
European and Mexican vs US diagnostic extracts of Bermuda grass and cat in skin testing

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Background: Laboratory testing of various diagnostic extracts has shown lower potencies for several European and Mexican extracts relative to the US Food and Drug Administration (FDA) reference (10,000 BAU/mL). Quantitative skin prick testing (QSPT) with *Dermatophagoides pteronyssinus* extracts have previously shown a similar picture.

Objective: To compare European and Mexican Bermuda grass (BG) and cat diagnostic extracts against an FDA-validated extract using QSPT.

Methods: Six diagnostic BG and cat extracts (1 reference FDA extract, 3 European extracts, 1 imported nonstandardized extract from the United States, and 1 Mexican extract) were tested with quadruplicate QSPT, as a concentrate and as 2 serial 2-fold dilutions, in cat and BG allergic individuals.

Results: BG showed good dose response in wheal size for the concentrate (1:2–1:4 dilutions; steep part of the curve). Cat showed poorer dose response. The Wilcoxon test for linked random samples was used to investigate whether the distribution of the reference differed from each of the test extracts to a statistically significant degree (2-sided asymptotic significance, $\alpha = .05$). All BG and 2 cat extracts were statistically less potent than the 10,000 BAU/mL US reference. European BG extracts were 7,700, 4,100, and 1,600 BAU/mL, and cat extracts were 12,500, 4,400, and 5,100 BAU/mL.

Conclusions: The potency of some diagnostic extracts of BG and cat used in Europe, Mexico, and the United States differs, with the US extracts being generally more potent. On the basis of provocation tests, optimal diagnostic concentrations should be determined. Similar comparisons using other manufacturers and therapeutic extracts might be interesting.

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INTRODUCTION

Even though there has been an enormous development in the field of in vitro diagnostic testing, today the skin prick test (SPT) is still the most important diagnostic tool to detect IgE-mediated disease.¹ The reliability of the results of a SPT heavily depends on the technique and the materials used. As such, the quality and potency of the diagnostic extracts directly relate to the sensitivity and specificity of the SPT outcome. The potency of the allergen extracts may not differ too much between manufacturers within the United States because the US Food and Drug Administration (FDA)

through its Center for Biologics Evaluation and Research (CBER) dictates reference tests and substances to be used for extract quality control before batch release. However, in Europe each company has its own in-house reference standard.^{2,3} Apart from the US and European extracts, in some Latin American countries nonstandardized extracts are commercialized, which means that extracts are bought from raw material providers in the United States as lyophilized products and conditioned locally for sale. Finally, another group consists of extracts locally harvested and processed.⁴ A potency comparison of extracts from each of these 4 groups has not yet been made but might be important in light of the growing worldwide exchange of information on subcutaneous and sublingual immunotherapy and the appearance of global guidelines in which dosing issues are discussed.^{5–7}

The current trial has been part of a group of extract comparison studies. The first assays, performed in vitro on diagnostic extracts from European, Mexican, and US manufacturers,⁸ demonstrated that US commercial extracts of 10,000 BAU/mL were at least twice as potent as the European ones for the 3 allergens tested (Table 1). In follow-up trials, fresh lots of the same diagnostic extracts and manufacturers were compared in vivo using quantitative skin prick testing (QSPT). Recently, we reported in this journal on the results of the QSPT comparisons of *Dermatophagoides pteronyssinus* extracts, confirming the in vitro data.⁹ In this publication, the

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Table 1. Analytical Results of In Vitro Testing of Diagnostic Allergen Extracts for *Dermatophagoides pteronyssinus*, Bermuda Grass, and Cat From European and Mexican Sources^a

Extract source	Relative potency of house dust mite (<i>D pteronyssinus</i>) ^b	Relative potency of Bermuda grass (<i>Cynodon dactylon</i>) ^b	Fel d 1 content of cat (<i>Felis domesticus</i>), U/mL ^c
US reference	1.000	1.000	18
Eur1	0.360	0.089	29.4
Eur2	0.444	0.246	2.1
Eur3	0.398	0.083	2.6
US-Mex1 (Mex 1 ^d)	0.411	0.287	4.4
US-Mex2	0.337	0.028	1.6
Mex1	0.082	0.090	< 0.6
Mex2	0.005	0.049	1.3
Mex3 (Mex 2 ^d)	0.034	0.046	1.7

^a Adapted from Larenas-Linnemann et al. European and Mexican diagnostic allergen extracts perform differently from US (CBER/FDA) extracts in vitro. *Allergol Immunopathol.* 2010;38:170–173.

