CLINICAL STUDY

PRODUCT: LIVARIZ ANTIVARICOSE TREATMENT

LABORATORY

Induchem
S. de R.L. de C.V.

CLIENT: Marcas de Renombre S.A. de C.V.

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PRODUCT DESCRIPTION

PRODUCT NAME: LIVARIZ ANTIVARICOSE TREATMENT

PRODUCT INFORMATION: Helps to reduce the symptoms of varicose vines and optimizes micro-circulation through a new cryogenic technology applied by a spray. Totally eliminates the syndrome of “fallen asleep” legs in seconds.

PRODUCT PRESENTATION: 125ml. spray in an aluminum tube – 103.2ml cream in a plastic tube

MADE BY: Marcas de Renombre S.A. de C.V. (MEXICO)

DATE: September-2009

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1. The Circulatory System.

The circulatory system can be considered as composed of two parts: the systemic circulation, which serves the body as a whole except for the lungs, and the pulmonary circulation, which carries the blood to and from the lungs. The organs of circulatory system consist of vessels that carry the blood and a muscular pump, the heart, muscular organ that pumps blood to all parts of the body.

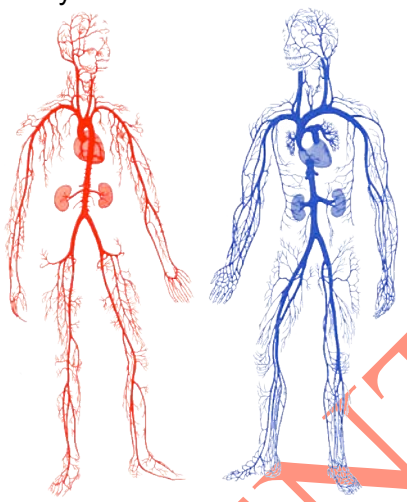


Fig. 1 Arterial and venous systems

In the pulmonary circuit, blood leaves the heart through the pulmonary arteries, goes to the lungs, and returns to the heart through the pulmonary veins.

In the systemic circulation, which serves the body, except for the lungs, oxygenated blood from the lungs returns to the heart from two pairs of pulmonary veins, a pair from each lung. It enters the left atrium, which contracts when filled, sending blood into the left ventricle (a large percentage of blood also enters the ventricle passively, without atrial contraction). The bicuspid, or mitral, valve controls blood flow into the ventricle. Contraction of the powerful ventricle forces the blood under great pressure into the aortic arch and on into the aorta. The coronary arteries stem from the aortic root and nourish the heart muscle itself.

Thus there are two circuits. Arteries always carry blood away from the heart and veins always carry blood toward the heart.

Three major arteries originate from the aortic arch, supplying blood to the head, neck, and arms. The other major arteries originating from the aorta are the renal arteries, which supply the kidneys; the celiac axis and superior and inferior mesenteric arteries, which supply the intestines, spleen, and liver; and the iliac arteries, which branch out to the lower trunk and become the femoral and popliteal arteries of the thighs and legs, respectively.

1.1 The Blood Vessels

There are three types of vessels – arteries, veins, and capillaries.

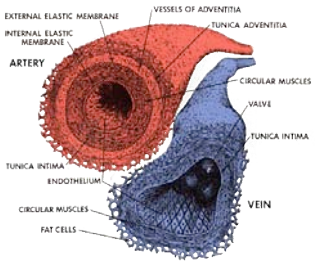


Fig. 2 Blood vessel anatomy

Fig. 3 Capillary Bed

The arterial walls are partially composed of fibromuscular tissue, which help to regulate blood pressure, force exerted by the blood upon the walls of the arteries. The pressure in the arteries originates in the pumping action of the heart, and pressure waves can be felt at the wrist and other points where arteries lie near the surface of the body and flow.

At the far end of the network, the capillaries converge to form venules, which in turn form veins. The inferior vena cava returns blood to the heart from the legs and trunk; it is supplied by the iliac veins from the legs, the hepatic veins from the liver, and the renal veins from the kidneys. The subclavian veins, draining the arms, and the jugular veins, draining the head and neck, join to form the superior vena cava. The two vena cavae, together with the coronary veins, return blood low in oxygen and high in carbon dioxide to the right atrium of the heart.

Arteries have the property of elasticity, they can expand to accept a volume of blood, then contract and squeeze back to their original size after the pressure is released.
Veins do not have as many elastic fibers as arteries. Veins do have valves, which keep the blood from pooling and flowing back to the legs under the influence of gravity. When these valves break down, as often happens in older or inactive people, the blood does flow back and pool in the legs. The result is varicose veins, which often appear as large purplish tubes in the lower legs.

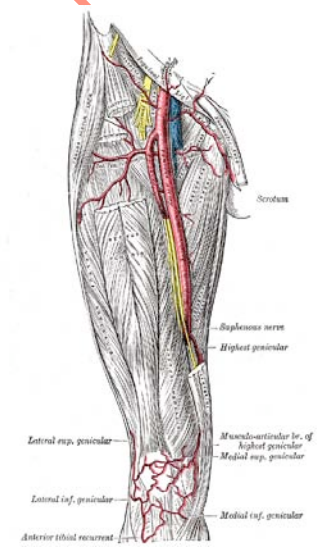


Fig.4 The femoral Artery

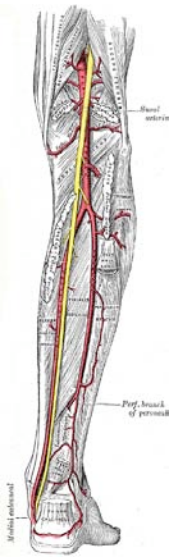


Fig. 5 The popliteal, posterior tibial and peroneal arteries.

2. Causes of blood circulation disorders in the legs

Blood circulation disorders are not due to only one cause but to a combination of factors.

- Extrinsic factors:

  -Environmental temperature. Since heat worsens leg tiredness, this disorder is often noticed during the summer. Sunbaths, saunas and hot baths worsen this condition.
  -Unhealthy diet
  -Wearing tight clothes
  -Sedentary; standing or sitting for long time periods

- Intrinsic factors:
  -Gender. In general, women are more likely to suffer this condition because of hormonal factors and pregnancy
  -Age. The incidence of leg tiredness increases with age.
  -Inheritance: antecedents of impaired blood circulation in the family
  -Pathologies: obesity, cardiac illness, hypertension, diabetes.

