Thyroid Protocol:

Title:	"Clinical Evaluation of Opto-Acoustic Tomography for Detection and Diagnostic Differentiation of Thyroid Nodules"
Protocol Number:	Thyroid-01
Device:	Imagio™ Imaging System
Sponsor:	Seno Medical Instruments, Inc.
Sponsor Contact:	Shaan Schaeffer Vice President of Clinical Operations Seno Medical Instruments, Inc. 5253 Prue Road, Suite 315 San Antonio, TX 78240 210-615-6501

Date: 2/6/2018

Thyroid Protocol Version 10.0

Confidentiality Statement

PROTOCOL SIGNATURE PAGE

Shaan Schaeffer
Vice President of Clinical Operations

2/6/2018
Date

A. Thomas Stavros, MD
Medical Director

8-FEB-2018
Date

Ann Waterhouse
Vice President of Regulatory and Quality Assurance

7Feb 2018
Date

Steve Miller
Vice President of R&D

2/6/18
Date

REVISION HISTORY

Version: 2.0 Date: 12/7/2016	K. Imler changed to P. Taige as sign off for Regulatory Section 6.2 corrected “breast” to thyroid Removed section 6.3 as it applies to breast
Version: 3.0 Date: 1/12/2017	T. Umbel added to replace P. Taige as sign off Section 3.2 – Study Duration – study duration changed from 12-24 months
Version: 4.0 Date: 1/30/2017	Page 42/42- Updated Appendix D Thyroid Scanning Protocol from Rev 01 to Rev 02
Version: 5.0 Date: 4/25/2017	Replaced all ® with ™ when followed after Imagio throughout protocol Replaced CEO Tom Umbel as signatory with Ann Waterhouse VP of Regulatory and Quality Assurance; Added signature of Steve Miller, VP of R&D Updated /corrected List of abbreviations for ADE, CRO, IUS, and OA/US Updated all instances of OA acronym to OA/US throughout the protocol and general formatting Section 1.2-Updated Photo of Imagio with OA/US probe only Section 1.3- Removed last sentence: This device is not intended to be used as a screening device or for definitive pathologic diagnosis. Section 1.4 removed statement: The majority of the population was white (n – 1,558; 79.0%). Section 3.2-Removed paragraphs describing image annotation requirements; removed

	paragraph describing scanning protocol; added the collection of initials, DOB and subject number requirement to study images. All will be included in Core Lab Manual. Section 4.2-Clarified Exclusion Criteria 4 to include multiple lesion presence requirement of minimum separation of 1 cm between study lesion(s) from any other lesion(s) Section 5.1-Study Device description updated to appropriately describe Imagio as an investigational device in the US Section 6.2-Removed-Contamination due to insufficient cleaning materials due to it being listed in User Manual Section 6.3 moved heading and first following statement. Section 7.5-Updated Enrollment Failure definition to include Imaging Core Lab review and removing "subjects who have incomplete or unusable study device scans" Section 14.1-Removal of –"IRB committee and will provide appropriate therapy and follow-up of treated subjects" from last sentence. Page 42/42- Updated Appendix D Thyroid Scanning Protocol from Rev 02 to Rev 03.
Version: 6.0 Date: 7/18/2017	Section 4.2: Updated Inclusion Criteria #2 and extended window for standard of care imaging from 45 days to 90 days. Updated Exclusion Criteria # 10 to exclude previous FNA of target nodule of interest only if FNA was performed within 6 months of baseline IMAGIO scan. Section 7.1 - Table 1 Schedule of Events footnote #2 was updated from 45 days to 90 days to match inclusion criteria #2 update.

Version: 7.0 Date: 9/8/2017	Section 4.2: Updated Inclusion Criteria #2 and extended window for standard of care imaging from 90 days to 365 days. Section 7.1 - Table 1 Schedule of Events foot note #2 was updated from 90 days to 365 days to match inclusion criteria #2 update.
Version: 8.0 Date: 11/01/2017	Section 3.2 Removed exclusion language limiting "up to two suspicious nodules" to be biopsied from the thyroid with "a maximum of 1from each lobe". Added lesion numbering format to include thyroid and lymph node designation. Section 4.2: Modified Inclusion #5 and removed limitation of "no more than 2 (one in each lobe) thyroid nodules". Removed Exclusion #4: Have greater than 2 nodules (1 in each lobe) recommended for biopsy at baseline, study nodules cannot be touching any other nodules with a minimum separation of 1 cm; Modified Exclusion #9 (Previously #10) to reduce previous biopsy of target nodule of interest occurring prior to baseline Imagio scan from 6 months to 3 months. Section 5.1 Modified Imagio "Instructions for Use" to "User Manual" to reflect correct document name Section 7.2.1 Updated CDU imaging window from 45 days to 365 days to stay consistent with version 7.0 changes Updated Appendix D Thyroid Scanning Protocol from Rev 03 to Rev 05.
Version: 9.0 Date: 11/30/2017	Updated Appendix C: ACR TIRADs to reflect latest released ACR TIRADs Chart.
Version: 10.0 Date: 2/6/2018	Updated Section 4.2 Inclusion Criteria 2 Modified to:

	Have an undiagnosed suspicious solid or mostly solid thyroid nodule. Exclusion Criteria 9 modified to shorten window to 45 days Section 9- Eliminated language that is included in Imaging Core Lab Documents. Updated Scan Procedure to v06

Table of Contents

Table of Figures

Table of Tables

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ACR	American College of Radiology
ADE	Anticipated Adverse Device Event
CDU	Conventional Diagnostic Ultrasound
CDI	Color Doppler Imaging
CI	Confidence Interval
CNB	Core Needle Biopsy
CRF	Case Report Form
CRO	Clinical Research Organization
DVAB	Directional Vacuum – Assisted Biopsy
eCRF	Electronic CRF
EC	Ethics Committee
EDC	Electronic Data Capture
EOS	End of Study
FDA	Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
FN	False Negative
FNA	Fine Needle Aspiration
FNAB	Fine Needle Aspiration Biopsy
FP	False Positive
FPR	False Positive Rate
GCP	Good Clinical Practice
ICL	Imaging Core Lab
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ISO	International Organization for Standardization
ITD	Intention to Diagnose
IUS	Imagio Ultrasound/Internal Ultrasound
LOM	Laser Optical Movie
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
MRMC	Multi-reader, Multi-case
NDU	Negative Diagnostic Ultrasound
OA/US	Opto-Acoustic
NLR	Negative Likelihood Ratio
NPV	Negative Predictive Value
OR	Odds Ratio

PDI	Power Doppler Imaging
PI	Principal Investigator
POM	Probability of Malignancy
PLR	Positive Likelihood Ratio
PPV	Positive Predictive Value
QAR	Quality Assurance Reviewer
SADE	Serious Adverse Device Effect
SAP	Statistical Analysis Plan
Se	Sensitivity
Sp	Specificity
SE	Strain Elastography
SWE	Shearwave Elastography
TI-RADS	Thyroid Imaging Reporting and Data System
TN	True Negative
TP	True Positive
TPR	True Positive Rate
UADE	Unanticipated Adverse Device Effect
USADE	Unanticipated Serious Adverse Device Effect
WHO	World Health Organization

1. INTRODUCTION

1.1. Background of Thyroid Cancer

The incidence of thyroid nodules has increased tremendously in recent years [1]. The reasons for this increase are likely multifactorial but are largely attributed to widespread application of high-resolution ultrasound to the thyroid and the frequent incidental detection of nodules on other imaging modalities. In distinction to palpation, which demonstrates nodules in only 5% to 10% of the population, autopsy and sonography detect them in at least 60% [2-4]. Although common, malignancy in nodules remains relatively low ranging from 1.6% to 12%. Ultrasound is superior to other modalities in categorizing thyroid nodules however a definitive diagnosis is arrived at through the use of fine-needle aspiration (FNA), core biopsy or surgery. This places a burden on the health care system and anxiety may occur in many patients. In addition, long-term studies by Ito et al [5] showed no difference in outcomes between patients with biopsy-proven differentiated papillary thyroid carcinomas <1 cm undergoing thyroidectomy and those followed with no surgical intervention.
The structure of the thyroid and the nature of thyroid cancer present a great challenge for imaging technologies to detect malignant nodules reliably and to differentiate them from benign nodules. Seno's imaging technology addresses this challenge by utilizing 2 very different real time temporally fused modalities, opto-acoustic (OA/US) and diagnostic ultrasound(US), thus providing co-registered and temporally interleaved real time functional and anatomical information. This combination has the potential to add diagnostic information to help the radiologist better evaluate suspicious thyroid nodules in an efficient manner.

