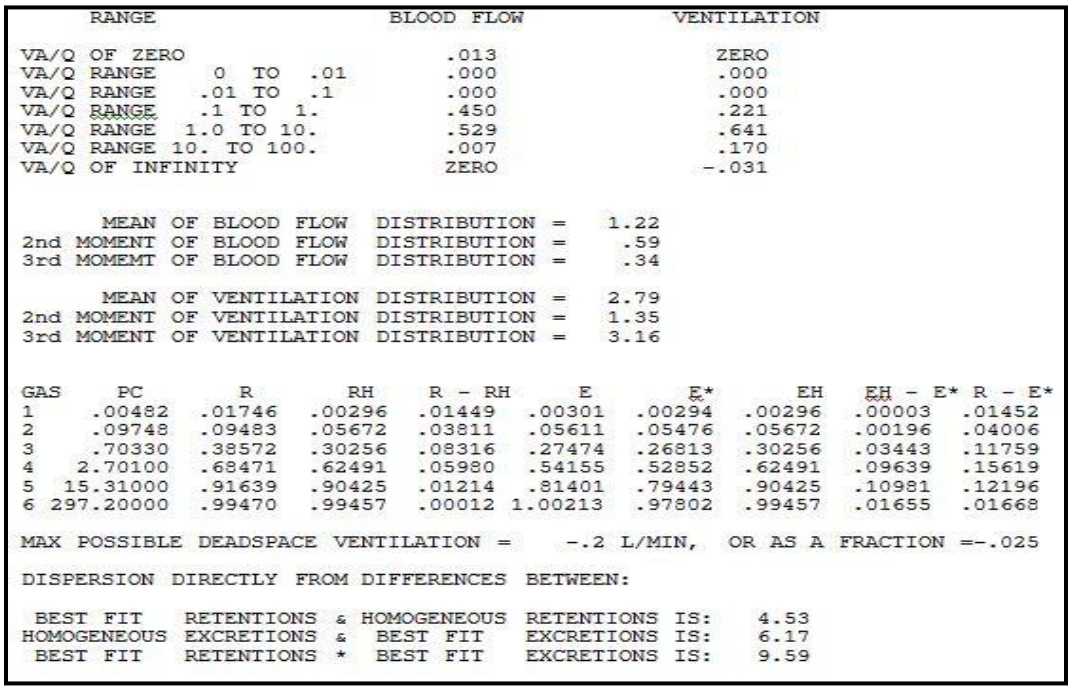
ITERATION NUMBER = 1
TOTAL BLOOD FLOW = .999999

PC	RETENTIONS	BEST FIT	ERROR	RAW DATA	ERROR
.0048	8.486	8.247	.239	.01796	-.00051
.0975	16.429	17.203	-.774	.09056	.00427
.7033	27.055	27.158	-.104	.38424	.00147
2.7010	53.973	53.462	.510	.69125	-.00653
15.3100	201.279	201.102	.177	.91720	-.00081
297.2000	3116.503	3116.644	-.140	.99465	.00004
.0000	20000.000	19999.980	.020	1.00000	.00000

REMAINING SUM OF SQUARES = 9.79E-01

RANGE	BLOOD FLOW	VENTILATION
VA/Q OF ZERO	.013	ZERO
VA/Q RANGE 0 TO .01	.000	.000
VA/Q RANGE .01 TO .1	.000	.000
VA/Q RANGE .1 TO 1.	.450	.221
VA/Q RANGE 1.0 TO 10.	.529	.641
VA/Q RANGE 10. TO 100.	.007	.170
VA/Q OF INFINITY	ZERO	-.031

Figure 8. The remaining sum of squares (RSS) defines the fit of the data to the model and serves as quality control for the whole set of data. In the case of high RSS values, the source of error can be identified by looking at the "Error" column between the "Best Fit" and "Raw Data" columns. In this case, the cause of the error may be narrowed down to the culpable gas.