Several research studies have demonstrated the influence of such factors on leg blood circulation.
Callejas et al. (2004) published a study carried out in 606 primary care medical centers across Spain. Table 1 shows their results.

	Patients suffering from this condition (%)					
	Symptoms of the tired legs syndrome					
	Heaviness	Itching	Pain	Cramps	Edema	Changes in the skin
Influence of age						
< 45 years	83,1	50,2	38,3	32,3	38,3	10,9
45-65 years	86,7	56,7	51,4	44,2	53,1	20,7
> 65 years	86,0	56,1	51,5	42,2	61,0	30,1
Antecedents						
With antecedents	88,0	57,7	51,3	41,5	55,6	23,5
Without antecedents	83,1	51,9	42,6	37,0	46,4	17,0
Body mass index (Kg./m^2)						
< 25	83,3	51,1	38,0	33,8	38,6	13,1
25-30	85,6	56,1	48,9	41,8	53,4	29,9
> 30	89,2	58,5	55,1	46,5	63,4	27,7
Physical activity						
Low	87,9	55,0	47,8	39,6	54,5	22,4
Moderate/intense	82,2	53,7	44,6	38,6	45,7	15,8

Table. 1. Study of the influence of several factors on tired legs syndrome-associated symptoms.

Symptoms were found to occur more frequently in subjects with lower physical activity, higher body mass index, older age and impaired blood circulation antecedents.

3. Blood Circulation disorders in the legs.

Disorders in the circulatory system are most likely to occur in the lower limbs because blood must return against gravity.

Several mechanisms in the circulatory system ensure efficient blood return.

- The endothelium in the blood vessels' walls is **elastic**.
- Contractions of the muscles surrounding the blood vessels pump the blood, thus promoting blood return.
- Veins contain pocket-valves that allow blood flow **only** towards the heart.

Blood circulation disorders in the lower limbs affect increasing proportions of the human population. It is estimated that 80% adults suffer from the tired legs syndrome at any time of their lives.
The most frequent symptoms are:

- leg heaviness
- tiredness
- leg and ankle swelling
- pain
- itching, cramps and varicose veins (severe cases)

3.1. The tired legs syndrome

The tired legs syndrome seems to be associated to deficiencies in the return blood circulation due to impaired elasticity and tone of the endothelial and the muscular tissue, respectively. The outcome is **slower blood circulation**, which may produce blood **stagnation** in calves and ankles. Increased hydrostatic pressure in these areas promotes fluid movement towards the tissues outside the circulatory system eventually producing swelling or **edema**.

Varicose veins

Varicose veins are enlarged veins. Any vein may become varicose, but the veins most commonly affected are those in legs and feet. That is because standing and walking upright increases the pressure in the veins of the lower body.

For many people, varicose veins and spider veins — a common, mild variation of varicose veins — are simply a cosmetic concern. For other people, varicose veins can cause aching pain and discomfort. Sometimes varicose veins lead to more-serious problems. Varicose veins may also signal a higher risk of other circulatory problems.

3.2. Prevention and cosmetic treatment

Improving circulation and muscle tone can reduce risk of tired legs syndrome, developing varicose veins, or getting additional ones.

Measures that can help prevent or decrease the discomfort of tired legs syndrome and help improve swelling include:

Blood circulation stimulatory activity
By improving the elasticity of the blood vessels and the muscular tone of the muscles surrounding vessels, helps veins and leg muscles move blood more efficiently.

Anti-edema activity
By Improving capillary resilience and reducing capillary permeability

Anti-inflammatory and calming component
Inhibiting lipoxygenase an enzyme involved in allergic and inflammatory diseases and the formation of prostaglandins as inflammation mediator.

4. LIVARIZ INFORMATION

Complex with plant active ingredients, that help relieve leg tiredness and heaviness due to its blood circulation promoting and swelling reducing actions, which provides relaxation and wellness.
Its activity results from the combined actions of its active principles: naringin, hesperidin, rutin and ruscogenin, which stimulate blood circulation and reduce edema. While troxerutin provides anti-inflammatory properties.

4.1 Active Ingredients

Livariz Spray: **Troxerutin; Ac. Esencial de Lavandin** (*lavandula hybrida*).

Livariz Crema: **Legactif** 7234 [Goldenrod extract (*Solidago virgaurea L.*) (rutin), Lemon peel extract (*Citrus limonum L.*) (naringin and hesperidin),Butcher's broom extract (*Ruscus aculeatus L.*) (ruscogenin)]; **Lavandin essential oil** (*Lavandula hybrida*); **Peppermint essential oil** (*Mentha piperita*).

4.2 Propiedades de los Activos:

- **Goldenrod (Rutin)**: Anti-edema
- **Lemon (Narangin and Hesperidin)**: Anti-edema
- **Butcher's broom (Ruscogenin)**: Blood circulation stimulator.
- **Troxerutin (Nature based bioflavonoid)** [tri-O(ß-hydroxyethyl)rutoside]: Blood circulation enhancing properties combined with anti-inflammation activities.
- **Lavandin essential oil:** (lavandula hybrida): Increases Circulation and reduces redness.
- **Mentha piperita essential oil:** acts as an analgesic and reduces pain.

Göbel H, Schmidt G, Dwoshak M, et al. Essential plant oils and headache mechanisms. *Phytomedicine* 1995;2:93–102.

5. ACTION MECHANISM

The action mechanism of **LIVARIZ** is based on the combined actions of **Legactif** active principles. The biological activities of naringin, hesperidin, rutin and ruscogenin, as well as their influence on the relief of leg tiredness, and the **Troxerutin** benefits: Lipoxygenase inhibitor, anti-inflammatory activity, Increase of capillary resistance, regulation of microcirculation and antioxidant.

5.1 Blood circulation stimulatory activity

A substance is considered to stimulate or enhance blood circulation if it improves the elasticity of the blood vessels and the muscular tone of the muscles surrounding vessels, consequently improving return circulation.
Butcher's broom is considered one of the main plant activators of blood circulation. Such a stimulating action is exerted by **ruscogenin** through an adrenergic type mechanism (= responding to adrenalin-like neurotransmitters) (Alonso J, 2004).

The general effects of ruscogenin have been evaluated in different studies:

- In a study with 100 volunteers suffering from several symptoms, such as varicose veins and haematoma, a 2-months treatment with 2-3 daily ruscogenin applications on the affected areas showed 82% average improvement.
- A recent study also evidenced the efficacy of a ruscogenin treatment on 148 volunteers with impaired leg circulation (Alonso J, 2004).

