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Natural Rubber Latex-specific IgE Antibodies in Non-healthcare Workers: Comparison of Two FDA-cleared *in vitro* Kits*†

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Latex-specific IgE antibody immunoassays are heavily relied upon in the diagnosis of latex allergy in the United States. The goal of this study is to compare anti-latex IgE levels measured by two U.S. Food and Drug Administration (FDA)-cleared kits (CAP® System and AlaSTAT® Microplate) in sera obtained from employees in non-healthcare industries. Sera were obtained from 381 workers employed in several, non-healthcare industries over the past 10 years, and stored frozen. All 381 coded sera were analysed for latex-specific IgE using the Diagnostic Products Corporation microplate AlaSTAT® and the Pharmacia-UpJohn CAP® System. Concordance between methods and intra- and inter-assay reproducibility were evaluated. Twenty-six sera gave positive results using the AlaSTAT® assay (26/381, 6.82%), while 24 yielded CAP® positive results (6.30%). There were no significant differences (p = NS) between the assays' measurements of latex-specific IgE antibody levels for all 381 sera, yielding 0.28 ± 0.19 kU L^{-1} and 0.34 ± 0.59 kU_A L^{-1}, respectively. AlaSTAT® and CAP® assays agreed on the positive status of 9 (9/381, 2.4%) sera, and the negative status of 340 sera (340/381, 89.2%). The assays yielded discordant results on some individual sera. CAP® discordant results occurred in 17/26 sera (65.4%) of AlaSTAT® positive sera, while AlaSTAT® discordant results were found in 15/24 (57.7%) of the CAP® positive sera. The CAP® System, for instance, detected 0.39–2.3 kU_A l^{-1} (1.03 ± 0.59, [mean ± SD]) of latex-specific IgE in the serum from 15 individuals that were all AlaSTAT® negative. In contrast, the AlaSTAT® detected 0.36–1.6 kU l^{-1} (0.62 ± 0.31, [mean ± SD]) of IgE anti-latex in the serum from 17 subjects that were all CAP® negative. These data indicate that the *a priori* seroprevalence of latex-specific sera IgE is about 6%–7% in non-healthcare workers and that the CAP® and AlaSTAT® assays agree on the positive or negative status of the majority of sera (91.6%). However, caution should be exercised when applying FDA-cleared *in vitro* assays for latex-specific sera IgE in populations known to have potentially low concentrations of latex-specific IgE antibodies, as there appears to be a finite possibility for these assays to misclassify sera as being positive or negative for latex-specific IgE antibodies.

Introduction

The prevalence of latex hypersensitivity in the latex-exposed health care workforce has been reported to range from around 6% to 17%.[1–5] The general population exhibits a lower rate of natural rubber latex (NRL) sensitization (approximately 1% to 6%).[6,7] These prevalence statistics are based on seroprevalence (with a variety of assays) or puncture skin test (PST)

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reactions to a variety of skin test reagents. Recent evidence suggests that the PST prevalence of latex sensitization in occupationally unexposed groups is quite low (<1%).[8] The marked discrepancies in seroprevalence rates among studies of both healthcare workers and non-healthcare workers could be due to many factors including the non-specificity of the *in vitro* assays used, technical issues regarding the assays themselves (i.e. changes in format from tube to microplate), changes in exposure patterns over time, cross reactions with foods, plant defence-proteins[9] and pollens,[10] to suggest just a few. PST has been regarded as a primary confirmatory test for the assessment of patients for IgE-mediated latex disease, although the absence of an FDA-licensed *Hevea brasiliensis* latex extract in the USA has restricted its use in the diagnosis of latex allergy. Because of this, serological tests have become critically important in diagnosis. We have shown marked differences in the diagnostic performance of these serological tests when compared with either clinical history (Hx) or the results of PST with a well characterized skin test reagent.[11] Data from that study demonstrated that under the best of circumstances (PST-positive prevalence of 42%), the currently FDA-cleared latex IgE assays produce false-negative and false-positive IgE antibody results.[11] Theoretically, if the prevalence of PST positives in a study population were lower than the 42% cited in the previous study,[11] differing predictive values and possibly, test sensitivities and specificities should be expected.[12] In this study, we have analysed sera from 381 workers from many industries who were unlikely to have occupational exposure to latex using two of the three FDA-cleared latex IgE assays.