RANGE	BLOOD FLOW	VENTILATION
VA/Q OF ZERO	.013	ZERO
VA/Q RANGE 0 TO .01	.000	.000
VA/Q RANGE .01 TO .1	.000	.000
VA/Q RANGE .1 TO 1.	.450	.221
VA/Q RANGE 1.0 TO 10.	.529	.641
VA/Q RANGE 10. TO 100.	.007	.170
VA/Q OF INFINITY	ZERO	-.031

MEAN OF BLOOD FLOW	DISTRIBUTION =	1.22
2nd MOMENT OF BLOOD FLOW	DISTRIBUTION =	.59
3rd MOMEMT OF BLOOD FLOW	DISTRIBUTION =	.34
MEAN OF VENTILATION	DISTRIBUTION =	2.79
2nd MOMENT OF VENTILATION	DISTRIBUTION =	1.35
3rd MOMENT OF VENTILATION	DISTRIBUTION =	3.16

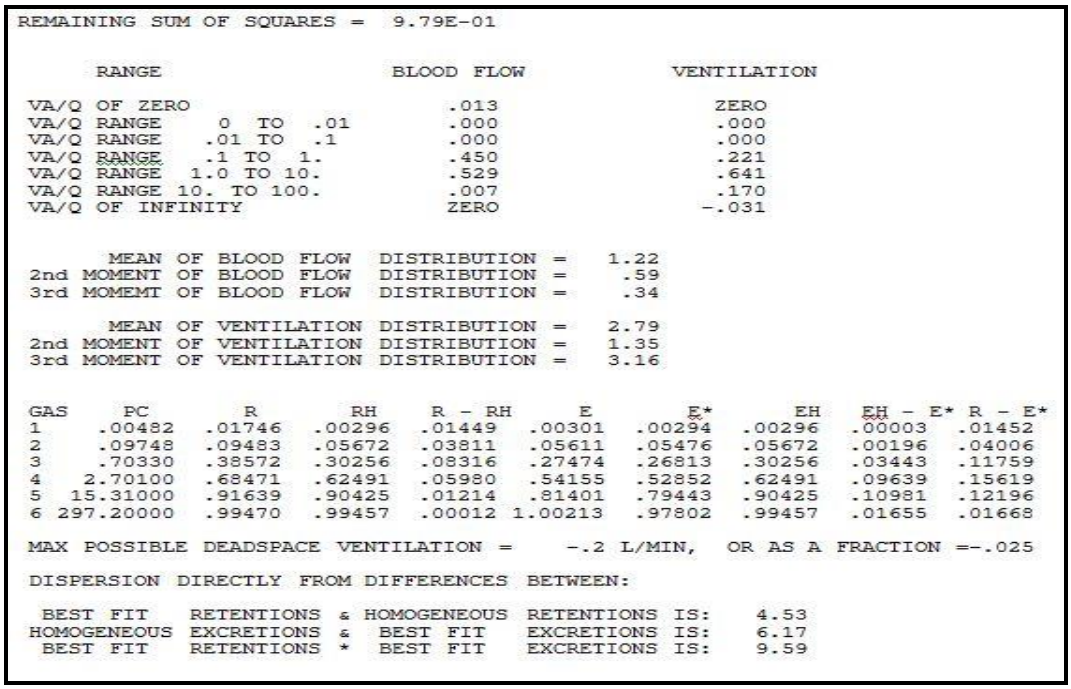
GAS	PC	R	RH	R - RH	E	E*	EH	EH - E*	R - E*
1	.00482	.01746	.00296	.01449	.00301	.00294	.00296	.00003	.01452
2	.09748	.09483	.05672	.03811	.05611	.05476	.05672	.00196	.04006
3	.70330	.38572	.30256	.08316	.27474	.26813	.30256	.03443	.11759
4	2.70100	.68471	.62491	.05980	.54155	.52852	.62491	.09639	.15619
5	15.31000	.91639	.90425	.01214	.81401	.79443	.90425	.10981	.12196
6	297.20000	.99470	.99457	.00012	1.00213	.97802	.99457	.01655	.01668

MAX POSSIBLE DEADSPACE VENTILATION = -.2 L/MIN, OR AS A FRACTION =-.025

DISPERSION DIRECTLY FROM DIFFERENCES BETWEEN:

