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# Relative potency of allergenic extracts from mites in patients with asthma and allergic rhinitis

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## KEYWORDS:

Dermatophagoides pteronyssinus; Dermatophagoides farinae; Dermatophagoides siboney; Blomia tropicalis; allergenic potency, Cuba.

## Abstract

**Introduction:** Skin tests for specific diagnosis of allergies require high quality extracts, which can be measured by the determination of allergenic potency, utilizing different concentrations of extracts from a manufacturer.

**Objective:** To compare allergenic mite extracts manufactured by laboratories in Spain, Argentina, and Cuba by skin prick test response.

**Methods:** A cutaneous puncture was performed on 56 patients aged 2 to 15 years with asthma and allergic rhinitis with extracts of *Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides farinae* (Df), *Dermatophagoides siboney* (Ds) and *Blomia tropicalis* (Bt) produced by DIATER (Argentina), ALK-Abelló (Spain) and BIOCEN (Cuba).

**Results:** In 84.6% of patients there was an association of symptoms with seasonal changes and house dust exposure. The diameter of wheals from ALK allergen extract was larger than extracts from other sources, but without any significant difference ( $p > 0.05$ ). The ratio between wheals and allergen concentration with BIOCEN products showed lineal dependence between the diameter averages and the concentration logarithm, ranging from 4000 to 100 000 UB/mL. The skin prick tests with diameters  $\geq 3$ mm showed a larger proportion of Dp and Df from ALK in comparison to BIOCEN and DIATER.

**Conclusion:** We found that dust mite extracts produced and standardized by DIATER, ALK-Abello and BIOCEN were similar with respect to allergenic potency.

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**PALABRAS CLAVE:**

*Dermatophagoides pteronyssinus*; *Dermatophagoides farinae*; *Dermatophagoides siboney*; *Blomia tropicalis*; potencia alergénica, Cuba.

## Potencia relativa de extractos alergénicos de ácaros en pacientes con asma y rinitis alérgicas

### Resumen

**Introducción:** Las pruebas cutáneas para el diagnóstico específico de enfermedades alérgicas requieren extractos alergénicos de alta calidad, estas pruebas pueden evaluarse por la determinación de la potencia alergénica, utilizando diferentes concentraciones de extractos de un fabricante.

**Objetivo:** Comparar extractos alergénicos de ácaros elaborados por laboratorios en España, Argentina y Cuba mediante la respuesta de pruebas cutáneas por punción.

**Material y métodos:** Se aplicaron pruebas cutáneas por punción en 56 pacientes de 2 a 15 años de edad, con asma y rinitis alérgicas, con extractos de *Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides farinae* (Df), *Dermatophagoides siboney* (Ds) y *Blomia tropicalis* (Bt) producidos por DIATER (Argentina), ALK-Abelló (España) y BIOCEN (Cuba).

**Resultados:** En 84% de los pacientes hubo una asociación de los síntomas con cambios estacionales y exposición al polvo casero. El diámetro de las ronchas producidas por los extractos de ALK-Abelló fue mayor que los extractos de los otros laboratorios, pero sin significancia estadística ( $p > 0.05$ ). La relación entre las ronchas y la concentración de los extractos alergénicos de BIOCEN, mostró una dependencia lineal entre los promedios de los diámetros y la concentración logarítmica, con variaciones entre 4 000 y 100 000 UB/mL. Las pruebas cutáneas con diámetros  $> 3$  mm mostraron una mayor proporción con Dp y Df de ALK-Abelló en comparación con los extractos de BIOCEN y DIATER.

**Conclusión:** Encontramos que los extractos alergénicos de ácaros del polvo producidos y estandarizados por DIATER, ALK-Abelló y BIOCEN fueron similares en relación a su potencia alergénica.

## Introduction

A recent study confirms the impact of the association between asthma and allergic rhinitis on the quality of life; which requires adequate treatment that will reduce the morbidity of asthma.<sup>1</sup> In order to reach an adequate and efficient treatment, an etiologic diagnosis is needed to identify the cause; this is basically identified by skin tests. For achieving reliable skin tests results is necessary to use high quality extracts produced in laboratories that fulfill the proper conditions for human use. The quality of the extracts can be measured, using different methods including specific IgE determination of allergens and skin reaction to inoculation of standardized products, according to the method used by each laboratory. The comparison of allergenic extracts potency from different manufacturers done by *in vitro* techniques showed less potency of *Dermatophagoides pteronyssinus* (Dp) in Mexico and Europe than those produced in the United States.<sup>2</sup> On the other hand, patients allergic to Dp with specific IgE  $>0.70$  KU/L, revealed