^b Relative potency reported with respect to the US reference extract, determined by IgE competition enzyme-linked immunosorbent assay (Center for Biologics Evaluation and Research recommended methods).

^c Fel d 1 content determined by the Center for Biologics Evaluation and Research radial immunodiffusion assay.

^d US-Mex1 from this study is Mex1 in the present article; Mex3 from this study is Mex2 in the present article.

results of the same QSPT comparisons of the Bermuda grass (BG) and cat extracts are presented.

METHODS

Trial Design

A double-blind, single-center trial was undertaken to compare the biological activity of diagnostic allergen extracts of BG and cat of different manufacturers by QSPT. BG was tested in the Hospital Universitario de Nuevo Leon during the rainy season of 2007, and cat was tested in Hospital Juarez in 2007–2008.

Patient Selection

Patients 18 to 55 years old with clinical symptoms of rhinitis (and mild asthma) were preselected as candidates for the trial from the outpatient clinic of both hospitals. Candidates had to have rhinitis symptoms with exposure to BG or cat to be included in the study. Moreover, BG and cat allergy were documented by a positive SPT result (defined as a major wheal diameter of at least 3 mm above the negative control) during a routine SPT with a basic panel, including 36 allergens: house dust mite, cat, dog, tree pollen, grass pollen, weed pollen, and molds (10,000 BAU/mL, NELCO, Burlington, Massachusetts, and 1:20 [wt/vol], Allerstand, Mexico City, Mexico, in the BG and cat centers, respectively). Moreover, a vehicle reaction (negative control, 50% glycerin) of

less than a 3-mm major wheal diameter led to recruitment in this trial. Patients signed an informed consent document before inclusion in the study. Exclusion criteria were medications that interfere with skin reactivity,¹⁰ pregnancy, and a prior history of immunotherapy. Data from the patients with a positive reaction to the negative control (50% glycerin) of the study extracts (wheal ≥ 5 mm²) were eliminated once statistical analysis was performed. The study protocols and informed consent forms were approved by the institutional review board of the corresponding study centers.

Allergen Extracts

The extracts compared were the glycerinated, phenolated saline solutions commercialized as diagnostic SPT extracts of BG and cat. The commercial extracts were obtained from 4 different groups of allergen manufacturers: American (US), European (Eur1-Eur3), US-Mexican (products acquired from a large-scale raw material provider in the United States and without standardization commercialized in Mexico) (Mex1), and Mexican (Mex2). The US extracts used as reference in this study are the commercial diagnostic extracts for BG and cat of Greer Laboratories Inc (10,000 BAU/mL; Lenoir, North Carolina), standardized according to FDA/CBER recommendations. The 3 European allergen manufacturers whose products we used are all members of the European Allergen Manufacturers Group and were selected for inclusion in the study because their extracts are licensed for use in human beings in Mexico: diagnostic extract from Laboratories Leti (30 HEP/mL; Madrid, Spain), diagnostic extract from IPI-ASAC (standardized in UBE/mL; Madrid, Spain), and Soluprick (10 HEP/mL; ALK-Abelló, Madrid, Spain; manufactured in Spain and commercialized throughout Europe). From here on these are referred to as Eur1, Eur2, and Eur3 in a random order. The US-Mexican extracts are from Alerquim (1:20 wt/vol; Mexico City, Mexico) and from here on are called Mex1. The Mexican products tested are the diagnostic extracts from Rocel (1:20 wt/vol; Puebla, Mexico) and from here on are called Mex2. Moreover, 6.75 mg/mL of histamine (Alerquim, Mexico City, Mexico) and 50% glycerin were used as the positive and negative controls, respectively.

Skin Prick Testing

All extracts were tested with a DuoTip lancet (Lincoln Diagnostics, Decatur, Illinois) by a single allergist in each center, as a concentrate and as 2 serial 2-fold dilutions (concentrate, dilution 1:2, and dilution 1:4), on the patient's back in 2 sessions per patient. As such, all extracts were tested in quadruplicate, in accordance with the Nordic guidelines¹¹ as recommended by the European Academy of Allergy and Clinical Immunology position paper on allergen standardization and skin tests.¹² Tracing of the wheal circumference the extracts produced on the patient's skin 20 minutes after puncture was followed by the calculation of the wheal surface, electronically, at the statistical department. In this assay the wheal surfaces were used as the marker of biological activity for each allergen solution.

