**Research Protocol: The Effect of Surgical Humidification on Local and Systemic inflammation and Peritoneal Trauma in Colorectal Cancer Surgery: a Randomised Controlled Trial**

**Prepared for the Epworth Healthcare Human Research Ethics Committee and the Peter MacCallum Human Research Ethics Committee**

**Prepared by**

**Dr Meara Dean – Colorectal Fellow**

**Associate Professor Craig Lynch- Consultant Surgeon**

**Prof Robert Ramsay- Senior Research Fellow**

**Mr Alan Herschtal- Senior Biostatistician**

**13th April 2015**

**Table of Contents**

1. Background 3

2. Aims 4

3. Primary Objectives 4

4. Secondary Objectives 4

5. Settings and Participants 5

6. Study Methodology and Techniques 6-8

7. Recruitment Strategy 8

8. End Points and Data Collection Plan 9-11

9. Statistical and other Analysis 12

10. Ethics 15

11. Timeline 15

12. Budget 15

13. Research Findings and Outcomes 15

14. References 16

1. **Background**

Abdominal cancer surgery is performed via two approaches: the most common open or laparotomy, and the increasingly frequent minimally invasive or laparoscopic technique. Advances in technology and techniques have meant minimally invasive procedures have wider applications, and become more complicated and prolonged. This is especially applicable in the area of colorectal cancer surgery. Variations of minimally invasive surgery have also been developed in recent years, including single incision laparoscopic surgery (SILS) and robotic surgery.

All minimally invasive surgery relies on pumping gas (insufflation) into the abdominal cavity (pneumoperitoneum) to create a working space. Cold, dry carbon dioxide (CO2) insufflation at 21oC and 0% relative humidity has been the traditional approach, and is still the most frequently used insufflation worldwide.

Adverse local and systemic effects of cold, dry CO2 insufflation have been reported, including perioperative hypothermia, postoperative pain and peritoneal injury with peritoneal surface desiccation and acidification. Morphological alterations of the peritoneal surface are evident after 2 hours of insufflation with CO2 in animal studies (1) and 42 minutes in human trials (2). Exposure of the open surgical wound to the dry, cold air of the operating room causes similar effects.

To alleviate these detrimental effects, devices have been developed to warm (to 35oC) and humidify (to 95%) CO2 insufflation. These devices can be used in laparoscopic and open surgery. In Australia the available device is the HumiGardTM, made by Fisher and Paykel Healthcare (F&PH).

The clinical effects of surgical humidification have been investigated with previous studies finding benefits of reduced perioperative hypothermia, post-operative pain and inflammation and structural damage to the peritoneum in laparoscopic surgery [1-5] and open surgery [6]. Pre-clinical mouse model data from Peter MacCallum Cancer Center (Peter Mac) has shown that humidified, warm CO2 reduces peritoneal damage and tumour cell adhesion[7], a finding with potentially profound consequences for cancer patients in terms of the prevention of tumour recurrence and metastasis.

This study protocol has been developed in collaboration with Assoc. Prof Craig Lynch and Prof. Alexander Heriot, both colorectal surgeons working at Peter Mac and Epworth Health care with Prof. Rob Ramsay, a lab based researcher at Peter Mac who has developed the relevant pre-clinical cancer mouse models that have tested the hypothesis that humidified, warm CO2 insufflation reduces peritoneal cancer development. F & PH has been providing funding to Ramsay’s laboratory to conduct these studies. We now need to advance this work by collecting clinically relevant data in open and laparoscopic cancer surgery, adding further data on the effects of surgical humidification.

1. **Aims**

The aim of this study is to evaluate the effects of warming and humidification of insufflation CO2, which is thought to be beneficial in laparoscopic and open abdominal colorectal cancer surgery. This multicentre, prospective, randomized controlled trial will enrol patients undergoing elective colorectal cancer surgery to determine effects on local and systemic inflammation and peritoneal damage.

1. **Primary objectives**

The primary objectives will be to determine if insufflation of warmed, humidified CO2 results in:

* 1. **Reduction in markers of local inflammation, as measured by examining the following markers of peritoneal inflammation:**
* Cytokines (IL 6, IL 8, IL 10)
* TNF –a
* COX-2
* VEGF-A
* Macrophage activity
  1. **Reduced rates of peritoneal tissue damage, as measured by the following peritoneal morphological changes:**

- loss of microvilli

- mesothelial cell bulging and retraction

- widening of intercellular junctions

- exposed basal lamina

1. **Secondary objectives**

**4.1** **Effects of humidification**

The use of warmed, humidified CO2 may have wide ranging benefits for the surgeon, anaesthetist and healthcare system. Secondary objectives will test for the existence of a relationship between humidification and each of the following.

