
A comparison of two skin test methodologies and allergens from two different manufacturers

Robert Rhodius, MD*; Kristin Wickens, PhD†; Soo Cheng, BsC Honst; and Julian Crane, MD†

Background: Skin prick tests (SPTs) are a frequently used method for evaluation of atopy. A variety of standard allergen preparations are available, together with a number of different methods of application.

Objective: The objective of this study was to compare SPT reactivity 1) using Soluprick SQ allergens (ALK Allergologisk Laboratorium A/S, Hørsholm, Denmark) and Bayer allergens [Bayer Corporation, West Haven, CT], and 2) using two common methods of application, a standard prick lancet and a Quintest, both produced by Bayer.

Methods: SPTs were undertaken on 22 adult volunteers (mean age 40 years, 17 female, 5 male). Wheal size was recorded as mean diameters (mm) and area (mm²).

Results: Bayer allergens produced larger mean diameters and areas to dust mites than ALK allergens, with the differences significant when allergens were applied with the lancet. There was a tendency for the ALK cat allergen to produce larger reactions than the Bayer product. The method of application also influenced the wheal size, with the lancet producing significantly larger mean diameters than the Quintest for the histamine and allergens from both manufacturers, except the ALK cat allergen. There were similar differences between methods of application for reactions measured as an area.

Conclusions: SPTs that use different allergens or different methods of application will not provide comparable assessments of atopy.

Ann Allergy Asthma Immunol 2002;88:374-379.

INTRODUCTION

Skin prick testing for sensitivity to common allergens is an accepted method of determining individual atopic status, especially useful in population surveys. However, across-study comparisons in the prevalence of atopy may be compromised because of differences in the methods or allergens used to determine individual atopic status. Many studies have examined this potential bias by comparing the many different prick devices on the market,¹⁻⁶ although few studies have included the more recently produced

Quintest manufactured by Bayer Corporation (West Haven, CT).⁵⁻⁸ Further, we found only one paper⁹ comparing skin prick test (SPT) reactivity to a panel of standardized extracts produced by different manufacturers and applied using the lancet recommended by each manufacturer. The findings of fewer positive reactions for the Dome/Hollister-Stier (Spokane, WA) allergens may thus have reflected the different lancet used. A second paper¹⁰ which compared the potency of conventional and standardized allergens included extracts from two different manufacturers of conventional allergens and showed that each conventional allergen produced similar wheal sizes for 7 of the 8 allergens tested.

Until recently, atopy has generally been measured in New Zealand population studies using the allergens and SPT lancet produced by Bayer. For the purposes of this study, this method will be considered the reference against

which ALK (Allergologisk Laboratorium A/S, Hørsholm, Denmark) allergens and the Quintest method of allergen application will be evaluated.

MATERIALS AND METHODS

Study Population

Staff at local schools at the hospital in Hawkes Bay and staff at the University of Otago, Wellington, New Zealand were approached and invited to undergo a screening test to determine whether they were eligible to participate in this study. Participants were eligible if they responded positively to a screening test of ALK or Bayer *Der-matophagoides pteronyssinus* or cat allergen using the Bayer prick lancet. A positive reaction was at least one wheal with a mean diameter of ≥ 3 mm after 10 minutes. Of the 57 who volunteered, 22 satisfied these eligibility requirements. All participants were aged between 24 and 60 years (mean age 40) and 17 were female.

SPTs

For the study, the ALK allergens used were *D. pteronyssinus*, *D. farinae*, and *Felis domesticus*, all with a concentration of 10-HEP (10,000 biologic units¹¹). The Bayer allergens used were *D. pteronyssinus* and *D. farinae*, each with a concentration of 30,000 AU/mL, and cat pelt, with a concentration of 10,000 BAU/mL. Both Bayer and ALK allergen extracts were standardized and histamine concentrations from both companies were 1%.

The Bayer prick lancet is a 30-mm stainless steel disposable device with a 1-mm tip. The histamine and allergens are placed on the skin using wooden orange sticks and pricked through with the lancet at a 90° angle to the skin. The Quintest is a disposable plastic device in which five lancets with 1.2-mm tips are mounted 3 cm apart.

* Faculty of Medicine, University of Groningen, The Netherlands.

† Wellington Asthma Research Group, Wellington School of Medicine, New Zealand.

