ERCHONIA® LUNULALASER™

An Evaluation of the Effect of the Erchonia® LunulaLaser™ for the Treatment of Toenail Onychomycosis

ERCHONIA CORPORATION

Version 2.0
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STUDY INFORMATION

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PURPOSE OF STUDY
The purpose of this clinical study is to demonstrate the efficacy of the Erchonia LunulaLaser™, manufactured by Erchonia Corporation (the Sponsor) for the treatment of onychomycosis of the toenail.

The Sponsor intends to submit the data and analysis from this study via a 510(k) application to obtain FDA clearance to market the laser device for the intended indication.

LABELING
Once cleared for market in the U.S., the Erchonia LunulaLaser™ laser device will be labeled as prescription devices, per 21 CFR § 801.109.

INDICATION FOR USE
The results of this clinical study will be used to support the following indication for use statement: "The Erchonia LunulaLaser™ laser device is indicated for the treatment of onychomycosis of the toenail (dermatophytes *Trichophyton rubrum* and *T. mentagrophytes*,)."

REGULATORY HISTORY
On June 3, 2016, the FDA issued the following 510(k) clearance for the Erchonia® LunulaLaser™ under **K153164**: "The LunulaLaser™ device is indicated for use for the temporary increase of clear nail in patients with onychomycosis (e.g. dermatophytes Trichophyton rubrum and T. mentagrophytes, and/or yeasts Candida albicans, etc.)"

FDA RESOURCES UTILIZED

- **Medical Devices and Clinical Trial Design for the Treatment or Improvement in the Appearance of Fungally-Infected Nails;**
  Draft Guidance for Industry and Food & Drug Administration Staff;
  January 27, 2015.

  U.S. Department of Health and Human Services
  Food and Drug Administration
  Center for Devices and Radiological Health
  Office of Device Evaluation
  Division of Surgical Devices

- Feedback and recommendations made by the FDA through the review of Q-Subs Q161189 and Q161189/S001**.**

DEVICE INFORMATION: ERCHONIA LUNULA™LASER

REGULATORY HISTORY

510(k)# K153164: On June 3, 2016, the FDA issued the following 510(k) clearance for the Erchonia Corporation LunulaLaser

510(K) Number:	K153164
Device Name:	LunulaLaser™
Device Classification Name:	Lasers For Temporary Increase Of Clear Nail In Patients With Onychomycosis
Regulation Number:	878.4810
Classification Product Code:	PDZ
Decision Date:	06/03/2016

Indications For Use (IFU) Statement: "The LunulaLaser™ device is indicated for use for the temporary increase of clear nail in patients with onychomycosis (e.g. dermatophytes Trichophyton rubrum and T. mentagrophytes, and/or yeasts Candida albicans, etc.)"

DEVICE DESCRIPTION AND SPECIFICATIONS

The Erchonia® LunulaLaser™ to be evaluated in this clinical study is identical in appearance, technical specifications and output parameters to the device cleared under K153164.

The Erchonia® LunulaLaser™ is a dual-diode laser of 635 nm and 405 nm wavelength. The light emitting diodes are manufactured by DLC and classified by the Center for Devices and Radiological Health (CDRH) as Class II laser diodes.

The LunulaLaser™ is an easy to use, compact, all in one AC powered portable floor device that is easy to carry. The laser output heads are located in the enclosure, a fixed position that allows optimal coverage of the nail bed.

By design of the device, it is not possible for the patient to move the foot's position to any significant degree that would impact the intended exposure to the laser procedure, particularly considering that the laser beam is designed to cover the entire treated toe region. With the creative enclosure design, the laser light is confined to the treatment area, ensuring that there is no possible instance of residual laser energy or light beyond the enclosure.

The user interface is a touch screen which communicates with the PLC to initiate, stop, pause or set the energy flow to the diodes. The diode can only be on or off; there is no user interface that allows the end user to alter the diode output. The protocol software is factory set and cannot be altered by the end user.

The LunulaLaser™ has the following specifications:

Device

- Weight: 23lbs / 10.43 kg
- Height:16in/40cm, Width:12in/30cm, Depth: 10in/25.40cm (38cm with platform extended)
- Full Color TFT Touch Screen Control Center
- Chassis: Powder Coated Aircraft Aluminum Base Plate and Door

- Housing: Injection Molded Process with Non-Allergen Material/Plastic
- Applied Part: Type B

Laser

- 2 line generating diode modules
- Wavelength: 635 nm & 405nm ± 10nm
- Output: 635nm: 17.25 ± 1.25mW / 405nm: 23.00 ± 2.00mW
- Modulation: Constant Wave (CW)

Power

- Source: 100-240VAC, 50-60Hz
- Fuse Rating: T2AH 250V
- Treatment time: 12 minutes

The Erchonia LunulaLaser™ Laser is shown in Figure 1 below:

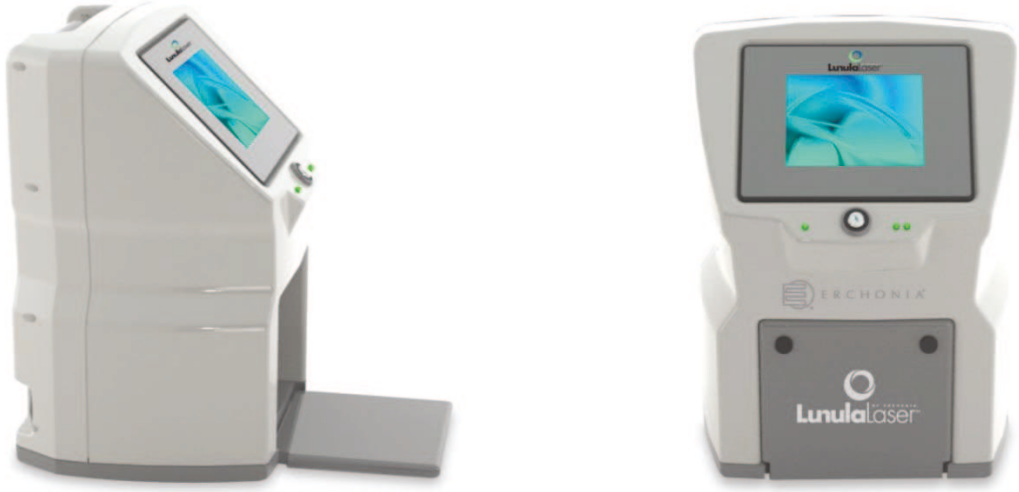


Figure 1: The Erchonia® LunulaLaser™

Placement of the target toenail inside the Erchonia® LunulaLaser™ during treatment administration is shown in Figure 2 to the right.

Figure 2: Placement of Toenail Inside the Erchonia® LunulaLaser™ During Treatment

The dimensions of the Erchonia® LunulaLaser™ are shown in Figure 3 below.

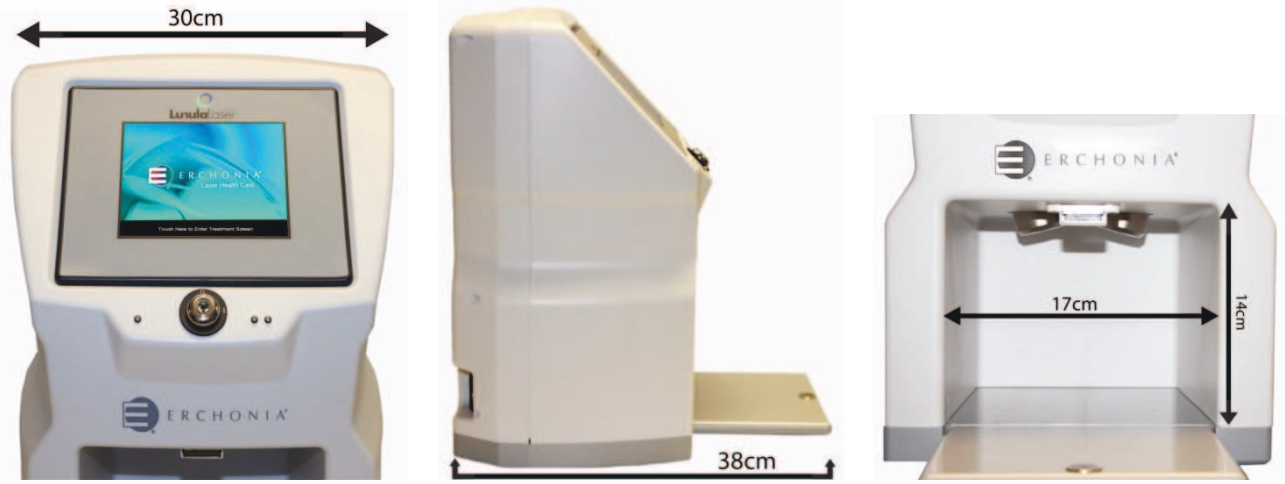


Figure 3: Dimension of the Erchonia® LunulaLaser™

DEVICE SYSTEM COMPONENTS

The Erchonia® laser package is comprised of (1) LunulaLaser™ device, (1) Power Cord, (2) Keys, (1) Patient Glasses and the Operation and Maintenance Manual.

The individual system components of the Erchonia LUNULA™ are displayed in Figures 4, 5 and 6 below, respectively, with each individual system component described in the associated text below the respective figure.

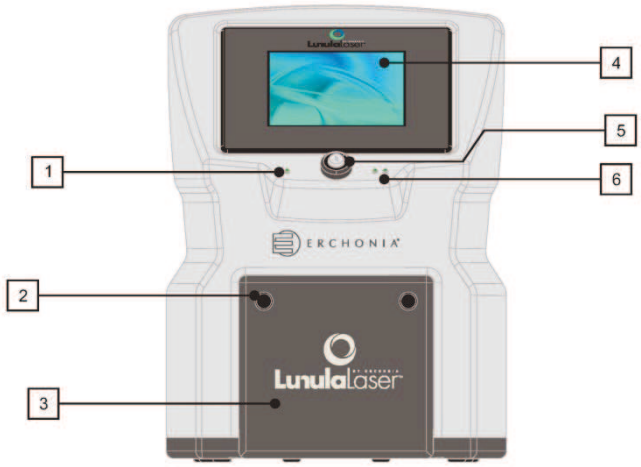


Figure 4: System components 1 through 6 of the Erchonia LunulaLaser™

1. Power Indicator Light
2. Pull Knobs / Stops
3. Door / Foot Platform
4. Touch Screen
5. Key Switch
6. Diode Light Indicator

1. **Power Indicator Light**
   When the LunulaLaser™ is ON, the power indicator light shows GREEN. When the device is OFF, the LED is NOT lit.