1.2. Imagio – Device Description

The Imagio provides the clinician with the display of 2-dimensional real-time images of the structures of the breast containing OA absorption and a co-registered B-mode ultrasound image. The Imagio hand-held OA transducer transmits predefined laser wavelengths (757nm and 1064nm) to illuminate the breast tissue. Internal light absorption, which occurs in the tissue, results in acoustic signals that can be detected by the multi-element wideband ultrasound transducer, a sub-assembly in the OA transducer. The Imagio software acquires and processes the return OA and B-mode ultrasound signals to form and log images containing the structural and functional information associated with breast abnormalities. This information is presented to the clinician as a co-registered and temporally interleaved display of OA and B-mode ultrasound images and can be compared to conventional ultrasound image displayed alongside OA/US images.
Separate from the OA imaging modality, the user can select conventional ultrasound modalities, i.e., B-mode ultrasound, Pulsed-Wave Doppler, Color/Power Doppler, and combined ultrasound modes, and thereby operate the Imagio in the same manner as a conventional ultrasound system. It is important to note that Doppler modes cannot be activated while in OA imaging mode. The B-mode ultrasound includes image enhancing features, and the ability to measure and annotate images. The system is DICOM compliant and includes PACS connectivity.
The Imagio is comprised of a system cart, which encloses the following sub-systems:

- Ultrasound
- Laser
- Data Acquisition and Processing (DAP) module
- Power Supplies
- Power Control

External to the cart, the user has access to the following elements:

- High Resolution Display Monitor with laser emission LED
- Console with Touch Screen with soft keyboard
- Optional QWERTY keyboard
- Master Power key switch On/Off Control (attached to Console)
- Emergency Power Off Control (attached to Console)
- Remote Interlock
- Foot switch
- Laser coolant port and filling system
- Calibration box
- OA Probe
- Ultrasound ports
- System wheel lock
- Protective eyewear

Figure 1: Imagio breast imaging system

The laser energy emitted by the Imagio is Class 3b. To protect users and subjects, several safety features are included in the Imagio. In order to turn on the device, a physical key is required to be inserted into the locked on/off switch and turned to the ON position (Figure 2). In order to enable the laser for possible emission, the user must enter a password. After 15 minutes of inactivity, the user must enter the password again. Protective eyewear is provided for the user(s) and the patient/subject. Prior to the start of key tasks (i.e., calibration and laser authorization), the user must actively click "ok" to confirm that protective eyewear is applied to all persons in the room. Emission is activated by the footswitch and is indicated by an icon on the Display Monitor, the LED at the top of the Display Monitor, and an audible tone. During clinical use, the laser will not emit if the OA Probe is not connected. An emergency stop is also provided (Figure 3).

Figure 2 Key Switch

Figure 3 Emergency stop

1.3. Intended Use

The Imagio system is a diagnostic functional and morphologic imaging system intended for the opto-acoustic (OA/US) evaluation of thyroid in men and/or women who have findings of thyroid nodules. The Imagio thyroid imaging system is intended to be used in the evaluation of thyroid nodules, regardless of the method or mechanism of initial detection, including both palpable and non-palpable nodules. Thyroid nodules may present as palpable nodules or may be detected incidentally on CT or MR scans of the neck or chest. They may also be detected as incidental findings on carotid doppler examinations. Occasionally they are detected as areas of increased activity on PET/CT scans.

The Imagio multi-modality system utilizes opto-acoustic and ultrasound technologies to acquire, process, display, and log co-registered structural and functional information about thyroid abnormalities. This information is presented to the user as fused and temporally interleaved opto-acoustic and ultrasonic images.

The Imagio system also includes real-time diagnostic ultrasound with color and pulsed Doppler. The grayscale ultrasound component may be used alone and includes image enhancing features, and the ability to measure and annotate images. The system is DICOM compliant and has PACS connectivity. Imagio is intended to be used by qualified healthcare professionals in the differentiation of benign from malignant thyroid lesions and to support them in their decisions.

1.4. Imagio Previous Studies

Early breast proof-of-concept studies provided the company with supporting data to modify and enhance diagnostic performance of the Imagio real time opto-acoustic functional imaging characteristics performed at the wavelengths of Nd:YAG and Alexandrite lasers. Imagio was also tested on gelatin phantoms simulating dense

breast with blood vessels having blood with various degrees of oxygen saturation. In-vitro experiments with various phantoms demonstrated that OA/US imaging is capable of not only visualizing shape and dimensions of blood vessels, but also differentiating deoxygenated blood from oxygenated blood.

PREVIOUSLY PERFORMED IN VIVO SENO BREAST STUDIES WITH IMAGIO

FEASIBILITY STUDY

A Feasibility Study was conducted using a total of 155 subjects to determine the ability of Imagio to help identify and define benign and malignant OA features. This study was conducted at CTRC and at South Texas Radiology Imaging Centers (STRIC). The Feasibility Study provided data to further develop the algorithm and to identify separate sets of features that characterize benign and malignant disease. The Feasibility Study included subjects with masses classified as either negative diagnostic ultrasound (NDU) results or positive diagnostic ultrasound (PDU) results. The masses had varying histopathologic diagnoses, sizes, locations, and depths. The Feasibility Study subjects also had varying ages, ethnicities, and races. These subject-specific characteristics are intended to identify any unusual subpopulations. To date, there are no such known exclusions other than subjects with obscured views and subjects on medications with the potential to activate photosensitive dermatologic conditions.

The Feasibility Study demonstrated 3 internal and 3 external OA features that correlate with benign vs. malignant mass histopathology [17-20]. The POM was significantly higher for malignant masses than for benign masses [21]. The specificity was 8% higher for Imagio than for CDU (24% for OA vs 16% for CDU) among those with benign disease, there was >40% relative reduction in the numbers with POM >2% in support of biopsy sparing [22]. There were no reported device related adverse events.

SYSTEM VERIFICATON STUDY

A subsequent System Verification Study was performed, enrolling a total of 44 subjects. The study was conducted at STRIC to evaluate the dual-wavelength performance of the Imagio breast imaging system in detecting suspicious lesions and for final tuning of the algorithm prior to embarking upon the pilot phase of the PIONEER study. The objectives of the System Verification Study were met and further conclusions could be drawn. There were no reported adverse events.

PIONEER STUDY

The Pioneer Study was a prospective multi-center trial with 2105 subjects enrolled across 16 US clinical sites including CTRC. Subjects were prospectively enrolled upon confirmation of being BI-RADS 3, BI-RADS 4, or BI-RADS 5, as confirmed by CDU evaluation of the suspicious mass(es). Images were collected at the clinical sites and sent to an Independent Imaging Core Lab who processed and checked image quality prior to images being processed and read by 7 independent readers.

Of the 1,972 subjects in the Safety Population, the overall mean age 49.4 years; 44.7 years for benign subjects and 58.4 years for malignant (cancer) subjects, consistent with the age distribution at cancer diagnosis. Seventy-two subjects (<4%) were noted to have more than one Study mass.

The study had 1 primary effectiveness endpoint and 5 secondary effectiveness endpoints, all of which were achieved with statistical significance after accounting for multiplicity testing.

There were no serious adverse device effects (SADEs), UADEs or deaths reported during the Study. Ten subjects (0.5%) reported 11 AEs that were considered related to the OA procedure. None were serious, all were mild in severity.

MAESTRO STUDY

Five centers in the Netherlands participated in this prospective, controlled, multi-center, observational and post market surveillance study. Between March 2015 and February 2016, 217 patients with 223 mass lesions were included in this study. Three patients were excluded due to technical failure (not exposed to OA) and 5 were excluded due to the absence of a biopsy (patients' choice not to undergo further investigation). Therefore, 209 patients with 215 lesions were included in the intent-to-diagnose population. The mean age of these patients was 47.8 years (range 19 to 84). Sensitivity was 95.5% versus 100% for CDU. 53.3% of CDU BI-RADS 4b masses were upgraded.

The primary objective of this study was to downgrade benign CDU BR 4a, 4b masses to OA BR 2-3 and to Not downgrade malignant CDU BR 4a, 4b masses to OA BR 2-3. Of the 146 benign lesions, 12.3% (18/146) were upgraded from BI-RADS 4a to 4b, 6.1% (9/146) were upgraded from BI-RADS 4b to 4c and 0.6% (1/146) was upgraded from 4b to 5. Among the 67 malignant lesions, 1.4% (1/67) was upgraded from BI-RADS 4a to 4b, 44.7% (30/67) were upgraded from BI-RADS 4b to 4c and 2.8% (2/67) were upgraded from 4b to 5. From the 215 lesions, 68.5% (146/215) were histologically as benign, 31.4% (67/215) were histologically malignant and 0.1% (2/215) were considered high-risk lesions histologically. Among the 146 histologically benign masses, 81.5% (119/146) were classified as BI-RADS 4a and 18.5% (27/146) were classified as BI-RADS 4b by CDU. Sixty benign lesions were correctly downgraded from BI-RADS 4a or 4b to 3 or 2 ($p < 0.0001$). From the benign lesions classified as BI-RADS 4a, 57 (48%) were downgraded to BI-RADS 3 or 2 according to OA ($p < 0.0001$), and from the 27 benign lesions classified as 4b, 6 (22%) were downgraded to BI-RADS 4a, 3 or 2 by OA ($p = 0.0471$). One adverse event was noted not related to OA which was a hematoma post biopsy.