Financial support was received from the Hawkes Bay Medical Trust and the Health Research Council of New Zealand.

Received for publication June 8, 2001.

Accepted for publication in revised form December 1, 2001.

After inserting the Quintest into wells in a tray to coat the tip of the lancets with the histamine and allergens, the five SPTs are performed simultaneously on the subject. The lancet was applied with moderate pressure and the Quintest with greater pressure in an attempt to ensure that small circles remained at the test site as an indication that the correct pressure had been applied (as recommended by the manufacturer). Antihistamines were withheld for at least 35 hours before skin prick testing. All allergic subjects were tested on the volar aspect of the forearm by the same investigator with both devices and both sets of allergens and histamine in one session. On the left arm the tests were performed with lancets, after applying ALK allergens on the medial side and Bayer on the lateral side. On the right arm the Quintest was used, with ALK allergens on the lateral side and Bayer allergens on the medial side. Allergen responses were measured at 15 minutes and the histamine control 10 minutes after application. The wheals were outlined with a thin felt-tip pen, and using micropore tape, a record of the results was transferred to paper.

Measurements of the wheals were recorded in two different ways. For each wheal, the longest and orthogonal diameter was measured and the mean diameter calculated. Because of the irregularity of some wheals, the area of the wheal was also determined by scanning the wheal outline into a computer and calculating the enclosed area.

Statistical Analysis

SAS version 8 (SAS Institute, Cary, NC) was used for the analysis. Most wheal diameter or wheal area distributions were not normally or log-normally distributed. Therefore a non-parametric test, the Wilcoxon rank sum test, was used to test for statistically significant differences between mean wheal diameters and between mean wheal areas for each allergen stratified by manufacturer and application method. In addition, Bland and Altman¹² plots were used to show the differences in allergen preparations (ALK or Bayer) and devices (lancet or Quintest) against mean wheal diameters for *D. pteronyssinus*. For this analysis the negative control was not subtracted from the allergen response, because the purpose was to compare responses between allergens and prick devices and not to define atopy. Sensitivity in determining atopy was calculated for each combination of method and allergen source compared with the reference method (Bayer allergens and lancets) with atopy defined as a mean wheal diameter of 3 mm or greater after subtraction of the negative control. A subsequent analysis defined atopy similarly but without the subtraction of the negative control.

Ethical approval was granted by the Hawke's Bay Ethics Committee.

RESULTS

Table 1 shows the numbers of participants who tested positive for the positive control and for allergens manufac-

tured by Bayer and ALK using the lancet and Quintest methods of application. The numbers of atopic participants (a positive reaction to any allergen after subtraction of the negative control) using each combination of allergen source and method are also shown. For the reference method (Bayer allergens applied with the Bayer lancet), of the 22 eligible subjects there was one subject who was classified as nonatopic after subtraction of the negative control. Using the Bayer lancet and the allergens produced by ALK only 16 were defined as atopic. Similarly, 16 were defined as atopic when the Quintest was used to apply the standard Bayer allergens. The use of the Quintest device with the ALK allergens further reduced the number atopic to 13. Compared with the reference method (Bayer allergens and lancets), the sensitivity of the ALK allergens applied with the lancet was 0.76, the sensitivity of the Quintest using Bayer allergens was 0.76, and the sensitivity of the Quintest using ALK allergens was 0.62. Five respondents had at least one reaction to a negative control, and these were twice as frequent with the Quintest than with the lancet (8 vs 4). We also analyzed atopy prevalence defined as at least one positive reaction without the subtraction of the negative control. The sensitivity of the Quintest, compared with the reference method, increased to 0.86 (19 of 22 atopic) when Bayer allergens were used, and to 0.77 (17 of 22 atopic) when ALK allergens were used; but there was little change in the sensitivity of ALK allergens applied with the lancet (0.77).