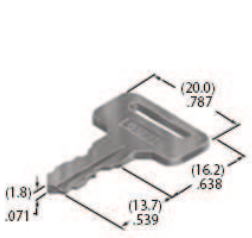
2. **Pull Knobs / Stops**
   On both sides of the door, there are protrusions that function as pull knobs when the door is closed. Pulling on these opens the door. Once the door is open, the protrusions function as stops, supporting the weight of the patient's foot.

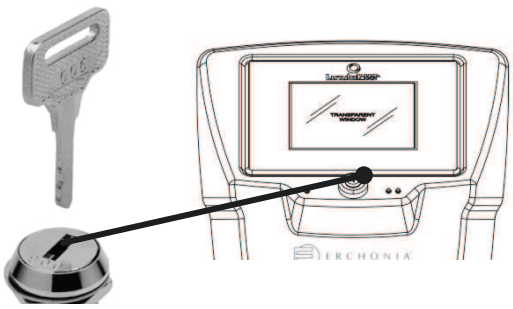
3. **Door / Foot Platform**
   In the closed position, this element is a door. The door / foot platform is multifunctional, when open, the inside of the door becomes the back half of the treatment platform.

4. **Touch Screen**
   The touch screen functions as a display screen and an input panel, providing information to the user and a means to operate the device by touching the appropriate icon.

5. **Key Switch**
   The Key Switch is the outwardly visible portion of an internal locking mechanism below the touch screen [4] that comes with an external key. Together they allow the end user to turn the device ON or OFF. ("O" = OFF and "|" = ON) In the OFF position the device is locked. From the locked position the external key can be removed. This is a code-regulated feature installed to ensure no unauthorized use of the laser device. The device will not operate if the key is in the OFF position. Turning the key to the OFF position while the device is in operation will immediately shut down the device. The key switch has a failsafe system which ensures the 110/240 voltage from a wall socket can never come in contact with the user. The device uses a 2 amp fuse, which will only require replacement if there is an issue.

NOTE: Two keys are included with the LunulaLaser™. The key is brass with Nickel plating, approximately 1.17 inch long. The device requires (1) key to operate; the key cannot be removed unless in the "O" OFF position.





6. **Diode Light Indicator**
   When the device is ON and in-treatment, these lights are lit, showing that each diode is emitting device light.

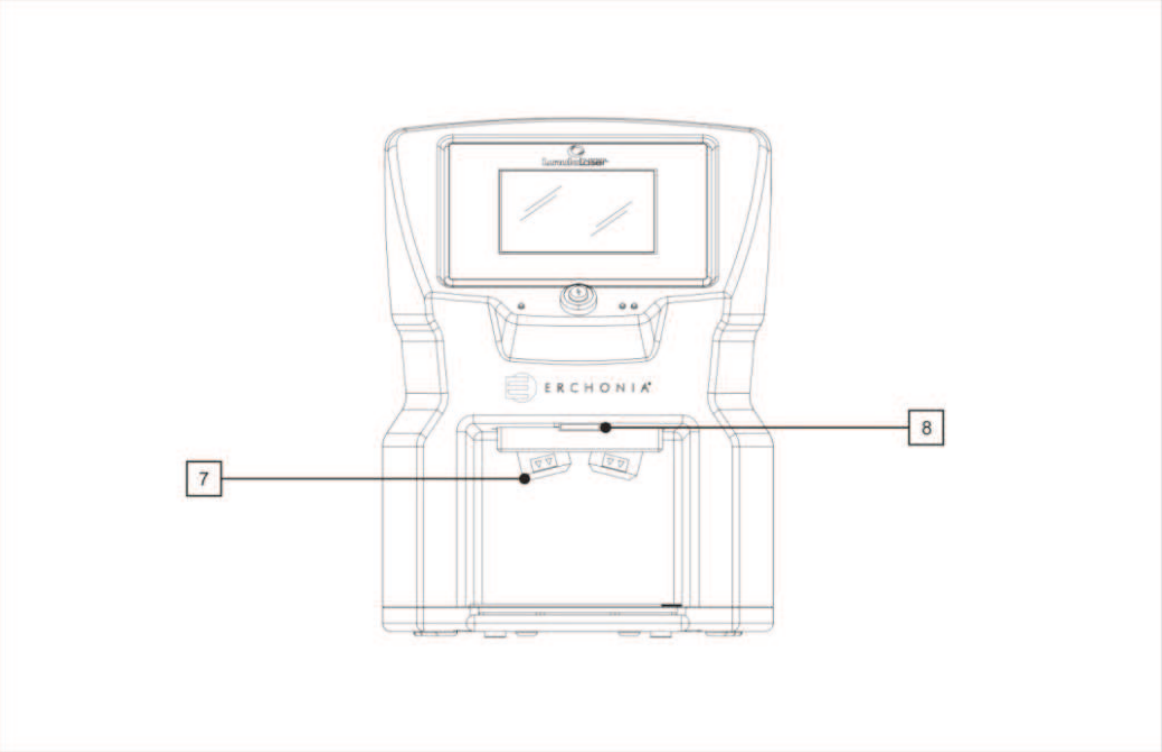


Figure 5: System components 7 and 8 of the Erchonia LunulaLaser™

7. Laser Output Heads
8. Magnetic Latch

7. Magnetic Latch

The Door / Foot Platform are held in the closed position by this magnetic latch.

8. Laser Output Heads

Each one of the two output heads emits laser light, one a 405nm violet beam and the other a 635nm red beam

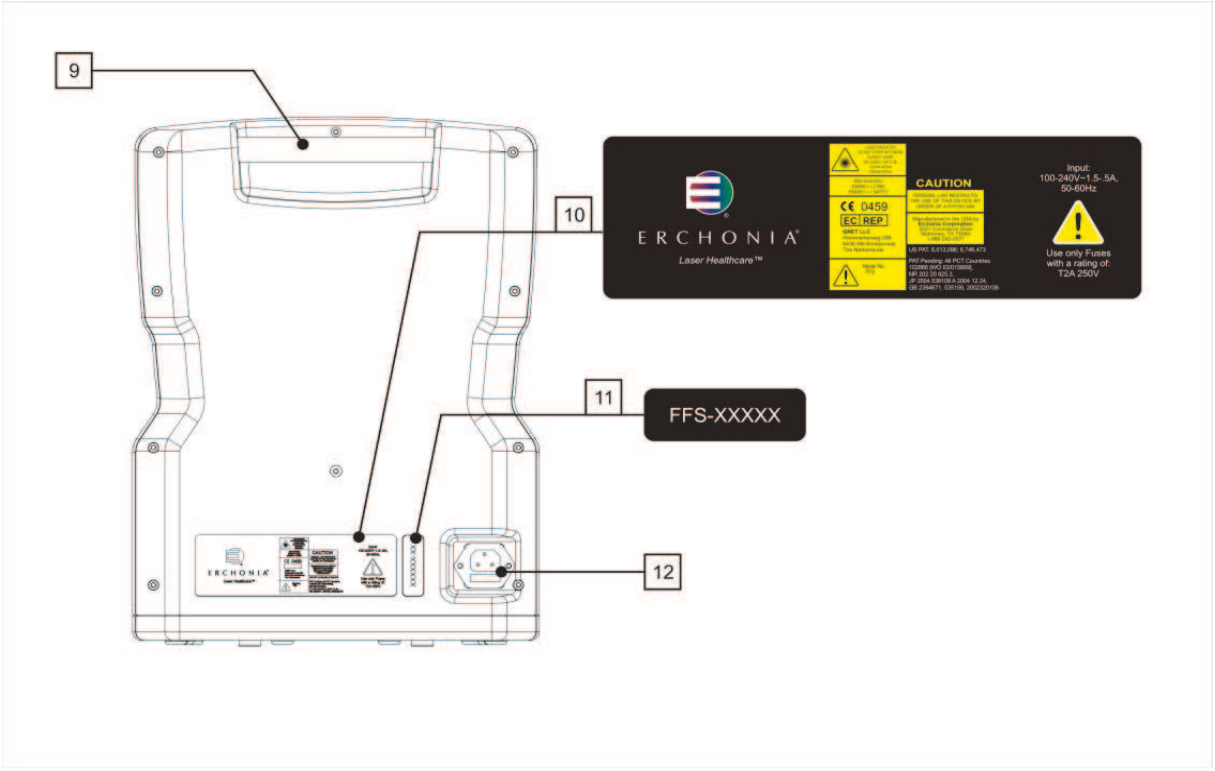


Figure 6: System components 9 through 12 of the Erchonia LunulaLaser™

9. Handle
10. Compliance Label
11. Serial Number
12. Power Inlet / Fuse Holder

9. Handle

The handle enables the user to pick up, carry and / or move the device with ease.

10. Compliance Label

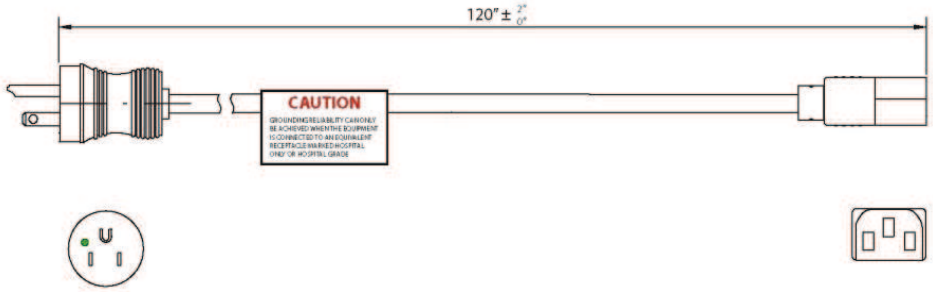
Contains all the governing agencies required information regarding the device, including but not limited to the US FDA device classification, EU classification, output information and power inlet symbols. Also includes the manufacturer name and address.

11. Serial Number

This is the unique identifier for the device. All information regarding this device is associated with the serial number.

12. Power Inlet Module/Fuse Holder

The device contains a flexible detachable power cord. **NOTE:** Make sure the power cord is plugged into device at this location prior to plugging into a wall socket. The power inlet module also contains a fuse holder. Replacing the fuses is the only service that can be conducted by the end-user. Fuses to be rated a T2AH 250V with an input to cover 100 – 240V~ .5-1.5A, 50-60 Hz.



The power cord does not contain any operator-serviceable components. If the power cord needs replacement, contact an Erchonia Corporation representative.