PREVIOUSLY PERFORMED STUDIES WITH IMAGIO ON OTHER BODY STRUCTURES INCLUDING THE THYROID

PLATFORM STUDY

The Platform Study is an on-going early feasibility study designed to determine the ability of the Imagio OA device to image various body structures, thyroid being one of them. A total of 15 subjects have been enrolled in the study, 13 of which have undergone thyroid OA scans. The 13 subjects were comprised of 3 males and 10 females. The races of the enrolled subjects included 4 African American subjects and 9 Caucasian subjects. The study population included subjects ranging in ages of 22-60. There were 3 mild thyroid scan AEs reported consisting of tingling, pulsing, slight shock sensation.

The purpose of this project is to evaluate the performance of the Imagio device in distinguishing malignant from benign thyroid nodules using the Imagio ultrasound (IUS) and opto-acoustic (OA) component of the device and to refine the device algorithm specific to thyroid nodule diagnosis and to develop guidelines for interpretation of thyroid OA studies. The results will be used to further develop the pivotal study objectives and endpoints.

2. STUDY OBJECTIVES

2.1. Primary Objective

The primary objective of this feasibility study is to provide an initial assessment of Imagio OA/US's ability to distinguish between benign and malignant thyroid nodules, and when appropriate, between benign and metastatic cervical lymph nodes in subjects.

2.2. Secondary Objectives

There are two secondary objectives.

- Complete tasks related to the development of the thyroid device algorithm, interpretation rules and estimators that will be used to determine clinical predictions.

- Obtain initial estimates of predictive value based on optimized clinical prediction rules including incremental improvement in specificity, percentage with correct downgrade in Thyroid Imaging and Reporting System (TI-RADS) Scores, percentage with correct upgrade in TI-RADS category, and negative likelihood ratio (NLR).

3. STUDY PLAN

3.1. Study Design

This is a prospective feasibility study

3.2. Overall Study Design and Plan: Description

This is a feasibility study to assess Imagio OA/US's ability to optimize the device algorithm specific to thyroid in order to detect the difference between benign and malignant thyroid nodules, and when appropriate, suspicious associated cervical lymph nodes. Imagio evaluation will be performed according to the system's instructions for use. The study will initially consent subjects with at least one nodule classified as suspicious enough to warrant recommendation for biopsy according to CDU. In subjects with nodules very suspicious for malignancy in which there is a suspicion for metastatic lymph node disease that have also been recommended for biopsy, OA/US imaging will also be performed on the suspicious lymph node.
All selected subjects will undergo an Imagio evaluation following consent and before any immediate fine needles aspiration biopsy (FNAB), core biopsy, or surgical excision. The decision whether to recommend and perform ultrasound guided FNAB or core needle biopsy will be based upon standard of care conventional diagnostic thyroid ultrasound findings. Imagio IUS or OA findings will not be used as the reason to perform or to defer a biopsy or surgery.

A total of approximately 110 nodules classified as TI-RADS 1-5 scheduled for biopsy will prospectively screened (subject signed inform consent) to get 100 evaluable nodules (acceptable OA images (via Seno Medical Director and/or Independent Core Lab (ICL) quality assurance process) collected with acceptable cytology/histology final diagnosis data. It is expected that 10% of nodules will be enrollment failure leading to target evaluable analysis set of 100 nodules.
Enrollment failures in the study would consist of subjects who did not have acceptable OA images collected and/or valid cytopathology cell counts or histopathology considered adequate for diagnosis. (Cytopathology insufficiency rates vary from about 1.5% - 20%). Thyroid nodules that have undergone FNAB, but have insufficient cellularity for cytopathologic assessment, cannot be used unless repeat FNAB is performed and shows adequate cellularity for diagnosis).

The study will include a minimum number of thyroid nodules (TI-RADS 1-5) with adequate cytopathology or histopathology for diagnosis that include at least: 10 papillary thyroid carcinomas minimum, 10 Follicular neoplasms, and 30 benign nodules that include at least 5 Hashimoto (chronic lymphocytic thyroiditis) nodules.

A significant percentage of FNABs result in insufficient cellularity for diagnosis. If the initial FNAB is deemed insufficient, either repeat FNAB or core biopsy that is sufficient for diagnosis will be required for the subject and thyroid nodule to be included in the study. An initial FNAB that is insufficient and is not repeated cannot be included in the study and will need to be replaced, unless the patient undergoes surgical removal of the nodule from which adequate histopathology is obtained. If both initial FNAB and repeat FNAB or core biopsy are insufficient, the subject and the thyroid nodule cannot be included in the study and the subject and nodule will

need to be replaced unless the patient undergoes surgical removal of the nodule that results in adequate histopathology for diagnosis.

The study will be explained to the subject after the biopsy decision has been made based upon conventional diagnostic ultrasound (CDU) and then will be prospectively evaluated with Imagio OA/US. The PI or Sub-PI from each site will evaluate all cases in an unblinded "real world" manner. The OA study will be performed after CDU with full knowledge of other imaging findings as available (CT, MRI, PET, CT/PET, carotid ultrasound), clinical history, and personal risk history. All Imagio studies will be recorded during prescribed probe sweeps through the nodule and surrounding tissues.

Additionally, the estimator generated in a previous study will be potentially developed to assist with scoring various features associated with the thyroid mass. Pathology data will be collected to assess histologic features of the nodule. The ultimate diagnosis depends on the cytopathologic examination of the FNAB thyroid nodule specimen by a cytopathologist or from a histopathologist in the case of a core needle or surgical excisional biopsy.

When available, genetic biomarkers that may help distinguish more aggressive thyroid malignancies that require more aggressive treatment from less aggressive malignant nodules that might warrant less aggressive treatment or even merely observation on long term follow-up should be made available. These will usually come from surgical procedures such as lobectomy or complete thyroidectomy with or without central lymph node dissection. Such biomarkers might include: presence or absence of BRAF and/or RET mutations in papillary carcinoma, RAS and/or PPARG mutations in medullary carcinoma, and TP53 and/or P13K/AKT1 mutations in anaplastic carcinoma. These biomarkers may be critical, because the diagnosis of malignant versus benign might not be as important as the distinction between aggressive or undifferentiated thyroid malignant nodules that require aggressive treatment and less aggressive differentiated malignant nodules that require less aggressive treatment or merely observation on long term follow-up. Studies have shown that up to 60% of papillary thyroid carcinomas do not change over 6 years on imaging follow-up and might merely warrant watchful waiting on ultrasound rather than surgery (23).

Study Duration

In order for a subject to be enrolled in the study, he/she must have the following:

- A Screening/Enrollment/Imaging Visit (Day 1)
- Biopsy Visit (up to 45 days from Screening/Enrollment/Imaging Visit)
- Second Biopsy Visit/ surgery visit (up to 45 days from First Biopsy Visit), if needed
- Final Surgery (up to 45 days from Second Biopsy) as applicable

The minimum duration of study participation for a patient is 48 hours: if the Screening Visit, Enrollment Visit, Imaging Visit, Biopsy Visit all occur in sequential order within 48 hours. In this scenario no further biopsy of thyroid or surgery is required.

The maximum duration of study participation for a patient is 135 days

The enrollment period will extend approximately 12-24 months.

Imaging Data Collected-

The Imagio field of view (both OA/US and IUS) will include at least longitudinal and transverse views of the nodule at a minimum. Views will be obtained with and without measurement calipers, as the presence of calipers may interfere with surface characteristics evaluation from standard diagnostic and Imagio gray scale images. The views obtained during the OA/US scan and the labeling of the OA views must precisely replicate the views and annotation used for IUS, for comparative purposes. In some cases, additional views may be necessary. The scan protocols will be those recommended in ACR or AIUM Accreditation Committees for thyroid ultrasound. The CDU classification system will be based upon the new ACR TI-RADS committee recommendations (26).

When more than one nodule is scanned and recommended for FNAB, the investigator should annotate the images to indicate which nodule is being designated nodule 1 and nodule 2. This will facilitate Histopathologist/Pathologist and potential Independent Readers interpreting the correct nodule. When a thyroid and lymph node (s) are both imaged with OA, the lymph node(s) should be assigned a number preceded by the letter T or L, respectively, (Ex. L-001).

For both the IUS and OA/US scans video sweeps will be obtained in addition to still images. Scan protocols are located in Appendix D.

SCANNING LYMPH NODES: Data from Imagio evaluation of lymph nodes will also be collected for research purposes. When the investigator deems it necessary to scan the lymph nodes, he/she will perform Imagio imaging of any suspicious lymph node for which FNAB or surgical excision is recommended. Scan protocols are located in Appendix D.