significant differences when compared by skin prick tests using extracts from Stallergenes (Antony, France), Lofarma Allergeni (Milan, Italy), and ALK- Abelló (Hørsholm, Denmark).<sup>3</sup> Efforts have been made in Cuba at attempting to define an optimal concentration of mite allergenic extracts, taking into account that at greater allergen concentration there is greater sensitivity but less specificity.<sup>4,5</sup> This study proposes that since domestic mite allergens are the leading cause of sensitization in patients with asthma and allergic rhinitis, a comparison must be made using allergenic extract products from laboratories in Spain, Argentina and Cuba by means of the skin prick tests to measure allergenic potency for Dp, *Dermatophagoides farinae* (Df), *Dermatophagoides siboney* (Ds) and *Blomia tropicalis* (Bt).

## Methods

Skin prick tests were performed in 56 patients between 2 to 15 years of age with allergic asthma and/or rhinitis; previously assessed by a family

physician and otolaryngologist then transferred to the allergy service of the Previsora Polyclinic in Camagüey City from January to April 2011.

Skin tests were carried out using *Dp*, *Ds* and *Bt*, produced and standardized by the *Centro Nacional de Biopreparados de Cuba* (BIOCEN) in dilutions of 4,000 UB/mL, 20,000 UB/mL, and 100,000 UB/mL. Extracts of *Dp*, *Df* and *Bt* produced and standardized by DIATER laboratories in Argentina were also used at 50 000 UB/mL. Extracts of the same species produced by ALK-Abelló from Spain were also used. These were standardized in HEB (1 HEB: 1000 UB). The extracts of *Dp* and *Df* were 30 HEP/mL and *Bt* at 10 HEP/mL. DIATER PRICK lancets sterilized by gamma radiation were used in all patients.

Statistical analysis was performed using the statistical functions and tools of Microsoft Excel.

## Results

The study group included 44 female patients (78.5%); of these, 35 presented rhinitis (62.5%); 15 had rhinitis and asthma, and 6 only had asthma. As for the patients' age the mode was 5 and the arithmetic mean was 9.5. Of the patients suffering only from rhinitis, in 78% this was moderate persistent and the rest was intermittent. Of the patients only with asthma or associated with rhinitis: 10 were mild, 8 moderate, and 3 severe. In 84.6%

of the study group their symptoms were related to changes in season and house dust exposure.

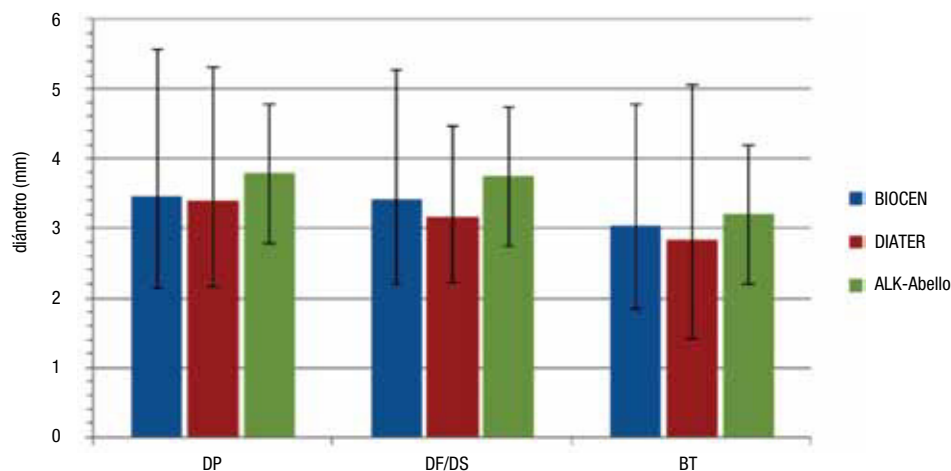
With respect to the wheal diameter, the size reactions were similar among all the extracts; somewhat greater for ALK-Abelló, but without reaching significant difference in any case (ANOVA,  $p > 0.05$ ) (**Figure 1**). Concerning the diameter of wheals and the allergen concentration used in the skin prick tests BIOCEN products showed linear dependence between diameter average and the logarithm of allergen concentration, when using concentrations between 4000 to 100 000 UB/mL (**Figure 2**).

Regarding the behavior of patients with positive skin prick tests (diameter  $\geq 3$  mm), a slightly higher proportion showed response for ALK-Abelló *Dp* and *Df* amounting to 85%, in contrast to BIOCEN and DIATER products (**Figure 3**).