The device includes a transformer which converts AC power to match the power output (i.e. 110V or 240V). Only a 3 plug prong adaptor is required (Hospital Grade Only). Once the adaptor is affixed to the plug end of power inlet, put into wall socket. Input: 1.5A/100VAC & 0.5A/240VAC, 50-60 Hz.

PROTECTIVE EYEWEAR

The Erchonia LunulaLaser™ is classified by the FDA/IEC as a Class 2 laser device. This designation represents a current standard for use in order to ensure the safety of the patient. A Class 2 laser is determined to have a chronic viewing hazard. Pointing the laser beam directly into the eye and maintaining it there for an extended period of time could prove to be damaging.

To ensure there is no possible instance of residual effect, a pair of specialty glasses (shown in Figure 7 below) is provided for use during in-office procedure applications with the Erchonia LUNULA™ laser device. These safety glasses are Kentek Corporation Filter #6101 light blue glasses with approximate VLT 63% that sufficiently and effectively block the laser light spectrum of the LUNULA™ laser device as follows: 405nm (OD 1.22) & 635nm (OD 2.07).

Figure 7: Kentek Corporation Filter #6101 Safety Glasses

PERFORMANCE STANDARDS

The LunulaLaser™ complies with FDA's performance standards for light-emitting products (21 CFR Part 1040).

COMPLIANCE WITH VOLUNTARY STANDARDS

The LunulaLaser™ complies with the following voluntary standards:

- ES60601-1:2005/(R) 2012 and C1:2009/(R)2012 and, A2:2010/(R)2012 (consolidated text) Medical Electrical Equipment - part 1: General Requirements for Basic Safety and Essential Performance (IEC 60601-1:2005, mod). (General II (ES/EMC))
- IEC 60601-1-2 Edition 3: 2007-03, medical electrical equipment - part 1-2: general requirements for basic safety and essential performance - collateral standard: electromagnetic compatibility - requirements and tests. (General II (ES/EMC))
- IEC 60825-1 Edition 2.0 2007-03, safety of laser products - part 1: equipment classification, and requirements [including: technical corrigendum 1 (2008), interpretation sheet 1 (2007), interpretation sheet 2 (2007)].

STUDY INDICATION AND RATIONALE, THEORY OF MECHANISM OF OPERATION, & SUPPORTING MATERIALS

STUDY INDICATION: TOENAIL ONYCHOMYCOSIS

Definition

An infection of toenail fungus, or onychomycosis, occurs when fungi infect the nail. A nail fungal infection may begin as a white or yellow spot under the tip of the toenail. As the nail fungus spreads deeper into the nail, it may cause nail discoloration, thickening and the development of crumbling edges, all of which can lead to an unsightly and potentially painful problem. Onychomycosis may be difficult to treat, and infections may recur easily. Toenail fungus affects approximately 23 million people in the US – about 10% of all adults.

Symptoms

The *primary symptoms* of onychomycosis are nails that are:

- thickened
- brittle, crumbly or ragged
- distorted in shape
- dull, lacking luster or shine
- a dark color, caused by debris building up under the nail

Additional symptoms may include:

- separation of the nail from the nail bed (onycholysis)
- pain in the toes
- a slightly foul odor.

Causes

Fungi are microscopic organisms that don't need sunlight to survive. Nail fungal infections are typically caused by a fungus that belongs to a group of fungi called dermatophytes, but may also be caused by yeasts and molds.

All of these microscopic organisms live in warm, moist environments, including swimming pools and showers. They can invade the skin through tiny invisible cuts or through a small separation between the nail and the nail bed. They cause problems only if the nails are continually exposed to warmth and moisture — conditions perfect for the growth and spread of fungi.

Infection with nail fungus occurs more commonly in toenails than in fingernails because toenails are often confined in a dark, warm, moist environment inside shoes — where fungi can thrive. Another reason may be the diminished blood circulation to the toes as compared with the fingers, which makes it harder for the body's immune system to detect and eliminate the infection.

Risk factors

- Onychomycosis is **more common among older adults** for several reasons, including diminished blood circulation and more years of exposure to fungi. Also, nails may grow more slowly and thicken with age, making them more susceptible to infection.
- Onychomycosis tends to **affect men more often than it does women**, particularly those with a family history of the infection.

- **Other factors** that can increase the risk of developing nail fungus include:
  - perspiring heavily
  - working in a humid or moist environment
  - having the skin condition psoriasis
  - wearing socks and shoes that hinder ventilation and don't absorb perspiration
  - walking barefoot in damp public places, such as swimming pools, gyms and shower rooms
  - having athlete's foot (tinea pedis)
  - having a minor skin or nail injury, a damaged nail or another infection
  - having diabetes, circulation problems or a weakened immune system

Potential Complications

The **potential complications** of onychomycosis include:

- pain in the nails
- permanent damage to the nails.
- leading to other serious infections that can spread beyond the feet for individuals with a suppressed immune system due to medication, diabetes or other conditions, such as leukemia and AIDS.
- For diabetes patients in particular, toenail onychomycosis can lead to impairment of the blood circulation and nerve supply to the feet and a greater risk for cellulitis, a potentially serious bacterial skin infection.

Current Available Treatments

Nail fungus can be difficult to treat, and repeated infections are common. The currently available treatments for onychomycosis are the following:

- *Oral antifungal medications*

  Studies have shown the most effective treatments to be terbinafine (Lamisil) and itraconazole (Sporanox). Oral antifungal medications help a new nail grow free of infection, thereby slowly replacing the infected portion of the nail. These medications are usually taken for 6 to 12 weeks, but it may take four months or longer to eliminate the infection and for the nail to grow back completely. Recurrent infections are possible, especially if the nails continue to be exposed to warm, moist conditions.

  Antifungal drugs may cause side effects ranging from skin rashes to liver damage. Therefore, doctors may not prescribe them for people with liver disease or congestive heart failure or for those taking certain medications.

- *Antifungal lacquer*

  The antifungal nail polish called ciclopirox (Penlac) is often prescribed for cases of mild to moderate onychomycosis. The polish is painted onto the infected nails and surrounding skin once a day. After seven days, the piled-on layers are wiped clean with alcohol and fresh applications are begun. Daily use of Penlac for about one year has been shown to help clear some nail fungal infections.

- *Topical medications*

  Other topical over-the-counter antifungal creams containing urea can be used to help speed up absorption. Topical medications usually don't provide a cure, but may be used in conjunction with oral medications.

- *Surgery*

If the nail infection is severe or extremely painful, the nail may be surgically removed. A new nail will usually grow in its place, though it will come in slowly and may take as long as a year to grow back completely. Sometimes surgery is used in combination with ciclopirox to treat the nail bed.

- *Photodynamic therapy*
  A laser is applied to irradiate the nail after it's been treated with an acid.

STUDY RATIONALE AND JUSTIFICATION: LOW LEVEL LASER THERAPY AS A TREATMENT FOR TOENAIL ONYCHOMYCOSIS

Presently, there is no perfect cure for toenail fungus. Even the most effective oral medications are successful only about half of the time. Topical medications are successful less than 10% of the time. As 10% of the adult population is affected by toenail onychomycosis, the need for a more effective and lasting cure is evident.

Recently, application of low level laser therapy to the affected toenail over a four-week period using the Erchonia® LunulaLaser™ device has been clinically demonstrated to effect increase in clear nail in onychomycosis-infected toenails, and the device subsequently received FDA clearance to be marketed for this indication (K153164) based on this clinical outcome.

Unlike medication-driven treatments for toenail fungus which can have many side effects including serious ones such as liver toxicity, low level laser therapy presents minimal risk of side effects. Laser therapy is applied to toenail onychomycosis by shining a laser light through the toenail. The laser light vaporizes the fungus while leaving the skin and surrounding tissue unharmed, in a simple, quick, non-invasive and pain-free manner.

The goal of this current study is to expand the present indication for use for the Erchonia® LunulaLaser™ of: “The LunulaLaser™ device is indicated for use for the temporary increase of clear nail in patients with onychomycosis (e.g. dermatophytes Trichophyton rubrum and T. mentagrophytes, and/or yeasts Candida albicans, etc.)” (K153164) from the aesthetic claim of changing the nail appearance to the medical claim of eradicating the underlying fungal infection. As such, this clinical study protocol design is intended to provide data to support an indication for use of ‘treatment of onychomycosis’ through application of the Erchonia® LunulaLaser™.

Prior clinical trials evaluating application of the Erchonia® LunulaLaser™ to toenail onychomycosis have reported negative culture results at completion of treatment administration, providing support and justification for conducting a controlled clinical trial to specifically assess and support a claim of treatment of onychomycosis for the Erchonia® LunulaLaser™ device.

THEORY OF MECHANISM OF OPERATION

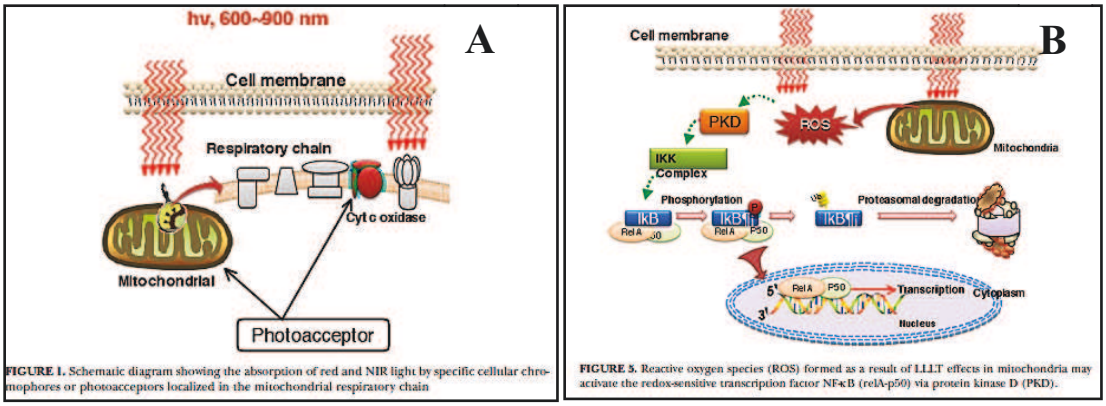
The most common form of onychomycosis, which is the subject of evaluation in this clinical study, is distal lateral subungual onychomycosis (DLSO), a condition wherein the fungus spreads from plantar skin and progresses towards the underside of the nail through the hyponychium or the distal lateral nail bed. An inflammatory response is quickly upregulated in the area of infection, generating the physical signs of DLSO. The onset of fungal infection is caused by three main classes of fungi: dermatophytes, yeasts, and nondermatophyte molds. The most common cause of onychomycosis worldwide is due to the infection of dermatophytes, including the genera *Epidermophyton, Microsporum*, and *Trichophyton*. There are two major

pathogens that account for a majority of onychomycosis cases, *T rubrum* and *Trichophyton mentagrophytes*.

