STUDY PROTOCOL

Official title: RAFA Trial: Radiofrequency Ablation of Adenomyosis

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Protocol for the RAFA Trial: Radiofrequency Ablation of Adenomyosis

UT Southwestern Medical Center
PI: Kimberly Kho MD, MPH
Department of Obstetrics and Gynecology

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Background and Purpose

Adenomyosis is a benign but progressing medical condition characterized by the growth of endometrial cells within the uterine wall and is prevalent in 20-35% of women in the general population[1]. The endometrial cells misplaced in the uterine wall are completely functional and will bleed during every menstrual cycle[2] thus causing pelvic pain and discomfort. Hormonal factors, parity, age, and uterine abrasion are thought to influence the development of adenomyosis. Patients with adenomyosis are most often between the ages of 35 and 50[3] and commonly report dysmenorrhea, menorrhagia, dyspareunia, chronic pelvic pain and infertility.

Adenomyosis can be focal or diffuse and is often associated with other uterine conditions such as fibroids, endometriosis, and/or endometrial polyps. Focal adenomyomas may be mistaken for a uterine fibroid during imaging. Upon imaging or surgical removal, the uterus generally appears larger than normal, particularly when the disease is diffuse where it is described as "bulky" or "heavy".[4]

The junctional zone, also called the "inner myometrium" is thought to play a role in the development and diagnosis of adenomyosis. Thickening, enlargement, invasion, or disruption of this junctional zone are "markers" for adenomyosis during magnetic resonance imaging (MRI) or transvaginal ultrasound (TVUS). In addition to this thickening, findings such as anterior/posterior wall asymmetry, echogenic linear striations, or myometrial cysts may be present.[4][5][6] Doppler ultrasound can also be used to look for circumferential blood vessels that are characteristic of fibroids vs adenomyomas that have vessels found within the lesion.[7][8] A definitive diagnosis for adenomyosis is obtained through the examination of uterine tissue following a uterine biopsy or hysterectomy. Under the microscope, the pathologist looks for clusters of endometrial tissue within the myometrium and if the depth of invasion is between 2.5 and 8mm, adenomyosis is confirmed.[4]

The definitive treatment for adenomyosis is hysterectomy though patients may respond to the administration of non-steroidal anti-inflammatory drugs for pain relief. Reproductive hormones and hormone modulators such as the Mirena IUD [9] may help to reduce menstrual pain and bleeding. Other hormonal treatments that have been used with some

level of pain relief and disease regression are oral contraceptives, progestins, and GnRH agonists.

The surgical options for treatment are generally classified as uterine-sparing or non-uterine sparing. The non-uterine-sparing approach is hysterectomy and, as stated above, is the only definitive treatment for adenomyosis. Uterine-sparing procedures such as uterine artery embolization (UAE) have been used to provide some improvement in menstrual bleeding and pain. However, 26% of women undergoing UAE later require a hysterectomy. [4] Other uterine-sparing surgical options include myometrial or adenoma resection, myometrial electrocoagulation, endometrial ablation, myometrial reduction, or magnetic-resonance focused ultrasound. [4]

One proposed method of focal ablation for the treatment of adenomyomas has been used successfully in the treatment of uterine fibroids, providing long term relief of fibroid-related symptoms: laparoscopic radiofrequency ablation, or LAP-RFA. [10][11]In the process of enrolling subjects in an FDA-regulated trial, investigators often encountered women who presented with adenomyomas or diffuse adenomyosis and who, based on exclusion criteria, were not eligible to be treated in the trial. [Personal communication] Several surgeons proposed LAP-RFA as a viable method of treating focal adenomyosis/adenomyomas. The objective of this study is to observe the effects of RFA on adenomyosis through the pathological analysis of the treated tissue that has been removed during a planned hysterectomy.

Experimental Procedures

At the time of the subject's trans-abdominal or laparoscopic hysterectomy, the ProVu System will be used to apply RF treatment to one or two adenomas, focal areas of adenomyosis, or diffuse adenomyosis. Women who require a hysterectomy do not normally undergo this procedure.

Endpoints

The endpoint of this study is the pathological analysis of the treated tissue after application of RF treatment.

Sample Size

Up to twenty (20) subjects will be expected to complete this study. We expect that enrollment of up to 40 subjects may be necessary due to the requirement for a baseline MRI to verify, locate and "map" the adenomyosis/adenomyomas.

Number of Sites

One site will participate. Site will be required to receive study approval from the Institutional Review Board (IRB) prior to study inception. Study enrollment will not begin until IRB approval is obtained.

Design

This is an open-label, prospective, non-randomized, non-controlled study designed to obtain pathology results from the treatment of adenomyomas and/or adenomyosis using a system designed for the ablation soft tissue including symptomatic fibroids.

A. Screening

The standard pre-screening for hysterectomy eligibility is expected to be completed before patients are referred to the investigator. Upon referral and following consent, the investigator will review the subject's files and images to determine if the initial inclusion/exclusion criteria have been met.

B. Blinding

Blinding will not be appropriate for this study. The subjects and investigators will be informed regarding the use of the system.

C. Randomization

Subjects will not be randomized.

D. Baseline measurements

After the initial screening of the patient's records including imaging results, both a pre-operative endometrial biopsy (standard of care) and Magnetic Resonance Imaging (MRI) will be performed (if not previously performed within 12 months) to verify enrollment criteria and locate/measure the adenomyosis/adenomyomas.

E. Exposure assignments

The length of time that the subject will be exposed to the ProVu System is as follows:

Period of exposure to the device	Number of subjects exposed
< 30 minutes	≤ 20

F. Follow-up

Subjects will be followed for safety postoperatively for 6 +/- 2 weeks. The subject's participation in this study ends at the conclusion of the postoperative visit.

Subjects

A. Selection Criteria

The criteria listed below shall be used to determine if a participant is eligible for entry into the study.