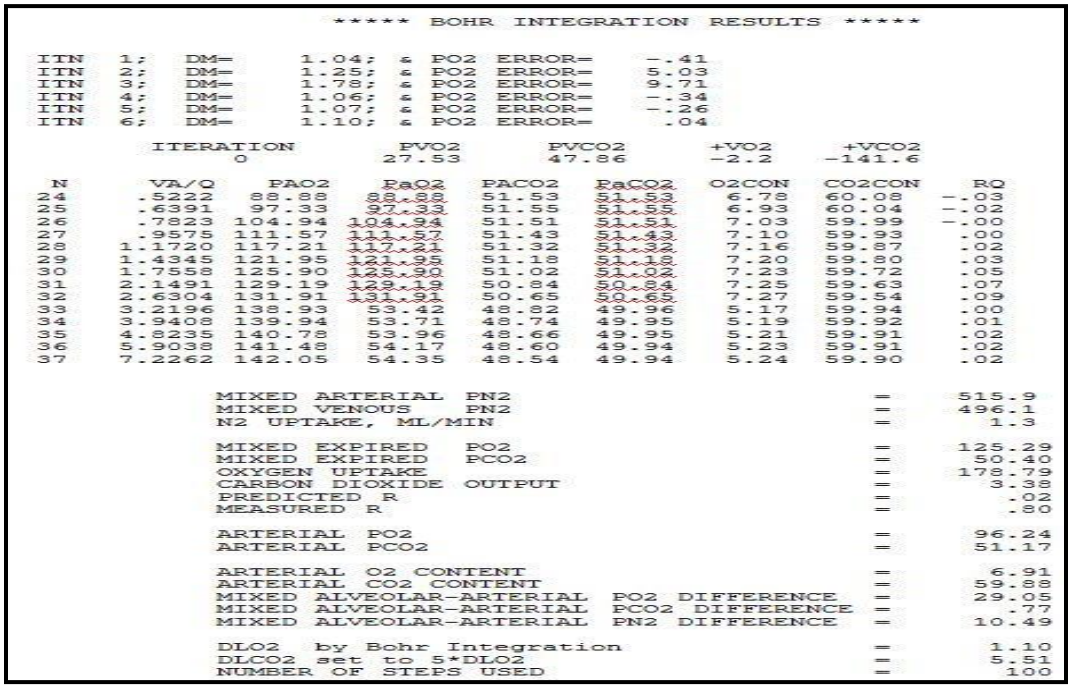
BEST FIT	RETENTIONS &	HOMOGENEOUS	RETENTIONS IS:	4.53
HOMOGENEOUS	EXCRETIONS &	BEST FIT	EXCRETIONS IS:	6.17
BEST FIT	RETENTIONS *	BEST FIT	EXCRETIONS IS:	9.59

Figure 9. This table provides the fraction distributions of blood flow and ventilation segregated into arbitrary ranges. In a normal lung, blood flow is fully contained within the range of V_A/Q = 0.1 to 10. Abnormally low V_A/Q is < 0.1. Abnormally high V_A/Q is > 10. Shunt is specified as blood flow with V_A/Q < 0.005, and dead space as V_A/Q > 100. The MIGET detects intrapulmonary and intracardiac shunting but does not account for shunt resulting from bronchial or Thebesian venous admixture.



```
REMAINING SUM OF SQUARES =  9.79E-01

     RANGE                    BLOOD FLOW          VENTILATION

VA/Q OF ZERO                      .013                ZERO
VA/Q RANGE     0  TO  .01         .000                .000
VA/Q RANGE    .01 TO  .1          .000                .000
VA/Q RANGE    .1 TO  1.           .450                .221
VA/Q RANGE   1.0 TO 10.           .529                .641
VA/Q RANGE 10. TO 100.            .007                .170
VA/Q OF INFINITY                  ZERO               -.031


      MEAN OF BLOOD FLOW  DISTRIBUTION =   1.22
2nd MOMENT OF BLOOD FLOW  DISTRIBUTION =    .59
3rd MOMEMT OF BLOOD FLOW  DISTRIBUTION =    .34

      MEAN OF VENTILATION DISTRIBUTION =   2.79
2nd MOMENT OF VENTILATION DISTRIBUTION =   1.35
3rd MOMENT OF VENTILATION DISTRIBUTION =   3.16


GAS     PC        R        RH     R - RH      E        E*       EH    EH - E* R - E*
1     .00482   .01746   .00296   .01449   .00301   .00294   .00296   .00003   .01452
2     .09748   .09483   .05672   .03811   .05611   .05476   .05672   .00196   .04006
3     .70330   .38572   .30256   .08316   .27474   .26813   .30256   .03443   .11759
4    2.70100   .68471   .62491   .05980   .54155   .52852   .62491   .09639   .15619
5   15.31000   .91639   .90425   .01214   .81401   .79443   .90425   .10981   .12196
6  297.20000   .99470   .99457   .00012  1.00213   .97802   .99457   .01655   .01668

MAX POSSIBLE DEADSPACE VENTILATION =    -.2 L/MIN,   OR AS A FRACTION =-.025

DISPERSION DIRECTLY FROM DIFFERENCES BETWEEN:

 BEST FIT    RETENTIONS & HOMOGENEOUS  RETENTIONS IS:    4.53
HOMOGENEOUS  EXCRETIONS &  BEST FIT    EXCRETIONS IS:    6.17
 BEST FIT    RETENTIONS *  BEST FIT    EXCRETIONS IS:    9.59
```