Therefore, butcher's broom is of great use to formulate cosmetic products with blood circulation stimulatory activity.

Troxerutin shows regulating features on the capillary resistance, thus supporting the microcirculation of blood and lymph.

Insufficient microcirculation is observed in conditions like e.g. heavy legs, couperose and cellulitis.

5.2 Anti-edema activity

A substance is considered to have anti-edema and decongestant activity if it improves the resilience and reduces the permeability of blood vessels. These combined actions reduce fatigue.

It has been found that the **citrus flavonoids** (**naringin** and **hesperidin**) have decongestant activity. Their biological activity counteracts blood vessels fragility. Actually, these flavonoids used to be called vitamin P for a long time, due to their effects on vessels permeability.

Hesperidin deficiency is known to produce blood circulation related problems in the lower limbs, such as edema, pain and night leg cramps. A number of studies have demonstrated that daily hesperidin intake improves blood circulation (Garg A., 2001).

The anti-edema effects of flavonoids are due to their high affinity for proline-rich proteins, such as collagen and elastin. These proteins are structural components in the blood

vessels. Degradation of these proteins by proteolytic enzymes weakens blood vessels, thus producing edema and lower limb swelling.

Hesperidin has been found to improve capillary resilience due to its inhibitory action on the hialuronidase enzyme (Garg A., 2001).

Rutin is also included in the flavonoids group. It has been used in pharmacy preparations to treat capillary fragility (Raj Narayana K. Raj., 2001) and has been useful to relief vein edema. Several studies have demonstrated that rutin is significantly efficient to reduce edema associated to impaired return circulation in the legs (PDR health).

Besides its important blood circulation stimulatory action, **ruscogenin** also has some decongestant activity. Several assays have shown that ruscogenin reduces capillary permeability and increases vessels resilience, thus yielding a characteristic vitamin P effect. The action mechanism is related to its antielastase activity, observed *in vitro* (Alonso J, 2004).

5.3 Other activities: anti-inflammatory and calming effects

Besides their direct actions on blood vessels, **LEGACTIF** active components have other complementary properties, which are beneficial to relief the tired legs syndrome.

Certain **citrus flavonoids**, such as **naringin** and **hesperidin**, have shown protective action against inflammatory disorders, *in vivo* as well as *in vitro* (Garg A., 2001). Furthermore, *in vivo* studies carried out with rats and mice have demonstrated that **hesperidin** has calming effects (Garg A., 2001), (Galati E.M.,1994).

The anti-inflammatory potential of **rutin** has been demonstrated in several animal studies (PDR health).
Anti-inflammatory and calming effects of Goldenrod have been observed in several experimental models e.g. carrageenan- and dextran-induced plantar edema in rats (ESCOP, 2003) (Alonso, J, 2004).

Some **ruscogenin** effects have been known for years, including its anti-inflammatory activity. This effect has been demonstrated by using the carrageenan-induced plantar edema test in mice (Alonso, J., 2004).

Rona Care® **Troxerutin** provides anti-inflammatory properties. Troxerutin is known to inhibit lipoxygenase, an enzyme involved in inflammation and allergic conditions.
The formation of inflammation mediators, is reduced to a great extent.
Alcaraz et al. (1986) Pharmazie 41, 299-300
Prostaglandins (1975) 10/6, 941-948

As phenolic compounds, bioflavonoids are antioxidants and metal chelators. As conjugated aromatic compounds, they act as screens protecting the plant cells against UV light and shorter-wave visible light.
Due to their chemical structure some bioflavonoids prevent viral, bacterial and fungal invasion by interfering with the growth, development and reproduction of the invaders.
Swain T: Plant Flavonoids in Biology and Medicine. 1 - 14. A. Liss. New York (1986)

Further evidence for the beneficial effects of flavonoids has been published:
Flavonoids influence the permeability of membranes;
Tukavkina NA, Rulenko IA, Kolesnik YuA: Voprosy Pitaniya 2, 33 - 38 (1996)
Bombardelli E, Morazzoni P: Chimicaoggi 25 - 28 (1993)

Flavonoids are inhibitors of a variety of enzymes, e.g. cyclooxygenase and lipoxygenase.
Havsteen B: Biochem. Pharmacol. 32, 1141 - 1148 (1983)

6. EFFICACY

Antioxidative power of Bioflavonoids

Many analytical methods are used to measure the antioxidative activity of substances.
As recently published elsewhere (Bünger et al., 2006), DPPH, TEAC and lipid assays have been identified as being the best methods for comparison of results as they are relatively robust and show good reproducibility both within a single laboratory and between laboratories. They were chosen therefore to evaluate the antioxidative power of bioflavonoids. The DPPH and TEAC assays measure the scavenging properties of an antioxidant, whereas the lipid assay reflects the protective properties of the antioxidant.

The following results were obtained:

Bioflavonoid	Key features	Antioxidative potential	Use level
RonaCare® Troxerutin	In addition to its antioxidant properties RonaCare® Troxerutin provides anti-inflammatory properties. Amongst the flavonoids troxerutin has the advantage of excellent solubility; therefore high concentrations can be achieved. This potentiates its effect. Troxerutin is one of the most stable bioflavonoids.		0,1 - 2 %

Antioxidative potential:
strong weak

6.1 LEGACTIF IN-VIVO EFFICACY

The goal of this assay was to evaluate **LEGACTIF** efficacy *in vivo* to relieve or eliminate the typical uneasiness associated to leg tiredness, under normal use conditions.
Its efficacy was objectively evaluated with instrumental measurements (Laser Doppler and ultrasonography) and centimetric measurements, as well as subjectively evaluated by means of a questionnaire. Measurements from a group of 10 volunteers treated with a formulation containing
LEGACTIF were compared with those from a group of 10 Placebo-treated volunteers. Table 2 lists the components of both formulations:

	Active formulation	Placebo formulation
Aqua (Water)	c.s.p.	c.s.p.
Acrylates C/10-30 Alkyl-Acrylate Crosspolymer	0.4%	0.4%
Glycerin	5%	5%
Triethanolamine (99%)	0.375%	0.35%
LEGACTIF	**1.5%**	**---**
Oleth-20	0.65%	0.65%
Parfum	0.15%	0.15%
Preservative	0.8%	0.8%

Table 2. Formulations used in the *in vivo* study

The experimental areas were calf and ankle. Each volunteer applied the product on one leg and one ankle while the contralateral areas remained untreated for control. Products were applied twice a day for 2 consecutive weeks.