Additional data obtained that is required, if performed and available, includes:

- CDU in all cases;
- Images of ultrasound-guided FNAB or core biopsy in all cases;
- Carotid Doppler examination if this is how the nodule was first detected
- CT, MRI, or PET/CT, PET if this is how the nodule was first detected

All images used for this study will be anonymized and deemed compliant. Subjects in this study will be assigned identifying subject numbers and their names and medical facility record numbers will be redacted from all accumulated images. The images will identify the subject only by subject initials, DOB and subject numbers. The subject number will be in the format of ###-###-###, with the first 3 digits being the country code, the second 3 digits representing the site code, and the third three digits representing the subject code. The database that links the subject number with the subject names, medical facility record numbers, and birth dates will be maintained by the PI and sub-investigator and access to this database will be limited to the investigators and Seno Medical Instruments, Inc. and designees.

4. STUDY POPULATION

4.1. Number of Subjects

A total of approximately 110 subjects with 110 nodules classified as TI-RADS 1-5 scheduled for biopsy will prospectively screened (subject signed inform consent) to get 100 evaluable nodules (acceptable OA images (via

ICL QA process) collected with acceptable cytology/histology final diagnosis data). It is expected that 10% of nodules will be enrollment failure leading to target evaluable analysis set of 100 nodules.

Enrollment failures in the study would consist of subjects who did not have acceptable OA images collected and/or valid cytopathology cell counts or histopathology considered adequate for diagnosis. (Cytopathology insufficiency rates vary from about 1.5% - 20%. Thyroid nodules that have undergone FNA, but have insufficient cellularity for cytopathologic assessment, cannot be used unless repeat FNA is performed and shows adequate cellularity for diagnosis).

4.2. Inclusion/Exclusion Criteria

Inclusion Criteria: Subjects must meet all of the following criteria to be included in the study:

1. Have been informed of the nature of the study and provided written informed consent, prior to initiation of any study activities;
2. Have an undiagnosed suspicious solid or mostly solid thyroid nodule.;
3. 18 years of age or older at the time of consent;
4. Are willing and able to complete all procedures and assessments in accordance with the clinical protocol; and,
5. Have received recommendation for and are scheduled for an ultrasound guided FNAB, ultrasound guided core biopsy, excisional biopsy, lobectomy or complete thyroidectomy of at least one thyroid nodule.

Exclusion Criteria: Subjects who meet any of the following criteria will be excluded from the study:

1. Are prisoners;
2. Have a condition or impediment (i.e., insect bites, poison ivy, open sores, chafing of the skin, scar, tattoos, moles, etc.); that could interfere with the intended field of view (within one probe length or 4 cm of the nodule),
3. Previous or on-going radioactive iodine treatment.
4. Nodule to be biopsied is greater than 3.0 cm in maximum diameter;
5. Is pregnant;
6. Have an acute or a chronic hematoma and/or acute ecchymosis of the thyroid;
7. Is experiencing photo-toxicity or photo-sensitivity or is undergoing treatment for a photo-sensitive condition such as porphyria or lupus erythematosus;
8. Patient has received chemotherapy for any type of cancer within 90 days from date of screening CDU;
9. Have had previous image guided FNAB or surgical biopsy of the target nodule of interest within the 45 days of baseline Imagio Scan;
10. Patient has participated in a clinical study of an investigational drug or device within 3 months prior to screening CDU that may have an impact on clinical outcomes; and,
11. Patient has previously participated in this study.

5. DESCRIPTION OF EXPERIMENTAL DESIGN AND METHODS

5.1. Study Device

The study will use Imagio to generate OA/US images. Imagio is an investigational device in the US.

Operator Credentials and Training

Experienced clinicians (or registered sonographers) will conduct all exams. Only radiologists or registered sonographers who have received approved training from Seno Medical Instruments, Inc. may perform the examinations. Clinician experience guidelines are included in the Imagio User Manual. The sponsor or a sponsor's representative may be present during the procedure to provide technical assistance, if required.

5.2. Cytopathology and/or Histopathology Review

All biopsied nodules will be analyzed by a designated Cytopathologist and/or Histopathologist. This may include the initial biopsy and/or any second core/surgical biopsy.

5.3. Comparators and Gold Standard

Imagio OA/US will be assessed as a diagnostic test in the classification of thyroid nodules.

The CDU will serve as the "control" for comparative purposes. The ACR-TI-RADS committee classification system will be used to assess the thyroid nodule and the decision to biopsy with no single diagnostic methodology used to make this decision. CDU represents the subject-specific diagnostic tests used to make the thyroid nodule classification and the decision to biopsy. Imagio OA may not be used to determine final thyroid nodule classification or the decision to biopsy. If available, any data for and/or carotid duplex sonography, CT, MRI, or PET/CT, PET will also be used for comparative purposes.

The histology obtained from FNAB, core biopsy, or surgical biopsy (at the nodule level) will serve as the gold standard.

5.4. Blinding

Subjects will not be blinded to best practice study procedures but will not have access to the Imagio OA evaluation result.

The site investigators will have access to all subject data including the Imagio images and videos, and will assess and document the results of the OA study by completing the electronic data entry form (eCRF).

The site investigators will have access to the background clinical information and to the subsequent histopathology report.

5.5. Treatment Compliance

The Imagio unit will be operated only by study personnel trained by Seno personnel or their representatives.

5.6. Packaging, Labeling, and Instructions for Use

Seno will ship Imagio devices to the participating site. Seno personnel will unpack and install the study device as well as conduct and document operator training for study personnel.

5.7. Storage and Accountability

Investigators will be responsible for ensuring that only authorized study personnel have access to the study device for study purposes in accordance with FDA guidelines.

6. RISKS AND BENEFITS OF THE INVESTIGATIONAL DEVICE AND CLINICAL INVESTIGATION

6.1. Anticipated Clinical Benefits

The Imagio thyroid imaging system was designed to provide quick and precise evaluation of suspicious nodules (including both palpable and non-palpable nodules) or imaging findings when the thyroid nodule and when appropriate, suspicious cervical lymph node was first detected as an incidental finding on CDU, CT or MRI of the chest or neck, carotid duplex sonography, or on PET/CT.

If proven valuable, the Imagio may contribute to a reduction in the number of biopsy procedures to be performed. Consequently, fewer patients will suffer from physical and emotional stress around the biopsy procedure and waiting period to get the result from the pathologist.

In this study, the results of the Imagio scans do not determine whether or not biopsies need to be performed. The study participants will therefore not experience direct benefit. We hope that this study will contribute in the future to a more accurate diagnosis of thyroid nodules.

6.2. Anticipated Adverse Device Effects

List of potential risks and discomforts:

- Tingling or warmth of the skin during the Imagio scan (0.4% or a 1 in 269 chance) that resolve at completion of the scan
- Exposure to the laser of the Imagio (to both skin and eyes)
- Exposure to the acoustic output
- Phototoxicity when applying fragrance or lotion to the skin prior to scanning

.

6.3. Risk/Benefit Rationale

Seno Medical Instruments, Inc. believes that any potential risk presented by this investigation has been minimized and that adequate testing, safeguards, and safety monitoring have been incorporated into the investigation to further minimize and mitigate the risks.

The laser energy will be set to a level that is known as being below known harmful levels, which is unlikely to harm the thyroid or give any discomfort.

Also, everyone in the examination room will be wearing protective eyewear in order to minimize risk of damage by laser light to the eyes

Seno Medical Instruments, Inc. firmly believes that the value of the knowledge to be gained by conducting this clinical investigation to demonstrate the clinical value of the Imagio™ Thyroid Imaging System outweighs the potential risks posed to participating subjects.

7. STUDY PROCEDURES

7.1. Study Procedure Overview

The study procedures and the sequence in which they will occur are defined below in Table 1 below and described in greater detail in Section 7.2:

Table 1: SCHEDULE OF EVENTS

Evaluation	Screening/ Enrollment/Imaging Visit[1]	1st Biopsy Visit (≤45 days after Imaging Visit)	2nd Biopsy Visit (≤45 days after 1st Biopsy Visit; as applicable)	Additional surgical procedure (≤45 days after 2nd biopsy as applicable)
Informed Consent	X			
Medical History	X			
I/E Criteria	X			
TI-RADS Score	X			
CDU thyroid images (Doppler as applicable) [2]	X			
Other images/studies PET, CT, MRI, PET/CT, and Carotid Doppler[2].	X			
Biopsy Decision	X			
Directed Thyroid History	X			
Imagio OA Evaluation of thyroid and lymph nodes (as applicable)	X			
Ultrasound Guided FNA and/or core biopsy images of thyroid and lymph nodes (as applicable)		X	X	
Images uploaded to Imaging Core Lab	X			
Adverse Event Evaluation	X			
FNA or Core Biopsy Procedure (s)		X	X	
Surgical Biopsy/Treatment (as applicable)		X	X	X
Histopathology/Cytology Samples Collected including biomarkers (as applicable)		X	X	X
End of Study		X[3]	X[3]	X[4,5]

[1]The Screening/Enrollment/ Imaging, and Biopsy Visits may take place on the same date as the discretion of the investigator.