Laser therapy operates under the principle of photochemistry with a photoacceptor molecule absorbing the emitted photons and inducing a biological cascade. Like our eukaryotic cell, fungi contain the highly complex organelle the mitochondria, which is responsible for the manufacturing of the energy molecule ATP. Within the inner mitochondrial membrane is cytochrome c oxidase, an identified photoacceptor molecule. It is believed that laser therapy could perhaps provide a means to photo-destroy the fungi responsible for onychomycosis by inducing the release of highly reactive superoxides. Moreover, laser therapy has been shown to promote superoxide dismutase (SOD), a process responsible for the destruction of foreign invaders. Extracellular release of low levels of mediators associated with SOD can increase the expression of chemokines, cytokines, and endothelial leukocyte adhesion molecules, amplifying the cascade that elicits the inflammatory response. The physiologic function of hydrogen peroxide, superoxide anion, and hydroxyl free radical is to destroy phagocytosed microbes. By enhancing the natural processes of the immune system and impacting the structural integrity of the fungi strain, it is believed that laser therapy may provide a means for clinicians to effectively treat onychomycosis without the onset of any adverse events.

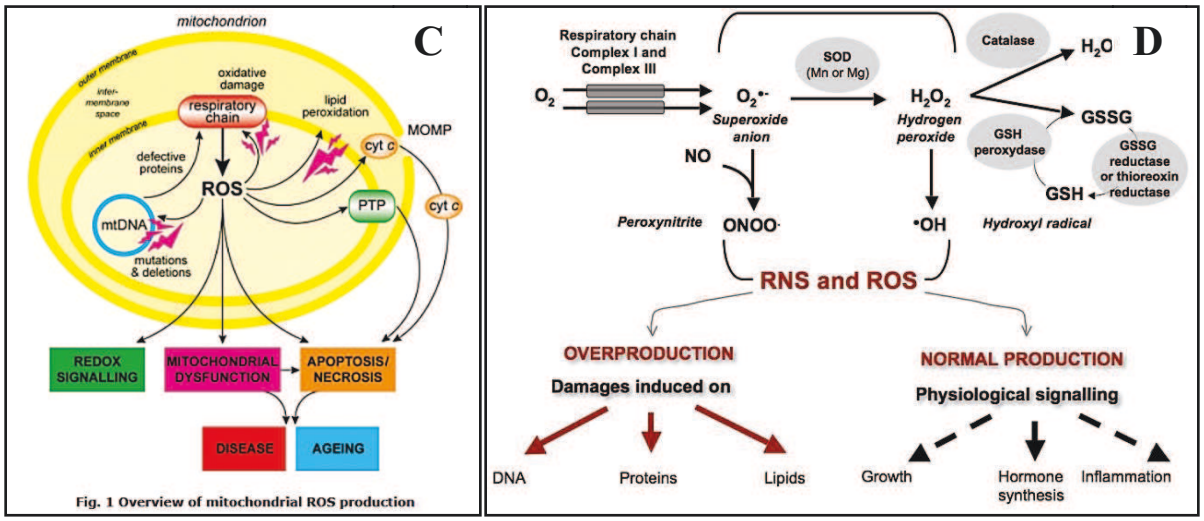
The wavelengths that will be utilized in this study will be a violet (405nm) and red (635nm) combination. The wavelengths were chosen based on the theory of electron volt stimulation. When studying the light spectrum chart, there is an inverse relationship between wavelength and the individual energy level of the photon (electron volt). As we move towards lower wavelengths, the energy of the emitted photon increases; for instance, a radio wave has far less electron volts (energy per photon) than the photons of an x-ray. Within the visible light spectrum, the highest electron volt levels are observed towards the lower end of the spectrum, therefore greater biological stimulation can occur with a 400 nm and 635nm wavelength.

Work from several laboratories has demonstrated that visible light and NIR photoirradiation improves various cellular functions and can accelerate wound healing and tensile strength in both normal and impaired tissues and that the effects are not related to alterations in tissue temperatures [1-18]. Increased cellular metabolism and ATP production have been shown to occur as a result of photostimulation of cytochromes and other cellular energy transport compounds [4-6, 10, 11], as well as through cellular responses induced by changes in reactive oxygen species (ROS), nitric oxide (NO) and other inflammatory mediators orchestrated by light exposure [6-8, 10-16]. Some of these pathways are illustrated in the figures below.



Source: Huang YY, Chen AC, Carroll JD, Hamblin MR: Biphasic Dose Response in Low Level Light Therapy. Dose-Response 2009; 7: 358-383.

Photoacceptors in the tissue cells' mitochondria absorb visible and NIR light inducing a cascade of events that results in the production of ROS, NO and RNS (Figures A & B).

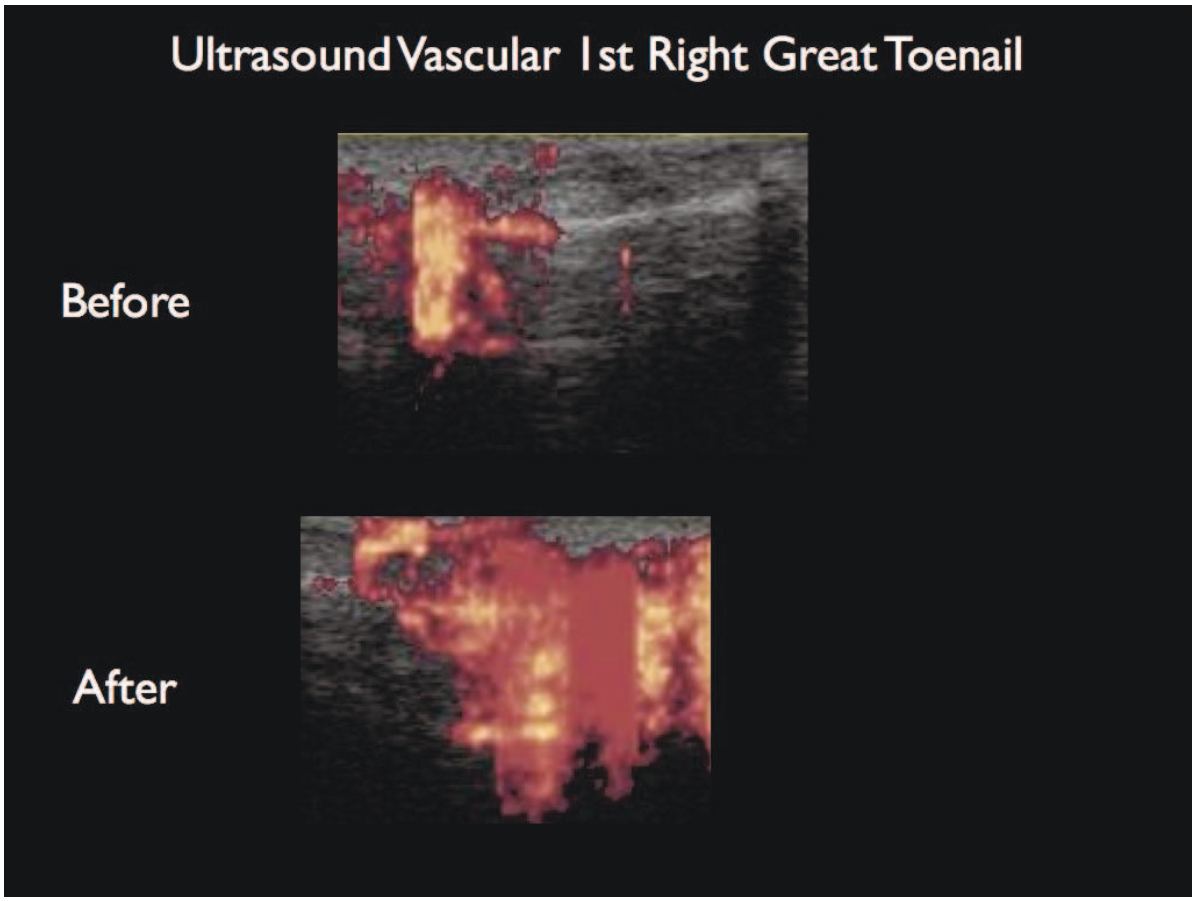


Source: http://www.mrc-mbu.cam.ac.uk/research/mitochondrial-dysfunction/measuring-mitochondrial-reactive-oxygen-species(C);
http://www.bioscience.org/2009/v14/af/3509/fig8.jpg (D)

These in turn cause a series of downstream events and effects. These ROS and RNS compounds are lethal to fungi and bacteria (Figures C & D), while promoting stimulatory, immune modulatory and anti-inflammatory effects in the adjacent tissues.

The Erchonia® LunulaLaser™ activates the mitochondria within the cells within the germinal layer which activates the production of nitric oxide which in turn produces vasodilation, Vasodilation evokes increased blood flow and local circulation to the targeted region. This results in an increase in oxygenation and nutrition to the germinal matrix layer and nail bed. Nitric oxide also decreases production of cytokines and other mediators affecting immune cells that promote inflammation that effects accelerated nail growth. This is a similar mechanism as that contributing to accelerated hair growth.

The local circulatory effects are demonstrated in the following flow Doppler ultrasound images of a great toe before and after LunulaLaser™ therapy. The blood flow in the before treatment image is noted at the level of the inter-phalangeal joint, and there is some vascular activity present in the germinal nail matrix which clearly ends where the nail plate begins. The after ultrasound image was taken just after treatment with the LunulaLaser™. It demonstrates the dramatic increase in circulation that encompasses the entire germinal matrix layer, the nail bed and nail. There is a marked increase in blood flow involving the entire toe.



This phenomenon induced by the LunulaLaser™ low level laser device, occurs without the generation of heat. This is unlike a Class 4 laser, such as the PinPointe® FootLaser™, which relies on heat for its clinical effect [5].

The target sites for treating onychomycosis are the nail plate, nail bed and nail matrix. Where a topically administered antifungal drug concentration typically only permeates through to the nail plate level, laser light has the ability to penetrate through to the nail matrix, thus providing therapeutic intervention across the entire nail barrier.

