2. SYNOPSIS

<table>
<tr><td colspan="2">Name of Sponsor / Company: Laboratorios LETI, S.L.Unipersonal</td></tr>
<tr><td>Name of Finished Product:</td><td>Prick Test Alternaria alternata LETI at 10, 1, 0.1 and 0.01 mg/ml
Positive control (histamine dihydrochloride {10 mg/ml})
Negative control (glycerinated phenol saline solution)</td></tr>
<tr><td>Name of Investigational product substance(s):</td><td>Experimental product:
Alternaria alternata allergen extract
Control product:
Histamine dihydrochloride (10 mg/ml)
Glycerinated phenol saline solution</td></tr>
<tr><td>Name of Non Investigational product substance:</td><td>Standarized PRICK TEST Alternaria alternata 30 HEP/ml (D.02949.02.1)</td></tr>
<tr><td colspan="2">Title of the study: Biological standardization of Alternaria alternata allergen extract to determine the biological activity in HEP units.</td></tr>
<tr><td colspan="2">Investigator(s):
Santiago Quirce Gancedo, MD, PhD
Hospital Universitario La Paz
Servicio de Alergia
Paseo de la Castellana, 261
28046 Madrid

Dr. José Damián López Sánchez, MD, PhD
Hospital Universitario Virgen de Arrixaca
Servicio de Alergia
Ctra. Madrid-Cartagena, s/n
30120 El Palmar-Murcia</td></tr>
<tr><td colspan="2">Study centres: 2 sites in Spain</td></tr>
<tr><td colspan="2">Publication (reference): None at the time of this clinical study report (CSR)</td></tr>
<tr><td colspan="2">Study period: First site initiated: 21st of February 2014
First patient first visit: 21st of February 2014
Last patient last visit: 26th of November 2014</td></tr>
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Name of Investigational product substance(s):	Experimental product: *Alternaria alternata* allergen extract Control product: Histamine dihydrochloride (10 mg/ml) Glycerinated phenol saline solution
Study phase: II	
Objectives: The objective of this study is to determine the biological activity of a *Alternaria alternata* 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 *Alternaria alternata* allergen extract, together with a positive and negative control, were tested in every patient in duplicate on 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 30 patients were enrolled and received the study medication. The wheal data of 26 patients were analysed (PP population), since 1 out of the 30 subjects voluntarily left the study after signing and 3 subjects did not meet eligibility criteria (n=1) or did not meet the Nordic Guidelines(1) statistical criteria for analysis (n=2).	
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	

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Alternaria alternata allergen extract. The skin prick test will be considered positive if the test results in a wheal major diameter of at least 3 mm. Positive skin prick test results are valid if performed within one year prior to the inclusion of the patient in the study.

5. A positive test for specific IgE to *Alternaria alternata* (CAP-RAST ≥ 2). IgE results are valid if performed within one year prior to the inclusion of the patient in the study.
6. Allergic symptoms during the pollen season of *Alternaria alternata.*
7. Mean of the forearm major diameters of the wheals provoked by histamine dihydrochloride (10 mg/ml) ≥ 3 mm.

- 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., grass group extracts).
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.
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 clinically relevant, both autoimmune and immune deficiencies.
9. Uncontrolled severe diseases (heart failure, severe or uncontrolled respiratory diseases,

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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.
14. Individuals who have received treatment with anti-IgE (Omalizumab).

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

Alternaria alternata allergen extract, vial 1: 10 mg/ml. Batch nº: G-9 *(Expiry date: 03/2014),* and H-7 *(Expiry date: 11/14)*.

Alternaria alternata allergen extract, vial 2: 1 mg/ml. It was obtained by diluting vial 1.

Alternaria alternata allergen extract, vial 3: 0.1 mg/ml. It was obtained by diluting vial 2.

Alternaria alternata allergen extract, vial 4: 0.01 mg/ml. It was obtained by diluting vial 3.

Solvent SC-2, SC-3, and SC-4: Batch no.: G-8 (*Expiry Date: April 2015*).

Negative control: Batch no.: 2470642 (*Expiry Date: April-2015*).

Positive control (Histamine dihydrochloride 10 mg/ml): Batch no.: 2475243 (*Expiry Date: May-2015)*.

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 *Alternaria alternata* allergen extract. Prick tests were placed in areas 5 cm from the wrist or 3 cm from the antecubital fossae. In

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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.

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 *Alternaria alternata* 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 *Alternaria alternata* 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 *Alternaria alternata* allergen extract that elicited a wheal size equivalent to that of histamine (10 mg/ml). The median of the concentrations of the *Alternaria alternata* allergen extract that elicits a wheal size

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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 *Alternaria alternata* allergen extract.	
Dose response result: 3.96 mg of *Alternaria alternata* allergen extract is equivalent to 10 HEP.	
Safety results: No adverse events were reported in the clinical trial.	
Conclusion: The biological activity of the *Alternaria alternata* allergen extract is equivalent to **10 HEP = 3.96 mg.** The administration of the study medication by prick testing was well tolerated and safe.	
Date of report: 24th Aug 2015	