Objective measurements - Laser Doppler

Blood flow was quantified by using a Periflux 5010 equipment (Perimed, Stokholm, Sweden). This equipment yields blood perfusion units results (BPU= "Blood Perfusion Units") based on the quantification of the microcirculation Doppler effect. Larger BPU readings mean more blood flow, in a certain area.

Measurements were instantaneous and consisted in the average of several instantaneous readings taken after obtaining a stable local record. Records were taken on all of the volunteers at the beginning and at the end of the study (D0 and D14).
Table 3 shows the averaged recorded values.

	% variation as compared to Control	
Day of the study	**LEGACTIF at 1.5%**	**Placebo**
D0	2.27	1.27
D14	30.94	-3.38

Table 3. Results of the laser Doppler flowmetry.

Figure 6 shows these results.

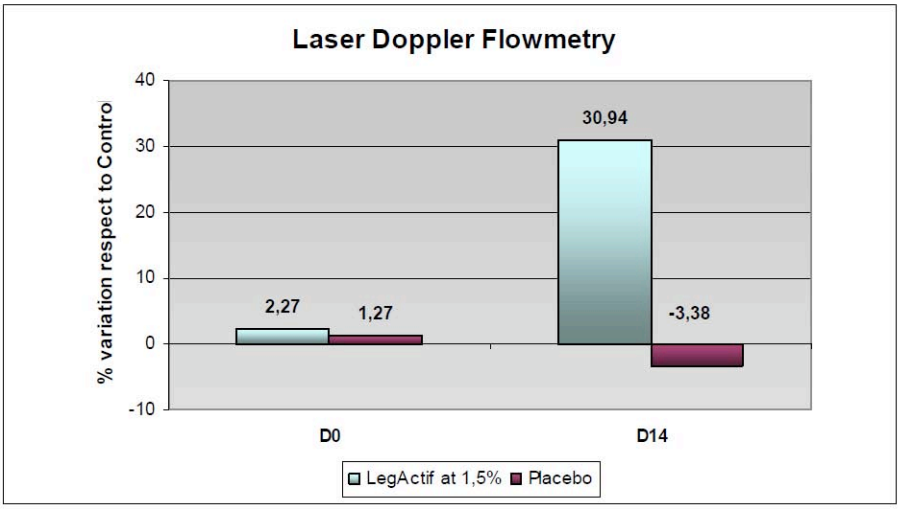


Fig. 6. Graphic plot of the results (laser Doppler).

LegActif-treated volunteers showed clear improvement in blood circulation. Blood flow increased by 31% as compared to Control.

Objective measurements – High resolution sonography

Skin sonographic images of the ankles were done with a Dermascan C® equipment (Cortex Technology,
Denmark). Sonographic images are produced by emitting and detecting ultrasound 17 MHz waves; results indicate the skin thickness in mm. In the tired legs syndrome, skin thickness is directly related to the degree of edema.

The most relevant images taken on D14 were recorded and compared with those taken on D0, in order to quantify edema reduction. This quantification was only carried out for volunteers who showed evident subcutaneous edema by the start of the study, namely 6 volunteers from the Placebo group and 4 volunteers from the **LEGACTIF** group.

Table 4 shows the averaged recorded values.

	Skin thickness (mm)	
Experimental area	**LEGACTIF at 1.5%**	**Placebo**
Treated (D14-D0)	-0.582	-0.082
Control (D14-D0)	-0.135	-0.104
Treated-Control	**-0.447**	**0.022**

Table 4. Results from the high resolution sonography.

Figure 7 illustrates these results.

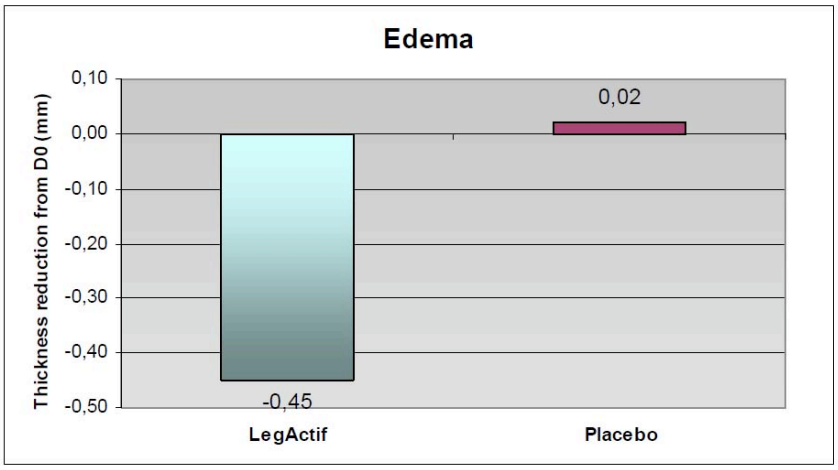
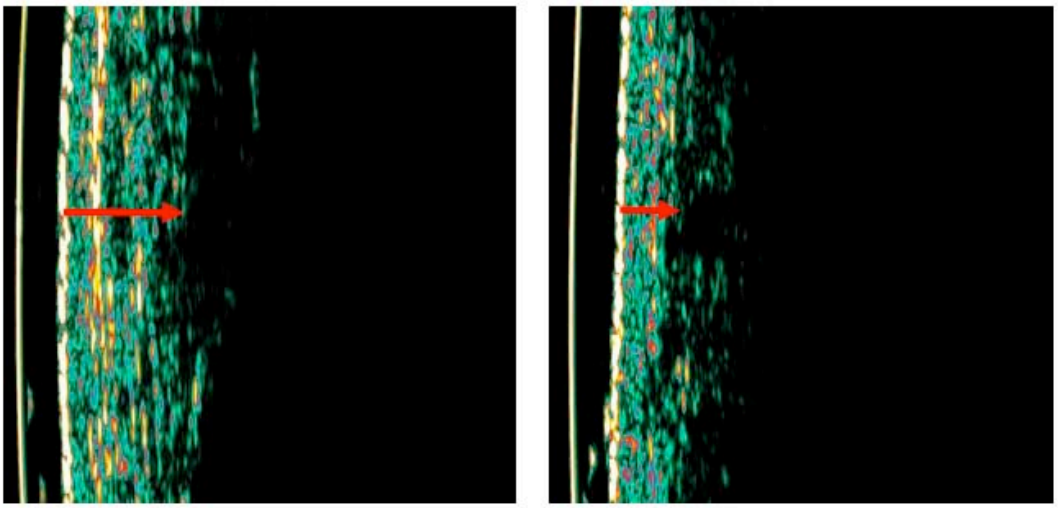


Fig 7. Graphic plot of the high resolution sonography results.

