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# Vitellin/Vitellogenin Is an Important Allergen in German Cockroach

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Allergen sources · Allergens · Asthma · Allergic rhinitis · Cockroach · ImmunoCAP · Indoor allergens

**Abstract**
***Introduction:*** German cockroach (GCr) aeroallergens are associated with allergic rhinitis and asthma. Vitellogenin (Vg) and vitellin (Vn) are abundant proteins in GCr blood and eggs (including egg cases), respectively, and are possible high molecular mass allergens. Prior efforts to purify Vg/Vn yielded amounts too small for subsequent studies. In this study, we report the affinity purification of Vg/Vn from whole-body defatted GCr powder and determination of the binding of Vg/Vn to anti-GCr IgE. ***Method:*** New Zealand white rabbits were immunized with pure Vg/Vn in Freund's adjuvant, and IgG was purified from the rabbit sera and conjugated to cyanogen bromide (CNBr)-activated Sepharose. Aqueous extracts from GCr powder were passed over the column. After extensive washing, putative Vg/Vn was eluted in low-pH buffer, neutralized, and analyzed by SDS-PAGE and liquid chromatography high-resolution mass spectrometry (LC-HRMS). IgE binding of Vg/Vn was evaluated by inhibition of IgE binding to GCr-ImmunoCAP(I6) in sera from 10 GCr-allergic individuals. In addition, Vg/Vn was biotinylated and bound to ImmunoCAP-streptavidin, and direct IgE antibody binding to the immobilized Vg/Vn was determined in sera from 26 GCr-allergic individuals. ***Results:*** Vg/Vn isolated by affinity chromatography was 91% pure by LC-HRMS; contaminants included Bla g 3 (0.9%), human keratin (6%), and rabbit IgG. Vg/Vn inhibited IgE binding to GCr-ImmunoCAP(I6) in 8 of 10 sera. In direct-binding experiments, 21/26 (80%) sera had anti-Vg/Vn IgE at >0.10 $kU_A/L$, while 11/26 (42%) sera were >0.35 $kU_A/L$. ***Conclusions:*** We affinity-purified Vg/Vn and demonstrated that Vg/Vn-specific IgE antibody is a major component of GCr-specific IgE.

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## Introduction

The German cockroach (GCr), *Blattella germanica*, produces potent indoor allergens associated with IgE-mediated asthma and rhinitis [1]. In the USA, GCr allergy can be diagnosed, in the context of a consistent clinical

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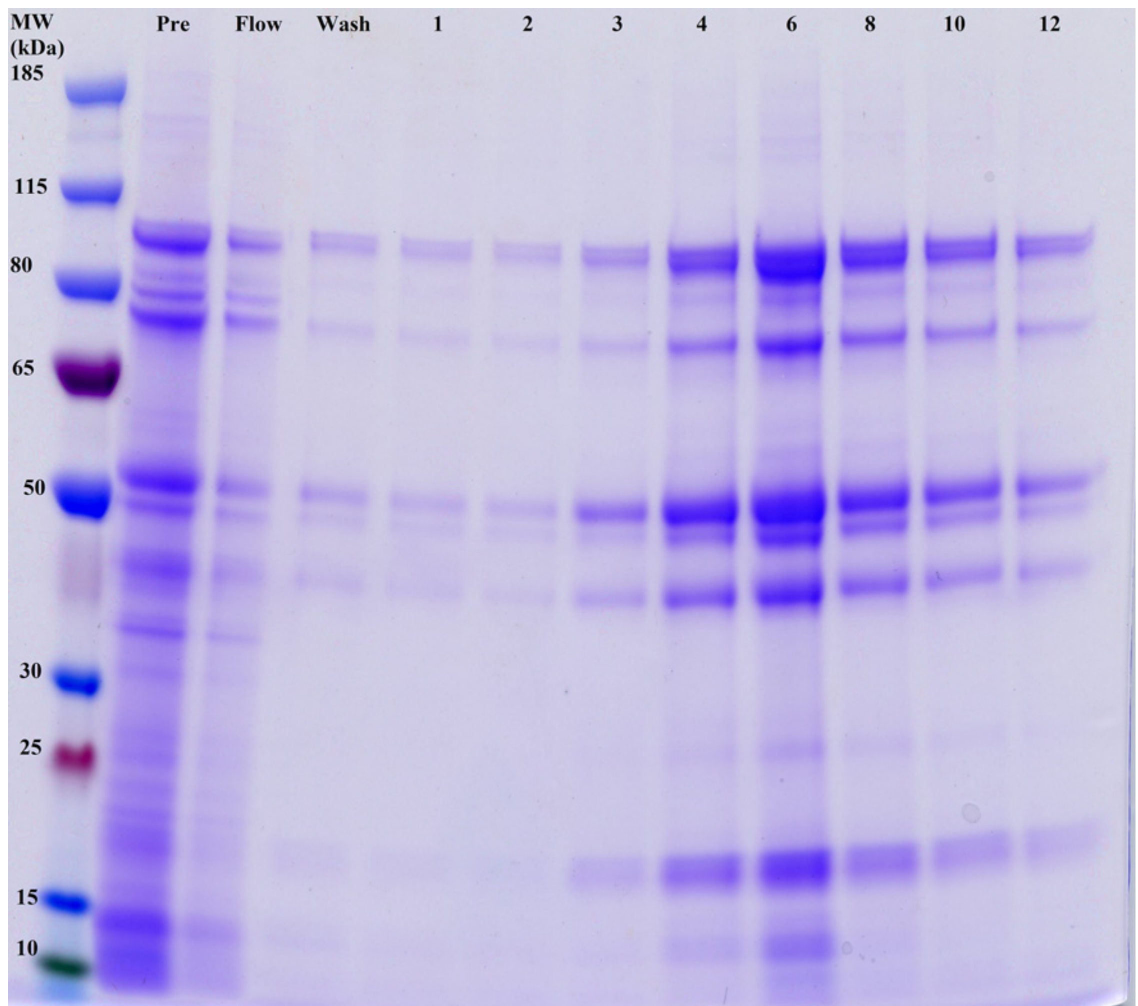