Figure 10. The first moment of the distribution (blood flow/ventilation on a log scale), is the mean abscissa value (V_A/Q) of each curve. The second moment for the distribution is the standard deviation for the blood flow and ventilation curves. This indicator only reflects the standard deviation if the distribution is logarithmically normal. In case of multi-modal curves, this parameter does not necessarily represent the standard deviation, but it can still be a useful parameter of dispersion. The third moment of distribution represents the skewness of the curves.



```
                    ***** BOHR INTEGRATION RESULTS *****

ITN   1;   DM=     1.04;  & PO2 ERROR=    -.41
ITN   2;   DM=     1.25;  & PO2 ERROR=    5.03
ITN   3;   DM=     1.78;  & PO2 ERROR=    9.71
ITN   4;   DM=     1.06;  & PO2 ERROR=    -.34
ITN   5;   DM=     1.07;  & PO2 ERROR=    -.26
ITN   6;   DM=     1.10;  & PO2 ERROR=     .04

        ITERATION          PVO2        PVCO2        +VO2       +VCO2
                0         27.53        47.86        -2.2      -141.6

 N       VA/Q      PAO2      PaO2     PACO2     PaCO2     O2CON    CO2CON      RQ
24      .5222     88.88     88.88     51.53     51.53      6.78     60.08    -.03
25      .6391     97.33     97.33     51.55     51.55      6.93     60.04    -.02
26      .7823    104.94    104.94     51.51     51.51      7.03     59.99    -.00
27      .9575    111.57    111.57     51.43     51.43      7.10     59.93     .00
28     1.1720    117.21    117.21     51.32     51.32      7.16     59.87     .02
29     1.4345    121.95    121.95     51.18     51.18      7.20     59.80     .03
30     1.7558    125.90    125.90     51.02     51.02      7.23     59.72     .05
31     2.1491    129.19    129.19     50.84     50.84      7.25     59.63     .07
32     2.6304    131.91    131.91     50.65     50.65      7.27     59.54     .09
33     3.2196    138.93     53.42     48.82     49.96      5.17     59.94     .00
34     3.9408    139.94     53.71     48.74     49.95      5.19     59.92     .01
35     4.8235    140.78     53.96     48.66     49.95      5.21     59.91     .02
36     5.9038    141.48     54.17     48.60     49.94      5.23     59.91     .02
37     7.2262    142.05     54.35     48.54     49.94      5.24     59.90     .02

                MIXED ARTERIAL  PN2                                =     515.9
                MIXED VENOUS    PN2                                =     496.1
                N2 UPTAKE, ML/MIN                                  =       1.3

                MIXED EXPIRED   PO2                                =     125.29
                MIXED EXPIRED   PCO2                               =      50.40
                OXYGEN UPTAKE                                      =     178.79
                CARBON DIOXIDE  OUTPUT                             =       3.38
                PREDICTED  R                                       =        .02
                MEASURED  R                                        =        .80

                ARTERIAL  PO2                                      =      96.24
                ARTERIAL  PCO2                                     =      51.17

                ARTERIAL  O2 CONTENT                               =       6.91
                ARTERIAL  CO2 CONTENT                              =      59.88
                MIXED ALVEOLAR-ARTERIAL  PO2 DIFFERENCE            =      29.05
                MIXED ALVEOLAR-ARTERIAL  PCO2 DIFFERENCE           =        .77
                MIXED ALVEOLAR-ARTERIAL  PN2 DIFFERENCE            =      10.49

                DLO2  by Bohr Integration                          =       1.10
                DLCO2 set to 5*DLO2                                =       5.51
                NUMBER OF STEPS USED                               =        100
```

Figure 11. The program allows for estimation of diffusion limitation. Compared to oxygen, the inert gases are minimally susceptible to diffusion limitation. Thus, despite the presence of oxygen diffusion limitation, the method will continue to estimate V_A/Q distribution. If the measured PO_2 is lower than the predicted PO_2, then a diffusion limitation is present. The value in the row labeled "DLO_2 by Bohr Integration" represents diffusion limitation.