Volunteers in the Placebo-treated group showed no variation in skin edema, while those in the
LEGACTIF treated group showed clear cut skin thickness reduction, which indicated **anti-edema effects** for this active compound.

Figure 8 shows the images recorded for a **LEGACTIF** treated volunteer.

- Edema reduction Ultrasound images:



Initial situation Final situation

Figure 8. Images recorded for a **LEGACTIF**-treated volunteer. The left image corresponds to the beginning and the right image to the end of the treatment. Skin edema reduction can be observed.

Objective measurements – Centimetric measurements

Edema was also calculated by taking centimetric measurements on both experimental areas: calf and ankle. These measurements were recorded for all of the volunteers at the beginning and the end of the study (D0 and D14).
Table 5 shows the averaged recorded values.

Centimetric value (cm)

Experimental area	LEGACTIF at 1.5%	Placebo
Calf (Treated-Control)	-0.50	0.00
Ankle (Treated-Control)	-0.30	0.05

Table 5. Centimetric values.

Figure 9 and 10 show the results for calves and ankles, respectively.

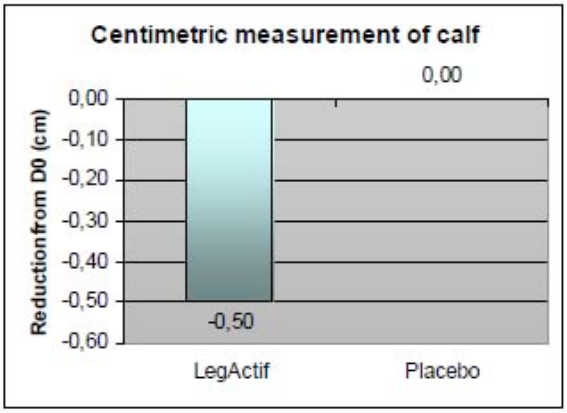


Fig. 9
Graphic plot of the centimetric results recorded on calf.

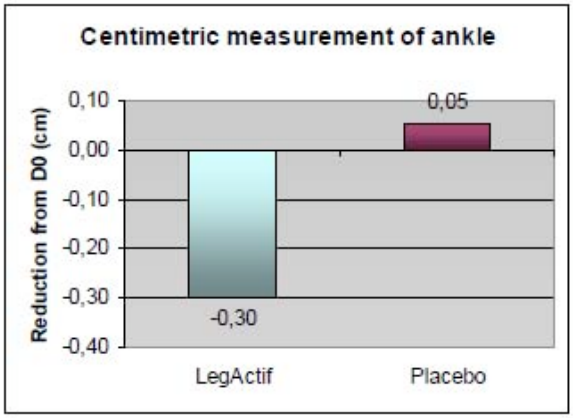


Fig. 10
Graphic plot of the centimetric results recorded on ankle.

Volunteers in the Placebo-treated group showed no variation in calf or ankle volume during the 14 days of the study. Conversely, those in the **LEGACTIF**-treated group showed clear cut edema reduction both in calves and ankles.

LegActif-treated volunteers showed clear 0.5 cm reduction in leg contour.

Figure 11 shows the results observed in a **LEGACTIF**-treated volunteer.

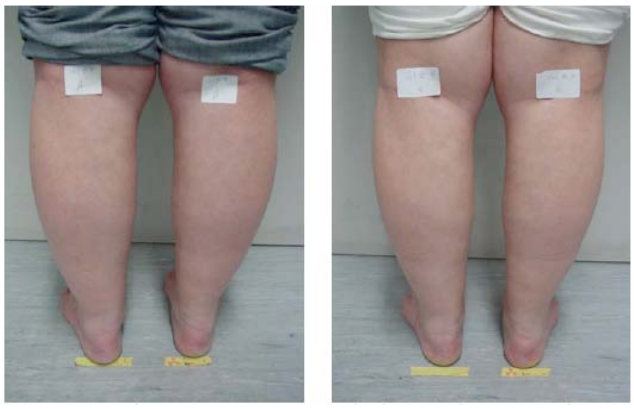


Figure 11. Photographs of a **LEGACTIF**-treated volunteer. The left image corresponds to the beginning and right image to the end of the study.

Subjective measurements – Questionnaire

After the termination of the treatment period, volunteers responded to a questionnaire about the cosmetic qualities and the efficacy of the tested product. Graphics 12 and 13 show the results.

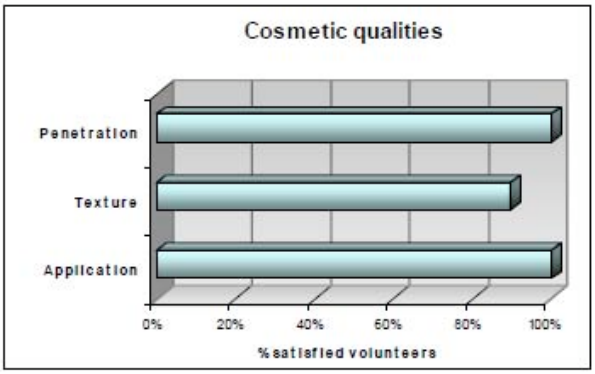


Fig. 12

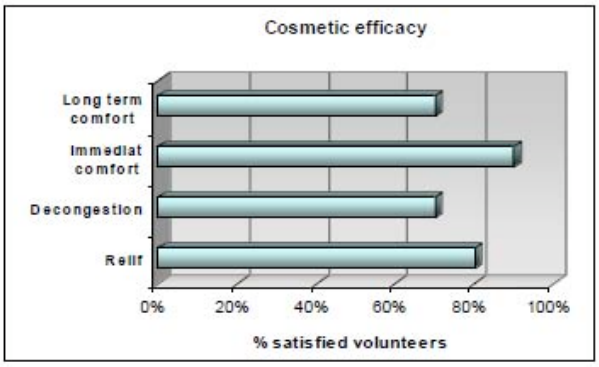


Fig. 13

Volunteers valued the product **LEGACTIF** highly, in relation to both cosmetic qualities and efficacy. In regards to efficacy, we would like to highlight that:

- **90%** volunteers reported **immediate comfort** after application.
- **80%** volunteers reported **relief of leg tiredness**.
- **70%** volunteers reported **decongestant effect**.
- **70%** volunteers reported **long term comfort**.