[2] CDU with or without Doppler (as available - CT, MRI, PET/CT, and carotid ultrasound, strain elastography and shear-wave elastography) within 365 days of baseline Imagio Scan

[3]For benign thyroid nodules, where cytopathology or histopathology is concordant with imaging findings, FNAB or core needle biopsy is the final procedure and End of Study.

[4]If biopsy reveals malignant cytology or histology, further surgical treatment will likely be the final procedure and End of Study. Data associated with additional surgical procedures will only be collected up until the time that the confirmatory biopsy/surgical procedure takes place.

[5]If a central lymph node dissection and/or lateral cervical lymph node dissection was performed during or after the thyroid procedure, surgical and histology data associated with those lymph node procedures will be collected.

7.2. Study Visits and Procedures

7.2.1. Screening/Enrollment/Imaging Visit- Day 1

The following procedures will be conducted at the Screening/Enrollment Visit:

- Perform informed consent;
- Review inclusion /exclusion criteria;
- verify from images and/or reports that a suspicious nodule was seen;
- Confirm that CDU has been performed, for which the site radiologist has recommended either ultrasound-guided FNA and/or US-guided core needle biopsy, and will fall within 365 days of Imagio OA procedure.
- Record demographics and directed thyroid history; and,
- TI-RADS CDU-based classification
- Imagio Imaging

Upon enrollment, subjects receive a subject number that will be used on all documentation for the subject throughout the study. Subject numbers should be assigned in ascending order, and numbers should not be omitted or reused. The subject number is coupled with the site identification number for unique identification of each subject.

Subjects will undergo the Imagio OA procedure. Subjects may be asked to return to the clinic to repeat the Imagio scan if (a) the image quality is not acceptable for review by A. Thomas Stavros, M.D. Seno's Medical Director and/or the ICLs quality assurance reviewers (QARs) and (b) they have not yet undergone a biopsy procedure. Any adverse device effects or unanticipated adverse device effects that occur during this visit will be reported to appropriate authorities such as the IRB and FDA. In order to provide technical support, representatives from Seno Medical Instruments, Inc. or their designees may be present during the procedure.

7.2.2. Biopsy Procedure

Patients will be scheduled for initial biopsy within 45 days of the Imagio OA/US procedure. Images of FNAB or Core Biopsy needle within the lesion are collected. If an FNAB is performed and results in insufficient cellularity for cytologic diagnosis and a repeat FNAB or core biopsy is required, this should be scheduled within 45 days after the first biopsy. The investigator is to ensure that the applicable site histopathology/cytology report is obtained. If a surgical procedure is applicable, it would be scheduled within 45 days after the second biopsy. Surgical and histology data will be collected.

7.3. Effectiveness Assessments

Primary Effectiveness Endpoint:

The primary metric for demonstration of feasibility is the incremental improvement in specificity. An improvement of approximately 5% would be indicative of a successful feasibility study.

Secondary Effectiveness Endpoints:

The percentage of correct downgrades is the next most important metric. Other metrics including sensitivity and negative likelihood ratios will be considered as supporting evidence.

7.4. Subject Withdrawal Criteria

Subjects may be discontinued from the study for the following reasons if deemed appropriate by the Sponsor and /or Investigator:

- Subject enters the study in violation of the protocol;
- Subject deviates from the protocol during the study;
- Pregnancy;
- Withdrawal of consent;
- If in the opinion of the investigator it is not in the subject's best interest to continue (reason for withdrawal must be specified); and,
- Termination of the study by the sponsor.

Protocol Deviations will be reviewed by the Sponsor on a routine basis and any deviation that results in the exclusion of the subject from the statistical analyses will be documented in the final clinical study report.

7.5. Enrollment Failures

Subjects deemed enrollment failures according to the quality assurance review or failure to complete the biopsy evaluations) will be replaced during the active enrollment period of the study. The following subjects will be considered enrollment failures and will be followed until all AEs are resolved:

- Subjects found to be ineligible after the scan is conducted;
- Subjects for whom the study device scans do not pass review (Seno Medical Director and/or Imaging Core Lab QAR review); and,
- Subjects who have incomplete or unusable biopsy results.

8. ADVERSE EVENTS, ADVERSE DEVICE EFFECTS, UNANTICIPATED ADVERSE DEVICE EFFECTS AND DEVICE DEFICIENCIES

For the purposes of this study, only Imagio device-related adverse events (AE), unanticipated adverse device effects, procedure-related AEs and device deficiencies will be collected. AE and device deficiency collection will commence once the subject has provided written informed consent and the patient has entered OA Imagio scanning room.

8.1. Definitions

8.1.1. Adverse Event and Adverse Device Effects

Adverse Event: any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device.

NOTE 1: This definition includes events related to the investigational medical device.
NOTE 2: This definition includes events related to the procedures involved.
NOTE 3: For users or other persons, this definition is restricted to events related to investigational medical devices.

Adverse Device Effect: adverse event related to the use of an investigational medical device.

NOTE 1: This definition includes adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device.
NOTE 2: This definition includes any event resulting from use error or from intentional misuse of the investigational medical device.

Unanticipated Adverse Device Effect: adverse event related to the use of an investigational medical device.

NOTE 1: This definition includes adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, installation, or operation, or any malfunction of the investigational medical device.
NOTE 2: This definition may include any event resulting from use error or from intentional misuse of the investigational medical device.

8.1.2. Device Deficiency

Device Deficiency: inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance.

NOTE: Device deficiencies include malfunctions, use errors, and inadequate labelling.

8.1.3. Serious Adverse Event and Serious Adverse Device Effects

Serious adverse event (SAE): an adverse event that

1. Led to death,
2. Led to serious deterioration in the health of the subject, that either resulted in
    a. a life-threatening illness or injury, or
    b. a permanent impairment of a body structure or a body function, or
    c. in-patient or prolonged hospitalization (>24 hours), or
    d. medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
3. Led to fetal distress, fetal death or a congenital abnormality or birth defect.

Note: Preplanned events of hospitalization or surgical interventions will not be recorded as an SAE.

Serious adverse device effect (SADE): adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

8.1.4. Unanticipated (Serious) Adverse Device Effect (U(S)ADE)

Anticipated serious adverse device effect (ASADE): is an effect which by its nature, incidence, severity or outcome has been identified in the risk analysis report.

Unanticipated serious adverse device effect (USADE): serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report and section 6.2.

8.2. Device relatedness

The potential relationship of the event to the investigational device or procedure will be judged by the investigator. The relation will be based on the following definitions:

- Related - Definite causal relationship
- Probable - Good reasons and sufficient documentation to assume a causal relationship
- Possible - A causal relationship is conceivable and cannot be dismissed
- Unlikely - The event is most likely related to an etiology other than the investigational product administration
- Unrelated - Definitely no causal relationship

Events classified as related, probable and possible are considered related and need to be reported. Events classified as unlikely and unrelated are not considered related and do not need to be reported. See section 8.4 for more details.

8.3. Severity

The assessment of severity is a clinical determination of the intensity of an adverse event. The severity assessment for a clinical adverse event should be completed using the following definitions as guidelines:

- Mild – Transient symptoms, no interference with the patient's daily activities
- Moderate – Marked symptoms, moderate interference with the patient's daily activities
- Severe- Considerable interference with the patient's daily activities, which is unacceptable

8.4. Reporting

8.4.1. Reporting Requirements

For the purposes of study, only device-related events ((S)(U)ADEs), procedure-related events and device deficiencies will be reported. (S)ADE, procedure-related events and device deficiency collection will commence once the subject has provided written informed consent and the patient has entered OA Imagio scanning room.

8.4.2. Reporting to Sponsor

All adverse events classified as serious adverse device effects or (SADEs) as well as serious unanticipated adverse device effects (SUADE) and possibly-, probably- or definitely-related to the device need to be reported to Seno within 24 hours of learning of the event, using the EDC system.

8.4.3. Reporting to Regulatory Authorities

Events will be notified to the IRB per IRB guidelines and to FDA in accordance with FDA Guidelines.

8.5. Emergency Contact Details

In the event discussion is necessary regarding the safety or treatment of a subject, investigators can call A. Thomas Stavros, MD at (office) 210-615-6501, ext. 142 or (mobile) 707-849-6548.

9. IMAGING CORE LAB

To ensure consistency, the assessment of all imaging studies including Imagio will be managed by an independent Imaging Core Lab (ICL) to perform quality assurance review of collected images.
The Imaging Core Lab participating in this study will be:

Medical Metrics
2121 Sage Rd. Suite 300
Houston, TX 77056
Phone 713-850-7500

10. MONITORING

Monitoring of the study will be performed in accordance with FDA guidelines. Monitoring including source data verification should be routinely performed prior to the transfer of the data to Data Management.

Site monitoring visits will be performed by the Seno or Seno's representatives on a regular basis in accordance with the Monitoring Plan. During monitoring visits, data entered in the eCRF will be source verified for accuracy and completeness. In addition, protocol compliance and compliance with FDA regulations will be verified. The monitor should have direct access to source data for verification purposes. The eCRF data will be compared with data in the subjects' medical records. Such verification is essential to quality control.

