Name of Company: Inmunotek SL	**INDIVIDUAL STUDY TABULAR FORMAT**
Name of Finished Product: *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae Prick Test*.	
Name of Investigational product substance(s): *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* allergenic extract	
Title of the study: *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* allergenic extract. Determination of the Allergenic Potency in Vivo in Histamine Equivalent Units (HEP)	
Investigators: Elena Rodríguez Plata, MD	
Study centre(s): Hospital Universitario Nuestra Señora de la Candelaria Tenerife, Spain	
Publication (reference): N/A	
Study period. **Date of first enrolment:** August 17, 2015 **Date of last completed**: August 7, 2018	**Phase of development**: II
Objectives: The main objective is to evaluate the concentration of an allergenic mixture of mites extract of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* necessary to produce a papule size equivalent to the one produced by a 10 mg/mL solution of histamine dihydrochloride.	
Methodology: Open, unblinded and non-randomized biological assay. The study design is a slight modification of the recommendations proposed by the Nordic Guidelines. [(1)].	
Number of subjects: 23	
Diagnosis: **Subjects with dust mites (Dermatophagoides pteronyssinus and Dermatophagoides farinae) allergy.**	
Criteria for inclusion:	

- Respiratory symptoms backgrounds (rhinitis / rhinoconjunctivitis / asthma) due to Dermatophagoides pteronyssinus or Dermatophagoides farinae allergy.
- A positive prick-test (average of the papule ≥ 3 mm diameter) with an extract of the same allergen and / or the presence of specific IgE against the allergen.
- The average area of the wheal obtained with Histamine dihydrochloride at 10 mg / mL should be ≥ 7 mm2.
- Age: over 18 years old.
- Both genders.
- Patients able to sign the informed consent.

Test product, dose, mode of administration, batch No.:

Two Investigational Medicine Products (IMP):

- A Dermatophagoides pteronyssinus allergenic extract concentrated at 300 µg/mL tested in four concentrations (300 µg / mL, 30 µg / mL, 3 µg / mL y 0.3 µg / mL) followed by a forearm skin Prick Test.

Batch No. EC0101P14C (Ex. 09/2017) and Batch No. AEC0203P17E (Ex. 12/2019)

- A Dermatophagoides farinae allergenic extract concentrated at 400 µg/mL tested in four concentrations (400 µg / mL, 40 µg/mL, 4 µg/mL y 0.4 µg /mL) followed by a forearm skin Prick Test.

Batch No. EC0101P15E (Ex. 12/2017) and Batch No. AEC0201P16N (Ex. 10/2019)

Reference therapy, dose, mode of administration, batch No.:

- Positive and negative controls were tested onto the forearm skin in the same way as the investigational product.
- The positive control contains 10 mg/mL histamine dihydrochloride in diluent solution. Batch No. 14S04P (Ex. 09/2017) and Batch No. A16N10P (Ex. 11/2019)
- The negative control contained the diluent: NaCl 0.9%, phenol 0.4% and glycerol 50%. Batch No. 14S03P (Ex. 09/2017) and Batch No. A16N11P (Ex. 10/2019)
- Diluent (same composition as the negative control). Batch No. 14C02DP (Ex. 09/2017) and Batch No. AEC0204P16N (Ex. 10/2019)

Criteria for evaluation:

Efficacy

The main endpoint was the measure of the skin papule produced by each of the different concentrations of the allergenic extracts in relation to positive (histamine) and negative controls, according to the prick test.

Safety

All adverse events were recorded. All these adverse reactions were individually evaluated to assess severity and classify them as probably related or unrelated to the study medication.

Statistical methods:

The statistical analysis was done by Inmunotek S.L. using Microsoft Excel ® software.

Statistical analysis used:

- The calcule of the geometric mean of the two wheals produced by each allergen concentration (mm2) in relating with the positive (Histamine 10 mg/mL) and negative control (diluent's solution).
- A linear regression analysis using the method of least squares was performed computing the constant a and b for each patient, to describe the relationship:

  log (m) = a+b log (C), in which:

  - m: Geometric mean of the two-wheal areas induced by each concentration of the allergen (mm2)
  - C: Allergen concentration (µg/mL)
  - a: intercept
  - b: slope

The calcule of the individual bioequivalent dose of each allergenic extract to achieve a wheal the same size as the positive control (individual 10 HEP) was performed for each patient. General descriptive statistics were calculated, including the median that was 10 HEP for the population sensitize to each allergen.

Following the Nordic Guidelines (1), by default, any wheal area having a value equal to 0 was replaced with wheal area equal to 1 mm2.

This procedure was repeated in all patients:

- The MEDIAN CH of all patients represents the concentration that corresponds 10 HEP/mL (95% confidence limits). This value indicates the biological potency.

Arithmetic mean, geometric mean, standard deviation, variance, etc. were also calculated for each allergen concentration equivalent to 10 HEP/mL.

The results accepted were only from patients that fulfil the following criteria;

- Geometric mean of the wheal's area reactions provoked by either of the two highest concentration of the allergen preparation ≥ 7 mm2
- Geometric mean of the wheal's area reactions provoked by Histamine 10 mg/mL ≥ 7 mm^2
- Geometric mean of the wheal reactions provoked by the negative control < 7 mm^2
- Regression line slope (b) ≥ 0. 1
- Coefficient of correlation(r) ≥ 0. 85

SUMMARY CONCLUSIONS

SAFETY RESULTS: No adverse events occurred during the trial.

CONCLUSION:

Dermatophagoides farinae

Following the Nordic Guidelines(1) and based on the population allergic to Dermatophagoides farinae, the biological potency of this experimental allergen extract equivalent to 10 HEP (Histamine Equivalent Prick) was 6.8 µg (95% IC:11.49 – 132.1).

Dermatophagoides pteronyssinus

Following the Nordic Guidelines and based on the population allergic to Dermatophagoides pteronyssinus, the biological potency of this experimental allergen extract equivalent to 10 HEP (Histamine Equivalent Prick) was 16.0 µg (95% IC: 12.93 – 167.29).