Methods

Human Sera

The CDC/NIOSH Human Subjects Review Board approved the study design. Subject's ($n = 381$) sera were stored frozen at −80°C for up to 10 years. The sera were obtained from non-healthcare workers who were subjects in studies designed to investigate the toxic effects of a variety of exposures (lead, ethical narcotics, isocyanates, industrial enzymes, *Stachybotrys chartarum*).[13–19] Whole blood was collected by venipuncture, allowed to clot, centrifuged and sera aliquoted, coded and frozen. Long-term frozen storage of sera does not appear to affect the measurement of IgE levels.[20]

Serologic Analyses

Natural rubber latex-specific IgE antibody was measured in the following two FDA-cleared immunoassays briefly described below.

The CAP® System RAST FEIA was performed by the Analytical Services Branch, HELD, NIOSH, Morgantown, WV, as per manufacturer's instructions using reagents purchased from Pharmacia and UpJohn Diagnostics (Uppsala, Sweden). The assay is a solid phase immunofluorometric assay in which IgE antibody is bound to latex allergosorbent (K82, sponge matrix) and detected with β-galactosidase-labelled rabbit polyclonal anti-human IgE and 4-methylumbelliferyl β-D galactosidase substrate. Results $\geq 0.35\ kU_A L^{-1}$ were considered positive. All sera analysed by the CAP® assay were run individually, as per the manufacturer's instructions. A subset of the sera samples ($n = 31$, 14 samples with $kU_A L^{-1} < 0.35$ and 17 with $kU_A L^{-1}$ values of ≥ 0.35) were re-run to evaluate reproducibility. All 14 of the CAP-assayed samples $< 0.35\ kU_A L^{-1}$ reproduced their values. The coefficient of variation (CV) for all CAP-repeated samples was 5.21 ± 6.60 (standard deviation, SD). The CAP® between-assay variation was 4.6% (mean level $5.97\ kU_A L^{-1}$, $n = 3$). NIOSH, DART (Cincinnati, OH) performed the AlaSTAT® Microplate Assay as per manufacturer's instructions using reagents purchased from Diagnostic Products Corporation (Los Angeles, CA). The assay is a liquid phase immunoenzymetric assay in which latex allergen (K82) that is coupled to soluble biotin-polymer/copolymer matrix binds antibody. The complex is then bound to biotin-coated microtitre plate wells and bound IgE is detected with peroxidase-labelled murine monoclonal anti-human IgE and 3,3′, 5,5′-tetramethylbenzidine substrate in buffered H_2O_2. Results $\geq 0.35\ kU\ L^{-1}$ were defined as positive. The AlaSTAT between-assay variation was 7.1% (mean level $1.67\ kU\ L^{-1}$, $n = 5$). For the AlaSTAT® assay, all test sera were run in duplicate, with a mean coefficient of variation (CV) of the duplicates of 3.91 ± 4.00 ($n = 381$; ±SD); the mean CV ± SD for duplicate samples where the anti-latex IgE concentration was $< 0.35\ kU\ L^{-1}$ was 4.84 ± 3.84, $n = 355$; the mean CV ± SD for duplicates of samples which were $\geq 0.35\ kU\ L^{-1}$ was 4.8 ± 4.0, $n = 26$). These latter two groups were statistically indistiguishable ($p > 0.05$, Mann-Whitney U test).

Statistical Analyses

For statistical calculations, results below the positive cut-off for all assays were adjusted by an averaging method.[21] All statistical tests were performed with SPSS for Windows, version 9.01 (SPSS, Inc, Chicago, IL). A type 1 error level of $p < 0.05$ was considered statistically significant. Non-parametric tests were used for all contrasts as no assumptions regarding the normality of the data distributions were made. Wilcoxon Signed-Rank Tests were used to evaluate the differences between paired samples, while when unpaired analyses were needed, the Mann–Whitney U test was

used. Correlations were evaluated using the method of Pearson.

