1. TITLE PAGE

STUDY TITTLE	Biological standardization of *Chenopodium album* allergen extract to determine the biological activity in HEP units.
PROTOCOL NUMBER	6062-PR-PRI-195
EUDRACT NUMBER	2012-001937-15
INVESTIGATIONAL MEDICINAL PRODUCT	Experimental product: *Chenopodium album* allergen extract Control product: Histamine dihydrochloride (10 mg/ml) Glycerinated phenol saline solution
INDICATION STUDIED	Diagnosis of *Chenopodium album* allergy
DESIGN	Non-randomised, open-label and multi-center clinical trial.
SPONSOR	Laboratorios LETI, S.L. Unipersonal C\ Sol, 5. 28760 Tres Cantos. Madrid. Spain Phone: +34 91.771.17.90 / Fax: +34 91.804.09.19 http://www.leti.com
PHASE OF STUDY	II
STUDY INITIATION DATE	31st of October 2012
STUDY COMPLETION DATE	22nd of May 2014
COORDINATING INVESTIGATOR	Not applicable
PRINCIPAL INVESTIGATOR	Dr. Jesús Garde Garde Hospital General Universitario de Elche Alicante (Spain) Dra. Cristina de Castro Centro Médico Adeslas C/ Francisco Rabal, 33 Córdoba (Spain)
MEDICAL DIRECTOR LETI GROUP	Rafael Levitch, MD
GCP STAMENT	This study was performed in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki (with amendments) and local legal and regulatory requirements
ARCHIVING	The study documents will be archived according to ICH GCP regulations
DATE OF REPORT	18. Abril.2016

For any questions of the study report please contact to Medical Department, Laboratorios LETI S.L.U (Medico@leti.com).

SIGNATURES:

STUDY TITLE: Biological standardization of *Chenopodium album* allergen extract to determine the biological activity in HEP units.

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

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2. SYNOPSIS

Name of Sponsor / Company: Laboratorios LETI, S.L. Unipersonal	
Name of Finished Product:	Prick Test *Chenopodium album* LETI at 20, 15, 2 and 0.2 mg/ml Positive control (histamine dihydrochloride {10 mg/ml}) Negative control (glycerinated phenol saline solution)
Name of Investigational product substance(s):	Experimental product: *Chenopodium album* allergen extract Control product: Histamine dihydrochloride (10 mg/ml) Glycerinated phenol saline solution
Name of Non Investigational product substance:	PRICK TEST Gänsefuss LETI (PEI D.02489.01.1) / Prick Test de *Chenopodium album* 30 HEP/ml
Title of the study: Biological standardization of *Chenopodium album* allergen extract to determine the biological activity in HEP units.	
Investigator(s):	
Study centres: 2 sites in Spain	
Publication (reference): None at the time of this clinical study report (CSR)	
Study period:	First site initiated: 31 of October 2012 100% sites closed: 22 of May 2014 First patient first visit: 7th of November 2012 Last patient last visit: 26th of March 2014
Study phase: II	
Objectives: The objective of this study is to determine the biological activity of a *Chenopodium album* allergen extract in histamine equivalent prick (HEP) units, in order to be used as in-house reference preparation (IHRP).	
Methodology: Titrated Skin Prick test biological study. Four concentrations of *Chenopodium album* allergen extract, together with a positive and negative control, were tested in every patient in duplicate on	

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the volar surface of the forearm. The tests were performed at the study sites and all patients remained in the medical rooms under observation at least 30 minutes after the application of the Titrated Skin Prick test.

Number of patients (planned and analysed): A total number of 48 patients were screened and 47 were enrolled and received the study medication. A total of 23 subjects out of 47 who received the study medication were excluded from the PP population since they did not meet eligibility criteria (see the Note in Patient inclusion criteria, n=15) and did not meet the Nordic Guidelines(1) statistical criteria for analysis (n=8). Therefore, the wheal data of 24 patients were analysed (PP population).

Diagnosis and main Criteria for inclusion and exclusion:

- Patient inclusion criteria:
  1. Patient has provided written informed consent, appropriately signed and dated by the patient (or legal representative, if applicable).
  2. Patient can be male or female of any race and ethnic group.
  3. Age ≥ 18 years and ≤ 60 years at the study inclusion day.
  4. Positive skin prick test with a standardised commercially available preparation of *Chenopodium album* allergen extract. The skin prick test will be considered positive if the test results in a wheal major diameter of at least 6 mm. Positive skin prick test results are valid if performed within one year prior to the inclusion of the patient in the study.
  5. Allergic symptoms during the pollen season of *Chenopodium album.*
  6. Clinical history positive to inhalation allergy (rhinitis and/or rhinoconjunctivitis and/or asthma) due to *Chenopodium album*
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- Patient exclusion criteria