Inclusion Criteria: Women who are/have

- planning to undergo an abdominal, laparoscopic, or robotic-assisted hysterectomy due to benign conditions,
- uterus ≤ 16 weeks gestational size if undergoing a laparoscopic or robotic procedure (no size limit for patients planning to undergo a transabdominal hysterectomy),
- at least one area of focal or diffuse adenomyosis or adenomyomas that is/are contralateral to any fibroids as determined by MRI,
- able to provide informed consent,
- suitable candidates for surgery (have passed a standard pre-operative health assessment),
- English speaking.

Exclusion Criteria: Women who:

- require emergent hysterectomy or vaginal hysterectomy,
- have a uterus > 16 weeks gestational size if undergoing a laparoscopic or robotic procedure (no size limit for patients planning to undergo a transabdominal hysterectomy)
- have fibroids in the proximity of the target adenomyosis (same side, similar location),
- are not appropriate surgical candidates as determined during pre-operative health assessment,
- are unable or unwilling to undergo a hysterectomy,
- are pregnant or lactating,
- are under the age of 18 years,
- have active pelvic inflammatory disease,
- have a history of gynecologic malignancy within the past 3 years,
- are unable to give informed consent, or
- have an implantable uterine or fallopian tube device for contraception
- are not English speaking.

If the subject has been found to have one or more exclusion criteria even after enrollment, the subject will be withdrawn from the study prior to the RF ablation procedure.

B. Identification

The investigator shall assign an identification code to each subject; all data forms and images shall identify the subject by this identification code only.

C. Informed Consent

If a subject meets the inclusion/exclusion criteria, the investigator will explain the conditions of the study. The informed consent documents shall be approved by the IRB prior to use. Informed consent shall be obtained under the conditions as follows:

1. The Principal Investigator or a member of the clinical staff to whom the Principal Investigator may have delegated this responsibility, will

provide the subject with a thorough explanation of the study design, its procedures, expected benefits and potential risks, and all pertinent aspects relative to protection of subjects' safety, wellbeing and confidentiality. During this process, the subject will be allowed to ask all the questions she may have and will receive answers to such questions to her satisfaction. The consenting process will be documented in the subject's study file with a physician's note.

2. the subject shall have sufficient opportunity to consider participation in the study,
3. informed consent shall be obtained without coercion or undue influence,
4. informed consent shall be written in English,
5. a subject will not be led to believe that she is waiving her rights as a subject.

D. Enrollment Timeline

Enrollment occurs when the subject successfully completes the pre-operative health assessment procedures, has met all inclusion/exclusion criteria, and has signed the informed consent document. Study participation ends when the subject has completed her hysterectomy and postoperative period (6 +/- 2 weeks) *or* if the subject withdraws or has been withdrawn from the study prior to her hysterectomy.

E. Treatment

1. All subjects will be under general anesthesia per institutional guidelines.
2. Standard sterile preparation and operative technique will be used.
3. Intraoperative ultrasound will be used to locate target areas of adenomyosis prior to treatment, measure the volume of tissue to be treated, and to guide the placement of the treatment probe.
4. The ProVu treatment probe will be energized and RF energy delivered in a pre-programmed, controlled method based on the predetermined size of the affected tissue.
5. Information on all treated tissue regarding location, morphology, size, deployment, generator mode, treatment temperature, and treatment time will be documented.
6. The treatment probe will be removed and the site assessed for hemostasis.
7. All hysterectomies will be performed according to standard surgical practice.
8. All tissue will be excised with minimal disruption at the time of surgery and will be sent to the hospital pathologist or a core laboratory for assessment.
9. All subjects will exit the study at the time of the postoperative follow up.
10. All other medical care shall be in accordance with accepted medical practice.

F. Subject Compliance

All subjects entering the study are expected to have agreed to a hysterectomy due to pre-existing conditions and to have passed a standard pre-operative health assessment.

G. Subject Discontinuation or Withdrawal

The investigator may withdraw the subject at any time if it is in the subject's best interest. Subjects may voluntarily withdraw from the study at any time without reason. The investigator shall complete a study exit case report form.

H. Compensation and Costs for Study Participation

Subjects will be offered compensation of $300 for their participation in the study. Subjects will not be required to pay for any of the study procedures or materials during the course of this study. The study grant will not cover non-medical costs or complications that are a result of the standard surgical procedure.

I. Contact for Scheduling

Phone contact for appointment reminders and/or mailed pre-operative instructions prior to surgery dates will be the primary methods of contact.

J. Visit Schedule

The initial screening will be conducted using patient records, prior imaging results, and visit notes. Once subjects have consented to be in the study, they will have an MRI from which the diagnosis of focal or diffuse adenomyosis/adenomyoma is confirmed and lesions are measured. All MRIs will be read by one central radiologist. An endometrial biopsy is standard of care and may have been performed prior to enrollment. (Refer to Table I.)

TABLE I: VISIT SCHEDULE:

	Screening/Enrollment Visit	Surgery Day	Follow Up 6 +/- 2 weeks Post Op
Pre-op Health Assessment Based on Medical Records, Review of Prior Imaging Results (TVUS and/or MRI)	X		
Informed Consent	X		
Magnetic Resonance Imaging (to verify and measure diffuse or focal adenomyosis/adenomyomas), if not performed within the last 12 months	X		
Endometrial Biopsy (standard of care) within 12 months	X		
Pregnancy Test (standard of care) within 24 hours	X	x	
Device Use at Surgery		x	
Adverse Event Form		x	x
Study Exit			x

Study Device and Procedure

The ProVu™ System (Hologic, Inc., Boston, MA) is designed and cleared by the FDA as a treatment method for soft tissue, including the treatment of symptomatic uterine fibroids. After locating the general region of the target tissue, a laparoscopic ultrasound transducer is placed on the serosal surface of the uterus to identify the size, location, and number of focal adenomyosis (or adenomyomas). Under ultrasound guidance, the treatment probe (handpiece) is inserted through the serosal surface and into the target tissue. The electrode array containing multiple thermocouples is then deployed according to the size of the target tissue and the position is verified using the ultrasound transducer. Once correctly placed, the surgeon initiates ablation by pressing the foot pedal. Continuous temperature feedback is displayed on the generator screen. For safety purposes, dispersive pads are placed on the patient's thighs to disperse electrical current. If the target tissue is irregular or large, the needle array is retracted and the probe repositioned within the same area under ultrasound

guidance. The ablation is repeated until the area of interest is ablated. Overlapping ablations may be required. At the conclusion of the final ablation, the surgeon retracts the array withdraws the probe through the serosal surface of the uterus while coagulating the track to avoid bleeding. Once hemostasis is confirmed and all target tissue has been treated, the surgeon proceeds with the planned hysterectomy and the tissue is examined by a pathologist to assess the ablation zones within the adenomyoma or focal adenomyosis.