* + 1. **Reduction in markers of systemic inflammation**

Humidification is expected to decrease the concentration of circulating cytokines and CRP.

* + 1. **Presence of systemic ctDNA**

Humidification is expected to decrease the frequency of systemic circulating tumour DNA.

* + 1. **Intra-operative and Post-operative core body temperature**

Humidification is expected to maintain perioperative normothermia.

4**.1.4 Length of stay (LOS)**

Humidification is expected to reduce the length of patient stay.

**4.1.5 Cost of intervention**

Humidification is expected to decrease the cost of intervention due to decreased overall surgical costs due to earlier discharge and decreased demand for pain medication. An incremental cost effectiveness ratio (ICER) will be calculated for length of stay.

* 1. **Effect of Surgical Technique**

An additional secondary objective is to test for a relationship between the surgical method (laparoscopic vs. open and humidified vs. non-humidified) and the presence of ctDNA in patients with non -metastatic, non-perforated cancer.

* 1. **Effect of transfer time on temperature change**

To test for an association between time from end of surgery until transfer to recovery room, and temperature change between the end of surgery and the transfer of the patient to the recovery room.

1. **Settings and participants**

All patients undergoing elective colorectal cancer surgery for any indication between April 2015 and December 2016 at Epworth Health and Peter MacCallum Cancer Centre will be screened for inclusion and invited to participate.

Inclusion criteria:

* Age 18 years or older
* Patients having colorectal cancer abdominal elective surgery
* Patients are able to provide written consent

Exclusion criteria:

* Patients under age 18
* Patients with known intra-abdominal sepsis
* Pre-operative steroid dependence
* Patients who are pregnant
* Prior diagnosis of Crohns or ulcerative colitis
* Inability to consent due to cognitive or language barrier
* Preoperative blood transfusion

1. **Study methodology and techniques**

**Study Structure**

The study design is a multicentre randomized controlled trial. As the effects of the intervention are apparent intra operatively, and the measures of the investigation are objective, there will be no attempt to blind the investigators. At all study sites clinicians are currently using either cold, dry CO2 and warm or humidified CO2 as methods of insufflation, therefore randomization will not have any effect on standard of care provided. Currently the used of warmed, humidified CO2 during open surgery is not widely in use, however standard of care is not expected to be affected by this intervention.

Participants in the study group will receive warmed (37o), humidified (98%) CO2 via insufflation in the minimally invasive group or wound insufflation in the open group. The control group will have surgery performed with traditional cold, dry CO2 in the minimally invasive group and no wound insufflation in the open group.

***This study will aim to enrol 240 patients in the following cohorts:***

Cohort 1: 60 patients undergoing CRC surgery using a laparoscopic approach with cold, dry CO2.

Cohort 2: 60 patients undergoing CRC surgery using a laparoscopic approach using humidified, warmed CO2

Cohort 3: 60 patients undergoing CRC surgery via laparotomy with no insufflation of CO2

Cohort 4: 60 patients undergoing CRC surgery via laparotomy with insufflation of CO2 into the open abdominal cavity

Interim analysis of serum and peritoneal biopsy samples will be performed after recruitment of 15 patients in each group.

**Study group: CO2 insufflation**

The HumiGardTM humidification system (MR860; Fisher and Paykel Health Care, Auckland, New Zealand) has been independently tested to confirm the effectiveness of gas humidification [8].

For laparoscopic surgery, pneumoperitoneum will be established after insertion of a 12mm port by open method. Further 10mm and 5mm ports will be inserted according to the type of laparoscopic procedure. The humidification system consists of a bacterial filter and a humidification chamber filled with 180 mL sterile water, attached to a humidifier controller that includes an integrated temperature and flow sensor. The outlet of the humidification chamber is connected to a thermally insulated 2.5m long heated insufflation tube that maintains temperature and humidity of the gas to its outlet. The control group received the usual cold, dry CO2 insufflation.

In the open group dry CO2 is delivered via a 6.35mm polyvinylchloride tube to the open surgery humidification system (F&P HumiGard, Fisher & Paykel Healthcare Ltd, Auckland, New Zealand). This is attached to flexible tubing and positioned inside the open abdominal wound cavity in the right upper quadrant, a depth of approximately 4 cm from the skin. Insufflation of warmed, humidified CO2 will be continued until just before abdominal wall closure.