It is also noted that treatment inhibits or destroys the fungus present which ultimately results in clearing of the nail as a result of changing the counterpoised outward growth of the nail versus the inward “growth” or progression of the fungal infection. The stimulation of nail growth and clearing is therefore due in part due to photostimulation of the nail matrix coupled with an antifungal effect.

References:

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SUPPORTING MATERIALS

Below are summaries of trial results that present both mycological and clinical evidence to support the anticipated efficacy of the Erchonia® LunulaLaser™ for the treatment of onychomycosis, to be demonstrated in this current clinical study as the presence of complete clear nail with simultaneous negative mycological testing following treatment administration with the Erchonia® LunulaLaser™.

- **AN EVALUATION OF THE EFFECT OF THE ERCHONIA LUNULA™ ON TREATING TOENAIL ONYCHOMYCOSIS CLINICAL STUDY*; Version 7.0; December 27, 2012; Erchonia Corporation: Mycology Results***

This study was pre-established as a positive mycology study such that as a condition of study enrollment, all subjects were required to have a positive mycology for onychomycosis as confirmed through toenail cultures taken during study qualification evaluation and sent to an independent laboratory to be grown in an agar solution used to clinically identify dermatophites that are consistent with a definitive and verifiable diagnosis of the presence of onychomycosis.

The mycology testing was performed at Lawrence Laboratories in Dublin, Ireland. It was established, registered and certified as a Manufacturer of pharmaceutical preparations on August 21, 1972 (Registration #38315), and was dissolved on December 16, 2013.

The methodology used for the mycology testing was the following:

- Core samples were obtained from under the nail plate as close as possible to viable tissue
- Cultures were then grown in a neutral dermatophie medium (peptone-glucose agar solution) with cycloheximide
- The presence of verifiable dermatophites was confirmed by the agar solution turning a pink color. The presence of verifiable dermatophites is considered consistent with the condition known as onychomycosis.
- The samples were monitored for up to 21 days, with evaluations recorded on Days 3, 7, 14 and 21, as applicable.

In all instances of enrolled toenails, the presence of dermatophites was confirmed by the agar solution turning a pink color. Furthermore, the onychomycosis strain of Trichophyton rubrum (T. rubrum) was present on almost all slides. T. rubrum is established as the most common cause of onychomycosis in Europe and North America.

Mycology testing was repeated for all enrolled subject toenails at 2 weeks post baseline evaluation. The results demonstrated the conversion from positive mycology to negative mycology as a result of the treatment administration with the Erchonia® LunulaLaser™ for all enrolled and treated study subject toenails.

Specifically, mycology testing results were as follows:

- *Baseline (Pre-Treatment) Evaluation*: Baseline results for all study toenails was a positive mycology for onychomycosis, denoted by detection of the presence of verifiable dermatophites consistent with onychomycosis. The culture results also indicated the positive presence of the Trichophyton rubrum strain for all samples. Trichophyton rubrum is the most common cause of onychomycosis in North America and Europe. The presence of

dermatophites was detected by day 3 or day 7, and the species identified as T. rubrum by day 7 or 14 in most cases, at which time the sample was destroyed.

- *Two Weeks Post-Baseline Evaluation*: All samples were found to have a negative mycology, consistent with the absence of onychomycosis. There was no change seen across all evaluation points, and the sample was destroyed following Day 21 evaluation.

The results summary of the culminating trial that resulted in the FDA's clearance for K153164 for the Erchonia® LunulaLaser™ is provided below:

- **A RETROSPECTIVE EVALUATION OF THE EFFECT OF THE ERCHONIA LUNULALASER™ ON THE INCREASE OF CLEAR NAIL IN PATIENTS WITH TOENAIL ONYCHOMYCOSIS;** *Version 1.0; September 29, 2015*

BACKGROUND: The purpose of this study was to demonstrate through retrospective analysis the efficacy of the Erchonia LunulaLaser™, manufactured by Erchonia Corporation, for the increase of clear nail in patients with toenail onychomycosis, when applying the LunulaLaser™ to the toenail for 12 minutes one time per week for a total of 4 procedure administrations.

STUDY DESIGN: This study was a retrospective analysis of a compilation of pre-procedure and six-month post-procedure photographs of fifty-four (54) great toenails with varying degrees of onychomycosis disease involvement selected from amongst an existing pool of photographs taken during three prior Erchonia Corporation research studies wherein 4 sequential weekly 12-minute procedures with the LunulaLaser™ were administered. The evaluating investigator was blinded to corresponding pre- and post-procedure photographs through application of a randomized numeric coding methodology.

STUDY MEASURES: The linear measurement of millimeter (mm) of clear nail from the proximal nail fold to the most proximal area of nail dystrophy was objectively measured from unmarked digital photographic images using the validated GNU Image Manipulation Program (GIMP 2.8) software system, a multi-platform image/photo manipulation software system, at baseline evaluation (prior to LunulaLaser™ procedure administration) and at 6 months following completion of the LunulaLaser™ procedure administration protocol.

STUDY PROCEDURE: Study great toenails had received 4 procedure administrations with the Erchonia LunulaLaser™ across a consecutive 3-week period: each procedure administration 7 days apart. Exposure time to the laser was 12 minutes, directed at and about 4 inches above the great toenail.

SUBJECTS AND SAMPLE: Fifty-four (54) treated great toenails of varying degrees of onychomycosis involvement at study entry were evaluated in this study. Subjects were 18 years or older with current bacterial/fungal infection classified by the investigator and confirmed through lab testing as positive for onychomycosis.

STUDY RESULTS

Primary Outcome Measure: Change in mm of Clear Nail from Baseline to Study Endpoint: The primary efficacy outcome measure in this study was the mm of clear nail growth at 6 months post procedure administration end relative to Baseline (pre-procedure administration). Individual toenail success was defined as 3 mm or more of clear nail growth at 6 months post-

procedure end relative to baseline. Overall study success was defined as an anticipated 60% of treated toenails meeting the individual toenail success criteria.

Sixty seven per cent (67%) of all study treated toenails evaluated in this study met the study individual toenail success criteria**,** exceeding the pre-established overall study success goal of 60% by 7%. The magnitude of the mean change in mm of clear nail from baseline to 6 months post-procedure for all treated toenails was an increase of 5.18 mm, 2.18 mm in excess of the pre-established 3 mm increase success criteria. A t-test for paired samples found this mean change of +5.18 mm in clear nail to be statistically significant ($t=-8.0$; $df=53$; $p<0.0001$).

ADVERSE EVENTS: No adverse event was reported or observed for any subject throughout any of the three clinical trials from which this study's retrospective sample was drawn.

CONCLUSION: The Erchonia LunulaLaser™ is an effective tool for increasing clear nail in toenails infected with onychomycosis, significantly increasing mm of clear nail over a 6 month period following completion of the 4-week procedure administration phase.

STUDY DESIGN

STUDY TREATMENT GROUP

There will be an active test treatment group only in this study such that all study subjects will receive the active laser treatments with the Erchonia® LunulaLaser™. The absence of a control group was stipulated by FDA through prior Pre-IDE reviews and was subsequently established and accepted through the Erchonia® LunulaLaser™ Retrospective Analysis Study whose results successfully supported clearance of the Erchonia® LunulaLaser™ for the increase in clear nail in patients with toenail onychomycosis, under K153164.

BLINDING DESIGN

Given that there is only an active treatment group in this study, there is no need for blinding of either the subject or the study investigator administering the study treatments with the Erchonia LUNULA™ device. All subjects and investigators will be aware that the study treatment being administered will be the true active laser treatment.

RANDOMIZATION

Given that there is only an active treatment group in this study; randomization to treatment group is not applicable.

SUBJECTS

Recruitment
The recruitment process will work as follows:

1. An individual voluntarily schedules an appointment at the physician's office (that in the context of this clinical study also functions as the investigator's test site) regarding his or her toenail fungus.
2. Through this visit, if the physician perceives that the patient may satisfy the study qualification criteria (i.e. there are no obvious indicators that may exclude him or her), then the physician will present the option of being a subject in the study.
3. If the patient is interested in possibly taking part in the study, the physician – now in the role of study investigator – will personally review the informed consent form with the individual and answer any questions. The individual may sign the informed consent form at that visit or he or she may think about it for a while and sign the informed consent form at a later time (taking as long as desired, from hours to days, to sign as long as study enrollment is continuing at the time the decision to sign is made) or he or she may refuse to participate.
4. An individual who chooses to sign the informed consent form and proceed with study participation will receive a subject ID and proceed to the study qualification evaluation phase.
5. An individual who decides not to participate in the study will continue to work with the physician to determine a treatment plan for his or her toenail onychomycosis.

Compensation
A subject will not be offered money or any other form of compensation to participate in the clinical study. However, he or she will also not be charged for the cost of the study treatments with the Erchonia® LunulaLaser™, the cost of the mycological lab testing or for the cost of any other directly-related evaluations or measurements that occur as part of his or her participation in the study.

Sample size

There will be 66 qualified subjects/great toenails enrolled in this clinical study across three test sites, approximately 22 subjects/great toenails per test site.

Rationale for sample size

The sample size of 66 subjects/great toenails was calculated based upon the 95% one-sided confidence interval (i.e. lower bound only) around the observed response rate of 70% being ≥ 50% (56%) yielding a pre-determined final sample size of 60 subjects/great toenails. From here, it is anticipated that about one-twelfth of subjects overall may withdraw or be terminated from the study prior to completion for various reasons. Therefore, the following formula is used to determine the final needed starting sample size for each procedure arm:

Final sample size = sample size X 1/(1-d); where d = # expected dropouts/# subjects enrolled
Final sample size = 60 X 1/(1-0.082)
Final sample size = 60 X 1/0.917 = 60 X 1.091 = 66 subjects

Therefore, a minimum starting sample size of 66 subjects is needed to insure that a sufficient number remains at the end of the treatment administration phase (60 subjects), assuming a drop-out rate of around one-twelfth, for a positive study outcome to be considered statistically valid, clinically meaningful and representative of the general population being sampled.

Subject Recruitment Advertisement

Toenail Fungus Research Study

This study is to see if the Erchonia LunulaLaser™, a non-invasive device that uses low-level laser light to grow new nail in toenails with toenail fungus can help to get rid of the fungus in the toenail altogether.

The study involves 12 visits to a test site over about 14 months.

Please contact
<Test Site/PI name> at <phone#> or
<e-mail> for details.

STUDY PROCEDURE

STUDY TEST BATTERY

The following assessment tools will be used in this study. For each study phase, the precise tool that will be employed will be specified.