Masking

Bottles of the extracts under investigation were bought and packed in masked vials. Personnel not involved in the study gave randomly drawn numbers to all the vials: those containing the dilutional series of each of the extracts and the vials with the negative and positive controls. Both the patient and the medical staff that applied the SPTs were masked to the extracts, the dilutions, and the positive and negative controls. Collaborators at the statistical center were masked at the moment of data entrance. After that, it was revealed which wheals belonged to the negative and positive controls and which wheals belonged together as dilutional series of each extract. In the last step, after statistical calculations were finished, the US reference and the origin of the extracts (European, US-Mexican, or Mexican) were unmasked but not the manufacturers.

Sample Size Calculation

On the basis of the results of a preceding study,¹³ which had a similar single-center design and 1 skilled technician performing the SPT, a sample size of 12 patients per center provides a statistical power sufficient to detect a 20% difference in wheal size among the extracts. Moreover, the quadruplicate measurements of each extract and each concentration in every individual would correspond to an inter-individual sample of approximately 50 patients per center if the tests had been applied a single time to each patient.

Data Transfer and Statistical Analysis

Translucent tape was used to transfer the pen drawing of the wheal contour into the case report form. At the statistical center, the wheal area was digitized and analyzed using the software Adobe Photoshop 6.0 and ImageJ1.40g. The geometric mean of the patient's 4 individual wheal areas (in square centimeters) for each concentration was determined and the log of the mean values was plotted. The 3 concentrations of each test extract were calculated based on the variables $\log_{10}$ wheal area and $\log_{10}$ allergen concentration for each patient. The Wilcoxon test for linked random samples was used in each group to investigate whether the distribution of the US reference extract differed from each of the test extracts to a statistically significant degree with a test level $\alpha = .05$.

The geometric mean of the wheal area of each test extract and its dilutions was compared with the results of the US extract of 10,000 BAU/mL and its 2 dilutions. The concentrated US extract was the comparative value for the concentrates of the 5 test extracts. To convert the geometric mean of the wheal surface to bioequivalent allergy units, a reference concentrate factor, 10,000 BAU/mL, was calculated. In the next step the test extract concentrates' results were divided by that factor to obtain their bioequivalent allergy units. The procedure is the same for all 1:2 and 1:4 dilutions, using the wheal surfaces of the 1:2 and 1:4 dilutions of the US extract as reference. For each extract the 3 bioequivalent allergy unit values of the dilutional series were plotted logarithmically to

extrapolate individual corresponding bioequivalent allergy unit values for the extract concentrates of each manufacturer.

RESULTS

Table 2 presents the demographic characteristics of the study patients. Four of the 25 recruited patients with allergic rhinitis to BG were not included in the final data set because their negative control gave wheal sizes bigger than 5 mm². Nine of the 21 remaining patients did not return to the last study visit, but their data were included in the analysis. Twelve patients had a complete data set.

Of the 18 cat allergic patients screened, 3 dropped out because of failure to return to the second study visit. Of the remaining 15 patients, 5 were not included in the final data set because their negative control gave wheal sizes bigger than 5 mm², leaving a total of 10 patients for the final cat analysis.

BG Skin Test Results and Relative Potency of All Extracts

The concentrate and its 2 dilutions of almost all extracts showed a good dose response in wheal surface (Fig 1). The US reference extract produced a geometric mean wheal size of 0.49 cm², whereas the wheal surfaces of the European extracts were between 0.08 and 0.38 cm², and Mex1 and Mex2 produced wheal sizes between 0.41 and 0.07 cm² (Table 3) (standard deviations varied from 0.051 to 0.610 cm²). Statistically significant differences could be found between the concentrates of the US extract and all other extracts (Fig 1) and among the concentrate extracts of some manufacturers (Table 3). In Figure 2 the bioequivalent allergy unit values for all extracts are calculated, using extrapolation of the log-plotted trend lines.