Device Training

All investigators will have completed training on the ProVu system prior to enrollment in this study.

Risk Assessment

A. ***Standard surgical risks***

Participation in the study does not eliminate the standard risks of hysterectomy. These include, (but are not limited to), nausea, vomiting, pain, bleeding, infection, poor healing, hernia formation, temporary change in bowel or bladder function, formation of adhesions, air in tissue or abdominal cavity, bleeding at incision sites(s) or abdominal wall, vasovagal reactions, heart attack, fever, infection, and inflammation in the abdomen. Unexpected reactions can occur from any drug or anesthetic that is administered. Unintended injury may occur to other pelvic or abdominal structures such as fallopian tubes, ovaries, uterus, bladder, ureter, bowel, or large blood vessels. Such injury may require immediate surgery or may require surgery at a later time. Dangerous blood clots may form in the legs or lungs. Participation in this study is not expected to alter the likelihood of occurrence or severity of any of these standard surgical risks.

B. ***Device risk***

A significant-risk (SR) device study is defined by the United States Food and Drug Administration (https://www.fda.gov/media/75459/download) as one that:

- Is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject;
- Is purported or represented to be for use supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject;
- Is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject;
- or otherwise presents a potential for serious risk to the health, safety, or welfare of a subject.

A Non-Significant Risk (NSR) device study is one that does not meet the definition for an SR device study. This study poses no significant risks to subjects due to the planned hysterectomy. Risks associated with the device include minimal bleeding

that is no greater than that experienced during pelvic surgery. Bleeding can be easily identified and corrected during the hysterectomy. If bleeding is encountered, it will be managed and the hysterectomy would proceed as planned. The risk of infection from this device or procedure is not expected to be any greater than the risk of infection following a hysterectomy.

Risks associated with the use of radiofrequency ablation in this study are minimal due to the laparoscopic or direct visualization technique utilized in this study. In addition, ultrasound is used to locate and observe regions within the uterus that are not easily visualized directly. The dispersive electrodes on the ProVu System are designed to monitor temperature at three locations of dispersive pad and therefore risks of skin burns are minimized.

C. ***Physical risks and discomforts.***
The subjects will undergo normal medical care in every respect except that additional pre-operative screening (MRI) will be provided and the ProVu System will be used immediately prior to the scheduled hysterectomy. The use of the ProVu System will add approximately 30 minutes of anesthesia time. The additional anesthesia time is not considered significant. This study is considered to be non-significant risk (NSR) because the hysterectomy has been planned and only 30 minutes of additional anesthesia time is required to complete the ablation procedures.

D. ***Other risks-confidentiality***
Every effort will be made to maintain subject confidentiality. Subjects will be identified primarily by their case report form (CRF) code in the records and databases kept by the company. All the CRFs in which study data are maintained will be coded to disguise any of the subjects' personal information that is unrelated to the study. We cannot guarantee complete confidentiality as subject data may need to be made available to treating medical personnel, , Hologic, Inc. or other authorized outside agencies such as the United States Food and Drug Administration (FDA).

E. ***Treatment and compensation for injury***.
If the subject is injured as a result of participating in the study, the subject will be treated appropriately until the resolution or stabilization of the injury. The investigator will immediately notify the manufacturer of the occurrence. If it is determined that the ProVu System is responsible for the subject's physical injury, the manufacturer may cover the cost of treatment for the injury. The manufacturer will not cover non-medical costs or indemnification other than may be required by law. Refer to the ProVu System "Instructions for Use" for all warnings and precautions.

Benefits

This is a feasibility protocol for research into the effects of radiofrequency ablation on adenomyosis in women who are already planning to have a hysterectomy. Therefore, there is no expected direct benefit to the subjects enrolled in this study. Other women may benefit from the use of the ProVu System in the treatment of adenomyosis in the future. Society will benefit if it is established that this System design is appropriate for use in a larger population.

Study Adverse Events

A. Definitions

An *adverse event* is any undesirable medical occurrence or untoward deviation in health away from baseline. An *unanticipated* adverse event is one that is not identified in nature, severity, or frequency in the current instructions for use and informed consent. A *device related* adverse event or adverse effect is one that is either definitely, probably, or possibly a result of device use. *A Serious Adverse Event* is any AE that is fatal, immediately life-threatening (i.e., presents an immediate risk of death at the time of the AE, not an AE that hypothetically might have caused death if it were more severe), requires or prolongs in-patient hospitalization, causes permanent or significant disability, requires medical or surgical intervention to prevent permanent sequelae, or any of the outcomes listed above.

B. Investigator Records

The investigator shall categorize adverse events according to the following scale:

[ ] 1. Definitely device related
[ ] 2. Probably device related
[ ] 3. Possibly device related
[ ] 4. Probably not device related
[ ] 5. Definitely not device related

C. Investigator Reports

The investigator shall report all adverse events that are unanticipated and are device related to the manufacturer by telephone immediately upon becoming aware of them. A follow-up written report shall be emailed to the manufacturer within 24 hours and shall include the following information:

i. Nature of adverse effect
ii. Statement as to why it is considered unanticipated
iii. Statement as to the degree to which it is considered device related, and why
iv. Results of any diagnostic tests that were performed
v. Description of any treatment implemented

vi. Statement of subject's current clinical status
vii. Investigator's signature and date

The investigator shall supply a copy of the report for adverse events that are serious or unanticipated and device related to the reviewing IRB.