**Fig. 1.** SDS-PAGE analysis of eluates from affinity purification of Vg/Vn. Molecular mass standards are on the left. "Pre": extract loaded onto the column. "Flow" and "Wash": material not bound by the column at pH 6.3–8.0. "1–12": twelve 2.0 mL fractions eluted at pH 2.5 (see Methods). Fractions 3 through 12 were collected for further analysis.

history, by skin testing with GCr extracts or measurement of GCr-specific IgE in serum. The efficacy of allergen reduction measures is uncertain [2, 3], and pharmacotherapy is the mainstay of treatment. Immunotherapy, a highly effective approach with other allergens, has yet to be clearly demonstrated as effective for GCr.

Known GCr allergens include Bla g 1 (midgut protein), Bla g 2 (inactive aspartate protease), Bla g 3 (hemocyanin), Bla g 4 (lipocalin), Bla g 5 (glutathione S-transferase), Bla g 6 (troponin C), Bla g 7 (tropomyosin), Bla g 8 (myosin light chain), Bla g 9 (arginine kinase), Bla g 11 (α-amylase), and Bla g 12 (chitinase) (www.allergen.org). Other GCr proteins also suggested as potential allergens include enolase, receptor for activated protein kinase, triosephosphate isomerase [4], and delta-class glutathione S-transferase [5] (allergome.org). In addition, vitellogenin (Vg), the precursor protein to egg storage protein vitellin (Vn), has been noted to bind IgE [4] and stimulate T-cells [6] from GCr-allergic individuals. We have also identified Vg in commercial GCr allergen extracts and some allergen source materials [7]. To determine the allergenicity of Vg/Vn[1], we describe here its

[1]Vg and Vn share proteomic signatures and epitopes and coexist in GCr. Since we made no attempt in this study to distinguish Vg, Vn, and their numerous subunits, multimers, and proteolytic products, we designate them as Vg/Vn throughout this manuscript.



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affinity purification from defatted GCr powder and the measurement of the binding of Vg/Vn to IgE antibodies from GCr-allergic individuals.

## Methods

*Materials*

Defatted GCr powder was from Greer (Lenoir, NC, USA) and protease inhibitor mini tablets were from Roche. For affinity purification, anti-Vg/Vn serum was raised in female New Zealand white rabbits by intradermal injection of electrophoretically isolated Vg/Vn with Freund's complete and incomplete adjuvants [8, 9][2]. Protein A Sepharose was from BioVision, CNBr-activated Sepharose 4B from Cytiva, and N-hydroxy-succinimide biotin (Biotin-X-NHS) from Sigma-Aldrich. Protein content and purity were determined by liquid chromatography high-resolution mass spectrometry (LC-HRMS) using a LUMOS Tribid Orbitrap mass spectrometer. IgE binding was determined using the ImmunoCAP system. ImmunoCAP (I6) (GCr) and ImmunoCAP-Streptavidin were from ThermoFisher. Residual deidentified human sera from allergic individuals, selected for elevated specific IgE to GCr by ImmunoCAP (I6), and control sera were from the Johns Hopkins DACI Laboratory.