Table 2 shows the mean wheal diameters, and the mean differences (mm; 95% confidence intervals) in the diameter of the wheal size between ALK and Bayer allergens, and between the lancet and Quintest. There were differences in house-dust mite wheal size, which were dependent on the allergen source, with these differences reaching significance for the lancet method, but there were no significant differences for the positive control and the cat allergen. Apart from cat,

Table 1. Number of Positive Responses (≥ 3 mm) to SPTs by Method of Application and Manufacturer of Allergen

	Lancet		Quintest	
	Bayer allergens	ALK allergens	ALK allergens	Bayer allergens
	n = 22	n = 22	n = 22	n = 22
Histamine control	22	22	11	16
<i>D. pteronyssinus</i>	21	16	11	16
<i>D. farinae</i>	19	12	5	12
Cat	11	10	7	3
Total number atopic	21	16	13	16
Sensitivity in defining atopy	REF	0.76	0.62	0.76

Table 2. The Differences (mm) in Mean Wheal Diameter (95% Confidence Intervals and 95% Lower and Upper Limits of Agreement (LLA, ULA)) between ALK and Bayer Allergens for Lancets and Quintests, and between Lancets and Quintests using ALK and Bayer Allergens

Method	Allergen	Mean wheal		Mean difference	Lower 95% CI	Upper 95% CI	P value	LLA	ULA
		ALK	Bayer						
Lancet	+ve	4.8	5.1	-0.30	-0.66	0.06	0.20	-1.89	1.30
	<i>D. pter.</i>	5.0	8.0	-3.00	-4.61	-1.39	<0.001	-10.10	4.10
	<i>D. farinae</i>	2.9	6.6	-3.75	-5.33	-2.17	<0.001	-10.74	3.24
	Cat	3.1	2.8	0.27	-0.30	0.85	1.00	-2.27	2.82
Quintest	+ve	2.7	3.3	-0.59	-1.33	0.15	0.42	-3.87	2.69
	<i>D. pter.</i>	2.4	3.9	-1.55	-2.68	-0.41	0.07	-6.57	3.48
	<i>D. farinae</i>	1.6	3.3	-1.73	-3.29	-0.17	0.10	-8.62	5.16
	Cat	2.1	1.1	0.98	-0.11	2.06	0.27	-3.82	5.77
		Lancet	Quintest						
ALK	+ve	4.8	2.7	2.07	1.15	2.99	<0.001	-1.99	6.13
	<i>D. pter.</i>	5.0	2.4	2.64	1.01	4.26	0.01	-4.54	9.81
	<i>D. farinae</i>	2.9	1.6	1.30	0.26	2.33	0.02	-3.28	5.87
	Cat	3.1	2.1	1.02	0.15	1.89	0.49	-2.82	4.87
Bayer	+ve	5.1	3.3	1.77	0.95	2.59	<0.001	-1.85	5.40
	<i>D. pter.</i>	8.0	3.9	4.09	2.74	5.44	<0.001	-1.86	10.05
	<i>D. farinae</i>	6.6	3.3	3.32	1.36	5.28	0.005	-5.36	11.99
	Cat	2.8	1.1	1.73	0.64	2.82	0.04	-3.09	6.54

P values are based on Wilcoxon rank sum test.

the Bayer allergens produced larger mean wheals than the ALK allergens for both methods of application. The lancet produced significantly larger mean wheals than the Quintest for the two dust mite allergens and the positive control, regardless of which company produced the allergen. The mean wheal size produced by the lancet tended to be approximately twice that produced by the Quintest. The lancet also produced a significantly larger mean wheal diameter for the Bayer cat allergen, but the differences did not reach significance for the ALK cat allergen.

Table 2 also shows the 95% limits of agreement¹² for each comparison. Dust mite allergens showed a greater magnitude of disagreement than the cat allergen or the positive control. For example, when the lancet was used and atopy was expressed as a mean wheal diameter, the limits of agreement between ALK and Bayer for *D. pteronyssinus* allergen ranged from -10.1 to +4.1 compared with the narrower limits of agreement between ALK and Bayer for the positive control, from -1.89 to +1.30.

Table 3 shows the mean differences (95% confidence intervals) in the area

of the wheal between ALK and Bayer allergens, and between the lancet and the Quintest. Significant differences were found in mean wheal area between the ALK and Bayer dust mite allergens using the lancet. There were no significant differences in mean area between any ALK and Bayer allergens when the Quintest was used. Apart from cat, ALK allergens produced consistently smaller wheal areas for both the lancet and the Quintest. The comparison between the lancet and the Quintest showed that the lancet consistently produced larger wheal areas and this was significant for the positive control and *D. pteronyssinus* using ALK allergens, and for the positive control and the two dust mite allergens when allergens were supplied by Bayer.