The pressure, flow rate and total volume of CO2 delivered will be recorded for both groups.

**Study Techniques**

**Body core and intra peritoneal temperature**

Anesthesia will be standardised. An external warming device (Bair Hugger, Augustine Medical, Eden Prairie, Minnesota, USA or Cocoon, Care Essentials, North Geelong, Victoria, Australia) will be used for all patients during surgery. Operating room temperature will be set at 20-22 degrees in both groups and recorded intra operatively. Body temperature will be recorded using ear probe in the anaesthetic room and on induction. Core temperature measured using an oesophageal probe (Thermistor 400 series; Mallinckrodt, Cornamaddy, Athlone, Ireland)

**Markers of local and systematic inflammation, peritoneal damage and cancer biomarkers:**

* Pathology: systemic blood samples will be tested for cytokine levels: Il-6, Il-8 and Il-10, TNF-a, CRP, VEGF-A, PGE2 and circulating tumour (ct) DNA. Blood samples will be collected pre operatively, at 2 hours and 4 hours post induction (intra operative), and post-operative at 24 and 72 hours. Samples will be analyzed using ELISA (Diagnostic Products Corporation Immunline System, Los Angeles, CA, USA). Portal blood will be also collected from the specimen (IMA) for ctDNA. Samples will be stored at temperature of -20c until the time of analysis. 10ml samples of blood will be taken. We will use an H-score that systematically evaluates the extent and intensity of inflammatory markers.

As pre and postoperative bloods are routine in colorectal cancer surgery, study participants will have two additional blood tests performed post induction/intra operatively (additional 20mls of blood).

Peritoneal fluid and biopsies: Peritoneal fluid and biopsies will be taken at the start of the operation, then 2-hourly intra-operatively. Peritoneal fluid will be sampled using a laparoscopic aspiration (laparoscopic) or 5 ml syringe (open) from an area remote to the dissection. Peritoneal biopsies (5x5x1mm) will be taken from 4 quadrants of the peritoneal cavity, remote from area of mechanical trauma due to retractors. The risk of biopsy is bleeding, which can be identified and treated with cautery at the time of biopsy.

Peritoneal fluid will be placed in Cryovial and place in dry ice. Peritoneal samples divided into two and placed into 1) formalin for IHC and 2) pre prepared fixative for SEM. All specimens will be labelled.

These will be transported to Peter Mac and processed and analysed by microassay technique, light microscopy, scanning electron microscopy (SEM) and immunohistochemistry (IHC). Peritoneal fluid will be analysed for levels of cytokines using an enzyme linked immunosorbent assay technique and tumor DNA.

Light microscopy will be performed to determine macrophage infiltration. IHC will be performed to measure COX-2 and VEGF-A. SEM will assess peritoneal surface morphology. Peritoneal damage will be defined as observed microvilli damage (early signs of damage), mesothelial cell morphology change with bulging and delamination with exposed basal lamina (later signs of damage). Using techniques developed in the preclinical mouse study [7], the percentage of remaining normal microvilli will be determined. In addition an ordinal scale of 1-3 will be used to grade severity of changes.

These measures will be performed blinded to the sample identification and by two experienced investigators. Sample analysis will be performed by Ms Shienny Sampurno with the assistance of Dr Jordane Malaterre and Dr Meara Dean. The lab is set up to do all the assays described and is familiar with the appropriate receipt, processing and storage of clinical material associated with trials.

1. **Recruitment Strategy**

Patients will be seen pre-operatively and the trial rationale and procedure explained verbally and on written participant information sheet. Patients will be given time to read through this sheet and to ask questions. It will be emphasized that participation is voluntary and the patient is allowed to refuse further participation in the protocol whenever he/she wants. Patients will be informed their medical records may be viewed for study purposes by the treating team. Written informed consent will be obtained for all patients.

1. **End Points and Data Collection Plan**

**Base Line Data:**

* Demographics: patient name, hospital UR, sex, age
* Weight in kg, height in cm
* ASA score
* Medical and surgical history
* Approach: Open or Laparoscopic
* Group: Control or Treatment
* Use of drain (Y/N)
* Intraoperative urine output

**Primary End Points**

* **Reduction in markers of local inflammation**
* **Reduced rates of peritoneal tissue damage**

Peritoneal fluid samples and biopsies: start of the operation, then 2-hourly intra-operatively. Peritoneal fluid will be sampled using a laparoscopic aspiration (lap) or 5 mm syringe (open). Peritoneal biopsies (5x5x1mm) will be taken from 4 quadrants of the peritoneal cavity, remote from area of mechanical trauma due to retractors. Samples will be handled and process as outlined above in methodology.