*GCr Extract Preparation*

Protease inhibitors were added to all buffers. GCr extract was prepared from defatted GCr powder in ammonium bicarbonate buffer, 50 mM, pH 8.0 (ABC), 1:10 (w/v), shaken overnight at 4°C, and centrifuged for 15 min at 3,500 × *g*. The supernatant was passed over grade 1 filter paper (Whatman).

*Affinity Column Preparation and Vg/Vn Purification*

IgG from rabbit anti-Vg/Vn was purified over protein A Sepharose, and purified IgG anti-Vg/Vn was conjugated to CNBr-activated Sepharose 4B. Affinity chromatography was performed at 4°C. Anti-Vg/Vn-conjugated Sepharose was equilibrated with ABC. GCr extract was loaded onto the column, which was then washed with 0.05 M Tris-HCl, 0.5 M NaCl, pH 8.0, containing 0.1% Triton X-100, followed by 0.05 M sodium phosphate, 0.5 M NaCl, pH 6.3, containing 0.1% Triton X-100. Putative Vg/Vn was eluted with 0.05 M glycine buffer pH 2.5, containing 0.15 M NaCl and 0.1% Triton X-100, and immediately neutralized with 1 M Tris-HCl, pH 9.0. Yield was estimated by BCA.

*Protein and Proteomic Analyses*

GCr extract and affinity chromatogram flowthroughs/eluates were analyzed by SDS-PAGE using 4–12% gradient Bis-Tris gels visualized with Imperial stain (ThermoFisher). Prior to LC-HRMS, neutralized eluate samples were denatured in 6 M urea containing dithiothreitol (0.1 M) and iodoacetamide (0.05 M). Sequencing-grade modified trypsin (Promega) was reconstituted in 4 mL of ABC buffer to a concentration of 5 μg/mL. In both in-gel and in-solution digestion methods, 24 μL of trypsin was added to 6 μg of a sample (1:50, w/w) at 37°C before loading it onto the instrument for LC-HRMS analysis.

*Inhibition Assay and Direct-Binding Assay to Assess IgE Binding*

Pooled eluate containing putative Vg/Vn was concentrated and exchanged into PBS using an Amicon Ultra-15 filtration unit (NMWL 3 kDa); for long-term storage at −20°C, glycerol was added to a final concentration of 33% (v/v). Inhibition of GCr-allergic serum binding to GCr-ImmunoCAP(I6) was determined with 10 sera pre-incubated with Vg/Vn as previously described [10]. To assess non-specific binding of Vg/Vn to human IgE, Vg/Vn was pre-incubated with anti-oak or anti-peanut sera before exposure to oak and peanut ImmunoCAPs, respectively. In both cases, Vg/Vn produced no inhibition (data not shown).

In addition, purified Vg/Vn was deglycerinated at 4°C using Slide-A-Lyzer G2 (3,500 MWCO) in PBS and biotinylated with biotin-X-NHS (8:1 w/w) [11]. Biotinylated Vg/Vn was loaded onto a freshly washed ImmunoCAP-streptavidin as previously described [12]. Sera from 26 GCr-allergic individuals (including the 10 sera used for the inhibition testing) were assessed for binding to ImmunoCAP-streptavidin-Vg/Vn as well as GCr-ImmunoCAP (I6) [13].

## Results

*Affinity Column and Analyses of Affinity Column Eluates*

The titer of the rabbit anti-Vg/Vn serum was determined to be $10^{-6}$ by ELISA. We purified IgG from 4 mL of the rabbit anti-Vg/Vn serum over a protein A column; the IgG was shown to be electrophoretically pure by SDS-PAGE under reduced and nonreduced conditions (not shown). We bound 30 mg of anti-Vg/Vn IgG to 4 mL of CNBr-activated Sepharose. On each passage of the GCr extract over the 4 mL affinity column, we recovered 4.5–6 mg of putative Vg/Vn. A total of 21 mg was recovered for further experiments.

Putative Vg/Vn from the affinity column eluate resolved into at least 8 identifiable bands on SDS-PAGE, at approximately 15, 20, 45, 50, 55, 75, 80, and 105 kDa (Fig. 1). LC-HRMS confirmed the presence of 211 distinct peptide sequences, and 91% of the measured total ion current mapped to Vg/Vn. The remaining sequences mapped to Bla g 3 (0.9%) and rabbit or human contaminants.