The limits of agreement in dust mite wheal area for ALK and Bayer allergens are wide if the lancet was used but less with the Quintest (Table 3). Similarly, there is large disagreement between the size of the dust mite wheal area produced by the lancet compared with the Quintest, although this is less for *D. farinae* produced by ALK. Compared with the dust mite allergens, the cat allergen and the positive control

showed better agreement between the ALK and Bayer allergens and between the two methods of application.

Bland and Altman plots (Fig 1) show the differences in mean diameter by the mean value of the measurements for the *D. pteronyssinus* comparisons between ALK and Bayer for each method of application (Fig 1, A and C) and lancet and Quintest for each manufacturer (Fig 1, B and D). The plots comparing the lancet and the Quintest (Fig 1, B and D) show that most values are positive, indicating that the lancet almost always produced a larger reaction than the Quintest. There was a preponderance of negative reactions for the comparison between ALK and Bayer allergens when the lancet was used (Fig 1 A), indicating in this case that the Bayer reactions are almost always the larger. However, with the Quintest, the scattering of the agreement between *D. pteronyssinus* Bayer and ALK allergens (Fig 1 C) is less clearly in one direction.

DISCUSSION

Despite the frequency with which SPTs are used to evaluate atopy in population surveys, surprisingly we

Table 3. The Differences (mm²) in Area (95% Confidence Intervals and 95% Lower and Upper Limits of Agreement (LLA, ULA)) between ALK and Bayer Allergens for Lancets and Quintests, and Between Lancets and Quintests Using ALK and Bayer Allergens

Method	Allergen	Area		Mean Difference	Lower 95% CI	Upper 95% CI	P value	LLA	ULA
		ALK	Bayer						
Lancet	+ve	17.7	18.0	-0.25	-4.20	3.71	0.50	-17.73	17.23
	<i>D. pter.</i>	25.8	48.0	-22.23	-36.28	-8.17	<0.001	-84.36	39.91
	<i>D. farinae</i>	9.2	36.3	-27.14	-41.47	-12.78	<0.001	-90.51	36.24
	Cat	14.1	11.5	2.65	-1.43	6.74	0.83	-15.41	20.72
Quintest	+ve	9.6	11.7	-2.07	-5.42	1.28	0.58	-16.88	12.74
	<i>D. pter.</i>	9.0	13.7	-4.78	-9.78	0.22	0.13	-26.89	17.33
	<i>D. farinae</i>	5.1	14.1	-8.79	-17.70	-0.26	0.15	-46.45	28.88
	Cat	8.7	2.8	5.38	-0.48	12.29	0.36	-21.03	31.78
		Lancet	Quintest						
ALK	+ve	17.7	9.6	6.31	2.20	14.07	0.002	-18.12	34.39
	<i>D. pter.</i>	25.8	9.0	16.80	-3.63	37.23	0.04	-73.52	107.12
	<i>D. farinae</i>	9.2	5.1	3.80	-0.53	8.13	0.17	-15.33	22.94
	Cat	14.1	8.7	5.33	-0.26	10.92	0.39	-19.39	30.05
Bayer	+ve	18.0	11.7	6.31	1.54	11.09	0.002	-14.81	27.44
	<i>D. pter.</i>	48.0	13.7	34.25	19.52	48.98	<0.001	-30.87	99.37
	<i>D. farinae</i>	36.3	14.1	22.15	5.85	38.45	0.003	-49.89	94.20
	Cat	11.5	2.8	8.05	1.14	14.96	0.09	-22.50	38.60

P values are based on Wilcoxon rank sum test.

found no previous studies that compared the independent effects of allergens and prick devices on the evaluation of atopy.

Previous studies have shown that there is a tendency for the Bayer Quintest to elicit smaller reactions compared with other devices.⁵⁻⁷ Our comparisons among a group of atopic adults, showing that significantly smaller wheals are produced by the Quintest than the lancet, reinforce these findings. However, whereas other studies suggest that these differences have little effect on the classification of atopy,^{6,7} we have shown that in our population almost 25% of subjects (5 of 21) would have been misclassified using the Quintest to apply Bayer allergens.