7. COSMETIC PROPERTIES AND APPLICATIONS

Cosmetic Properties	Efficacy	Cosmetic Applications
Blood circulation Stimulatory	31% increase in blood circulation (Doppler Laser flowmetry)	Blood circulation stimulatory Leg heaviness relief Vitalizing tonic for the legs
Anti-edema	Edema reduction: 0.45 mm reduction of skin thickness (sonographic images) Edema reduction: 0.5 cm and 0.3 cm centimetric reduction of calf and ankle, respectively	Decongestant Reduces fatigue Calming

Table 6. Summary of **LEGACTIF** properties, efficacy and cosmetic applications.

8. LITERATURE & REFERENCES

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CONTROLLED USE STUDY ANTI-VARICOSE VEIN TREATMENT

AMA Ref. No.: MS09.QUEST.L5004.REP.ETP

Date: July 31, 2009

Sponsor: Eternélle Pharma
Mariano Escobedo No. 510
3er piso, Interior 301,
Col. Anzures, C.P. 11590 México, D.F.

1.0 Objective:

The purpose of this study is to evaluate the efficacy of a topically applied regimen designed to improve varicose vein conditions on the leg area over a period of 6 weeks. Efficacy and tolerance were evaluated using panelist self-assessment via questionnaire responses.

2.0 Test Material:

2.1 Test Sample Description:

On May 22, 2009 two test products labeled Cryo-Action Spray, Step 1, Lot #: 190509 and Reactivating Cream, Step 2, Lot #: 160609, CAD0611 were received from Eternélle Pharma and assigned AMA Lab Nos. L-5005 and L-5004, respectively.

2.2 Handling:

Upon arrival at AMA Laboratories, Inc., the test material was assigned a unique laboratory code number and entered into a daily log identifying the lot number, sample description, sponsor, date received and test requested.

Samples are retained for a period of three months beyond submission of final report unless otherwise specified by the sponsor or if sample is known to be in support of governmental applications, in which case retained samples are kept two years beyond final report submission.
Sample disposition is conducted in compliance with appropriate federal, state and local ordinances.

2.3 Test Material Evaluation Prerequisite:

Prior to induction of a human test panel, toxicology, microbiology or in-vitro performance spectra may be required to assess the feasibility of commencement as dictated by an Institutional Review Board (IRB) described in Section 3.0.

2.3.1 Sponsor purports that prior to sample submission to AMA the following tests were conducted with no adverse results and that the test data are on file at their premises and have not been made available to AMA personnel:

- USP or CTFA Preservative Efficacy Test or equivalent
- 90 Day Accelerated Stability and Container Compatibility Study
- Fifty (50) person Repeat Insult Patch Test (RIPT) or equivalent

3.0 Institutional Review Board:

Reference: CFR Title 21 Part 56, Subparts A, B, C, and D. The IRB of AMA Laboratories, Inc. consists of 5 or more individuals, chosen from within the company for technical expertise and from the local community for lay interaction. The list of IRB members is kept on file at AMA Laboratories, Inc., and is available for inspection during the hours of operation.

4.0 Panel Selection:

4.1 Standards for Inclusion in a Study:

1. Subjects experiencing varicose veins on their legs.
2. Individuals who will complete a preliminary medical history mandated by AMA Laboratories, Inc.
3. Individuals who will read, understand and sign an informed consent document as required by Reference 21 CFR Ch. 1 Part 50, Subpart B. Consent forms will be kept on file and will be available for examination on the premises of AMA Laboratories, Inc., only.
4. Individuals in good health and free of any dermatological or systemic disorder, which would interfere with the results, at the discretion of the Investigator.
5. Individuals, who will be able to cooperate with the Investigator and research staff, be willing to have the test materials applied according the protocol and complete the full course of study.
6. Individuals with no known abnormal response to cosmetic products and who are willing to cooperate with the study requirements.
7. Individuals who will abstain from using any products intended to treat varicose veins other than the test products.

4.2 Standards for Exclusion from a Study:

1. Individuals who are under doctor's care.
2. Individuals taking medication which in the opinion of the Investigator would mask or interfere with the results.
3. Individuals with chronic skin allergies or skin conditions such as severe dermatitis, psoriasis, and/or eczema.
4. Individuals with uncontrolled diseases such as diabetes, hypertension, hyperthyroidism, or hypothyroidism.
5. Individuals with irritation or sensitivity to topically applied cosmetic products.
6. Female volunteers who indicate that they are pregnant or lactating.
7. Individuals with blemishes, nevi, sunburn, suntan, scars, moles, active dermal lesions or uneven pigmentation in the test sites.
8. Individuals participating in any clinical research study at another facility or with a doctor's office at the commencement and duration of the study.

4.3 Recruitment:

Panel selection is accomplished by advertisements in local periodicals, community bulletin boards, phone solicitation, electronic media or any combination thereof.

4.4 Informed Consent Document:

An informed consent was obtained from each volunteer prior to initiating the study describing reasons for the study, possible adverse effects, associated risks and potential benefits of the treatment and their limits of liability. Panelists signed and dated the informed consent document to indicate their authorization to proceed and acknowledge their understanding of the contents. Each panelist was also given a copy of the informed consent for his records. Each subject was assigned a permanent identification number and completed an extensive medical history form and screening form. These forms, along with the signed consent forms, are available for inspection on the premises of AMA Laboratories, Inc., only. Reference 21 CFR Ch.1 Part 50, Subpart B.

5.0 Population Demographics:

Number of subjects enrolled		10
Number of subjects completing study		10
Age Range		45 - 58
Sex	Female	7
	Male	3
Race	Caucasian	9
	Asian	1

6.0 Procedure:

7 female and 3 male subjects were inducted into this study. The demographic data is shown in Section 5.0. All subjects completed a screening form, medical history form, and informed consent document prior to commencement.

The study was conducted according to the sponsor requested design wherein all subjects were instructed to apply the test materials according to the following sponsor supplied use instructions:

USE INSTRUCTION:

L-5005
1. Apply on thighs and ankles at distance of approximately four inches (10 cm).
2. Cover entire area desired to reduce symptoms. Spray can be applied various times a day, preferably apply it in the morning and at night.
3. The spray causes a cold sensation upon making contact with the skin. This is normal.
4. In seconds, the legs will feel a restful sensation.