References

1. Wagner, P. The Multiple Inert Gas Elimination Technique (MIGET). *ICM*. 34(6) : 994-1001, 2008.
2. Wagner, P., H. Saltzman, J. West. Measurement of continuous distributions of ventilation-perfusion ratios: theory. *J Appl Physiol*. 36(5) : 588-599, 1974.
3. Wagner, P., P. Naumann, R. Laravuso. Simultaneous measurement of eight foreign gases in blood by gas chromatography. *J Appl Physiol*. 36 (5) : 600-605, 1974.
4. Wagner, P. Calculating the distribution of ventilation-perfusion ratios from inert gas elimination data. *FASEB*. 41(1) : 136-139, 1982.
5. Fortune, J. R. Mazzone, P. Wagner. Ventilation-perfusion relationships during hemorrhagic hypotension and reinfusion in the dog. *J Appl Physiol*. 54(4) : 1071-1082, 1983.
6. Wager, P. G. Hedenstierna, G. Bylin, L. Lagerstrand. Reproducibility of the multiple inert gas elimination technique. *J Appl Physiol*. 62(4) : 1740-1746, 1987.
7. Rodriguez-Roisin, R., P. Wagner. Clinical relevance of ventilation-perfusion inequality determined by inert gas elimination. *ERJ*. 3(4) : 469-482, 1990.
8. Roca, J. P. Wagner. Contribution of multiple inert gas elimination technique to pulmonary medicine. 1. Principles and information content of the multiple inert gas elimination technique. *Thorax*. 49 : 815-824, 1994.
9. Wagner, P., F. Lopez. Techniques in the life sciences. P4/I. Gas chromatography techniques in respiratory physiology. *Respir Physiol*. P403 : 1-24
10. Wager, P. R. Laravuso, E. Goldzimmer, P. Naumann, J. West. Distribution of ventilation-perfusion ratios in dogs with normal and abnormal lungs. *J ApplPhysiol* 38(6) : 1099-1109, 1975
11. Wagner, P., R. Laravuso, R. Uhl, J. West. Distributions of ventilation-perfusion ratios in acute respiratory failure. *Chest*. 65(Suppl) : 32S-35S, 1974.
12. Shimazu, T., M. Kishikawa, T. Sugimoto, T. Yukioka, A. Johnson, Jr., A. Mason, Jr., B. Pruitt, Jr. Application of a gas chromatography-mass spectrometer (GC-MS) to the multiple inert gas elimination technique: multiple inert gas measurement with a GC-MS at trace level. *Kokyu To Junkan*. 37(10) : 1083-1087, 1989.
13. Shimazu, T., T. Yukioka, H. Ikeuchi, A. Mason, Jr., P. Wagner, B. Pruitt J. Ventilation-perfusion alterations after smoke inhalation injury in an ovine model. *J Appl Physiol*. 81(5) : 2250-2259, 1996.
14. Ogura, H. W. Cioffi, Jr., P. Offner, B. Jordan, A. Johnson, Jr., B. Pruitt, Jr., F. Moore, T. Billiar, K. Messmer. Effect of inhaled nitric oxide on pulmonary function after sepsis in a swine model. *Surgery*. 116(2) : 313-321, 1994.
15. Ogura, H., D. Saitoh, A. Johnson, Jr., A. Mason, Jr., B. Pruitt, Jr., W. Cioffi, Jr. The effect of inhaled nitric oxide on pulmonary ventilation-perfusion matching following smoke inhalation injury. *J Trauma*. 37(6) : 893-898, 1994.
16. Ogura, H., P. Offner, D. Saitoh, B. Jordan, A. Johnson, Jr., B. Pruitt, Jr., W. Cioffi, Jr., The pulmonary effect of nitric oxide synthase inhibition following endotoxemia in a swine model. *JAMA Surgery*. 129(12) 1233-1239, 1994.
17. Ogura, H. W. Cioffi, Jr., C. Okerberg, A. Johnson, Jr., R. Guzman, A. Mason, Jr., B. Pruitt, Jr. The effects of pentoxifylline on pulmonary function following smoke inhalation. *J Surg Res*. 56(3) : 242-250, 1994.