The Sponsor expects that, during monitoring visits, the study coordinator and Principal Investigator will be available, the source documentation will be available and that a suitable environment will be provided for review of study documents.

11. AUDITS AND INSPECTIONS

Independent auditors from the Seno or Seno's representative may request access to all study records, including source documents for inspection, audit and/or copying in keeping with applicable regulations. The Principal Investigator should immediately notify the sponsor of an upcoming regulatory authority inspection.

12. STATISTICAL METHODS

12.1. Study Hypotheses

The overall hypothesis motivating this pilot feasibility study is that Imagio OA can provide incremental improvements in the clinical classification of thyroid nodules into benign and not benign and into finder subclasses. To evaluate this hypothesis, a heterogeneous sample of at least 50 patients will evaluated and steps will be taken to optimize software parameter and clinical guidelines. Initial evaluation of reliability and validity will be obtained by having three independent readers make predictions first with conventional gold standard methods and then augmented with Imagio OA. These results will be summarized using conventional measures of diagnostic utility, with emphasis on incremental improvement in specificity, the percentage of correct down grades, the percentage of correct upgrades, and the negative likelihood ratio. When appropriate, statistical estimates will be reported along with 95% confidence intervals.

12.2. Analysis Populations

All analyses will be performed with the intention to diagnose (ITD) study population which consists of all subjects coming in for their scheduled Imaging Visit, having Imagio OA data declared evaluable, and having a biopsy of at least one nodule with evaluable cytology/histology results.

12.3. Demographics and Baseline Characteristics

The age, race, ethnicity, will be presented using descriptive statistics for the overall ITD population, by sites diagnostic status (benign, malignant).

12.4. Sample Size Discussion

Based on experience with feasibility studies performed for evaluation of breast nodules, the sample size for this feasibility study is driven by software and clinical rule development needs. From this perspective, it was concluded that the reference set should include a minimum of 50 thyroid nodules with adequate cytopathology or histopathology for diagnosis that include: 10 papillary thyroid carcinomas minimum, 10 Follicular neoplasms, and 30 benign nodules that include at least 5 Hashimoto nodules. Therefore, a total of 50 thyroid nodules will form the initial software development reference set. Based on the rate of non-benign nodules, it is expected that about twice the number of patients needed will have to be evaluated to obtain the minimum sample sizes noted above. Therefore, the target evaluable sample size is N=100. The 'extra' benign nodules enrolled are important because they will increase the statistical precision of key measures of predictive value including the increment improvement in specificity. It is estimated that roughly 10% of enrolled patients may have thyroid nodules for which FNAB cytopathology is inadequate (after initial and potential subsequent FNA) and in whom surgical excision is not performed cannot be included in the study and will be replaced. To account for this N=110 nodules will be enrolled. As discussed below, this study will rely on the TI-RAD risk categorization (categories 1-5). After 50 nodules are identified as evaluable, the clinical status will be summarized, and if it is projected that there will be too few non-benign nodules, subsequent enrollment will be restricted to TI-RAD categories 3, 4, and 5.

12.5. Analyses

The following outlines the steps to be taken to develop and tune the image creation and colorization algorithm and to develop the guidelines and interpretations rules that will be subsequently applied by three independent readers. The steps will be performed collaboratively by the software engineer and Medical Director, with potential input at earlier stages from the site radiologists.

DEVELOPMENT OF GUIDELINES, INTERPRETATION RULES AND ESTIMATORS

1. Images and data will provide a basis for algorithm finalization, potential instrument improvements, the development of guidelines for interpretation of OA studies of thyroid nodules. Permit Imagio OA optimizations with respect to specific parameters (e.g. illumination and detection conditions, laser pulse repetition rate, sound propagation characteristics, etc.). Optimizations may be different for thyroid nodules and for suspicious cervical lymph nodes.

2. Compare in vivo opto-acoustic images with those obtained from standard diagnostic thyroid ultrasound, color Doppler imaging (CDI), power Doppler imaging (PDI), strain elastography (SE), or shear wave elastography (SWE).

3. Compare the in vivo opto-acoustic features with those obtained from standard diagnostic thyroid ultrasound color Doppler imaging (CDI), power Doppler imaging (PDI), strain elastography (SE), or shear wave elastography (SWE).

4. Compare findings of conventional imaging modalities and those of opto-acoustic imaging with biopsy results for nodule size, location, type, and stage.

5. Develop Imagio-based estimators for thyroid use.

Once the above steps are completed, quantitative analyses will be performed similar those performed during previous pilot studies of the Imagio OA in breast imaging. The predictive and clinical classification results based on the optimal results determined from the process above will be summarized, as will the results based on application of the preliminary guidelines by three independent raters. The primary summary of results will include five by five cross-tabulation of initial TI-RAD category by TI-RAD category based Imagio OA stratified by clinical status (e.g., benign vs not benign and papillary thyroid carcinomas vs Follicular neoplasms, and benign nodules. These tables will be used to determine the percentages of correct downgrades (among benign nodules) and correct upgrades (among non-benign nodules). Specificity will be determined as the percentage of benign nodules with a final TI-RAD score of 1 or 2 since these risk categories generally would not result in further invasive evaluation unless patient requested. The improvement in specificity comparing initial TI-RAD to TI-RAD after Imagio OA is considered a primary endpoint in this study. Sensitivity will be similarly evaluated. Where appropriate, 95% confidence intervals will be determined to evaluate statistical precision of the estimates. The results will also be expressed in terms of negative likelihood ratios (NLR) at specific TI-RAD categories. The NLR is the false-negative fraction divided by the true negative fraction at each level of TI-RAD. For dichotomous tests, this is (1-sensitivity) divided by specificity. NLRs as well as positive likelihood ratios represent an effective way to summarize the predictive capacity of diagnostic tests. Pre-test probabilities are converted to pre-test odds (Pre-test Odds = Probability/1-Probability). Post-test Odds for a negative test = Pre-test Odds times NLR. Post-test probability = Post-test Odds / (1 + Post-test Odds). Analyses will be performed for each independent rater and for a consensus rule based on pooling predictions across raters. Weighted Kappa statistics and measures of overall agreement will be used to summarize prediction reliability across rates.

12.5.1. Acceptability Metrics

The primary metric for demonstration of feasibility is the incremental improvement in specificity. An improvement of approximately 5% would be indicative of a successful feasibility study. The percentage of correct downgrades is the next most important metric. Other metrics including sensitivity and NLR will be considered supported evidence.

13. ADMINISTRATIVE CONSIDERATIONS

13.1. Investigators and Study Administrative Structure

Each site will have a designated investigator and one or more study coordinators collectively responsible for the conduct of the study inclusive of referral, screening, enrollment, evaluation, documentation, and subsequent biopsy in accordance with FDA guidelines.

13.2. IRB Committee

The protocol and the informed consent form, and any other institution-specific documents, must have the approval of a properly constituted IRB Committee responsible for approving clinical studies. The signed IRB

Committee approval letter must specify the date of protocol and informed consent form approval and identify the documents approved including the investigator's name, the protocol version, date and title. Any subject materials or advertisements used to recruit subjects should also be reviewed and approved by the IRB Committee.

13.3. Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles outlined within FDA and GCP regulations as well as local IRB requirements.

13.4. Informed Consent

A signed Informed Consent Form (ICF) must be obtained from the subject prior to performing any study-related procedures. The investigator or investigator's designee will provide background information on the study, including the benefits and risks of all study-related procedures to the subject. The investigator or investigator's designee will also encourage the prospective subject to ask questions about the study and will provide the subject with sufficient opportunity to consider whether or not to participate. After written consent has been documented, previously obtained clinical radiographic data can be used for the study.

Original signed informed consent forms must be filed in the subject records at the site. A copy of the signed consent/assent form should also be provided to the subject.

The ICF for this study will be prepared by the study investigator for conformance with the government and institution's requirements for this class of diagnostic imaging. However, all changes requested by an investigator or an Ethics Committee, even those that may not be considered substantial and/or do not affect the rights, safety or welfare of a subject, must be approved by Seno. If Seno determines that the revisions are substantial and/or affect the rights, safety or welfare of a subject, the ICF must be reviewed and approved by both Seno and the Institutional Review Board before the ICF can be utilized.

13.5. Subject Confidentiality

Confidentiality will be maintained in accordance with FDA and IRB regulations. Subject names must not be revealed to the sponsor or sponsor's representatives. Only the subject identifier (number) will be recorded in the eCRF and if the subject's name appears on any other document, it must be redacted and replaced with the subject identifier before a copy of the document is supplied to the sponsor or sponsor's representatives. Study findings stored on a computer will be stored in accordance with local data protection laws. In the event of inadvertent communication of such information, immediate steps to redact the information from all study files will be implemented, with appropriate documentation in the subject study file.

13.6. Case Report Forms and Study Records

Data will be recorded using an electronic data capture (EDC) system, according to FDA regulations. The database is considered validated when the expected results are the same as the actual results, and the end users verify that the database performs according to the requirements.