Timing of sampling will be recorded.

The following factors that may affect peritoneal samples will be recorded:

* Incisions: number and type
* Type of retraction: Alexis, Fixed metal (Balfour/Omni-Tract)
* Pressure, flow rate and total volume of CO2 delivered

Secondary End Points

* **Reduction in markers of systemic inflammation**

Blood samples: Blood samples will be collected pre operatively, at 2 hours and 4 hours post induction

Factors that may affect the systemic response the surgery will be recorded:

* Incisions: number and type
* Type of retraction: Alexis, Fixed metal (Balfour/Omni-Tract)
* Anaesthetic time (Time of induction and extubation), Operation time, insufflation time
* Known metastatic disease
* Intraoperative administration of steroids
* Intraoperative blood or blood product transfusion
* **Presence of systemic ctDNA**

Blood samples: Blood samples will be collected pre operatively, at 2 hours and 4 hours post induction

Factors that relate to the presence of ctDNA will be recorded:

* Histopathology report and TNM staging, tumour markers
* Presence of known metastatic disease
* Presence of perforated tumour
* **Intra operative and Post-operative core body temperature**

Core body temperature will be recorded preoperatively, intra-operatively at 15 minute intervals, at completion of procedure while in the operating room and in recovery (or ICU if patient transported directly) half hourly for two hours post operatively.

Other factors that may influence core body temperature will be recorded:

* Patient pre-warming
* Use of external warming device
* Use of Epidural anaesthesia
* Post-operative blood transfusion
* Body Mass Index (BMI)
* Operating room temperature, humidity, and airflow ventilation rate
* Anaesthetic time (Time of induction and extubation), Operation time, insufflation time, time in OT post completion of surgery, time in recovery
* Pressure, flow rate and total volume of CO2 delivered
* Volume and type of fluids administered intra operatively
* Intraoperative blood or blood product transfusion
* **Length of stay (LOS)**
* **Cost of intervention**
* Length of stay (post-operative day fit for discharge)
* Surgical site infection within 30 days (as defined by CDC[9])
* Complications (graded by the Clavien-Dindo classification[10])
* Day PCA ceased
* Day oral analgesia commenced
* Cancer recurrence/metastasis/survival for 3 year follow up

For the first primary objective, the following markers of local inflammation will be recorded for each patient.

* IL-6, IL-8, IL-10 – all continuous valued
* Cox-2, VEGF-A, PGE2 – all Boolean valued (present/absent)

For the second primary objective, the following indicators of peritoneal tissue damage will be recorded for each patient.

* loss of microvilli, mesothelial cell bulging and retraction, widening of intercellular junctions – all ordinal, scale from 0-3

The following variables will be recorded and considered as potential confounders when assessing the primary objectives.

* perforated tumour (all primary end points)
* perioperative use of steroid- markers of inflammation

**Secondary endpoints**

The following quantities will be recorded for all patients for assessing the secondary objectives.

* Circulating cytokines, CRP – continuous
* Presence of ctDNA – Boolean
* Intra-operative core body temperature
* intraperitoneal temperature
* LOS
* Cost of intervention
* Temperature at end of surgery
* Temperature upon transfer to recovery room
* Time between end of surgery and transfer to recovery room

The following quantities will be recorded and considered as potential confounders in assessing the various secondary objectives.

* Baseline temperature - control for in assessing diff in temp
* Epidural/Spinal anaesthesia - control for in assessing diff in temp
* Pre-warming – control for in assessing diff in temp
* Length of surgery - control for in assessing diff in temp
* BMI - control for in assessing diff in temp
* Presence of known metastatic disease – control for in analysing ctDNA
* Presence of perforated tumor – control for in analysing ctDNA

1. **Statistical analysis**

**Primary objectives**

*To determine if insufflation of warmed, humidified CO2 results in a reduction in markers of local inflammation*

The following continuous valued markers of local inflammation will be considered:

* IL6
* IL 8
* IL 10
* TNF-a

For each of the above continuous valued markers of local inflammation, a linear regression model will be constructed to test for a difference in the mean value of that marker of inflammation, between patients treated with humidification, and patients treated without humidification. Surgical technique will be controlled for in the model if necessary. The independent variables will be the presence/absence of humidification, and surgical technique (if necessary). The marker of inflammation of interest will be the dependent variable. The difference in mean value of the marker between patients treated with humidification, and patients treated without humidification, together with its 95% CI will be reported. The assumption of normally distributed residuals will be tested for, and a suitable monotonic transformation (e.g. log transformation) will be considered if necessary.