There are a number of possible reasons to explain this. The amount of allergen deposited at the site of penetration may be lower for the Quintest than for the standard lancet technique, or alternatively, it may be that the outer plastic rim of the Quintest, by producing a small indentation in the skin, is actually constraining the size of the subsequent wheal. The smaller mean wheal diameters produced by the

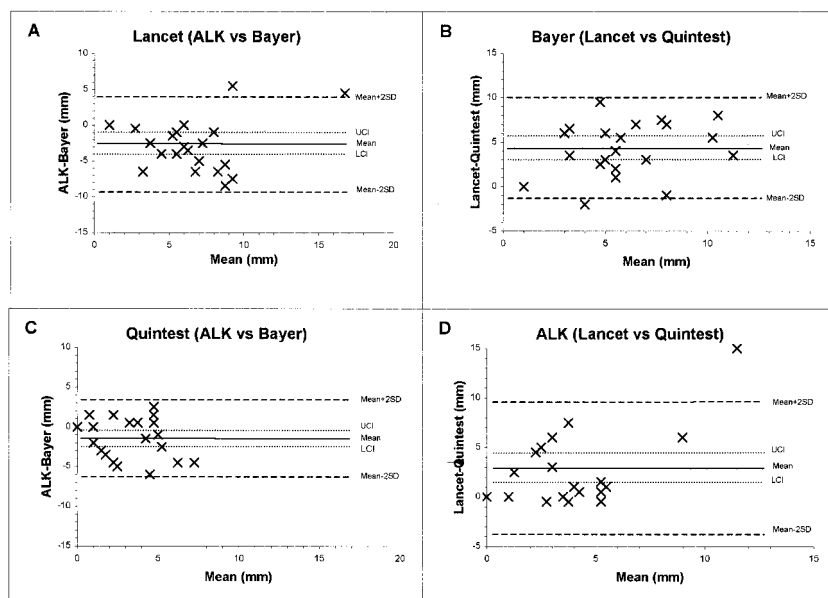


Figure 1. Bland-Altman plots showing differences between allergen preparations (A and C) and devices (B and D) against mean wheal diameters for *D. pteronyssinus*.

Quintest and the lack of statistically significant differences between the mean diameters produced by ALK and Bayer allergens when using the Quintest support the idea that the Quintest has a constraining effect. In contrast,

we have shown that the Quintest is twice as likely to produce a reaction to the negative control than the lancet, possibly because its rim causes an indentation in the skin that is indistinguishable from a histamine reaction.

The manufacturers recommend that the pressure applied should produce small wheals of approximately 4 mm in diameter. This may explain the larger number of reactions to the negative control with the Quintest compared with the lancet, and thus the smaller numbers defined as atopic using the Quintest. To evaluate the influence of negative control reactions on the prevalence of atopy, a subsequent analysis defined atopy without the subtraction of the negative control. Differences between the Quintest and the lancet lessened, although the Quintest remained a less sensitive device even though it was applied with greater pressure than the lancet. Further, the numbers positive to individual allergens were almost always lower with the Quintest regardless of the treatment of the negative control in the definition of atopy. Thus, our data suggest that the Quintest may both constrain the size of allergic reactions and be responsible for false-negative reactions.

A similar situation applies to the different allergens produced by Bayer and ALK, as here there seem to be differences in the allergen preparations themselves. For example, the Bayer cat preparations always produced a smaller mean reaction than the ALK cat preparation, although this did not usually reach significance possibly because small numbers of people were allergic to cat. In contrast, the two house-dust mite allergens from Bayer consistently produced larger reactions than the dust mite allergens from ALK. This potential bias is a concern in New Zealand where dust mite allergen dominates the atopic profile and, thus, the prevalence of atopy may be largely determined by the prevalence of dust mite allergy.

The different units that Bayer and ALK use to express the concentration of their extracts reflect the different methods used to standardize extracts.¹¹ Thus, the relative potency of their extracts is difficult to compare. Further, the major allergen content of the extracts we used differs between manufacturers (personal communication with manufacturers, July 2001). For *D.*

pteronysinus, the concentration of the major allergen, Der p 1 is reported to be approximately 19.6 $\mu\text{g/mL}$ from ALK and 182.2 $\mu\text{g/mL}$ (coefficient of variation, 24.1%) from Bayer. The concentration of the major allergen from *D. farinae*, Der f 1, is approximately 27.6 $\mu\text{g/mL}$ from ALK and 293.7 $\mu\text{g/mL}$ (coefficient of variation, 23%) from Bayer. Each manufacturer reports similar concentrations of Fel d 1: 73 $\mu\text{g/mL}$ for ALK and 60 $\mu\text{g/mL}$ for Bayer. Differences in the concentration of Der p 1 and Der f 1 between manufacturers parallel the differences in the size of SPT response to these allergens that we found in this study. In contrast, the differences between the SPT response to ALK and Bayer cat allergens were mostly nonsignificant, reflecting the similarity of the Fel d 1 concentration in their cat extract.