BASELINE VARIABLES

BASELINE TARGET TOENAIL VARIABLES

(i) Location of target toenail: right foot; left foot.
(ii) Duration of onychomycosis in target toenail: months/years since observed onset
(iii) Notation of any other toenail(s) on the target foot that appear to be infected with onychomycosis.

PRIOR TREATMENT APPROACHES

Recording of any and all prior treatments that the subject has tried to manage his or her onychomycosis of the target toenail, including:

(i) Over-the-counter (OTC) medications and treatments (oral and topical)
(ii) Prescription medications and treatments (oral and topical)
(iii) Herbal supplements (oral and topical)
(iv) Traditional therapies (e.g. surgery, YAG laser therapy)
(v) Alternative therapies (e.g. acupuncture)

Noting, for each medication/treatment/therapy tried:
(i) Date(s) of use/application
(ii) Duration of use/application
(iii) Perceived effectiveness

CONCOMITANT MEDICATIONS

Recording pf any and all current medications that the subject is taking for any reason, including:

(i) Over-the-counter (OTC) medications and treatments
(ii) Prescription medications and treatments
(iii) Herbal supplements

Noting, for each medication:

(i) Indication for use
(ii) Dosage and frequency of use
(iii) Duration of use

SUBJECT DEMOGRAPHICS

(i) Gender
(ii) Age
(iii) Ethnicity

CLINICAL AND OUTCOME EVALUATION TOOLS

MYCOLOGICAL EVALUATION: BIOMED INTRAY™ DM CULTURE

The BioMed InTray™ DM Culture will be used to determine the presence or absence of fungal organisms and to identify the type of dermatophyte in the case of a positive fungal determination.

The BioMed InTray™ DM Culture device allows for simultaneous detection and observation of the dermatophyte fungal group, including detection of dermatophytes of *Microsporum*, *Epidermophyton* and *Trichophyton (T. rubrum), as well as C.albicans*.

The InTray™ DM Culture testing will be performed from the same target toenail for each subject at each administration. For cultures positive for the presence of fungal organisms, the type of dermatophyte identified will also be recorded.

PHOTOGRAPHIC DOCUMENTATION

A photograph of the study great toenail will be taken with the great toe placed in a horizontal-vertical *mm scale measurement recording device*. This device is designed to maintain the great toenail in the same position at each photographic time point to ensure consistency of measurements across all evaluation time points and all toenails, and to provide a mm reference scale in the event that measurement of mm of clear nail is necessitated.

An image of the mm scale recording device and its application is shown below in Figure 8.

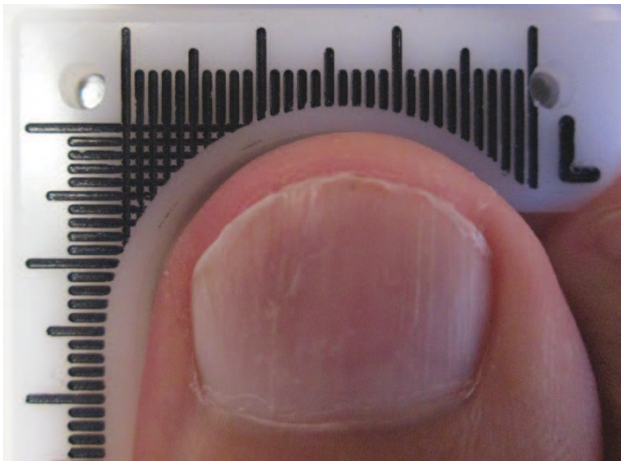


Figure 8: Positioning of the great toenail in the mm scale recording device

The photograph of the study great toenail will be taken using a high resolution digital camera, following a standardized methodology, ensuring consistency of equipment, lighting, distance, resolution, etc. between and across all subjects, test sites and measurement time points

MM OF CLEAR NAIL MEASUREMENT TOOL: THE GNU IMAGE MANIPULATION PROGRAM (GIMP 2.8)

The GNU Image Manipulation Program (GIMP 2.8) will be employed in this study for the measurement of the linear millimeters (mm) of clear nail bed.

The GNU Image Manipulation Program (GIMP 2.8) was originally released in January 1996 as the *General Image Manipulation Program, having been* created by Spencer Kimball and Peter Mattis as the result of a college project at the University of California, Berkeley. In 1997, the definition of the acronym 'GIMP' was changed to mean the *GNU* Image Manipulation Program, by permission of Richard Stallman, to also reflect its existence under the GNU Project.

The GNU Image Manipulation Program (GIMP 2.8) is a multi-platform image/photo manipulation software. It is a raster editor, such that it performs operations directly on the pixels that make up the image and not a vector editor.

GIMP is suitable for a variety of image manipulation and analysis tasks, including photo retouching, image composition, and image construction. It has many capabilities. It can be used as a simple paint program, an expert quality photo retouching program, an online batch processing system, a mass production image renderer, an image format converter, etc. It is designed to be augmented with plug-ins and extensions to do just about anything. The advanced scripting interface allows everything from the simplest task to the most complex image-manipulation procedures to be easily scripted.

Complete details of GIMP, its specifications, operations and applications can be found on the website: www.gimp.org.

Erchonia Corporation conducted a validation study of the GIMP 2.8 software to validate both the method/tool and the measurement methodology for the specific application of the measurement of linear mm of clear nail prior to the initial LunulaLaser™ trial. The results of the validation study have been reviewed and accepted by the FDA as a validated means of measuring linear mm of clear nail in onychomycosis-infected toenails.

In this study, GIMP 2.8 will be used to measure the linear mm of clear nail of the onychomycosis infected great toenail from the digital toenail images captured, defined as the distance from the proximal nail fold to the most proximal area of nail dystrophy. A straight line will be created between the two points, and the GIMP 2.8 system software will formulate a measurement of that line. An example is shown in Figure 9 to the right, demonstrating how total nail length and linear measurement of mm of clear nail from the proximal nail fold to the most proximal area of nail dystrophy is determined.

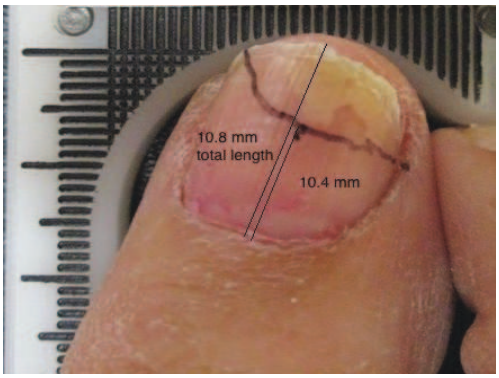


Figure 9: Application of the GIMP 2.8 to measurement of mm clear nail length

The 'GIMP 2.8 Software Measurement Methodology Instruction Sheet' contained in **Appendix A** details the instructions for operation and application of the GIMP 2.8 software system for measurement of linear mm clear nail measured from the proximal nail fold to the most proximal dystrophic component that will be followed by the study investigators.

NAIL ETCHING

A shallow etched horizontal line will be placed in the target study toenail, at the proximal-most area of dystrophy and aligned parallel to the horizontal portion of the proximal nail fold. This line will serve as both a marker for measuring nail growth rate as well as distance from the proximal nail fold to the most proximal point of the nail dystrophy.

PERCENTAGE OF CLINICAL ONYCHOMYCOSIS INVOLVEMENT DETERMINATION

Visual estimation of the % of clinical onychomycosis disease involvement in the target great toenail will be determined by the investigator based upon his or her professional and clinical knowledge and experience.

SUBJECT DAILY DIARY

On each day of the study, the subject will record the following measures in a Daily Diary supplied by the investigator at the test site on the day of the first treatment administration.

- ✓ Compliance with abstinence from other (non-study) treatments for toenail fungus
- ✓ Compliance with abstinence from the use of nail cosmetics on study toes
- ✓ Any changes in medication use and/or therapies engaged in from those reported at baseline evaluation
- ✓ Potential perceived adverse events, as described in the 'Adverse Events' evaluation tool listed below.
- ✓ Any study-related comments, if and as desired

ADVERSE EVENTS EVALUATION

Prior clinical trials applying the Erchonia® LunulaLaser™ to toenail onychomycosis have not yielded any adverse events or reactions. Numerous additional clinical trials applying Erchonia® low level laser for various different indications have also not yielded any adverse events or reactions. Therefore, it is unlikely and not expected that any adverse events will result from implementation of this clinical study protocol.

However, potential adverse events that may feasibly occur from application of the Erchonia LunulaLaser™ include, but are not necessarily limited to: skin irritation, discoloring, rash, indentations and re-infection.

Adverse events evaluation will include formal evaluation of the treatment area and surrounding skin area by the investigator at each subject test site visit as well as review of any potential events reported or recorded in the Subject Daily Diary by the subject. Adverse events evaluation will be conducted for all toenails on the foot with the study treated toenail.

Any and all observation(s)/report(s) of potential adverse events, whether observed by the investigator or reported by the subject, whether anticipated or unanticipated, will be recorded on the Adverse Events case report form and subsequently evaluated by the investigator for its relation to the study procedure and whether or not any corrective action needs to be taken. All potential adverse events recorded will be appropriately reported to the governing IRB, as applicable.

STUDY PROCEDURE PROTOCOL

PRE-TREATMENT ACTIVITIES

STUDY QUALIFICATION EVALUATION: PROGRESSIVE EVENTS

SIGNING OF INFORMED CONSENT FORM

The PI commences by presenting and reviewing in detail the items in the informed consent form with the individual and answering any questions he or she may have. To proceed, the individual must willingly sign the informed consent form.

ASSIGNMENT OF SUBJECT IDENTIFICATION NUMBER

The subject is assigned a unique subject identification number based upon his or her order of entry into the study. Additional information about the informed consent and subject ID number assignment is contained in a later section of the protocol titled, "SAFETY AND CONFIDENTIALITY ISSUES."