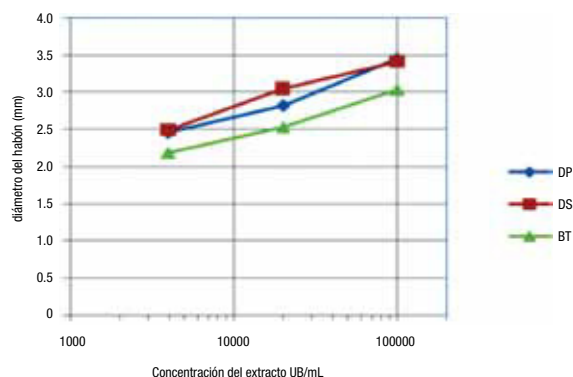
## Discussion

The average wheal diameter was similar among the different extracts which indicate a biological similarity; however, different results were obtained by foreign authors.<sup>2,3</sup> The precision of the *in vitro* method does not show the assumed biological activity differences in such a limited study group. Furthermore, the *in vitro* method is dependent on the degree of allergic sensitization of a given population and the patient selection. Therefore,

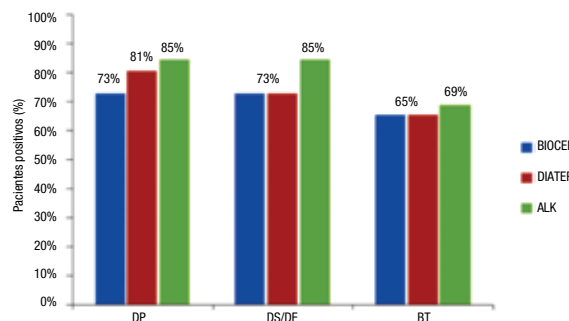
◇ **Figure 1.** Average diameter of the wheals using different products (BIOCEN, DIATER, ALK); Vertical bars show 95% confidence interval.



◇ **Figure 2.** Association between wheal diameter and allergen concentration used in skin prick tests with BIOCEN products.



◇ **Figure 3.** APatients with positive skin prick tests (diameter  $\geq 3$  mm) with the three extracts.



activity values determined *in vitro* with different geographic populations cannot be completely reproduced. BIOCEN products allowed for a linear dependence between wheal diameter average and the logarithm of allergen concentration when using concentrations of 4000 to 100 000 UB/mL. As expected there was similar dependency among the three products. These results match a study done in Cuba, with the same products.

Slightly larger wheals were observed for *Dp*, *Ds* and *Df* than for *Bt*, matching the findings of other Cuban studies; this suggests a minor degree of sensitization to this mite in Cuban territory.<sup>5-8</sup> Authors in different latitudes found a significant degree of association between wheal diameter and specific IgE values for the mite.<sup>10</sup>

The ratio of positive patients was slightly larger for ALK- Abelló and fairly similar for BIOCEN and DIATER, with exclusion of *Dp*. The comparison made among products demonstrates a larger proportion of patients are positive to *Dp* or *Ds* than to *Bt*; showing similar behavior among the three manufacturers. Allergenic extracts are complex mixtures of protein; these products are made by different manufacturers and are not identical. Differences can be appreciated if extracts are made from whole culture (which contain more fecal matter and Group 1: Der p 1, Der f 1, Der s 1 allergens) or from isolated mite bodies (which contain more Group 2 allergens, also important allergens). Differences found in the study might be somewhat

explained by this distinction since BIOCEN and DIATER are both whole culture extracts but ALK products are made from isolated mite bodies.

Although there was high similarity and cross reactivity between *Df* and *Ds*,<sup>11</sup> there are small differences that might have a diagnostic consequence on some patients.

There still is not a full spectrum of allergens for *Bt* and there might be geographical differences between species and cultures from different countries; this might have had some effect on the results.

Another factor that might have influenced the results is that the diagnosis is based fundamentally on clinical features. It has been observed that allergic rhinitis (AR) and nonallergic rhinitis (NAR) share some immunopathological characteristics. Patients with AR have specific IgE to allergens that can be found systemically, positive skin tests, and local IgE produced in the nasal mucosa. Studies in patients with NAR using nasal provocation with allergen have revealed positive nasal provocation results with negative skin test results.<sup>12</sup>

Reaching a diagnosis of allergic rhinitis or asthma is difficult in children without objective proof of allergenic indicators, which might show that some infants and children who begin to walk have nasal allergies. In addition some infants and children occasionally have episodes of wheezing associated with viral infection; most are not diagnosed with asthma in growth.<sup>13</sup>

## Conclusion

The relative potency of mite allergenic extracts produced and standardized by DIATER (Argentina), BIOGEN (Cuba), and ALK-Abelló (Spain) is similar in all three products and provide an appropriate result for a specific diagnosis.

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