The data will include collecting images and videos plus the Laser Optical Movie (LOM) to be sent to the Seno and/or the Central Imaging Core Lab as applicable.

The validation report, design procedures, testing results, source code, and test eCRF cases are filed in the central files. Database maintenance will be provided throughout the study, as well as user support and administration (access and site user rights set-up, removal, etc.)

The MedDRA dictionary will be used to code adverse device effects.

The investigator is responsible for maintaining adequate and accurate medical records from which information will be transferred into the study database.

13.7. Clinical Quality Assurance

The sponsor, or the sponsor's representative, may conduct audits at the investigational sites. Audits may include, but are not limited to, device supply, presence of required documents, the informed consent process, and comparison of eCRFs with source documents (original medical records, images). The investigator agrees to participate with audits conducted at a reasonable time, in a reasonable manner.

13.8. Protocol Amendments

Any amendment to the study protocol must be approved by the sponsor. A protocol amendment may not be implemented until after it has been approved by the IRB, unless immediate implementation of the change is necessary for subject safety. In this case, the protocol change must be documented in an amendment.

Once a protocol amendment has received approval from the sponsor, it will be submitted to the IRB Committee for written approval.

13.9. Protocol Deviations

Protocol deviations may occur in two ways.

1. Deviations from the protocol, contrary to protocol specifications (i.e., deviations from the protocol procedures, eligibility, instructions for use, visit windows, etc.).
2. Deviations affecting the endpoint outcome not previously specified in the protocol (i.e., deviation that had not been previously considered in the protocol or eligibility criteria, but having a clear impact on the primary outcome measure)

The investigator is not allowed to deviate from this protocol except as specified in FDA regulations. The Investigator should not implement any deviation from or changes to the protocol without approval by Seno and prior review and documented approval/favorable opinion from the IRB of a protocol amendment, except where necessary to eliminate immediate hazards to study subjects, or when the changes involve only logistical or administrative aspects of the study (e.g., change in monitors, change of telephone numbers). While every effort should be made to avoid protocol deviations, should a deviation be discovered, Seno must be informed immediately. Any protocol deviation impacting subject safety must be reported to A. Thomas Stavros, M.D. Seno's Medical Director immediately.

Deviations will be documented in the eCRF. Investigators will also adhere to procedures for reporting investigation deviations to their ethics committee in accordance with their specific reporting policies and procedures.

Prior to final database lock, all protocol deviations will be reviewed and categorized as major (i.e., those that affect measurement or interpretation of the primary endpoint or related to safety) or minor (those not affecting

the primary endpoint). The presence of a major deviation will exclude subjects from the Intention to Diagnose (ITD) analysis population.

13.9.1. Corrective and Preventive Actions

Seno Medical and her representatives will evaluate protocol deviations during monitoring visits. Individual event corrective and preventive actions may be recommended at that time. In addition, deviations occurring across investigational sites will be reviewed by Seno Medical on a periodic basis to determine if more global preventive actions may be required.

13.9.2. Investigator Disqualification Criteria

Seno Medical reserves the right to terminate an investigator/investigational site for any of the following reasons:

- Failure to secure subject informed consent including protection of personal data prior to enrollment.
- Failure to report unanticipated adverse device effects within 24 hours of discovery (to Seno Medical) and to the ethics committee within its required reporting time after learning of the event.
- Failure to report serious adverse device effects within 24 hours of discovery.
- Repeated investigational plan deviations.
- Repeated failure to appropriately complete case report forms.
- Failure to enroll an adequate number of subjects.
- Loss of or unaccounted for investigational product inventory.

13.10. Retention of Data

All records, including compact disks of images, must be retained at each clinical site and by Seno or Seno's representatives for a period of two years after the latter of the following two dates: the date the study is closed or terminated or the date of the last approval of a marketing application and records are no longer needed to support a regulatory submission. All study records may be relevant to regulatory inspection.

In the event that the Principal Investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility and the sponsor must be notified.

Seno or Seno's representatives should be contacted in advance if there is a desire to discard or relocate any records or if there are any questions regarding record retention.

Seno or Seno's representatives will maintain all records related to this study, according to FDA and IRB regulations.

Required regulatory reports will be submitted per FDA guidelines.

13.11. Statement of Compliance

This clinical investigation will be conducted in compliance with the principles that have their origin in the Declaration of Helsinki, this clinical investigation plan, requirements of the approving ethics committee, FDA and GCP Regulations and other applicable regulatory requirements.

This clinical investigation will not be initiated until approval has been obtained from the IRB committee. Any additional requirements imposed by the IRB committee will be followed. No deviation from the protocol will be implemented without the prior review and approval of the IRB committee except where it may be necessary to eliminate an immediate hazard to a subject. In such case, the deviation will be reported to the IRB committee as soon as possible.

13.12. Financial Disclosure

Investigators will be asked to provide financial disclosure prior to authorization to begin the study as well as after the study is completed. Investigators will also be expected to share any situations which could introduce site-specific bias.

13.13. Publication and Disclosure Policy

The results of the study are the property of Seno Medical Instruments, Inc. All publications (manuscripts, abstracts or other modes of presentation) must be submitted at a time determined by Seno Medical Instruments, Inc. and must be reviewed and approved in writing by Seno Medical Instruments, Inc., in advance of submission. Co-authorship with any Seno Medical Instruments, Inc. personnel will be discussed and mutually agreed upon before submission of a manuscript to a publisher.

14. SUSPENSION OR PREMATURE TERMINATION

14.1. Criteria for Premature Termination

Seno Medical reserves the right to terminate an investigator/investigational site for any of the reasons provided in Section 13.9.2 of this study protocol: Investigator Disqualification Criteria. In addition, Seno Medical may choose to suspend or prematurely terminate the investigation for the following reasons:

- Device deficiency or malfunction
- Administrative decision

Seno Medical will promptly notify the investigators.

Reference List

1. Grant E, Tessler F, Hoang, J, et al. Thyroid Ultrasound Reporting Lexicon: White Paper of the ACR Thyroid Imaging, Reporting and Data System (TIRADS) Committee. J Am Coll Radiol 2015;12:1272-1279.
2. Ezzat S, Sarti DA, Cain DR, Braunstein GD. Thyroid incidentalomas. Prevalence by palpation and ultrasonography. Arch Intern Med 1994;154:1838-40.
3. Smith-Bindman R, Lebda P, Feldstein VA, et al. Risk of thyroid cancer based on thyroid ultrasound imaging characteristics: results of a population-based study. JAMA Intern Med 2013;173:1788-96.
4. Nam-Goong IS, Kim HY, Gong G, et al. Ultrasonography-guided fine-needle aspiration of thyroid incidentaloma: correlation with pathological findings. Clin Endocrinol (Oxf) 2004;60:21-8.
5. Ito Y, Miyauchi A, Inoue H, et al. An observational trial for papillary thyroid microcarcinoma in Japanese patients. World J Surg 2010;34:28-35.
6. Oraevsky AA: Optoacoustic tomography of the breast, Chapter 33 in "Photoacoustic imaging and s pectroscopy", ed. by L. Wang, Taylor and Francis Group, New York, 2009.
7. Ermilov SA, Fronheiser MP, Nadvoretsky V, Brecht HP, Su R, Conjusteau A, Mehta K, Otto P, Oraevsky AA: Real-time optoacoustic imaging of breast cancer using an interleaved two-laser imaging system coregistered with ultrasound, in "Photons Plus Ultrasound: Imaging and Sensing", San Jose , Ca, January 24, 2010, *Proc. SPIE* vol. 7564: 75641W, pp.1-7.
8. Ermilov SA, Khamapirad T, Conjusteau A, Lacewell R, Mehta K, Miller T, Leonard MH, Oraevsky AA: Laser Optoacoustic Imaging System for Detection of Breast Cancer, *J Biomed Opt*. 2009; 14(2): 024007 (1-14).
9. Kruger RA, Lam RB, Reinecke DR, Del Rio SP, Doyle RP: Photoacoustic Angiography of the Breast, Med Phys; 37 (11); 6096-6100.
10. Brecht HP, Su R, Fronheiser M, Ermilov SA, Conjusteau A, and Oraevsky AA: Whole body three-dimensional optoacoustic tomography system for small animals, *J. Biomed. Optics* 2009; **14**(6), 0129061-8.
11. Ku G, Wang XD, Xie XY, Stoica G and Wang LHV: Imaging of tumor angiogenesis in rat brains in vivo by photoacoustic tomography, *Applied Optics* 2005; **44**(5), 770-775.
12. Wang XD, Xie XY, Ku G, Wang LHV, and Stoica G: Noninvasive imaging of hemoglobin concentration and oxygenation in the rat brain using high-resolution photoacoustic tomography, *Journal of biomedical optics* 2006; **11**(2), 024015.
13. Esenaliev RO, Karabutov AA, Oraevsky AA: Sensitivity of laser optoacoustic imaging in detection of small deeply embedded tumors, *IEEE J. ST Quant. Electr*. 1999; **5**(4):981-988.
14. Andreev VG, Karabutov AA, Oraevsky AA: Detection of ultra wide-band ultrasound pulses in optoacoustic tomography, *IEEE Trans. UFFC* 2003; **50**(10): 1383-1391.
15. Stavros AT, Lerner A, et al. Combining B-Mode Ultrasound and Opto-Acoustic Imaging to Evaluate Breast Lesions. ASBS; 2013 May 1-5; Chicago IL.
16. Stavros AT, Lerner A, et al. Opto-acoustic breast imaging, a new technology. NCBC; 2013 Mar 23-27; Las Vegas NV.
17. Zalev J, et al. Real-time opto-acoustic imaging system for clinical assessment of breast lesions. SPIE; 2013 Feb 2-7; San Francisco CA.
18. Butler RS, Stavros AT, et al. Opto-acoustic breast imaging - A novel fusion of functional and morphologic imaging. RSNA; 2013 Dec 1-6; Chicago IL.
19. Otto P, Kist K, et al. Functional Images of Hemoglobin and Blood Oxygen Saturation Co-registered with Ultrasound Provide Accurate Differentiation of Breast Tumors. AIUM; 2013 Apr 6-10; New York NY.
20. Otto P, Kist K, et al. Improved Differentiation of Breast Tumors using Novel Imaging System based on Co-registered Opto-acoustic Tomography and Ultrasound. ESR; 2013 Mar 7-11; Vienna AT.
21. Otto P, Kist K, et al. Downgrading BI-RADS 4a and 4b benign masses using functional images of

