A Study of the Variability of the in Vitro Component-Based Microarray ISAC CDR 103 Technique

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Specific immunoglobulin (Ig) E determination against allergens using the in vitro component-resolved diagnostic microarray technique (ImmunoCap ISAC CRD 103; Phadia, Uppsala, Sweden), has improved diagnostic accuracy [1-4], but few studies have analyzed the reproducibility of this semi-quantitative technique [5].

Reproducibility is analyzed in successive determinations carried out using the KS11 control serum provided with the test kit. This serum contains different concentrations of specific IgE against 10 allergens: rApi g 1, rBet v 2, nBos d 4, nGal d 1, nGal d 2, nGal d 3, rHev b 8, rPhl p 5, rPhl p 6, and rPhl p 7. The resulting data are then used to generate a standard curve that relates the fluorescence signal of the ISAC CRD103 microarray acquired by a laser scanner (LuxScan 10K/A, CapitalBio, Beijing, China) to known concentrations of specific IgE measured in ISAC standardized units (ISUs).

The microarray assay was performed according to the manufacturer´s instructions. The KS11 serum was analyzed for intraslide variability (4 times), intra-assay variability (8 times), and interassay variability (12 times). The reproducibility of the technique was analyzed by calculating the intraclass correlation coefficient (ICC) for overall variability using the software package SPSS 15.0 and the coefficient of variation (CV) for the variability of each of the 10 allergens using Microsoft Excel 97.

According to the classification of Fleiss [6], the ICCs were almost perfect for all 3 tests, with a score of 0.998 for intraslide variability ($P<.0001$), of 0.997 for intra-assay variability ($P<.0001$), and of 0.989 for interassay variability ($P<.0001$).

For the intraslide analysis, 7 of the 10 allergens detected by KS11 had CV values of 10% or less, and for the intra-assay analysis, 5 allergens had CV values of 15% or less. In the interassay analysis, all of the allergens had CV values of over 20%, three allergens (nBos d 4, rPhl p 5, and rPhl p 6) had values of between 20% and 30%, while 5 (rBet v 2, nGal d 1, nGal d 2, rHev b 8, and rPhl p 6) had values of under 40%. The remaining 2 allergens, rApi g 1 and nGal d 3, had values of 44% and 51%, respectively (Table).

Table. Coefficients of Variation (CV) for the 10 Allergens Detected by the Control Serum KS11 in the ISAC CRD 103 Microarray for Intraslide, Intra-Assay, and Interassay Variability Assessment

Control Serum (KS11)	Intraslide CV, %	Intra-assay CV, %	Inter-assay CV, %
rApi g 1	21	27	44
rBet v 2	7	12	32
nBos d 4	10	8	20
nGal d 1	9	39	39
nGal d 2	4	11	36
Gal d 3	117	130	51
Hev b 8	7	19	31
Phl p 5	7	10	23
Phl p 6	23	28	33
Phl p 7	3	9	26

While excellent results were observed for overall intraslide, intra-assay, and interassay variability, rApi g 1, nGal d 3, and rPhl p 6 all showed high variability in the individual analyses. Jahn-Schmid et al [5] reported similar results for rPhi p6. Moreover, in our study, nGal d3 had the highest CV. This suggests the existence of a technical problem related to the adhesion of the allergen to the slide, highlighting the need to validate each allergen individually. This requirement should be even stricter for allergens used to establish the standard curve, and to define specific IgE levels for all the allergens in the ISAC CRD 103 microarray.

Moreover, the fact that the variability of the data in the interassay analysis can be improved suggests that this technique can be used to assess sensitization profiles but is not appropriate for monitoring sensitization.

On the basis of our results, it can be concluded that, overall, the semi-quantitative ISAC CDR 103 method is a reproducible technique. However, the high variability detected for certain allergens suggests that this in vitro tool is valid for an initial study but probably not for follow-up or monitoring studies, or for establishing therapeutic decisions. In such cases, we recommend the use of quantitative tests such as specific IgE determination.