After the planned analysis, we also performed a post hoc analysis in which too mild responders (wheal surface of the

Table 2. Characteristics of the Bermuda Grass and Cat Sensitive Patients Who Underwent Skin Prick Testing^a

Characteristics	Bermuda grass	Cat
Enrolled	25	15
Excluded (negative control, wheal >5 mm ²)	4	5
Studied	21	10
Only 1 data set	9/21	0
Female/male	9/12	7/3
Age, mean (range), y	26.9 (19-52)	32.8 (19-50)
Rhinitis	17/21	8/10
Rhinitis and asthma	4/21	2/10
Polysensitized ^b	21/21 (80% with perennial allergens)	10/10 (8 with perennial allergens)

^a Data are presented as number of patients unless otherwise indicated.

^b Positive result with at least 1 other allergen, apart from the allergen under investigation. A major wheal diameter of 3 mm² over the major wheal diameter of the vehicle (negative control) was considered a positive result.

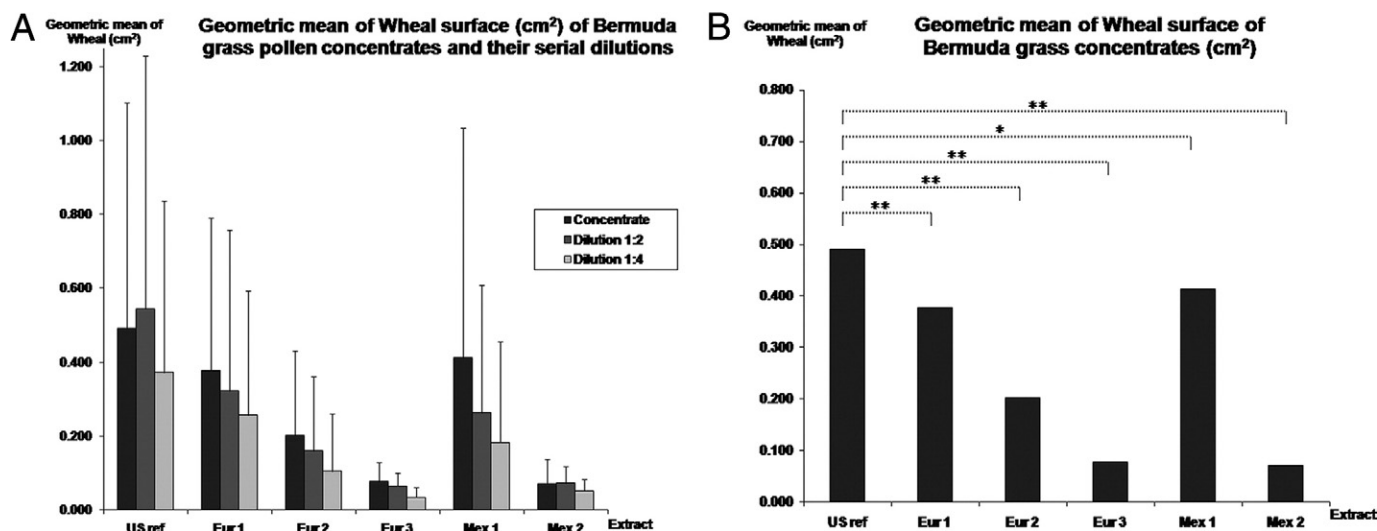


Figure 1. Geometric mean of the wheal surfaces produced by the diagnostic Bermuda grass pollen extracts of a US manufacturer (US), 3 European manufacturers (Eur1-3), a Mexican manufacturer that acquires unstandardized bulk extract from a US provider (Mex1), and a Mexican manufacturer that collects its own raw material (Mex2). A, Bar diagram of the concentrate and 2 serial dilutions of all Bermuda grass pollen extracts (1:2 and 1:4 dilutions) and standard deviations. B, Bar diagram of the concentrates with horizontal lines between bars indicating the presence of a statistically significant difference. * $P < .05$, ** $P < .01$, *** $P < .001$.

US concentrate less than 0.1 cm²) and patients with an incomplete data set were omitted. Similar results were obtained as those presented here. Only a reduction in the SDs was observed, as can be expected (data not shown).