hemoglobin and blood oxygen saturation co-registered with ultrasound provide differentiation of breast tumors. RSNA; 2013 Dec 1-6; Chicago IL.
22. Lavin P, Stavros AT, et al. Fusion Imaging Using Opto-acoustics and Ultrasound to Differentiate Breast Mass Histopathology. SABCS; 2013 Dec 10-14; San Antonio TX.
23. Ito Y, Uruno T, Nakano K, Takamura Y, Miya A, Kobayashi K, et al. An observation trial without surgical treatment in patients with papillary microcarcinoma of the thyroid. Thyroid. 2003;13 (4): 381-7
24. ACR Practice Guideline for the Performance of a Thyroid Examination. 2011.
25. Kwak J, Han K, Yoon J et al. Thyroid Imaging Reporting and Data System for US Features of Nodules: A Step In Establishing Better Stratification of Cancer Risk, Radiology*. 260: 2011; 892-899.*
26. Tessler F, Middleton W, Grant E, Hoang J, Berland L, Cronan J, Beland M, Desser T, Frates M, Hammers L, Hamper U, Langer J, Scoutt L, Stavros T, Teffey S, ACR Thyroid Imaging, Reporting and Data System (TI-RADS): White Paper of the ACR TI-RADS Committee. 2016.
27. Hoang JK, Langer JE, Middleton WD, Wu CC, Hammers LW, Cronan JJ, et al. Managing incidental thyroid nodules detected on imaging: white paper of the ACR Incidental Thyroid Findings Committee. J Am Coll Radiol. 2015;12(2):143-50.

APPENDIX A ACR GUIDELINES

Revised 2013 (Resolution 16) *

The American College of Radiology, with more than 30,000 members, is the principal organization of radiologists, radiation oncologists, and clinical medical physicists in the United States. The College is a nonprofit professional society whose primary purposes are to advance the science of radiology, improve radiologic services to the patient, study the socioeconomic aspects of the practice of radiology, and encourage continuing education for radiologists, radiation oncologists, medical physicists, and persons practicing in allied professional fields.

The American College of Radiology will periodically define new practice parameters and technical standards for radiologic practice to help advance the science of radiology and to improve the quality of service to patients throughout the United States. Existing practice parameters and technical standards will be reviewed for revision or renewal, as appropriate, on their fifth anniversary or sooner, if indicated.

Each practice parameter and technical standard, representing a policy statement by the College, has undergone a thorough consensus process in which it has been subjected to extensive review and approval. The practice parameters and technical standards recognize that the safe and effective use of diagnostic and therapeutic radiology requires specific training, skills, and techniques, as described in each document. Reproduction or modification of the published practice parameter and technical standard by those entities not providing these services is not authorized.

APPENDIX B: PROTOCOL SIGNATURE PAGE

I have read the protocol and agree that it along with the Clinical Trial Agreement contain all of the details necessary to carry out the study. I will conduct this study according to the protocol and required FDA and GCP regulatory requirements and will complete the study in the time agreed. Potential additions or modifications to the study will be by mutual written agreement between the Sponsor and me and will be documented and filed, if required, with the Institutional Review Board and the Food and Drug Administration.

I will provide copies of the protocol and other pertinent information to all individuals responsible to me who will assist in the study.

The Sponsor and applicable regulatory authorities will have direct access to source documentation from which case reports have been generated to conduct monitoring and audit checks as needed.

_____

Printed Name of Investigator

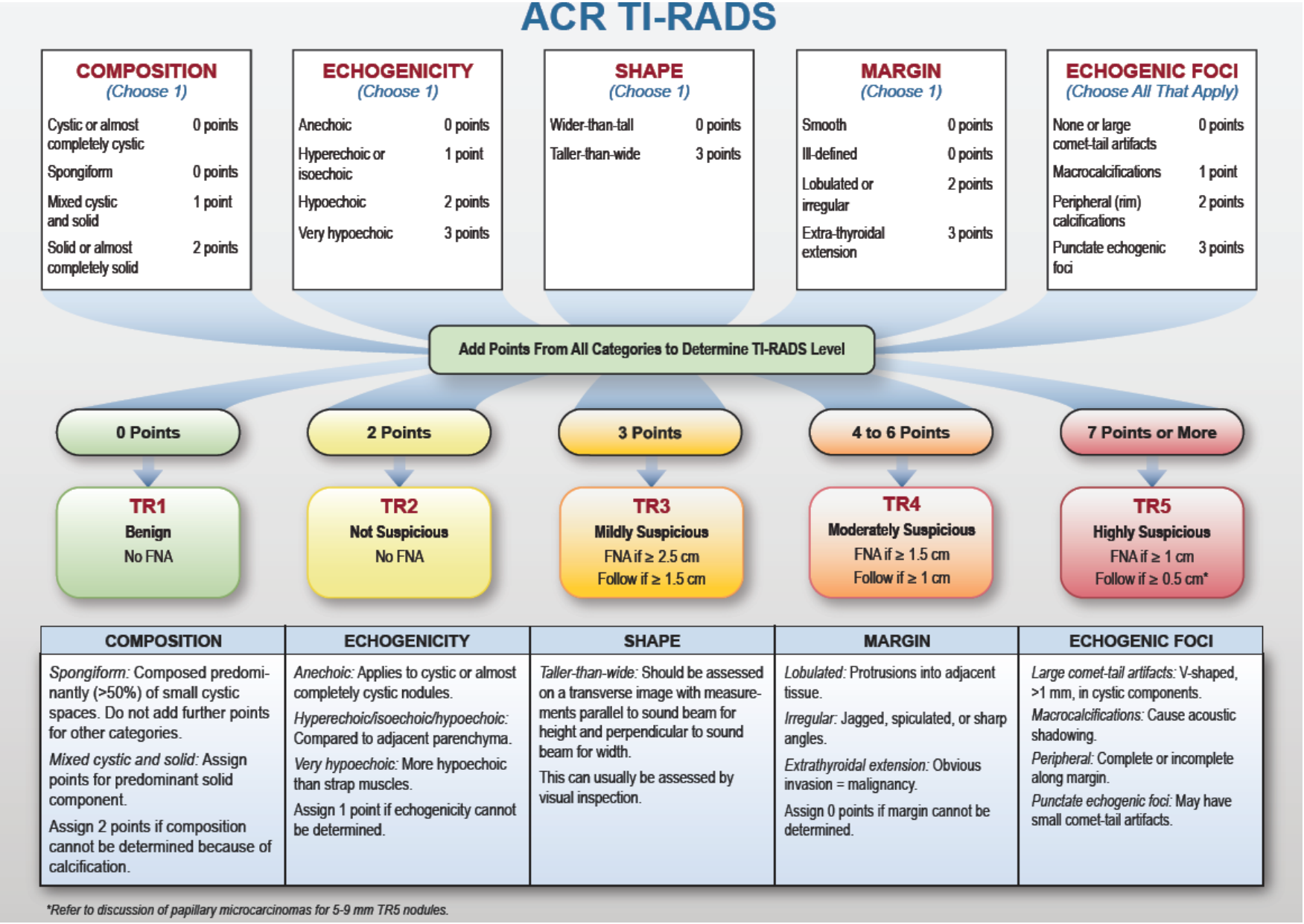
_____

Signature of Investigator

_____

Date

APPENDIX C ACR TI-RADS:



APPENDIX D- THYROID SCANNING PROTOCOL REV. 06 (SID-0000002854)