The investigator shall continue to clinically monitor the adverse effect, with laboratory tests if appropriate, until it is resolved, stabilized or returns to baseline.

Confidentiality

Research records are marked by subject number and initials. Subject research files, including signed consent forms, will be kept in locked cabinets at the study site. All information provided to the Investigator by the manufacturer, or its designates, including non-clinical data, protocols, CRFs, and verbal and written information, will be kept strictly confidential and confined to the research staff involved in conducting the study. It is recognized that this information may be related in confidence to the IRB. Subjects' records will be made available to regulatory authorities as required.

Documentation

Accurate, complete and timely documentation is essential to the successful conduct of this study.

A. Investigator Responsibility

The investigator is responsible for maintaining the following documentation as updated and complete as possible at all times:

1. Informed consents.
2. Case report forms.
3. Adverse effect reports to the manufacturer.
4. Adverse effect reports to IRB.
5. Photographs, videos, images, if any.
6. Material control logs.
7. Screening logs.
8. IRB approval for protocol.
9. IRB approval for informed consent.
10. Final report to the IRB and manufacturer, in addition to any other reporting requirement that may be requested.
11. Records of deviations, violations and amendments.
12. Protocol (including all revisions).
13. Regulatory Binder.
14. Agreement Letter and Study Site Contract.
15. All correspondence relating to study.

Quality Assurance:

A. Data Quality Assurance

Steps to be taken to assure the accuracy and reliability of data include review of protocol procedures with the investigator and associated personnel prior to the study. Case report forms will be reviewed for accuracy and completeness as compared with the source documents.

B. Monitoring

Monitoring will be performed according to Good Clinical Practices (GCP) guidelines established by the United States Food and Drug. A Contract Research Organization (CRO) may be used to monitor the study. Monitoring will be scheduled in accordance with recruitment/enrollment rates and site performance requirements.

C. Data Management

After monitoring of the data is complete, data will be entered into the database by a third-party data manager.

Data Analysis

Data analysis will consist of the evaluation of the ProVu System performance relative to the proposed use, prescribed treatment, pathology results and adverse device effects. Descriptive statistics (including those derived from pathology results, see Appendices A-C) will be provided as dictated per protocol. A full statistical analysis will not be appropriate considering the size and intent of this study.

Personnel Responsibilities

The investigator will assign a study coordinator to assist during the conduct of this study. There are no other required site personnel.

Return of Unused Inventory

All unused System handpieces shall be returned to the manufacturer. Used electrodes will be disposed of according to hospital standards for biohazard materials. Any generators, cables, and other non-disposable parts that are donated for use during this study will be returned to the manufacturer at the conclusion of the study.

References

1. https://en.wikipedia.org/wiki/Adenomyosis
2. Gunther R, Walker C (2020). "Adenomyosis" StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jun 24.PMID 30969690.

3. Brosens I, Gordts S, Habiba M, Benagiano G (December 2015). "Uterine Cystic Adenomyosis: A Disease of Younger Women". J Pediatr Adolesc Gynecol. **28** (6): 420–6. doi:10.1016/j.jpag.2014.05.008. PMID 26049940.
4. Struble, Jennifer; Reid, Shannon; Bedaiwy, Mohamed A. (2016). "Adenomyosis: A Clinical Review of a Challenging Gynecologic Condition". Journal of Minimally Invasive Gynecology. **23** (2): 164-185. doi:10.1016/j.jmig.2015.09.018. PMID 26427702.
5. Kepkep K, Tuncay YA, Göynümer G, Tutal E (2007). "Transvaginal sonography in the diagnosis of adenomyosis: which findings are most accurate?". Ultrasound Obstet Gynecol. **30** (3): 341–5. doi:10.1002/uog.3985. PMID 17659649. S2CID 26333703.
6. Sakhel K, Abuhamad A (May 2012). "Sonography of adenomyosis". J Ultrasound Med. **31** (5): 805–8. doi:10.7863/jum.2012.31.5.805. PMID 22535729. S2CID 37627898.
7. Dartmouth, Katherine (2014-08-01). "A systematic review with meta-analysis: the common sonographic characteristics of adenomyosis". Ultrasound. **22** (3): 148–157. doi:10.1177/1742271X14528837. ISSN 1742-271X. PMC 4760530. PMID 27433212.
8. Sharma, Kaveri (2015). "Role of 3D Ultrasound and Doppler in Differentiating Clinically Suspected Cases of Leiomyoma and Adenomyosis of Uterus". Journal of Clinical and Diagnostic Research. **9** (4): QC08–12. doi:10.7860/jcdr/2015/12240.5846. PMC 4437118. PMID 26023602.
9. Bragheto A.M.; et al. (2007). "Effectiveness of the levonorgestrel-releasing intrauterine system in the treatment of adenomyosis diagnosed and monitored by magnetic resonance imaging". Contraception. **76** (3): 195–9. doi:10.1016/j.contraception.2007.05.091. PMID 17707716.
10. Berman JM, Guido RS, Garza Leal JG, et al. (2014). "Three-year outcome of the Halt trial: a prospective analysis of radiofrequency volumetric thermal ablation of myomas." J Minim Invasive Gynecol.21(5):767-774. PMID: 24613404
11. Krämer B, Hahn M, Taran FA, Kraemer D, Isaacson KB, Brucker SY. Interim analysis of a randomized controlled trial comparing laparoscopic radiofrequency volumetric thermal ablation of uterine fibroids with laparoscopic myomectomy. *Int J Gynaecol Obstet.* 2016 May;133(2):206-11. PMID: 26892690 ClinicalTrials.gov Identifier: NCT01750008

Pathology

The hospital pathologist will be responsible for issuing separate standard of care anatomic pathology reports for subsequent patient care and that will not be incorporated into this study. As tissues required for patient care will be separately preserved for evaluation by the hospital pathologist, any tissues utilized / submitted in this study will be for investigational use only.