Cat Skin Test Results and Relative Potency of All Extracts

The concentrate of the US reference produced a mean wheal surface of 0.29 cm². In general, the wheal size for cat extracts was smaller than with BG, and the relationship between concentration and wheal size is less clear. Wheal sizes produced by the extracts under investigation are given in Table 3, with standard deviations ranging from 0.037 to 0.413 cm².

In Figure 3 the statistical significance of the differences between the US concentrate and 2 test extracts' concentrates, Eur2 and Mex2, is marked. The concentration of Eur3 is also less than that of the US extract, but this difference did not reach statistical significance (2-sided asymptotic Wilcoxon test). For cat, 1 European extract produced a larger wheal than all other extracts, including the US reference. This same extract, Eur1, also performed stronger in the previous study using laboratory testing (Table 1).

Just as with the BG extracts, the extrapolated bioequivalent allergy unit values were calculated for the concentrates of all

Table 3. Geometric Mean of the Wheal Surface of Concentrate Extracts Tested, Their Relation to the US Reference Extract, and Statistically Significant Differences Among the Test Extracts for Bermuda Grass and Cat

			Statistical significance of comparison of wheal surfaces ^a				
			Eur1	Eur2	Eur3	Mex1	Mex2
Bermuda grass							
Eur1	0.378	77					
Eur2	0.202	41	.002				
Eur3	0.077	16	.004	.01			
Mex1	0.413	84	.83	.005	.01		
Mex2	0.072	15	.008	.008	.89	.02	
Cat							
Eur1	0.356	125					
Eur2	0.126	44	.005				
Eur3	0.146	51	.005	.33			
Mex1	0.277	97	.06	.047	.01		
Mex2	0.056	20	.005	.14	.01	.007	

^a Geometric mean of the wheal surfaces in the patients studied.

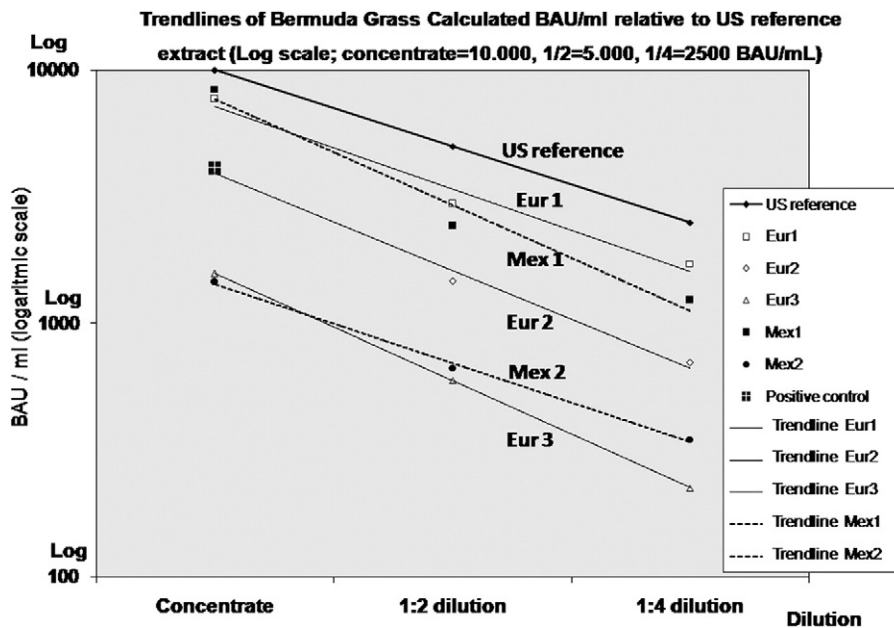


Figure 2. Trend lines of log bioequivalent allergy units as calculated from the geometric mean of the wheal surfaces, produced by diagnostic Bermuda grass extract concentrates and their 2 serial 2-fold dilutions, from a US manufacturer (US), 3 European manufacturers (Eur1-3), a Mexican manufacturer that acquires unstandardized bulk extract from a US provider (Mex1), and a Mexican manufacturer that collects its own raw material (Mex2). The US extract and its dilutions were taken as the references (10,000, 5,000, and 2,500 BAU/mL) to assign the relative potency values in BAU/mL for the other extracts and their dilutions. Bold black line, US extracts; fine black lines, European extracts; interrupted lines, Mexican extracts. Equations of the trend lines are as follows: Eur 1: $y = 15175e^{-0.748x}$, with a calculated value for the concentrate of 7,132 BAU/mL; Eur 2: $y = 15175e^{-0.884x}$, with a calculated value for the concentrate of 3,891 BAU/mL; Eur 3: $y = 15175e^{-0.973x}$, with a calculated value for the concentrate of 1,580 BAU/mL; Mex 1: $y = 15175e^{-0.961x}$, with a calculated value for the concentrate of 7,604 BAU/mL; Mex 2: $y = 15175e^{-0.715x}$, with a calculated value for the concentrate of 1,428 BAU/mL.