INCLUSION/EXCLUSION CRITERIA

MYCOLOGICAL CRITERIA EVALUATION

The BioMed InTray™ DM culture will be performed on the intended study great toenail:

Inclusion Criteria: The subject will continue to the Clinical Criteria Evaluation phase if the results of the InTray™ DM culture results satisfy both of the following inclusion criteria:

- Detection of the presence of fungal infection
- Identification of Trichophyton rubrum (T. rubrum) or other common dermatophyte

Exclusion Criteria: The subject's participation in the study will end at this time if the results of the InTray™ DM culture satisfies either or both of the following exclusion criteria:

- Lack of detection of the presence of fungal infection
- Identification of a fungal species other than Trichophyton rubrum (T. rubrum) or other common dermatophyte, such as C. albicans or mixed dermatophyte/Candida infection or non-fungal organisms such as mold or bacteria

CLINICAL CRITERIA EVALUATION

Inclusion Criteria: The subject will continue to the Baseline Assessment phase if all of the following clinical inclusion criteria are satisfied:

- Visual clinical presentation of onychomycosis in the target great toenail is distal subungual onychomycosis (DSO), visualized as a nail with normal surface texture and thickness but variable "bays" of white nail that extend from the distal nail tip proximally into the area of the nail bed
- Clinical involvement of onychomycosis in the target toenail is between 20%–50%
- Subject is willing and able to refrain from employing other (non-study) treatments (traditional or alternative) for his or her toenail onychomycosis throughout study participation.
- Subject is willing and able to refrain from the use of nail cosmetics such as clear and/or colored nail lacquers throughout study participation
- Male or female
- 18 years of age or older

Exclusion Criteria: The subject's participation in the study will end at this time of any one or more of the following clinical exclusion criteria are satisfied:

- Visual clinical presentations of onychomycosis in the target great toenail that are inconsistent with the clinical presentation of distal subungual onychomycosis (DSO), in whole or in part (i.e. indicative of mixed etiology); specifically visual clinical presentations consistent with one or more of the following:
  - *Proximal subungual onychomycosis (PSO)*: visualized as a white discoloration below the nail plate at the base of the nail, near the lunula. The distal nail retains normal appearance and texture. PSO involves infection near the matrix, deep to the nail. It may be associated with trauma to the nail or to immune compromise
  - *Superficial white onychomycosis (SWO)*: visualized by the appearance of a white coating on the nail surface that can be eliminated by filing or buffing the surface of the affected portion of the nail
  - *Complete dystrophy*: Nails which are 100% dystrophic are manifested by yellowing and thickening of the entire nail unit
  - *Other nail changes*: Nails that visually present with changes that appear as parallel lines, small pinpoint depressions, brown spots, black or brown linear streaks, complete yellowing of all nails without textural change, green debris below the nail, or notches in the nail margin
- Less than 2mm clear (unaffected) nail plate length beyond the proximal fold
- Presence of dermatophytoma (thick masses of fungal hyphae and necrotic keratin between the nail plate and nail bed)
- Infection involving lunula e.g., genetic nail disorders, primentary disorders
- Severe plantar (moccasin) tinea pedis
- Psoriasis of the skin and/or nails, lichen planus, or other medical conditions known to induce nail changes
- Onychogryphosis
- Trauma from ill-fitting shoes, running, or overly-aggressive nail care
- Previous toenail surgery
- Uncontrolled diabetes mellitus
- Peripheral vascular disease
- Recurrent cellulitis
- Lymphatic insufficiency
- Immune compromise (whether due to underlying medical disorders or immunosuppressive treatments)
- Other compromised states of health
- Known photosensitivity disorder
- Use of oral antifungal drugs in the prior 6 months
- Use of topical treatment of the skin or nails within the prior 2 months
- Any abnormality of the toenail that could prevent a normal appearing nail from occurring if clearing of infection is achieved.
- Current trauma, open wound on or about the treatment area
- Deformity of the target toe/toenail secondary to fungal infection/onychomycosis due to prior injury, surgical procedures or another medical condition
- Pregnant or planning pregnancy prior to the end of study participation
- Serious mental health illness such as dementia or schizophrenia; psychiatric hospitalization in the past two years

- Developmental disability or cognitive impairment that would preclude adequate comprehension of the informed consent form and/or ability to follow study subject requirements and/or record the necessary study measurements
- Involvement in litigation and/or receiving disability benefits related in any way to the parameters of the study
- Participation in a clinical study or other type of research in the past 30 days.

BASELINE ASSESSMENT

BASELINE VARIABLES

- Baseline Target Toenail Variables
- Prior Treatment Approaches
- Concomitant Medications And Therapies
- Notation of any other toenail(s) on the target foot that appear to be infected with onychomycosis.
- Subject Demographics

CLINICAL AND OUTCOME EVALUATIONS

- Nail Etching
- Photographic Documentation
- Measurement of mm of Clear Nail
- Percentage Of Clinical Onychomycosis Involvement Determination

TREATMENT ADMINISTRATION PHASE

GENERAL ASSESSMENT CONDITIONS

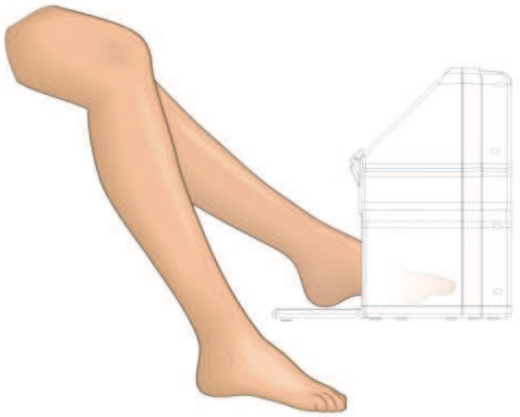
Subjects must agree to not partake in any non-study treatment(s) for toenail onychomycosis (including oral medications and nail lacquer, non-alternative therapies such as acupuncture and home remedies), or to use any toenail cosmetics throughout the course of study participation.

TREATMENT ADMINISTRATION PROTOCOL

- The treatment administration phase of the study will last 11 months, comprised of an initial weekly treatment administration phase followed by an every two month maintenance treatment administration phase.
- The treatment administration phase of the study will commence on the same day as the Baseline Assessment visit, following completion of all activities related to the Baseline Assessment visit.
- The initial weekly treatment administration phase will last three weeks and involve four in-office treatment administrations with the Erchonia® LunulaLaser™, each one week (7 days) apart (i.e. Days 1, 8, 15 and 22).
- The maintenance treatment administration phase involves the subject returning to the test site one time every eight weeks (2 months) following the final weekly treatment administration for a single maintenance treatment administration with the Erchonia® LunulaLaser™ (i.e. end of months 3, 5, 7, 9 and 11).
- Each treatment administration lasts 12 minutes.
- Each treatment administration is administered by the study investigator at the test site.

The treatment administration protocol for each of the Erchonia® LunulaLaser™ laser treatment administration is as follows:

1. The subject is seated comfortably with the LunulaLaser™ placed on the floor in front.
2. The subject is fitted with the safety glasses.
3. The subject places the foot with the infected great toenail to be treated in this study on the treatment platform inside the Erchonia LunulaLaser™ laser device – heel on the back, toes on the front platform under the laser output heads, as shown in the figure below.



4. The Erchonia LunulaLaser™ is activated such that the laser light is directed at the great toenail at a distance of approximately 4 inches above the toenail.
5. The dual wavelengths of 405 nm and 635nm are activated simultaneously for 12 minutes of total treatment administration time.

   By design of the device, it is not possible for the subject to move the foot's position to any significant degree that would impact the intended exposure to the laser procedure, particularly considering that the laser beam is designed to cover the entire treated toe region. The exception would be if the subject withdrew his or her foot entirely from the laser device mid-treatment administration, in which case, the foot would be re-inserted at once and the remaining minutes of exposure completed such that the total maximum exposure time in one session would not ever exceed the pre-determined 12-mintues. If the subject was unable or refused to re-insert his or her foot for any reason, the subject would be disqualified from further study participation at that time.

6. The subject removes his or her foot from the Erchonia LunulaLaser™.
7. The laser treatment administration is complete.
8. The subject removes the safety glasses, and the treatment procedure administration protocol is completed.

STUDY EVALUATION TIMELINE AND MEASURES

The evaluation time points and associated measures (as outlined in the STUDY TEST BATTERY section above) to be evaluated during the course of this study are as follows:

DAILY COMMENCING ON THE DAY OF THE FIRST TREATMENT ADMINISTRATION THROUGH TO STUDY END

- Subject Daily Diary

AT EACH TREATMENT ADMINISTRATION AND EVALUATION VISIT

- Adverse Events Evaluation

AT EACH TREATMENT ADMINISTRATION VISIT COMMENCING WITH THE FOURTH WEEKLY TREATMENT ADMINISTRATION (END OF WEEK 4, MONTHS 3, 5, 7, 9, AND 11)

- Photographic Documentation
- Measurement of mm of Clear Nail
- Percentage of Clinical Onychomycosis Involvement Determination
- Mycological Evaluation: InTray™ DM Culture

STUDY ENDPOINT EVALUATION: 12 MONTHS FOLLOWING THE INITIAL TREATMENT ADMINISTRATION

- Photographic Documentation
- Measurement of mm of Clear Nail
- Percentage of Clinical Onychomycosis Involvement Determination
- Mycological Evaluation: InTray™ DM Culture

TWO MONTHS AFTER STUDY ENDPOINT EVALUATION POST-TREATMENT EVALUATION

- Photographic Documentation
- Measurement of mm of Clear Nail
- Percentage of Clinical Onychomycosis Involvement Determination
- Mycological Evaluation: InTray™ DM Culture

TABLE OF SUBJECT EVENTS

The following table provides a progressive summary of subject events throughout this study.

PRE-TREATMENT ACTIVITIES

STUDY QUALIFICATION EVALUATION

- A potentially well-suited and interested candidate for participation in the study attends the investigator's office.
- The investigator reviews the informed consent form with the candidate.
- If the candidate continues to be interested and voluntarily signs the informed consent form, Study Qualification Evaluation is performed: Mycological Evaluation followed by Clinical Evaluation criteria.
- A fully qualified subject progresses to the Baseline Assessment phase.

BASELINE ASSESSMENT

BASELINE VARIABLES

- Baseline Target Toenail Variables
- Prior Treatment Approaches
- Concomitant Medications and Therapies
- Notation of all infected toenails on the target foot
- Subject Demographics

CLINICAL AND OUTCOME EVALUATIONS

- Nail Etching
- Photographic Documentation
- Measurement of mm of Clear Nail
- Percentage of Clinical Onychomycosis Involvement Determination

TREATMENT ADMINISTRATION PROTOCOL

Each participant receives 4 12-minute treatment administrations with the Erchonia® LunulaLaser™ over 4 consecutive weeks, each 7 days apart, followed by one treatment every two months thereafter. Each treatment is administered at the test site.