The intact uteri will be received fresh in pathology within four hours of completion of the in vivo hyperthermic treatment and two hours of completion of the hysterectomy. Care needs to be taken to ensure that the specimen surfaces do not dry and are not overly hydrated in the operating room, during hyperthermic treatment or during transportation to the pathology laboratory.

The uteri will be received labeled with the patient's name, hospital number, study number, specimen number, test article and test system. Upon receipt in pathology, the specimen will be accessioned in preparation for evaluation. The gross tissue evaluation findings will be recorded on the Gross Pathology Case Report Form (Appendix A).

The anterior and posterior uterine surfaces with attached cervix and adnexal organs (when present) will be digitally photographed as an intact organ block or bivalved. All orienting photographs will contain a scale and specimen identifier.

The uterus will be weighed and measured. After removing the adnexa (if present), the uterus with attached cervix will be anatomically oriented. Using different marking inks for correlation with the marking sutures, the individually ablated area will be focally inked for identification during the sectioning process. A midline anterior black ink strip will be used to mark the anterior serosal side of each tissue slice.

The uterus will then be serially sectioned in the sagittal plane into approximately 5-8mm macroscopic tissue slices (Figure 1). An attempt will be made to adjust the sectioning so that the cuts pass though the center of each thermal lesion in the sagittal plane.

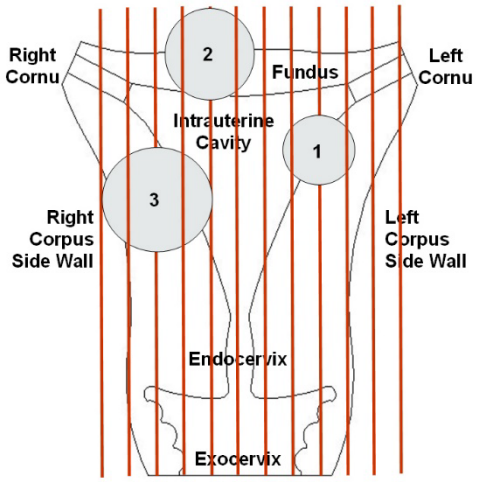


Figure 1. Serial uterine sectioning through the treated areas (grey circles).

The tissue slices will be serially laid out and digitally photographed on both sides (Figure 2). All orienting photographs will contain a scale and specimen identifier.

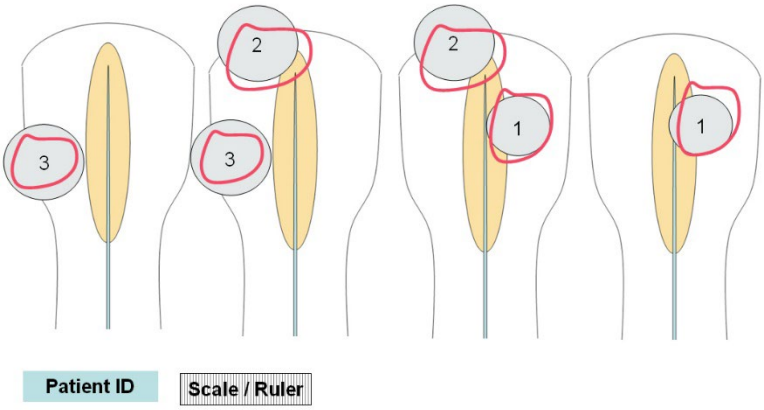


Figure 2. Serial unstained uterine tissue sections through the treated areas (grey circles). Variable hyperemic rings demarcate the ablation region zones. The number of slices thought a particular tissue zone will vary with its size.

The uterus will be examined for pre-existing intrauterine lesions. The number of leiomyomata, adenomyosis-like lesions and/or other gross lesions will be recorded. The number, size range (diameter) and location of the lesions will be recorded. Untreated lesions numbering greater than four (4) will be recorded as "multiple." Treated lesions must be identified as shown above in the grey circles.

In appropriately labeled plastic container(s), the uterine tissue slices will be macroscopically triphenyltetrazolium chloride (TTC) stained to assess for regions of

thermal enzyme inactivation (viability stain). The staining protocol is outlined in Appendix B. The thermal-treated regions will be assessed with TTC staining within four hours after completion of the thermal treatment. The presence of a pink-maroon color change in the tissue will be interpreted as having functional enzyme activity (diaphoreses / NADH; TTC-positive). The absence of a color change and preservation of native tissue coloration will be interpreted as having non-functional enzyme activity (TTC-negative).

The TTC-stained tissue slices will be digitally photographed following fixation of the TTC-stain with 10% neutral buffered formalin. The macroscopic TTC-stained tissue slices will be fixed in 10% neutral buffered formalin for at least 15 minutes but less than 24 hours prior to measurements and photography.

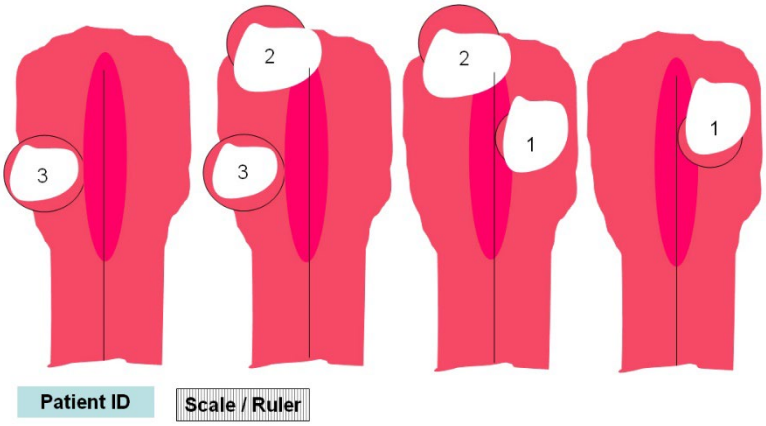


Figure 3. Serial TTC-stained uterine tissue sections through the treated tissue (circles). White zones represent TTC-negative ablation regions involving the treated zones and to varying extents the adjacent tissues.