The following Boolean valued markers of local inflammation will be considered

* COX-2
* VEGF-A

For each of the above markers of local inflammation, binary logistic regression will be used to test for a difference in the proportion of patients with that marker of inflammation, between patients treated with humidification, and patients treated without humidification. Surgical technique will be controlled for in the model if necessary. The independent variables will be the presence/absence of humidification, and surgical technique (if necessary). The marker of inflammation of interest will be the dependent variable. The odds ratio for humidification together with its 95% CI will be reported.

*To determine if insufflation of warmed, humidified CO2 results in reduced rates of peritoneal tissue damage*

The following measures of peritoneal tissue damage will be considered

* loss of microvilli
* mesothelial cell bulging and retraction
* widening of intercellular junctions
* exposed basal lamina

The percentage of remaining normal microvilli will be calculated.

Each of the above measures of peritoneal tissue damage is ordinal valued and may take values in the range 1 to 3. For each measure of peritoneal tissue damage, statistical analysis will proceed as follows. An ordinal logistic regression model will be constructed with the measure of peritoneal tissue damage as the dependent variable and the presence/absence of humidification, and surgical technique (if necessary to control for confounding) as the independent variables. The odds ratio of humidification corresponding to a one level change in the measure of peritoneal tissue damage, together with its 95% CI will be reported.

**Secondary objectives**

1. *To test for the existence of a relationship between humidification and each of the following.*

* *Concentration of circulating cytokines – continuous valued*
* *CRP – continuous valued*
* *Presence of ctDNA – Boolean valued*
* *Intra-operative core body temperature – continuous valued*
* *intraperitoneal temperature – continuous valued*
* *Length of hospital stay (LOS) – continuous valued*
* *Cost of intervention – continuous valued*

For each of the continuous valued measures, a linear regression model will be constructed with humidification and surgical technique (if necessary to control for confounding) as the independent variables and the continuous valued measure of interest as the independent variables. The difference in mean value of each continuous valued measure between levels of humidification status will be reported together with its 95% CI. For each of the temperature measures, the method will be repeated for temperature measured at both 1 hour and 4 hours after the start of surgery.

In addition, a mixed effects model will be constructed using temperature (measured at 15 minute intervals during surgery) as the dependent variable, time since start of surgery, surgical technique (if necessary to control for confounding) and humidification as fixed effects, and patient as the random effect. By including a term for the interaction between time and humidification, differences in change in temperature over time as a function of humidification will be tested for.

In order to test for a difference between humidification and presence of ctDNA (Boolean valued), a binary logistic regression model will be built, analogous to that for the first primary objective. Presence of ctDNA will be the dependent variable, and humidification and surgical technique (if necessary to control for confounding) will be the independent variables. Perioperative use of steroids and presence of metastatic disease will be considered as additional potential confounders and included in the model if necessary. The odds ratio corresponding to humidification and its 95% CI will be reported.

1. *To test for a difference between laparoscopic and open surgery in the proportion of patients with ctDNA detected.*

In order to test for a difference between surgical technique and presence of ctDNA (Boolean valued), a binary logistic regression model will be built, analogous to that for the previous secondary objective. As previously, presence of ctDNA will be the dependent variable. However in this case, the prime independent variable of interest will be surgical technique, and humidification, perioperative use of steroids and presence of metastatic disease will be included in the model to control for confounding if necessary.

1. *To test for association between time from end of surgery until transfer to recovery room and temperature change between end of surgery and transfer to recovery room.*

A simple linear regression model will be constructed with time from end of surgery until transfer to recovery room as the independent variable, and temperature change between end of surgery and transfer to recovery room as the dependent variable. The slope of the regression line of best fit and its 95% CI will be reported.

***Power Calculation:***

For a comparison of proportions between arms in a two arm trial, a sample size of 120 per arm is sufficient to detect a fairly small effect size 0f 0.36 with power of 0.8. If the overall proportion across both arms is in the vicinity of 0.5, this is enough to detect an approximate 18% difference in proportions between the arms.

***Local Inflammatory markers:***

COX-2: The largest difference is at the 8h mark, and significant differences were observed with only 3 individuals per group, so a sample size of 60 patients will be adequate for this marker[7].