STUDY EVALUATION TIMELINE AND MEASURES

DAILY COMMENCING ON THE DAY OF THE FIRST TREATMENT ADMINISTRATION THROUGH TO STUDY END

- Subject Daily Diary

AT EACH TREATMENT ADMINISTRATION AND EVALUATION VISIT

- Adverse Events Evaluation

AT EACH TREATMENT ADMINISTRATION AND EVALUATION VISIT COMMENCING WITH THE FOURTH WEEKLY TREATMENT ADMINISTRATION

- Photographic Documentation
- Measurement of mm of Clear Nail
- Percentage of Clinical Onychomycosis Involvement Determination
- Mycological Evaluation: InTray™ DM Culture

STUDY ENDPOINT EVALUATION: 12 MONTHS FOLLOWING THE INITIAL TREATMENT ADMINISTRATION

- Photographic Documentation
- Measurement of mm of Clear Nail
- Percentage of Clinical Onychomycosis Involvement Determination
- Mycological Evaluation: InTray™ DM Culture

TWO MONTHS AFTER STUDY ENDPOINT EVALUATION POST-TREATMENT EVALUATION

- Photographic Documentation
- Measurement of mm of Clear Nail
- Percentage of Clinical Onychomycosis Involvement Determination
- Mycological Evaluation: InTray™ DM Culture

SAFETY AND CONFIDENTIALITY

ADVERSE EVENTS

At each evaluation and measurement point throughout the clinical study (as outlined in the STUDY EVALUATION TIMELINE section above), and at any other time throughout the duration of the clinical trial that is necessary, any and all potential adverse events reported by a subject or observed by an investigator will be recorded on the case report form, and subsequently evaluated by the investigator for its relation to the study procedure and whether or not any corrective action needs to be taken. All potential adverse events recorded will be applicably and appropriately reported to the governing IRB.

It is unlikely and not expected that any adverse events will result from implementation of this clinical study protocol. Prior clinical trials using Erchonia® low level lasers, including the LunulaLaser™ have not yielded any adverse events or reactions. However, potential adverse events that may feasibly occur from application of the Erchonia LunulaLaser™ and that will be specifically evaluated during scheduled adverse events evaluations include, but are not necessarily limited to: skin irritation, discoloring, rash, indentations and infection. Adverse events evaluation will be conducted for all toes/toenails on the study treated foot.

PRIVACY AND CONFIDENTIALITY

Records for each subject in this clinical study will be maintained in separate files in a locked filing cabinet at the respective test site. The investigator at the test site will be responsible for ensuring that all records for a subject pertaining to his or her participation in the clinical study are stored in that subject's file at all times other than when information is being recorded on them.

Copies of all of the subject case report forms will be made and supplied to Regulatory Insight, Inc. and Erchonia Corporation. Regulatory Insight, Inc. and Erchonia Corporation will maintain these copies in a separate clinical study file that is kept in a locked filing cabinet on their respective premises. The original records will be maintained at the respective test sites.

Subjects' identities will be kept confidential by assigning each subject a subject ID upon acceptance into the study. The subject ID will comprise the investigator's two initials (first and last name initials) and a three-digit number that will be based upon the subject's order of entry into the clinical study. Each test site will be assigned a unique range of numbers. Test site #1 will be assigned numbers 001 to 100. Test site #2 will be assigned numbers 101 to 200, and so on. For example, the eighth subject to be enrolled at test site #2 with Principal Investigator John Black would have a subject ID of JB108. Neither the study Sponsor nor Regulatory Insight, Inc. will receive any additional identifying information about a subject and will therefore have no way of linking a subject ID to a particular subject and his or her results.

MONITORING OF THE CLINICAL STUDY

Training, set-up and monitoring of the clinical study will be according to all of the applicable protocols and procedures contained within the Erchonia Corporation Clinical Trials Monitoring Plan, Version 2.0, March 15, 2015, contained in **Appendix E** of this clinical study protocol document.

Furthermore, monitors will train the study investigators to the following document: Erchonia Corporation Clinical Trial Investigator Responsibilities Procedure, Version 1.0, April 3, 2015, contained in **Appendix F** of this clinical study protocol document.

STATISTICAL ANALYSIS PLAN

The aim of this study is to determine if there is a treatment effect of application of the Erchonia® LunulaLaser™ for individuals with onychomycosis of the great toenail.

PRIMARY EFFICACY OUTCOME EVALUATION

The primary efficacy outcome measure in this study is the Complete Cure Rate at study endpoint.

Individual Subject Success Criteria: 'Complete Cure' criteria is defined as a subject/toenail satisfying both the 'Clinical Cure' and the 'Mycological Cure' criteria in order to be considered a study responder, defined as follows:

(i) Clinical Cure: Measurement of clear nail increase as the following:

- at least 12 mm increase in clear nail of the great toenail, with evidence of distal growth of the affected area, 12 months after the first treatment;

or

- complete clearance 12 months after the first treatment if less than 12 mm distal nail was involved prior to treatment.

The response should be progressive in at least 2 sequential timepoints that are at least 3 months apart, with projected increase of at least 1 mm per month.

(ii) Mycologic Cure: Negative Fungal Culture results

Overall Study Success Criteria is defined as both of:

(i) a minimum 70% Clinical Cure Clear Nail Responder Rate;

AND

(ii) Among toenails which are deemed responders based on the Clinical Cure Clear Nail criteria, at least 80% will also demonstrate Mycological Cure.

STUDY END POINT EVALUATION

The study end point evaluation at which Individual Great Toenail Success and Overall Study Success will be evaluated is at 12 months following initiation of the treatment administration phase.

HYPOTHESES

- ***Null Hypothesis***: The Responder Rate for subjects/toenails attaining Clinical Cure will be 70% or greater and 80% or more of those subjects will also attain Mycological Cure.
- ***Alternative Hypothesis***: The Responder Rate for subjects/toenails attaining Clinical Cure will be less than 70% and/or fewer than 80% of those subjects will also attain Mycological Cure.

PRIMARY EFFICACY OUTCOME STATISTICAL EVALUATION METHODS

- **Intent to Treat (ITT) Principle**: Primary efficacy analysis will be according to the intent to treat (ITT) principle; wherein subjects will be included in the analysis if they had a valid Baseline Assessment visit.

- **Missing data** will be handled through *Last Observation Carried Forward (LOCF)*: by carrying forward the last recorded observation to fill in the subsequent missing value.

  Subjects who drop out or are lost to follow-up will be considered as treatment failures (non-responders) in the statistical evaluation.

- **Per-Protocol Analysis** will also be performed for the set of all subjects who completed the study according to the full protocol.

- **Primary analysis of efficacy** according to intent to treat (ITT) analysis will be through determination of satisfaction of the Overall Study Success Criteria.

SECONDARY EFFICACY OUTCOME EVALUATION

The pre-specified secondary efficacy endpoints are
(i) Clinical Efficacy Rate at week 52, defined as < 10% affected target nail area
(ii) Mycological Cure Rate at week 52 (negative culture), and
(iii) Unaffected New Nail Growth at week 52 (change from baseline in healthy nail measurement).

Secondary endpoints will be measured in sequential order.

ADDITIONAL SUPPORTIVE OUTCOME EVALUATIONS

- Responder Rates across progressive evaluation visits
- Percentage of clinical onychomycosis involvement determinations across progressive evaluation visits
- Change in mm clear nail across progressive evaluation visits.

SUBGROUP AND COVARIATE ANALYSES

Study outcome will also be performed for the following subgroups and covariates, as applicable:

- Study test site
- Category of % of baseline toenail onychomycosis involvement:
  - 20%-34% toenail onychomycosis involvement
  - 35%-50% toenail onychomycosis involvement

- Baseline Fungal Culture Results Category according to type of organism identified.
- Baseline % of toenail onychomycosis disease involvement
- Right versus left great toenail
- Duration of toenail onychomycosis at baseline
- Age
- Gender
- Ethnicity

EVALUATION OF SUBJECT DAILY DIARY MEASURES

The information recorded by the subjects in the Subject Daily Diary on each day of the study evaluation period will be evaluated as follows:

- The impact on study outcome of reported notable non-compliance with other (non-study) toenail fungus treatment abstinence.
- The impact on study outcome of reported notable non-compliance with abstinence from use of toenail cosmetics on the study great toenail(s)
- Any changes in medication and therapy use across study progression relative to that reported at baseline and the impact on study outcome.
- Any potential perceived adverse events will be reported and evaluated
- Any comments provided by subjects will be reported and evaluated.

SAFETY ANALYSES

Safety analyses will be based on all subjects who signed the informed consent form and were enrolled in the study. Safety will be assessed by evaluating the incidence, frequency and severity of observed and/or reported adverse events. Specific safety evaluation will be made with respect to re-infection rate, as applicable. Safety evaluation will be conducted for all toenails on the study treated foot, as applicable.

INFORMED CONSENT

- Informed consent will be an agreement between the individual investigator and each subject, having the capacity to understand and make an informed decision. Consent will be obtained prior to each potential subject's participation in this clinical study.
- Each subject participating in this clinical study will be made aware of the fact that his or her participation involves research and the intent of the research, the expected duration of his or her participation and a description of the procedures that will be followed.
- Each subject will be made aware of the reasonably expected benefits he or she might receive, as well as any risks or potential discomfort that are involved.
- Each subject will also be made aware of alternative treatments available to him or her.
- Each subject will be made aware that his or her records will remain confidential, but that the FDA and the IRB has the right to inspect his or her records.
- Each subject will be told that his or her participation in the clinical study is voluntary, without force or influence from the investigator or sponsor.
- Each subject will be given the name and method of contacting the appropriate person(s) to answer his or her questions about the research and in the event of a research-related injury.

RESEARCH CONSENT FORM

TITLE:	An Evaluation of the Effect of the Erchonia LunulaLaser™ for the Treatment of Toenail Onychomycosis
STUDY IRAS ID	277609
SPONSOR:	Erchonia Corporation Melbourne, Florida United States
INVESTIGATOR:	Dr. Robin Stones
SITE(S):	**Blemish Clinic** **40 Market Street** **Edenfield, Cheshire BL00JN**
STUDY-RELATED PHONE NUMBER(S):	01706822689

- I confirm that I have read the information sheet dated June 11rd, 2020 (version 4..0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

  ☐ Yes ☐ No

- I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.

  ☐ Yes ☐ No

- I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from Erchonia Corporation, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

  ☐ Yes ☐ No

- I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers.

  ☐ Yes ☐ No

- I agree to my General Practitioner being informed of my participation in the study.

  ☐ Yes ☐ No

- I agree to take part in the above study.

  ☐ Yes ☐ No

_____

Subject Name (printed)

CONSENT SIGNATURE:

_____ _____

Signature of Subject (18 years and older) Date