The three-dimensional size (cranial/caudal, anterior/posterior and right/left axis), type (submucosal, intramural, or subserosal) and location (fundus, right cornu, left cornu, anterior corpus, posterior corpus, right corpus, left corpus, anterior lower segment or posterior lower segment) of each treated area will be recorded. The closest distance of the treated zone to the uterine serosa and intrauterine cavity will be recorded. Measurements will be made with calipers to within ±0.1 mm.

Using the TTC-stained tissue slices, the three-dimensional size of the devitalized TTC-negative primary ablated region will be measured in the cranial/caudal, anterior/posterior and right/left axis. The location of the lesion within the adenomyosis will be documented (centered of eccentric). The approximate percentage of the target area treated by the ablated volume will be estimated. The closest distance between the TTC-negative primary thermal lesion and the uterine serosa and intrauterine cavity will be recorded. Measurements will be made with calipers to within ±0.1 mm.

Representative tissue blocks from the central ablation zone section will be taken from the 10% neutral buffered formalin fixed tissue. The submitted tissue blocks will be routinely processed and subsequently paraffin-embedding at the local Institution. The formalin-fixed / paraffin-embedded blocks will be faced and five micron hematoxylin and eosin stained sections prepared for review. Under fixed blocks will require reprocessing prior to preparation of the hematoxylin and eosin-stained sections. One slide will be prepared per block and labeled with specimen, treated area, and location identifiers.

As the tissue was treated on the same day as the hysterectomy (Day 0 specimen), the histological manifestations of cell injury (coagulative necrosis) will not be morphologically evident. While subtle thermal change can be seen in the treated tissue, a discreet boundary of devitalize versus viable tissue cannot be reliably ascertained. Therefore, the hematoxylin and eosin stained slides will be reviewed and a global brief microscopic summary prepared for each slide set. Microscopic measurements will not be made on the hematoxylin and eosin stained slides. Specific unique hematoxylin and eosin microscopic findings will be reported by location identifier, if present.

Appendix A – Gross Pathology Case Report Form

Gross Uterine Pathology Evaluation	*Hologic, Inc.* *Study/Protocol Number*
Subject ID	Institution / Site Number:
Uterus ID Number (Hosp. Number): Test Article: ProVu Test System: In Vivo Human Adenomyosis Specimen Type: Human Uterus	Surgery Date: (DD/MMM/YYYY) Time of Receipt: (HH:MM) Dissection Date: (DD/MMM/YYYY)

1. Digital Anterior/Posterior Photographs Prior to Dissection with Adnexa:

(include identifying tags and scale)

☐ ***Yes:*** *Total Number* _____ ☐ ***No:*** *Comment :*_____

2. Uterine Weight and Measurements:

a. *Uterine weight without adnexa:* _____ *grams*
b. *Uterine length (fundus to cervix):* _____ *cm*
Uterine thickness (anterior/posterior): _____ *cm*
Uterine widths (right/left):
Uterotubal / fundus region _____ *cm* *Mid corpus region* _____ *cm*
Lower segment region _____ cm

3. Ovaries and Fallopian Tubes:

a. Right Ovary*:*

☐ *Absent*

☐ *Present - Size: ___ x ___ x ___ cm;*

*Description:*_____

b. Right Fallopian Tube*:*

☐ *Absent*

☐ *Present - Size: ___ x ___ x ___ cm;*

*Description:*_____

c. Left Ovary:

☐ *Absent*

☐ *Present - Size: ___ x ___ x ___ cm;*

*Description:*_____

d. Left Fallopian Tube*:*

☐ *Absent*

☐ *Present - Size: ___ x ___ x ___ cm;*

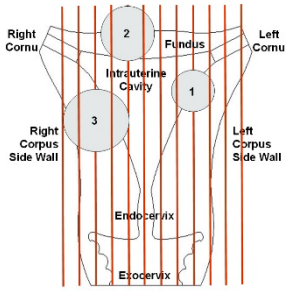
*Description:*_____

4. Other External Observation(s):

_____
_____
_____

5. Macroscopic Tissue Sectioning:

Using different marking inks for correlation with the marking sutures, the one to three individually ablated areas will be focally inked for identification during the sectioning process. A midline anterior black ink strip will be used to mark the anterior serosal side of each tissue slice. The uterus will then be serially sectioned in the sagittal plane into approximately 5-8mm macroscopic tissue slices. An attempt will be made to adjust the sectioning so that the cuts pass though the center of each thermal lesion in the sagittal plane.

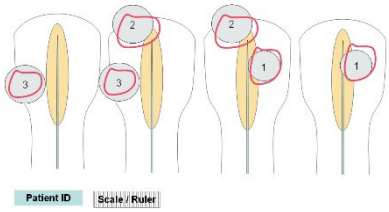


Marking Inks:

Treatment Site 1: ☐ N/A ☐ Color_____
Treatment Site 2: ☐ N/A ☐ Color_____
Treatment Site 3: ☐ N/A ☐ Color_____

Digital Photographs of Unstained Uterine Tissue Slices:
(include identifying tags and scale)

☐ ***Yes:*** *Total Number* _____
☐ **No:** Comment :_____



Uterine Lesions and Other Findings:

a. Leiomyomata:

☐ *Absent*

☐ *Present – Number: ____ Size Range: ___ - ___ cm;*

*Location:*_____
_____

b. Adenomyosis -

☐ *Present – Size: ___ x ___ x ___ cm; treated___ untreated__*

☐ *Present – Size: ___ x ___ x ___ cm; treated___ untreated__*
☐ *Present – Size: ___ x ___ x ___ cm; treated___ untreated__*
☐ *Present – Size: ___ x ___ x ___ cm; treated___ untreated__*
☐ *Present – Size: ___ x ___ x ___ cm; treated___ untreated__*
☐ *Present – Size: ___ x ___ x ___ cm; treated___ untreated__*