***Randomisation:***

Randomisation will be performed separately for each site and by surgical approach (laparoscopic/open). Randomisation will be blocked to achieve even numbers in each group of warmed, humidified and cold dry. Using computer based random number generator ([www.random.org](http://www.random.org)) will be used to randomize patients. Allocation to study or control group will occur intra-operatively by envelope.

1. **Ethics**

This study protocol will be submitted to Epworth and Peter Mac Ethics Human Research and Ethics Committee (HREC) for approval.

1. **Timeline**

Patients will be recruited July 2015 - December 2016. Interim analysis will be performed after 60 patients (15 in each group), in approximately 10 months. Publications will be generated as results on various outcomes become available.

1. **Budget (incomplete)**

HumiGardTM humidifier is available at Epworth and Peter Mac operating theatres. The sterile tubing packs are $ per unit and will be provided by F&PH.

Blood and peritoneum samples will be processed by the Peter Mac laboratory, with techniques of microassay, SCM, IHC, pathology review, focused sequence analysis of ctDNA. This will involve a 0.5 EFT highly trained research scientist. The cost recovery and processing costs for the microscopy samples are estimated to be $ 65 000 Samples will be couriered from Epworth Richmond and Epworth Eastern at estimated cost of $5000. The study will involve a research nurse to help with data collection.

1. **Research findings/outcomes**

This trial will add to the knowledge about the effects from the use of heated humidified CO2 insufflation in laparoscopic and open surgery. If this clinical trial supports the pre-clinical mouse model data, showing that warmed, humidified CO2 can reduce peritoneal damage and inflammation it will have a major impact on patient care.

The study protocol has been built upon many years of collaboration between laboratory researchers, colorectal surgeons and F&P Healthcare. F&P Healthcare have a 40 year history of taking research outcomes and developing them into established medical devices which act to improve patient care. Further information regarding the benefits of warmed, humidified CO2 will influence opinions regarding the uptake of this device. Peter MacCallum and Epworth Health care are the ideal sites to conduct this translational research project, as well as the environment to implement and lead changes in clinical practice to improve patient outcomes.

**References**

1. Sammour T, K.A., Hill A.G., *Meta-analysis of the effect of warm humidified insufflation on pain after laparoscopy.* British Journal of Surgery, 2008(95): p. 950-956.

2. Sajid M, M.A., Rimpel J et al. , *Effect of Heated and Humidified Carbon Dioxide on Patients After Laparoscopic Procedures: A Meta-analysis.* SUrg Laparosc Endosc Percutan Tech, 2008. **18**(6): p. 539-545.

3. Benavides R, W.A., Nguyen H, *Improved Outcomes for Lap-Banding Using the Insuflow Device Compared with Heated-Only Gas.* JSLS, 2008. **13**: p. 302-305.

4. Klugsberger B, S.M., Rothe A, *Warmed, humidified carbon dioxide insufflation versus standard carbon dioxide in laparoscopic cholecystectomy: a double-blinded randomised controlled trial.* Surg Endosc, 2014. **28**: p. 2656-2660.

5. Peng Y, Z.M., Ye Q et al. , *Heated and Humidified CO2 Prevents Hypothermia, Peritoneal Injury, INtra-Abdominal Adhesions During Prolonged Laparoscopic Insufflations.* Journal of Surgical Research, 2009. **151**: p. 40-47.

6. Frey J, J.M., Syanfeldt M et al. , *Intraoperative Local Insufflation of Warm Humidified CO2 Increases Open Wound and Core Temperature During Open Colonic Surgery: A Randomised Clinical Trial.* International Anaesthesia Research Society, 2012.

7. Carpinteri S, S.S., Bernardi M et al. , *Peritoneal Tumorigenesis and Imflammation are ameliorated by Humidified-Warm Carbon Dioxide Insufflation in the Mouse.* Annals of Surgical Oncology On line, 2015.

8. Sammour T, K.A., Hill A.G., *Indepdent testing of the Fisher and Paykel Healthcare MR860 Laparoscopic Humidification System.* Minim Invasive Ther Allied Technol, 2010. **19**(4): p. 219-23.

9. Horan T, G.R., Martone W, *CDC Definitions of Nosocomial Surgical Site Infections, 1992: A modification of the CDC definitions of surgical wound infections.* Infection Control and Hospital Epidemiology, 1992. **13**(10): p. 606-608.

10. Dindo D, D.N., Clavien P, *Classificaiton of Surgical Complications.* Annals of Surgery, 2004. **240**(2): p. 205-213.