18. Tasaki, O., C. Goodwin, D. Saitoh, D. Mozingo, S. Ishihara, W. Brinkley, W. Cioffi, Jr., B. Pruitt, Jr. Effects of burns on injury. *J Trauma*. 43(4) : 603-607, 1997.
19. Tasaki, O., C. Goodwin, D. Mozingo, W. Cioffi, Jr., S. Ishihara, W. Brinkley, M. Dubick, R. Smith, O. Srivastava, B. Pruitt, Jr. Selectin blockade worsened lipopolysaccharide-induced lung injury in a swine model. *J Trauma*. 46(6) : 1089-1095, 1999.
20. Tasaki, O., D. Mozing, S. Ishihara, W. Brinkley, A. Johnson, Jr., R. Smith, O. Srivastava, A. Mason, Jr., B. Pruitt, Jr., W. Cioffi, Jr. Effect of Sulfo Lewis C on smoke inhalation injury in an ovine model. *CCM*. 26(7) : 1238-1243, 1998.
21. Batchinsky, A., W. Weiss, B. Jordan, E. Dick, Jr., D. Cancelada, L. Cancio. Ventilation-perfusion relationships following experimental pulmonary contusion. *J Appl Physiol*. 103(3) : 895-902, 2007.
22. Batchinsky, A., D. Martini, B. Jordan, E. Dick, J. Fudge, C. Baird, D. Hardin, L. Cancio. Acute respiratory distress syndrome secondary to inhalation of chlorine gas in sheep. *J Trauma*. 60(5) : 944-957, 2006.
23. Evans, J., P. Wagner. Limits of VA/Q distributions from analysis of experimental inert gas elimination. *J Appl Physiol.* 42(6) : 889-898, 1977.
24. Guyton, A., J. Hall. *Textbook of Medical Physiology*. Philadelphia: Saunders Elsevier, 2011. Print.
25. McGee, W., J. Headley, J. Frazier. *Quick Guide to Cardiopulmonary Care*. Edwards Critical Care Education: Edwards Lifesciences, Irvine, CA 92614.
26. Swan, H., W. Ganz, J. Forrester, H. Marcus, G. Diamond, D. Chonette. Catheterization of the heart in man with use of a flow-directed balloon-tipped catheter. *N Engl J Med*. 283(9) : 447-451. 1970.
27. Raper, R., W. Sibbald. Misled by the wedge? The Swan-Ganz catheter and left ventricular preload. *Chest*. 89(3) : 427-434. 1986.
28. Kronberg, G., S. Quan, R. Schlobohm, J. Lindauer, P. Goodman. Anatomic locations of the tips of pulmonary-artery catheters in supine patients. *Anesthesiology*. 51(5) : 467-469. 1979.
29. Chatterjee, K. The Swan-Ganz catheters: past, present, and future. *Circulation*. 119 : 147-152. 2009.
30. Kelly, C., L. Rabbani. Pulmonary-artery catheterization. *N Engl J Med*. 369 : e35. 2013. DOI: 10.1059/NEJMvcm1212416.
31. Aberman, A., J. Cavanilles, J. Trotter, D. Erbeck, M. Weil, H. Shubin. An equation for the oxygen hemoglobin dissociation curve. *J Appl Physiol*. 35(4) : 570-571. 1973.
32. Powell, F., F. Lopez, P. Wagner. Effects of acetone in heparin on the multiple inert gas elimination technique. *J Appl Physiol*. 58(4) : 1143-1147, 1985.
33. Wagner, P., C. Smith, N. Davies, R. McEvoy, G. Gale. Estimation of ventilation-perfusion inequality by inert gas elimination without arterial sampling. *J Appl Physiol*. 59(2) : 376-383, 1985.
34. Ferrer, M., E. Zavala, O. Diaz, J. Roca, P. Wagner, R. Rodriguez-Roisin. Assessment of ventilation-perfusion mismatching in mechanically ventilated patients. *ERJ*. 12(5) : 1172-1176, 1998.