*Description:*_____

_____

_____

***c. Other Findings**:*

☐ *Absent*

☐ *Present -*

*Description:*_____

_____

_____

_____

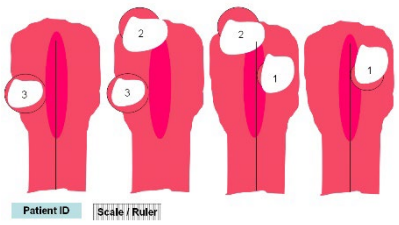
6. Macroscopic TTC-Staining Evaluation:

The size and location of each treated area will be recorded with the minimum " to serosa" and "to intrauterine cavity" distances. Using the TTC-stained tissue slices, the size of the TTC-negative region will be measured. The involved tissues will be documented. The lesion's location within the area of adenomyosis will be documented. The approximate percentage of treated area will be estimated. The closest distance between the TTC-negative lesion and the serosa and intrauterine cavity will be recorded. Measurements will be made with calipers to within ±0.1 mm.

Digital Photographs of TTC-Stained Uterine Slices:
(include identifying tags and scale)

☐ *Yes: Total Number* _____

☐ No: Comment :_____



Treated Region #1 TTC-Staining Results:

☐ Section Not Applicable

Adenomyosis Area: ___ x ___ x ___ mm (C/C x A/P x R/L)

Location: □ *Fundus* □ *Cornu* □ *Corpus* □ *Lower Segment*
□ *Anterior* □ *Posterior* □ *Right* □ *Left*

TTC-Staining Results
- □ *TTC-Negative Lesion Not Identified*
- □ *TTC-Negative Lesion Identified*

Ablation Zone Size: _____ x _____ x _____ mm (C/C x A/P x R/L)

Ablation Zone Relationship to Target: □ *Central* □ *Eccentric*

Approximate % of Ablated Tissue: _____ %

Minimum Ablation to Cavity: _____mm *to Serosa: _____mm*

Comment: _____
_____
_____

Treated Region #2 TTC-Staining Results:

□ Section Not Applicable

Adenomyosis Area: ___ x ___ x ___ mm (C/C x A/P x R/L)

Location: □ *Fundus* □ *Cornu* □ *Corpus* □ *Lower Segment*
□ *Anterior* □ *Posterior* □ *Right* □ *Left*

TTC-Staining Results
- □ *TTC-Negative Lesion Not Identified*
- □ *TTC-Negative Lesion Identified*

Ablation Zone Size: _____ x _____ x _____ mm (C/C x A/P x R/L)

Ablation Zone Relationship to Target: □ *Central* □ *Eccentric*

Approximate % of Ablated Tissue: _____ %

Minimum Ablation to Cavity: _____mm *to Serosa: _____mm*

Comment: _____
_____
_____

Treated Region #3 TTC-Staining Results:

□ Section Not Applicable

Adenomyosis Area: ___ x ___ x ___ mm (C/C x A/P x R/L)

Location: □ *Fundus* □ *Cornu* □ *Corpus* □ *Lower Segment*
□ *Anterior* □ *Posterior* □ *Right* □ *Left*

TTC-Staining Results
- □ *TTC-Negative Lesion Not Identified*
- □ *TTC-Negative Lesion Identified*

Ablation Zone Size: _____ x _____ x _____ mm (C/C x A/P x R/L)

Ablation Zone Relationship to Target: □ *Central* □ *Eccentric*

Approximate % of Ablated Tissue: _____ %

Minimum Ablation to Cavity: _____mm to Serosa: _____mm

Comment: _____
_____
_____

Treated Region #4 TTC-Staining Results:

□ Section Not Applicable

Adenomyosis Area: ___ x ___ x ___ mm (C/C x A/P x R/L)

Location: □ *Fundus* □ *Cornu* □ *Corpus* □ *Lower Segment*
□ *Anterior* □ *Posterior* □ *Right* □ *Left*

TTC-Staining Results

□ *TTC-Negative Lesion Not Identified*
□ *TTC-Negative Lesion Identified*

Ablation Zone Size: _____ x _____ x _____ mm (C/C x A/P x R/L)

Ablation Zone Relationship to Target: □ *Central* □ *Eccentric*

Approximate % of Ablated Tissue: _____ %

Minimum Ablation to Cavity: _____mm to Serosa: _____mm

Comment: _____
_____
_____

TTC-Negative Possible Satellite Thermal Lesions (submit tissue for histology):

☐ *Not Identified*

☐ *Present – Describe size and locations:* _____

_____

_____

_____

7. Representative Tissue Formalin-Fixation and Paraffin-Embedding for Histologic Evaluation:

Representative tissue blocks from each thermal lesion and any possible satellite lesions will be submitted for fixation in 10% neutral buffered formalin and routine paraffin-embedding. Each block will be labeled with the protocol number and location identifier. The blocks will be embedded at the local institution without slide preparation.

Submitted Histology Cassettes (Embed Only at Local Institution):

Location	**Cassette Letter Identifiers (A,B,C…)**	
Lesion #1 Cassette Map of Lesion:	_____	☐ Not Submitted
Lesion #2 _____ Cassette Map of Lesion:		☐ Not Submitted
Lesion #3 Cassette Map of Lesion:	_____	☐ Not Submitted
_____	_____	☐ Not Submitted

_____	_____	☐ Not Submitted
_____	_____	☐ Not Submitted
_____	_____	☐ Not Submitted
_____	_____	☐ Not Submitted

8. Additional Observations and Comments:

_____
_____
_____
_____
_____
_____
_____
_____

Print Pathologist Name	_____
Signature of Pathologist	_____
Date	_____/_____/_____ (DD/MMM/YYYY)

Appendix B – TTC Staining Protocol

TTC (Triphenyltetrazolium Chloride) Staining protocol for Macroscopic Tissue Viability Assessment

Principal

Regions of thermal necrosis will be identified using a dehydrogenase enzyme and cofactor based reaction that converts the tetrazolium salt to a formazan pigment within viable tissues. This conversion results in a cytochemical color change from colorless to red-maroon in tissues with preserved or partially preserved enzymatic activity (viable vs partially viable tissues). Tissues that lack intact enzymatic activity to metabolize the tetrazolium salt do not stain and remain their native color.