1. Immunotherapy in the past 5 years with an allergen preparation known to interfere with the allergen to be tested (e.g., Chenopodiacea; *Atriplex latifolia*, *Beta vulgaris*, *Salsola kali, Amaranthus retroflexu*).
2. Use of drugs that may interfere with the skin reactions (e.g., antihistamines).
3. Treatment with any of the following medications: tricyclic or tetracyclic antidepressants, β-blockers or corticosteroids (> 10 mg/day of prednisone or equivalent).
4. Pregnancy or breastfeeding and women with a pregnancy test positive in the visit 2, prior to the prick test.
5. Dermographism affecting the skin area at the test site at either study visit.
6. Atopic dermatitis affecting the skin area at the test site at either study visit.
7. Urticaria affecting the skin area at the test site at either study visit.
8. Diseases of the immune system relevant clinically, both autoimmune and immune deficiencies.
9. Uncontrolled severe diseases (heart failure, severe or uncontrolled respiratory diseases, endocrine diseases, liver or kidney diseases clinically relevant or hematologic diseases).
10. Participation in another clinical trial within the last month.
11. Patients suffering from diseases or conditions which limit the use of adrenaline (coronary heart disease, severe hypertension,...).
12. Severe psychiatric, psychological, or neurological disorders.
13. Abuse of alcohol, drugs or medication in the previous year.

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Test product, dose and mode of administration, batch number:

Chenopodium album allergen extract, vial 1: 15 mg/ml. Batch nº: F-22 *(Expiry date: 11/2013),* G-2 *(Expiry date: 04/2014)*

Chenopodium album allergen extract, vial 2: 20 mg/ml: Batch nº: F-20 *(Expiry date: 11/2013),* G-3 *(Expiry date: 04/2014)*.

Chenopodium album allergen extract, vial 3: 2 mg/ml. It was obtained by diluting vial 2.

Chenopodium album allergen extract, vial 4: 0,2 mg/ml. It was obtained by diluting vial 3.

Solvent SC-2 and SC-3: Batch no.: G-8 (*Expiry Date: 04/ 2015*) and F-19 (*Expiry Date: 02/2014)*.

Negative control: Batch no.: 2357762 (*Expiry Date: 07/2014*) and 2206734 (*Expiry Date: 02/ 2014)*

Positive control (Histamine dihydrochloride 10 mg/ml): Batch no.: 2254065 (*Expiry Date: 06/ 2014), 2168905* (*Expiry Date:01/ 2014*),

The investigational medicinal products were applied on the skin by the prick test method:

The investigator or designee placed a drop on the patient´s volar side of the forearm (in duplicate) of the positive control, the negative control and the 4 concentrations of *Chenopodium album* allergen extract. Prick tests were placed in areas 5 cm from the wrist or 3 cm from the antecubital fossae. In addition, test drops were allocated at least 3 cm apart to avoid wheal interferences.

For the Titrated Skin Prick test response evaluation, test sites were inspected and the responses recorded 15-20 min after application. The contours of the wheals (and not the erythema) were outlined with a skin marking pen, ensuring no red skin around the wheal was inside the encircled area. Using translucent tape (pressing it gently against the wheal), the contours were transferred into the corresponding page of the Case Report Form.

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Duration of study: The clinical trial consisted of 1 or 2 site visits per patient of approximately 30 minutes, depending on the possibility to assess eligibility criteria during visit 1.	
Criteria for evaluation: Wheal size area (mm^2) on the skin at the site of the puncture during the immediate phase.	
Statistical methods: The wheal areas (mm^2) resulting on the skin as a consequence of the exposure to *Chenopodium album* allergen extract at different concentrations, positive and negative controls, by the Prick-test procedure were measured. For every patient, the geometric means of the wheal areas provoked by the *Chenopodium album* allergen extract and the histamine were calculated. A logarithmic transformation of them were performed subsequently. For each patient, a linear regression analysis, using the method of least squares, was performed. Then it was checked if the patients fulfilled the Nordic Guidelines(1) criteria for analysis. For each valid patient, the value of the geometric mean of the wheal area provoked by histamine (10 mg/ml) was inserted into the equation to derive the corresponding concentration of the *Chenopodium album* allergen extract that elicited a wheal size equivalent to that of histamine (10 mg/ml). The median of the concentrations of the *Chenopodium album* allergen extract that elicits a wheal size equivalent to that of histamine (10 mg/ml) of the valid patients corresponds to 10 HEP/ml. This value indicates the *in vivo* biological activity of the *Chenopodium album* allergen extract.	
Safety results: 1 adverse event was reported in this clinical trial (Late local reaction with moderate intensity)	

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Conclusion: The biological activity of the *Chenopodium album* allergen extract equivalent to **10 HEP/mL** is obtained using **1.08 mg/mL** of this allergen**.** The administration of the study medication by prick testing was well tolerated and safe.	
Date of report: 18.April.2016	