*Binding of Human IgE-Anti-GCr to Vg/Vn*

Binding of Vg/Vn to IgE-anti-GCr was assessed in two ways: selective inhibition by pre-incubation of each serum with Vg/Vn or buffer followed by measurement of

[2] Rabbit antisera were generated by one of us (CS) in 1996 for another project and stored at −80°C.

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Table 1. Vg/Vn inhibition of IgE binding to immobilized GCr

| Serum identifier | Original analysis, $kU_A/L$ | + Vg/Vn, $kU_A/L$ | + buffer, $kU_A/L$ | Inhibition, % |
|---|---|---|---|---|
| U3056 | 71.8 | 75.3 | 78.4 | 4 |
| U3171 | 73.9 | 79.2 | 85.5 | 7.4 |
| U3520 | 13.5 | 10.8 | 11.9 | 9.2 |
| U6769 | 19.3 | 23.3 | 24.7 | 5.7 |
| U6821 | 27.7 | 16.7 | 18.5 | 9.7 |
| U8772 | 11.6 | 17 | 16.6 | −2.4 |
| U9313 | 33 | 20.8 | 27.2 | 23.5 |
| U9378 | 25.6 | 20 | 25.2 | 20.6 |
| V1984 | 20.3 | 20.3 | 23.8 | 14.7 |
| V2491 | 13.6 | 13 | 14 | 7.1 |

Serum from GCr-allergic individuals was pre-incubated with Vg/Vn (5 μg), and binding to GCr-ImmunoCAP (I6) was determined. Of the 10 sera tested, inhibition >5% was detected in 8. Vg/Vn, vitellogenin/vitellin; $kU_A/L$, kilo unit (allergen)/liter; GCr, German cockroach.

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the level of IgE binding to GCr-ImmunoCAP(I6) and direct binding of IgE antibody from GCr-allergic individuals to immobilized biotin-Vg/Vn. In the first approach, 10 GCr-sensitized sera (IgE-anti-GCr range: 11–74 $kU_A/L$) were pre-incubated for 2 h with 5 μg of Vg/Vn before adding to GCr-ImmunoCAP (I6). IgE-anti-GCr binding was inhibited in 8 sera by >5% and in 2 sera by >20% (Table 1).

In the second approach, biotinylated Vg/Vn was bound to ImmunoCAP-streptavidin, and sera from 26 GCr-allergic individuals were tested for direct binding to both GCr-ImmunoCAP(I6) and ImmunoCAP-streptavidin-Vg/Vn. Of the 26 sera from GCr-allergic individuals, 21 sera (80%) had specific IgE levels >0.1 $kU_A/L$ (the analytical sensitivity of ImmunoCAP), and 11 (42%) contained levels >0.35 $kU_A/L$. Among those sera in which IgE-anti-Vg/Vn was detected, it was present at 2–27% of the level of IgE-anti-GCr (Table 2).

## Discussion

GCr produces important allergens associated with IgE-mediated asthma and rhinitis. GCr infestations have been associated with substantial exposure to secretory debris including dead adult females and eggs [14] that contain Vg/Vn. Recent critical assessments of environmental control strategies for management of allergic disease have yielded conflicting outcomes [2, 3], although the effect of pest control on the initial development of allergy and asthma remains unevaluated. Thus, allergen immunotherapy to GCr [15] is an important goal, but this goal is complicated by at least two scientific obstacles: the complexity of the immune response to GCr allergens, with no clear immunodominant allergens [16], and the fact that GCr allergen extracts in the USA have a highly variable allergen content [7, 17, 18]. A more complete description of GCr allergens in the indoor environment will facilitate progress toward that goal by directing investigators, manufacturers, and regulators to target allergens that are most immunopathogenic.

Vn is the major egg storage protein in GCr with cross-reactivity to Vn's in other oviparous insects and birds, and Vg is its precursor. Insect Vg's are phospholipoglycoproteins synthesized in fat bodies, secreted into hemolymph, and taken up by developing oocytes through receptor-mediated endocytosis, where Vg is cleaved to Vn [19]. Prior investigators have raised the possibility that Vg/Vn is an allergen: Chuang [4] identified Vg/Vn as IgE-reactive in proteome-mining using sera from GCr-allergic individuals, and Pomes [6] confirmed that it elicits T-cell reactivity as well. Likewise, in our early proteomic screens [20] and mass spectrometric work [7] with GCr, we identified Vg/Vn as a potential allergen. We found Vg/Vn peptide sequences in commercial allergen extracts and in GCr female bodies and oothecae (egg cases) [7].