cat extracts. The biological activity of Eur1, Eur2, and Eur3 ranged from 4,132 to 12,572 BAU/mL, the biological activity of Mex1 was 11,257 BAU/mL, and the biological activity of Mex2 was 2,047 BAU/mL (Fig 4).

Adverse Events

All patients were observed for at least 1 hour after testing. All adverse events reported were classified as certainly or probably related to the skin testing. Two patients in the cat group presented with cough after testing, which in 1 of them was accompanied by wheezing. Both were given an oral antihistamine and an inhaled bronchodilator, which caused the symptoms to disappear within 30 minutes, and both were dismissed without any further problems within 2 hours. One of the BG patients developed acute urticaria and was withdrawn from the study. There were 3 local reactions, which in fact were normal responses to the QSPT but more severe than usual. All consisted of intense pruritus at the site of the SPT, 1 in the BG group and 2 in the cat group (4% of the tests); a local corticosteroid cream and an oral antihistamine relieved the discomfort.

DISCUSSION

In titrated SPTs the diagnostic extracts of BG from a US manufacturer produced a statistically significant greater wheal surface than the extracts from all 3 European manu-

facturers evaluated, as well as in comparison to the local Mexican extract, Mex2. For the cat extracts the situation was less straightforward. One European and the Mex1 cat extracts produced a bigger wheal surface than the US extract, although the difference was not statistically significant. The wheal surfaces of another European extract and of the local Mexican extract were smaller than the US extract, with statistical significance. The mean wheal surfaces varied from 16% to 77% in the European BG extracts and from 44% to 125% in the cat extracts in relation to the 10,000-BAU/mL US extract. The Mex1 extracts are in fact American extracts sold by a Mexican provider that obtains raw material in the United States, which might explain why the wheal surface produced by the Mex1 allergens is comparable to that produced by the US extracts.

This is the first study, to our knowledge, to show that major differences exist between the potency of some diagnostic extracts of BG from manufacturers in different locations. Just as a recent QSPT study demonstrated in this journal for diagnostic *D pteronyssinus* extracts,⁹ we show also for BG that the US extracts produce larger wheal surfaces. For the cat extracts, the picture is much less clear. In general, the cat extracts tested in this study produced a smaller wheal than the BG extracts. Moreover, they showed a strong variation in potency among themselves but without relation to their

