Measurement of human IgG subclass antibodies to allergens with new research ImmunoCAP assays

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ASSAY DEVELOPMENT
New research assays for IgG1, IgG2 and IgG3 antibodies were developed for ImmunoCAP and the automated Phadia 100 instrument. All incubations are at 37°C and total assay time for 48 samples is 2.5 hours. The ImmunoCAP solid phase is a cellulose polymer to which proteins can be covalently immobilized. A heterologous calibration system allows true quantitative measurement of antibodies against various antigens/allergens.

SPECIFICITY
The specificity of the assays were validated with purified myeloma paraproteins and Calibrator ImmunoCAP tests.

CALIBRATION
Calibrators with myeloma paraproteins were adjusted to the WHO international reference preparation IRP67/86, and calibration curves were generated for automatic calculation of antibody concentrations in samples. LLOQ, Lower limit of quantitation.

CONCLUSION
• New quantitative research assays for human IgG1, IgG2 and IgG3 antibodies were developed for ImmunoCAP and used with available standard assays for IgG, IgG4 and IgE antibodies in a pilot study which indicate:
  • A strong and stable IgG4 antibody response to beta-lactoglobulin in healthy individuals
  • A high IgG1 and even higher IgG2 antibody response to house dust mite in sensitized and non-sensitized subjects
  • A mixed IgG subclass response to venom allergens in allergic patients with increasing IgG4 antibody levels during venom immunotherapy

IgG SUBCLASS RESPONSE TO 3 DIFFERENT ALLERGENS
Measurement of pan-IgG, IgG1, IgG2, IgG3 and IgG4 antibodies against beta-lactoglobulin from cow’s milk (A), house dust mite extract (B) and bee and wasp venom (C) was done in a pilot study consisting of 3 sets of human samples. Filled circles represent IgE-sensitized subjects. Dotted lines represent assay cut-offs. Analysis of additional serum samples from the subjects in Sample set A collected 85 days after the initial sample collection revealed similar antibody concentrations to beta-lactoglobulin on individual level, indicating a stable antibody response (data not shown).

Characteristics of the new ImmunoCAP research assays for human IgG1, IgG2 and IgG3 antibodies

<table>
<thead>
<tr>
<th>Research ImmunoCAP assay</th>
<th>Specific IgG1</th>
<th>Specific IgG2</th>
<th>Specific IgG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measuring range</td>
<td>1.0 – 400 mg/l</td>
<td>4.6 – 100 mg/l</td>
<td>0.04 – 21 mg/l</td>
</tr>
<tr>
<td>Recovery of calibrators**</td>
<td>100%</td>
<td>97%</td>
<td>83%</td>
</tr>
<tr>
<td>Intra-assay precision</td>
<td>6.3 %CV</td>
<td>3.0 %CV</td>
<td>4.2 %CV</td>
</tr>
<tr>
<td>Inter-assay precision</td>
<td>8.8 %CV</td>
<td>9.4 %CV</td>
<td>5.0 %CV</td>
</tr>
<tr>
<td>Interdilutional precision</td>
<td>13.8 %CV</td>
<td>12.3 %CV</td>
<td>8.3 %CV</td>
</tr>
</tbody>
</table>

**Calibrator concentrations measured using the standard assay ImmunoCAP Specific IgG

*P<0.05; **P<0.01; Significant differences are compared to Before VIT (Wilcoxon matched-pairs signed-rank test)