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Selective Hypersensitivity With Positive Immediate Skin Tests to Nimesulide

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In a recent report of selective hypersensitivity to nimesulide with urticarial reactions [1], skin tests (skin prick testing [SPT] and intradermal testing [IDT]) with nimesulide and Sepharose-radioimmunoassay were unable to demonstrate the existence of an immunoglobulin (Ig) E–dependent mechanism.

In July 2008, a 44-year-old man with a history of mild allergic rhinitis and documented sensitivity to *Dermatophagoides* species consulted for systemic urticaria in January and urticaria with acute diarrhea in April. Both reactions had appeared within 15 minutes of taking a 100-mg tablet of nimesulide for a headache. The patient had taken these tablets at least 10 times in the previous 2 years, without adverse reactions. The symptoms disappeared spontaneously within 40 minutes, and the patient had since tolerated diclofenac and acetylsalicylic acid (ASA).

The patient and 3 healthy nonatopic individuals, who usually tolerated nimesulide, underwent parallel SPT with 1, 5, 10, and 20 mg/mL concentrations of nimesulide in distilled water. The tests were negative in the controls but induced a positive skin response with a dose-related wheal-and-flare reaction in the patient (Figure 1A).

The patient agreed to undergo a series of single-blind placebo-controlled oral provocation tests (OPTs), 1 week apart, with increasing doses of nimesulide, celecoxib, and ASA. Under controlled clinical conditions, the OPTs were performed by administering, at 90-minute intervals, 2 consecutive placebo doses (talc) followed by the drugs at a dose of l:100, 1:20, 1:10, and 1:3, and finally at the remainder of the therapeutic dose. ASA and celecoxib did not induce any adverse clinical reactions, but the nimesulide OPT was stopped after the second dose because the patient experienced diffuse urticaria, vomiting, and an associated 25% decrease in basal systolic blood pressure after 10 minutes. Intramuscular epinephrine 1 mg, intramuscular chlorpheniramine, and intravenous methylprednisolone were administered, and the symptoms resolved within 40 minutes.

In March 2009, according to Empedrad et al [2], we carried out an in vivo skin test study in 30 healthy volunteers, all regular users of nimesulide with no adverse reactions, to identify the highest concentrations of nimesulide that did not produce skin irritation. Due to the poor solubility of nimesulide in water (0.014 mg/mL), we performed the tests with a solution of nimesulide (Fingrange-Pharma, London, UK) in polyethylene-glycol 400 (PEG 400; ST-Trading- LLC, New York, USA), a semi-polar solvent with an optimal solubility of 63.120 mg/mL for nimesulide [3].

The nonirritating concentrations of nimesulide-PEG 400 solutions were identified as 20 mg/mL for SPT and 1 mg/mL for IDT. We then retested our patient with nimesulide- PEG 400 solutions at increasing concentrations and the previously effective nimesulide-water solutions. Of interest, in the case of the nimesulide-water solution, only IDT at a concentration of 20 mg/mL was positive. In the case of the PEG 400 solution, SPT was positive only at a concentration of 20 mg/mL, whereas IDT was positive from 0.2 mg/mL upwards; there was, however, no increase in the wheal-and-flare reaction with increasing concentrations of the drug up to the maximum nonirritating dose (Figure 1B).

In October 2009, skin tests performed with the nimesulide-water and nimesulide-PEG 400 solutions were negative. The spontaneous modulation of skin reactivity observed in the patient suggests that skin tests for nimesulide should be performed early because specific skin sensitivity to this drug seems to decrease quickly after the adverse drug reaction, in a similar manner to that reported for ß-lactam antibiotics [4].

The positive skin tests using nonirritating concentrations of nimesulide and the selective OPT strongly suggest a selective allergic hypersensitivity to nimesulide.