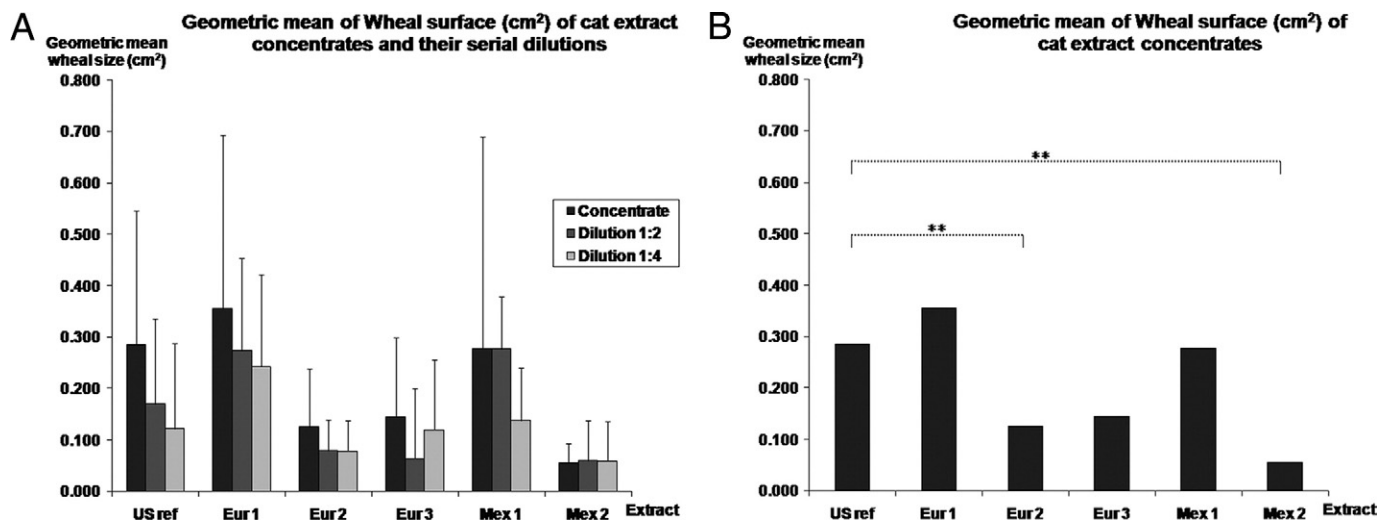


Figure 3. Geometric mean of the wheal surfaces produced by the diagnostic cat extracts of a US manufacturer (US), 3 European manufacturers (Eur1-3), a Mexican manufacturer that acquires unstandardized bulk extract from a US provider (Mex1), and a Mexican manufacturer that collects its own raw material (Mex2). A, Bar diagram of the concentrate and 2 serial dilutions of all cat extracts (1:2 and 1:4 dilutions) and standard deviations. B, Bar diagram of the concentrates with horizontal lines between bars indicating the presence of a statistically significant difference. * $P < .05$, ** $P < .01$, *** $P < .001$.

geographic origin. These observations of some diagnostic extracts being 4 to 6 times more potent than others lead directly to the question of which concentration would be ideal for a diagnostic extract. Together with the device used and the wheal size that is considered a positive reaction,^{14,15} the extract concentration is the third variable that defines the SPT result. When a stronger extract is used, the SPT has a high sensitivity, but less sensitive people might produce a wheal as well. This kind of extract is useful for epidemiologic studies in which investigators aim for a high positive predictive value and a low number of missed cases. However, in the situation where patients are preselected, as in the allergist's office, the specificity of a testing tool becomes more important. Extracts with lower potency might better fulfill this requirement to enable the allergist to only select the most important allergens for treatment, especially in those cases where the clinical history is unable to differentiate between allergens. The current results call for a subsequent set of trials to establish the optimal diagnostic concentration of allergen extracts tested against the "gold standard" of allergy diagnosis—organ challenge.

We used QSPT to establish and compare the biological activity of extracts because this method had been shown to be effective in a previous study of European extracts.¹³ This technique resulted in a good dose response for the BG extracts, and confirming tests were performed on the steep part of the dose-response curve, as is recommended. Because of quadruplicate testing, 12 patients were enough to show statistically significant differences among the extracts. For cat, the less potent extracts, Eur2, Eur3, and Mex2, did not manifest a good dose response (horizontal part of the dose-response slope). Moreover, because the testing was

performed among patients of a regional government hospital who come from far away and in some cases could not attend the second visit, the final data set included only 10 patients, which reduces the power of the statistical analysis of the cat extracts.

In the US extract, potency is routinely tested by intradermal titration (ID₅₀EAL method).¹ This somewhat painful method is restricted to the testing of just a limited number of allergens simultaneously but might have been more suitable for the comparison of the cat extracts with lower potency. A trial in which both techniques are used with the same extracts on the same patients could give an answer to this assumption and would make it possible to establish how best to compare extracts of different potency in skin testing.

That cat extracts commercialized in the United States are less potent than grass extracts might also be inferred from the fact that the recommended dose of cat extract for immunotherapy is 15 μ g of Fel d 1,^{16,17} corresponding to 0.3 mL of a 10,000-BAU/mL extract (containing 50 μ g/mL of Fel d; ALK-Abelló; data available on request). The dose of grass pollen confirmed to give the best clinical improvement lies in the same range, 20 μ g Phl p 5 every 6 weeks.¹⁸ For the BG extract of 10,000 BAU/mL (containing 300 μ g/mL of major allergen; ALK-Abelló; data available on request), this would be only 0.05 mL, 6 times less than the volume needed of a cat extract.