The differences in SPT reactivity that we found in this study, which were independent of the device used, may reflect the different concentrations of the allergens in the extracts, and caution is needed in comparing atopy across studies in which allergens come from different sources.

We have shown that the Quintest and ALK allergens will each independently result in a lower prevalence of atopy, compared with our usual method (lancet with Bayer allergens). The low sensitivity of the Quintest used in combination with ALK allergens (0.62) highlights the differences that SPT methods can make to the prevalence of atopy.

It is worth noting that, as we defined our atopic population according to our usual practice, any other method would almost certainly underestimate the prevalence of atopy relative to this. However, the purpose of this study was to determine whether the smaller wheal sizes produced by allergens manufactured by a different company and the Quintest resulted in fewer people being defined as atopic, because this would impair our ability to make comparisons in the prevalence of atopy with previous New Zealand studies.

CONCLUSION

The SPT device and allergen source may influence prevalence estimates of atopy; thus, comparisons over time and between place are only valid for studies that use identical methods. A further comparison of each method against specific immunoglobulin E would be worthwhile.

ACKNOWLEDGMENTS

R.R. undertook this study while on a student elective in New Zealand. We are grateful to the Hawkes Bay Medical Trust who provided support toward R.R.'s accommodation, and to the Health Research Council of New Zealand who provide the Wellington Asthma Research Group with a program grant and salary support for Professor J. C.

REFERENCES

1. Illi S, Garcia-Marcos L, Hernando V, et al. Reproducibility of skin prick test results in epidemiologic studies: a comparison of two devices. *Allergy* 1998;53:353-358.
2. Adinoff A, Rosloniec DM, McCall LL, Nelson HS. A comparison of six epicutaneous devices in the performance of hypersensitivity skin testing. *J Allergy Clin Immunol* 1989;84:168-174.
3. Corder WT, Hogan MB, Wilson NW. Comparison of two disposable plastic skin test devices with the bifurcated needle for epicutaneous allergy testing. *Ann Allergy Asthma Immunol* 1996;77:222-226.
4. Nelson HS, Rosloniec DM, McCall LI, Ikle D. Comparative performance of five commercial prick test devices. *J Allergy Clin Immunol* 1993;92:750-756.
5. Nelson HS, Lahr J, Buchmeier A, McCormick D. Evaluation of devices for skin prick testing. *J Allergy Clin Immunol* 1998;101:153-156.
6. Carrozzi FM, Byth K, Katelaris CH. Comparison of the Quintest to the lancet in allergic skin testing. *Asian Pac J Allergy Immunol* 1998;16:149-154.
7. Mahr, TA, West R, Rahn LA. Comparison of the Quintest and Multi-test for allergy skin testing. *J Allergy Clin Immunol* 1997;99:S336.

-
-
8. Caruso K, Witkowski S, Slattery SM, et al. Comparison of performance of four different skin test devices in allergic and non-allergic subjects. *J Allergy Clin Immunol* 1997;99:S60.
 9. Nielsen N, Dirksen A, Mosbech H, et al. Skin prick testing with standardized extracts from 3 different manufacturers. A comparative randomized study. *Allergol Immunopathol* 1992;20: 246–248.
 10. Lavins, BJ Dolen WK, Nelson HS, Weber RW. Use of standardized extracts and conventional allergen extracts in prick skin testing. *J Allergy Clin Immunol* 1992;89:658–666.
 11. Turkeltaub P. Standardized extracts in practice. In: Creticos P, Lockey R, editors. *Immunotherapy: A Practical Guide to Current Procedures*. American Academy of Allergy and Immunology, 1994.
 12. Bland J, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307–310.
- Requests for reprints should be addressed to:
Dr. Kristin Wickens
Wellington Asthma Research Group
Wellington School of Medicine
P. O. Box 7343
Wellington South, New Zealand 6002
E-mail: kwickens@wnmeds.ac.nz*
-