Results

With the CAP® assay, 24 sera were anti-latex IgE positive ($\geq 0.35\ kU_A L^{-1}$), yielding a prevalence of 24/381 (6.30%). In the AlaSTAT® assay, 26/381 sera were anti-latex IgE positive ($\geq 0.35\ kU\ L^{-1}$), yielding a prevalence of 6.82%. These prevalence values were not statistically different (p=NS, Mann–Whitney test). Likewise, the mean anti-latex IgE values for all 381 sera were not significantly different (p=NS, Mann–Whitney test) when measured by either the CAP® or AlaSTAT® assay (data not shown). When only anti-latex IgE antibody positive sera were analysed, CAP® ($1.71 \pm 0.03\ kU_A\ L^{-1}$, SE) yielded significantly ($p < 0.05$) higher anti-latex IgE antibody concentrations than AlaSTAT® ($0.75 \pm 0.02\ kU\ L^{-1}$, SE). CAP® and AlaSTAT® anti-latex antibody concentrations were found to be significantly associated with each other, albeit with low correlation coefficients ($r = 0.270$, $p < 0.01$, Pearson, $n = 381$). Figure 1 presents the number of sera that had discordant positive/negative IgE anti-latex results between the CAP® and AlaSTAT® systems. The CAP® System, for instance, detected 0.39–2.3 $kU_A\ L^{-1}$ (1.03 ± 0.59, [mean $\pm$ SD]) of latex-specific IgE in the serum from 15 individuals that were all AlaSTAT® negative. In contrast, the AlaSTAT® detected 0.36 to 1.6 $kU\ L^{-1}$ (0.62 ± 0.31, [mean $\pm$ SD]) of IgE anti-latex in the serum from 17 subjects that were all CAP® System negative. Nine sera were positive by both the AlaSTAT® and CAP® assays with mean $\pm$SD latex-specific IgE levels of 1.00 ± 0.82 (range 0.38–2.71) and 2.84 ± 2.79 (range 0.63–9.44), respectively. The concordant IgE anti-latex mean sera levels of anti-latex IgE in the nine samples were not significantly different ($p > 0.05$, Wilcoxon signed-rank test). The discordant sera had significantly different mean latex-specific IgE levels ($p < 0.02$, Mann–Whitney test). The AlaSTAT® and CAP® systems could agree on the positive IgE anti-latex status of 34.6% and disagreed on 65.4% of samples positive by either test ($n = 26$). Overall agreement for all samples was 91.6%.

Discussion

The prevalence of latex-specific IgE antibodies in the general population excluding healthcare workers is presently the subject of much discussion[6,8,11] with levels being reported to range between 1% and about 6%. In a study of the prevalence of latex-specific IgE antibodies in sera from 1000 blood donors using the AlaSTAT® microplate assay, 6.4% were identified as being positive.[22] In a recent similar study of 1025 Italian blood donors[7] using the CAP® assay, 3.5% were identified as being positive. In the present study we found between 6.3% and 6.8% of non-healthcare workers to be positive for latex-specific IgE antibodies using two FDA-cleared assays. Of these workers' sera, the two assays agreed on the positive or negative status of 349/381 (91.6%) of the sera. Disturbingly, the two assays could only agree on the positive status of 9/381 (2.4%) sera. The additional positive sera were found latex IgE positive by either, but not both, of the two assays. We have shown discordance between the three presently FDA-cleared latex specific IgE assays ranging from 9%–25% (8), even in individuals who were puncture skin test positive with a well characterized latex skin testing reagent. It is important to note that clearance from the FDA is based on the requirements specified in the Safe Medical Devices Act of 1990 (SMDA 1990) and 21 CFR Part 807.92. This clearance means that the assay has been shown to perform in a substantially equivalent manner to an existing, previously cleared or marketed assay for latex-specific IgE antibody. FDA clearance does not guarantee diagnostic