Historically, there have been only a few publications on extract comparisons, apart from 1 review of existing data.² One of the first studies was an SPT comparison between 2 sublingual immunotherapy solutions in Europe.¹³ Sander et al¹⁹ reported on the in vitro potency of diagnostic Timothy extracts from several European manufacturers, including So-

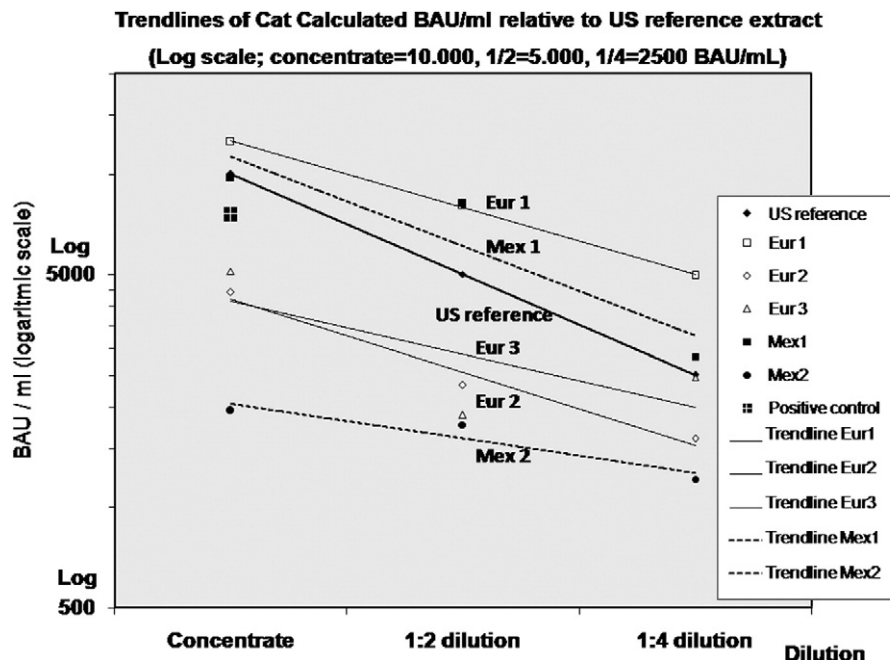


Figure 4. Trend lines of log bioequivalent allergy units as calculated from the geometric mean of the wheal surfaces, produced by diagnostic cat extract concentrates and their 2 serial 2-fold dilutions, from a US manufacturer (US), 3 European manufacturers (Eur1-3), a Mexican manufacturer that acquires unstandardized bulk extract from a US provider (Mex1), and a Mexican manufacturer that collects its own raw material (Mex2). The US extract and its dilutions were taken as the references (10,000, 5,000, and 2,500 BAU/mL) to assign the relative potency values in bioequivalent allergy units for the other extracts and their dilutions. Bold black line, US extracts; fine black lines, European extracts; interrupted lines, Mexican extracts. Equations of the trend lines are as follows: Eur 1: $y = 19955e^{-0.462x}$, with a calculated value for the concentrate of 12,572 BAU/mL; Eur 2: $y = 6977.5e^{-0.504x}$, with a calculated value for the concentrate of 4,187 BAU/mL; Eur 3: $y = 5989.3e^{-0.367x}$, with a calculated value for the concentrate of 4,132 BAU/mL; Mex 1: $y = 20846e^{-0.617x}$, with a calculated value for the concentrate of 11,257 BAU/mL; Mex 2: $y = 2591.9e^{-0.237x}$, with a calculated value for the concentrate of 2,047 BAU/mL.

luprick (ALK-Abell6), which was situated in the middle of the potency range. As already mentioned, diagnostic extracts of *D pteronyssinus* of American, European, and Mexican origin have been compared with the same QSPT protocol.⁹ Analogous to the present study, the European extracts were between 54% and 61% of the potency of the US reference extract of 10,000 BAU/mL.

In conclusion, diagnostic extracts of BG from selected European and Mexican manufacturers were generally less potent than those from the United States. For cat the potency of the extracts varied as well, with the US extract being of intermediate potency. Sensitivity and specificity studies will have to show in the future what the optimal diagnostic concentrations are for inhalant allergens. A similar in vivo potency comparison of therapeutic immunotherapy extracts from different parts of the world might yield interesting information.

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