L-5004
1. Apply activating cream generously over legs and ankles, giving a smooth upward massage, from the ankle to the calf.
2. Cover the entire area that had received product L-5005.
3. Cream can be applied various times a day, preferably apply it in the morning and at night.
4. In just a few seconds, the legs will feel a moisturizing sensation.

Subjective questionnaires addressing consumer perception were completed at baseline (before product application) and after 6 weeks of product use.

Clients are notified immediately in the case of an adverse reaction and a determination is made as to treatment program if necessary.

7.0 Results:

Please refer to attached Questionnaire Summary.

8.0 Observations:

No adverse effects or unexpected reactions of any kind were observed on any of the subjects.

9.0 Archiving:

All original samples, raw data sheets, technician's notebooks, correspondence files, copies of final reports and remaining specimens are maintained on the premises of AMA Laboratories, Inc. in limited access marked storage files. A duplicate DVD copy of final reports is separately archived in a bank safe deposit vault.

10.0 Conclusions:

Within the limits imposed by the conduct and population size of the study described herein, the test materials (AMA Lab Nos.: L-5005 and L-5004; Client Nos.: Cryo-Action Spray, Step 1, Lot #: 190509 and Reactivating Cream, Step 2, Lot #: 160609, CAD0611) were reported by the majority of test panelists to be effective anti-varicose vein treatment. 90% of test panelists would buy the test products and would recommend them to their family and friends. 90% of the test subjects noticed improvement in their varicose vein conditions. 90% of the panelists noted they could do their daily activities without physical discomfort after treatment.

Mayya Tatsene

Mayya Tatsene, M.D.
Study Director

Patrycja Bienias

Patrycja Bienias, M.S.
Technician

David R. Winne

David R. Winne, B.S.
Technical Director

7/31/09

Date

Note: All Services Undertaken Subject to the following General Policy: AMA Laboratories, Inc. Reports are submitted for exclusive use of the clients to whom they are addressed. Their significance is subject to the adequacy and representative character of the samples and to the comprehensiveness of the test, examination or surveys made. No quotations from AMA Laboratories, Inc., reports, or use of AMA Laboratories, Inc., name or names of staff members or sub-contractors is permitted except as expressly authorized in writing. The liability of AMA Laboratories, Inc. with respect to services rendered shall in no event exceed the amount of one hundred dollars. Any indemnification agreement attached to or included in the embodiment of this report shall, if sent by certified mail, return receipt requested, be deemed to be properly served, executed, notarized and accepted by virtue of the signature appearing on the return certified claim. Wherein this report is used to support commercial claims, the Sponsor is directed to provide said report in its entirety.

Pre-Test Product Questionnaire Summary

AMA Lab Nos.:	Client Nos.:
L-5005	Cryo-Action Spray, Step 1, Lot #: 190509
L-5004	Reactivating Cream, Step 2, Lot #: 160609, CAD0611

Section 1: QUESTIONNAIRE

Please circle the answer that best applies:

1. Do you have varicose veins?

YES	NO
100%	0%

2. How long do you have them?

1-5 years	6-10	11-20	More then 20
10%	40%	40%	10%

3. Are you able to do your daily activities without physical discomfort?

YES	NO
60%	40%

Pre and Post -Test Product Questionnaire Summary

AMA Lab Nos.:
L-5005
L-5004

Client Nos.:
Cryo-Action Spray, Step 1, Lot #: 190509
Reactivating Cream, Step 2, Lot #: 160609, CAD0611

Section 2: VAS (Visual Analog Scale)

In a scale from 0 to 10, please describe the intensity of perceived varicose veins related condition:

4. Amount of varicose veins:

	0 None	1	2	3	4	5	6	7	8	9	10 A lot
Baseline	0%	0%	10%	0%	0%	30%	10%	20%	30%	0%	0%
6 weeks	0%	10%	10%	0%	50%	0%	0%	30%	0%	0%	0%

5. An achy or heavy feeling in your legs:

	0 None	1	2	3	4	5	6	7	8	9	10 Severe
Baseline	0%	10%	10%	10%	0%	10%	10%	20%	30%	0%	0%
6 weeks	10%	10%	20%	10%	30%	0%	20%	0%	0%	0%	0%

6. Burning sensation in your legs:

	0 None	1	2	3	4	5	6	7	8	9	10 Severe
Baseline	0%	10%	20%	10%	10%	10%	10%	20%	10%	0%	0%
6 weeks	20%	10%	10%	10%	30%	0%	20%	0%	0%	0%	0%

7. Throbbing, muscle cramping and swelling in your legs:

	0 None	1	2	3	4	5	6	7	8	9	10 Severe
Baseline	0%	0%	10%	10%	10%	10%	20%	10%	20%	10%	0%
6 weeks	10%	20%	10%	10%	20%	0%	10%	10%	10%	0%	0%

Pre and Post -Test Product Questionnaire Summary

AMA Lab Nos.:
L-5005
L-5004

Client Nos.:
Cryo-Action Spray, Step 1, Lot #: 190509
Reactivating Cream, Step 2, Lot #: 160609, CAD0611

Section 2: VAS (Visual Analog Scale)

8. Itching around one or more of your veins:

	0 None	1	2	3	4	5	6	7	8	9	10 Severe
Baseline	10%	0%	20%	10%	0%	10%	10%	10%	10%	20%	0%
6 weeks	20%	10%	20%	0%	20%	10%	10%	10%	0%	0%	0%

9. Pain in your legs:

	0 None	1	2	3	4	5	6	7	8	9	10 Severe
Baseline	10%	0%	0%	10%	0%	10%	30%	10%	10%	20%	0%
6 weeks	10%	20%	10%	0%	30%	20%	0%	10%	0%	0%	0%

10. How relaxed and refreshed do your legs feel?

	0 Not at all	1	2	3	4	5	6	7	8	9	10 Very much
Baseline	20%	10%	0%	10%	20%	10%	0%	20%	10%	0%	0%
6 weeks	0%	10%	10%	10%	0%	10%	0%	10%	10%	40%	0%

11. How do you feel emotionally about having varicose veins?

	0 Extremely dissatisfied	1	2	3	4	5	6	7	8	9	10 Extremely satisfied
Baseline	60%	30%	0%	10%	0%	0%	0%	0%	0%	0%	0%
6 weeks	40%	10%	20%	0%	10%	20%	0%	0%	0%	0%	0%

12. How much do you want to reduce your varicose veins?

	0 Not at all	1	2	3	4	5	6	7	8	9	10 Very much
Baseline	0%	0%	0%	0%	0%	0%	0%	0%	0%	10%	90%
6 weeks	0%	20%	0%	0%	0%	20%	0%	0%	0%	0%	60%

Post-Test Product Questionnaire Summary

AMA Lab Nos.:	Client Nos.:
L-5005	Cryo-Action Spray, Step 1, Lot #: 190509
L-5004	Reactivating Cream, Step 2, Lot #: 160609, CAD0611

Section 1: QUESTIONNAIRE

Please circle the answer that best applies:

1. Would you buy this product?

YES	NO
90%	10%

2. Would you recommend the product to your family and friends?

YES	NO
90%	10%

3. Is there anything you disliked about the product?
   Subject No.: 52 5216: “Smell.”
   Subject No.: 50 1729: “Fragrance.”
   Subject No.: 38 5149: “Once can started to get low hard to spray on foot.”