Upon gross examination, the non-viable tissues appear pale pink-tan to yellow-grey in color depending on their baseline color. After staining, the boundary of the thermal necrosis, if present, can be assessed by identifying red-orange staining in the viable enzymatically active tissue. The protocol for preparing the TTC solution for tissue staining follows:

Specimen Requirements

Fresh tissue slices from the specimen maintained at room temperature after removal from the patient. Testing should be performed within two – four hours of tissue excision from the patient. The tissue should not be allowed to dry or become excessively wet. Avoid rinsing tissue slices after cutting with water.

Materials

Sigma Chemical - Tel. 1-800-325-3010 Fax 1-800-325-5052

Trizma HCl	Catalog #T3253-500g
Trizma Base	Catalog #T1503-100g
TTC	Catalog #T8877-100g

Distilled water

Stain Preparation

The following amount of each chemical will be brought into solution in 1000 ml of distilled water:

Trizma Base (Sigma #T 1503)	7.88 gram
Trizma HCl (Sigma #T 3253)	21.28 gram
T.T.C. (Sigma #T 8877)	10.00 gram

The stock staining solution must be stored in plastic non-metallic containers that are light-tight. The stock solution should be stored at 4ºC in the refrigerator. The stock solution expiration date should be no greater than 30 days from preparation. The stain can be filtered and re-used provided significant dilution of the solution with tissue fluids has not occurred.

In general, 1000 ml of stock staining solution should be sufficient for two to three uterine specimens. In general, no more than three uteri should be stained with 1000 ml of stock solution.

Procedure

1. The cut surfaces should be free from excessive blood and other tissue fluids prior to cutting. The cut surfaces should not be allowed to dry (cut on saline dampened toweling). The cut surfaces should not be rinsed with water or other fixatives.
2. Fresh cut tissue surfaces will be floated cut surface down in a non-metallic plastic container of the TTC staining solution. Staining will be performed at room temperature for a period of 60 minutes or optimal color change is achieved.
3. Following staining, the tissue will be fixed in 10% formalin to intensify and sharpen the stain (range: 5 min. up to 24 hours).
4. Digital photographs will be taken and the results measured.

Results

Staining Interpretation:
Viable tissue stains red-maroon
Non-viable tissue lacks staining.

As TTC staining can fade over time, macroscopic tissue evaluation should be made within 12 to 24 hours of staining. False negative staining can occur in regions where the tissue contacts the container or other surfaces during staining. TTC staining of fresh tissue does not affect formalin fixation, paraffin embedding and subsequent hematoxylin and eosin staining.

Appendix C – Microscopic Pathology Case Report Form

Microscopic Uterine Pathology Evaluation	*Hologic, Inc.*
Subject ID:	Institution / Site Number:
Uterus ID Number (Hosp. Number): Test Article: ProVu Test System: In Vivo Human Adenomyosis Specimen Type: Human Uterus	Surgery Date: (DD/MMM/YYYY) Microscopy Date: (DD/MMM/YYYY)

1. Received Histology Cassettes Embedded at Local Institution:

Labeled: _____ (Specimen Number)

Letter Identifier(s)	**Location**	**Status**	
_____	Lesion #1	□ Received	□ Not Received
_____	Lesion #2	□ Received	□ Not Received
_____	Lesion #3	□ Received	□ Not Received
_____	Lesion $4	□ Received	□ Not Received
_____	_____	□ Received	□ Not Received
_____	_____	□ Received	□ Not Received
_____	_____	□ Received	□ Not Received
_____	_____	□ Received	□ Not Received

2. Hematoxylin and Eosin Sections:

The received paraffin-embedded blocks will be faced and a 5 micron hematoxylin and eosin stained section will be prepared for review from each block. Microscopic measurements will not be made on these slides. A general summation will be provided with specific comments referenced by location ID (see figure).

General Summary:

_____
_____
_____
_____
_____
_____
_____
_____
_____

Slide Specific Comments:

ID Additional Finding(s) if present:

A. ☐ NA ☐ No Additional ☐

B. ☐ NA ☐ No Additional ☐

C. ☐ NA ☐ No Additional ☐

D. ☐ NA ☐ No Additional ☐

E. ☐ NA ☐ No Additional ☐

F. ☐ NA ☐ No Additional ☐

G. ☐ NA ☐ No Additional ☐

H. ☐ NA ☐ No Additional ☐

I. ☐ NA ☐ No Additional ☐

J. ☐ NA ☐ No Additional ☐

K. ☐ NA ☐ No Additional ☐

L. ☐ NA ☐ No Additional ☐

M. ☐ NA ☐ No Additional ☐

N. ☐ NA ☐ No Additional ☐

O. ☐ NA ☐ No Additional ☐

P. ☐ NA ☐ No Additional ☐

Q. ☐ NA ☐ No Additional ☐

R. ☐ NA ☐ No Additional ☐

S. ☐ NA ☐ No Additional ☐

T. ☐ NA ☐ No Additional ☐

U. ☐ NA ☐ No Additional ☐

V. ☐ NA ☐ No Additional ☐

W. ☐ NA ☐ No Additional ☐

_____

X. ☐ NA ☐ No Additional ☐

_____

Y. ☐ NA ☐ No Additional ☐

_____

Z. ☐ NA ☐ No Additional ☐

_____

3. Additional Observations and Comments:

_____
_____
_____
_____

Print Pathologist Name	_____
Signature of Pathologist	_____
Date	_____/_____/_____ (DD/MMM/YYYY)