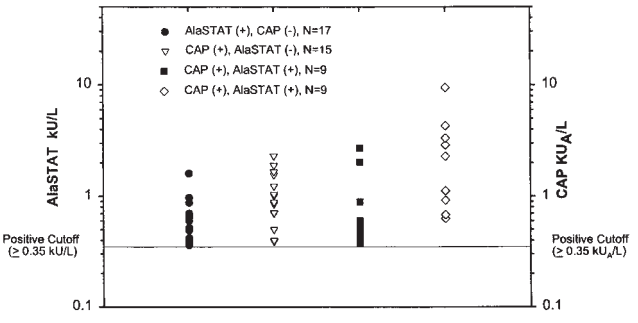

Figure 1. Scattergram of discordant IgE anti-latex results obtained in the analysis of 312 sera in the Pharmacia-UpJohn CAP® System (CAP®) and Diagnostic Products Corporation AlaSTAT® assays. The positive cutoff value for both CAP® and AlaSTAT® are given on the Figure. Anti-latex IgE antibody levels ($kU\ L^{-1}$) of sera which were AlaSTAT® positive, CAP® negative ($n = 17$) are given in the filled circles. Anti-latex IgE antibody levels ($kU_A\ L^{-1}$) of sera which were AlaSTAT® negative, CAP® positive ($n = 15$) are given in the open triangles. When CAP® and AlaSTAT® agreed on positive status, CAP® values are given in closed squares, while AlaSTAT® values are given in the open diamonds. CAP® anti-latex IgE values are reported as $kU_A\ L^{-1}$, while AlaSTAT® values are reported as $kU\ L^{-1}$.

performance, or as shown in the present and previous work,[11] concordance between FDA-cleared assays.

There are numerous reasons for this lack of concordance. IgE antibody assays may detect different IgE antibody specificities, possibly as a result of the differential epitope composition of their allergen-containing reagents.[23] Also, natural rubber latex contains more than 250 polypeptides, with approximately 60 of them showing IgE binding epitopes.[24,25] Different batches of source latex are known to vary up to 25 fold in their total allergen content as assessed by RAST inhibition simply as a result of normal seasonal variation among the *Hevea brasiliensis* trees.[26] Second, sensitized individuals produce specific IgE antibody to at least eight potent *Hevea sp.* allergens, Hev b1–Hev b8. Each of these allergens differs in its structure, size, net charge (pI), relative allergenicity and abundance in natural rubber latex which would be expected to be reflected in the source allergens used in the production of the kits. Other potential causes of allergen-containing reagent heterogeneity include variable stability during storage and variable binding of allergen to labels (e.g. biotinylated co-polymer in AlaSTAT) or solid supports (sponge in CAP®).[13] Cross-reactions between a variety of plant and food allergens and latex proteins have been reported,[9,10,27,28] and the results reported here may reflect exposure to these non-latex allergens. In a recent work, Chen *et al.*[29] have proposed that latex PST and challenge positive individuals with CAP and AlaSTAT negative serology may show significant RAST (radioallergosorbent test) binding to recombinant Hev b5. These investigators proposed that instability and possibly low concentrations of Hev b5 in NRL preparations may lead to it being absent or present in a non-immunologically active form in the capture antigens used in commercial assays, leading to false-negative results.

In the present work we present data suggesting the prevalence of latex-specific IgE antibodies in non-healthcare workers is in the 6%–7% range using two FDA-cleared tests. It is intriguing to speculate from discordant results between the two assays used (AlaSTAT® and CAP®), questionable reproducibility at low anti-latex IgE concentrations,[30] and the two assays finding different individuals 'positive', that the true seroprevalence of latex specific IgE antibodies in non-healthcare workers may be different to the 6%–7% we found with either assay alone. In fact, if the sera found positive by both assays is used in the prevalence calculations, a rate of 9/381 (2.4%) is found.

Finally, caution should be exercised when applying FDA-cleared *in vitro* assays for latex in populations known to have a potentially low prevalence of latex-specific IgE antibodies as there appears to be a significant chance for apparently false-positive or false-negative reactions,[30] and the correlation between the assays observed for split identical sera samples, although statistically significant, was low ($r = 0.270$).

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