4. Do you notice any improvement in your varicose vein conditions after using the treatment for 6 weeks?

No improvement	Improvement	Significant improvement
0%	90%	10%

5. Are you able to do your daily activities without physical discomfort?

YES	NO
90%	10%

6. How much did you like the product?

0 Not at all	1	2	3	4	5	6	7	8	9	10 Very Much
0%	0%	10%	0%	0%	0%	0%	0%	40%	30%	20%

11.0 Quality Assurance Statement:

This study was inspected in accordance with the Standard Operating Procedures of AMA Laboratories, Inc. To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the study records and report.

Report reviewed by:

Kamil Wojtowicz, M.S.
Quality Assurance Supervisor

7/31/09
Date

Quality Endorsed Company

CERTIFICATE OF REGISTRATION

AMA Laboratories, Inc.

216 Congers Road; Building 1, New City, New York 10956

complies with the requirements of

ISO 9001:2000

Quality Management Systems - Requirements

for the following capability

The Registration Covers the Quality Management System for Clinical and Consumer Research Testing Including Manufacturers of Internationally Renowned Brands, Suppliers of Raw Materials, Formulators, Medical Researchers, Consultants and Independent Teams Working in the Fields of Cosmetics and Cosmeceuticals, Pharmaceuticals, Nutraceuticals, and Botanicals, Household and Automotive Products, Food, Nutrition, Health and Beauty Aids.

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Certified Date: 19 June 2007
Expiry Date: 19 June 2010

Alex Ezrakhovich
General Manager Certification
for and on behalf of
SAI Global

Carl F. Blazik
Authorised Local Signatory
for and on behalf of
SAI Global

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Form Approved: OMB No. 0910-0045. Expiration Date: December 31, 2007. See OMB Statement on Reverse.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
REGISTRATION OF DRUG ESTABLISHMENT/ LABELER CODE ASSIGNMENT
(In accordance with Public Law 92-387)

FDA USE ONLY | FDA USE ONLY

NOTICE: This report is required by law (21 C.F.R. 207.20). Failure to report can result in imprisonment for not more than one year or a fine of not more than $1,000, or both. (FD&C Act, Section 303).

LABELER CODE: 054472 | REGISTRATION NUMBER: 2434339

SECTION A - SITE INFORMATION

REPORTING FIRM NAME: AMA Laboratories, Inc

STATE OF INC.: New Jersey, Iic New York

SITE ADDRESS *(No P.O. Box)*: 216 Congers Road, Bldg 1, 1A,4&5

SITE TELEPHONE NUMBER: (845) 634-4330

CITY	STATE	ZIP CODE	COUNTRY	BUSINESS CATEGORY
New City	NY	10956	USA	☒ HUMAN ☐ VETERINARY

SITE MAILING ADDRESS *(If different from site address)*

CITY	STATE	ZIP CODE	COUNTRY	SITE INTERNET/EMAIL ADDRESS

DOING BUSINESS AS (DBA) NAME OF FIRM *(if applicable)*

PARENT COMPANY NAME

REASON(s) FOR SUBMISSION
- ☐ Firm Registration
- ☐ Registration of Additional Site
- ☒ Re-Registration
- ☐ LC Assignment
- ☐ Name Change
- ☐ Address Change
- ☐ Merger/Buyout
- ☐ Reentry into Business with Same Name
- ☐ Out of Business

TYPE OF OWNERSHIP
- ☐ Sole Proprietorship
- ☐ Partnership
- ☐ Coop. Assn.
- ☒ Corporation
- ☐ Other _____

PERSON SUBMITTING DATA AND TELEPHONE: Gabriel J.Letizia, 845-634-4330

BUSINESS TYPE
- ☐ Manufacturer
- ☐ Repacker
- ☐ Relabeler
- ☐ Distributor*
- ☐ Foreign Country
- ☐ Analytical Lab
- ☒ Other Clinical Lab

SECTION B - FIRM COMPLIANCE MAILING ADDRESS for Annual Listing Report and/or Firm Correspondence

NUMBER AND STREET AND/OR P.O. BOX and ATTENTION LINE and/or Internal Mail Code: 216 Congers Road, Bldg 1,1A,4&5

TELEPHONE NUMBER: (845) 634-4330

CITY	STATE	ZIP CODE	COUNTRY	COMPLIANCE INTERNET/EMAIL ADDRESS
New City	NY	10956	USA	gabel@amalabs.com

SECTION C - ADDITIONAL FIRM AND SITE INFORMATION

NAME OF OWNER, PARTNERS OR OFFICERS	TITLE	POSITION
Gabriel J. Letizia	President	Executive Director

RECEIVED
JAN 2 8 2008
DRLS

OTHER FIRMS DOING BUSINESS AT THIS SITE

LABELER CODE	FIRM NAME	LABELER CODE	FIRM NAME

SECTION D - SIGNATURE

SIGNATURE OF AUTHORIZING OFFICIAL	TITLE	DATE
	President	120/30/2008

*DISTRIBUTOR'S CERTIFICATION: As a, Distributor, I am submitting product listing information to the FDA on my own behalf. I have provided a copy of this certification (Form FDA 2656) to the registered manufacturer(s). My signature and phone number are listed below.

RETURN THIS FORM TO:
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CDER/DRUG REGISTRATION AND LISTING (HFD-337)
5600 FISHERS LANE
ROCKVILLE, MD 20857
INTERNET: DRLS@FDA.HHS.GOV

SIGNATURE OF DISTRIBUTOR

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( )

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