Ascertaining that Vg/Vn is immunopathogenic in GCr-allergic individuals requires having adequate quantities of reasonably pure allergen, and our earlier attempts to purify Vg/Vn from oothecae (unpublished) and GCr powder by conventional methods [21, 22] were successful but with extremely low yield. The availability of a high-titer rabbit antiserum to GCr Vg/Vn made it

**Table 2.** Direct binding of IgE antibody from GCr-allergic individuals to GCr extract (GCr-ImmunoCAP [I6]) and to ImmunoCAP-streptavidin coated with biotinylated Vg/Vn

| Serum identifier | GCr(I6-CAP), $kU_A/L$ | Vg/Vn-CAP, $kU_A/L$ | Vg/Vn/GCr (I6-CAP) ratio, % |
|---|---|---|---|
| U9313 | 30.2 | 1.06 | 3.50 |
| U9378 | 25.9 | 1.2 | 4.60 |
| U9989 | 11.7 | 0.25 | 2.10 |
| V1984 | 22.9 | 0.81 | 3.50 |
| V4247 | 8.8 | 0.19 | 2.10 |
| V4497 | 6.5 | 0.82 | 12.60 |
| V5141 | 9.9 | 0.34 | 3.40 |
| V5740 | 9.9 | 0.26 | 2.60 |
| V5763 | 8.4 | 0.88 | 10.50 |
| V6236 | 12.1 | 0.37 | 3.10 |
| V6366 | 7.2 | 0.42 | 5.90 |
| V6461 | 41.8 | 2 | 4.80 |
| V6804 | 6.5 | <0.1 | ND |
| U6848 | 2.64 | <0.1 | ND |
| V6848 | 0.71 | <0.1 | ND |
| V6849 | 11.5 | 0.36 | 3.10 |
| V6860 | 1.94 | 0.15 | 7.70 |
| V6876 | 4.56 | 0.19 | 4.20 |
| V7069 | 1.07 | 0.21 | 19.60 |
| V7085 | 1.83 | <0.1 | ND |
| V7133 | 1.04 | 0.2 | 19.20 |
| V7212 | 7.24 | 1.23 | 17.00 |
| V7245 | 1.35 | 0.12 | 8.90 |
| V7267 | 9.18 | 0.7 | 7.60 |
| V7271 | 1.26 | <0.1 | ND |
| V7303 | 1.03 | 0.28 | 27.20 |

Specific IgE to Vg/Vn was detected in 21/26 sera at the detectable threshold of >0.10 $kU_A/L$, and in 11/26 sera at >0.35 $kU_A/L$. Vg/Vn, Vitellogenin/vitellin; $kU_A/L$, kilo unit (allergen)/liter; GCr, German cockroach; ND, Not determined.

possible to obtain much greater yields of pure material by affinity chromatography. Using this approach, we were able to obtain 21 mg of 91% pure Vg/Vn from an extract of GCr powder.

We found, in both inhibition and direct-binding experiments, that sera from 80% of GCr-allergic individuals retained in a Baltimore-based clinical laboratory had measurable IgE antibody that was specific to Vg/Vn. In some of those sera, Vg/Vn-specific IgE accounts for ≥10% of the GCr-specific IgE detected using the conventional GCr-ImmunoCAP(I6) solid phase as the reference.

Our Vg/Vn preparation co-purified with a small amount (0.9%) of Bla g 3. In previous work [23], we found that pure recombinant Bla g 3 inhibited binding of pooled sera from GCr-allergic individuals to GCr extract by up to 40% at concentrations up to 100 μg/mL. Therefore, co-purified Bla g 3 is unlikely to be contributing to the inhibitions noted in Table 1.

Definitive evidence for allergenicity is the generation of IgE-mediated responses by the candidate allergen, and that is not provided in the current account. However, we confirm that Vg/Vn-specific IgE, in some GCr-allergic individuals, accounts for a substantial portion of their GCr-specific IgE antibody response. Thus, Vg/Vn is an important component of GCr allergen extracts and should be considered in the design of future GCr allergen immunotherapy trials.

## Statement of Ethics

This study protocol was reviewed and approved by North Carolina State University's Institutional Animal Care and Use Committee, approval number 96-059B. The rabbit antisera were raised in 1996 for another study as a contract service at the North Carolina State University College of Veterinary Medicine (https://cvm.ncsu.edu/). The study protocol was reviewed and approved by North Carolina State University's Institutional Animal Care and Use Committee, approval number 96-059B. Residual deidentified

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human sera were determined by the FDA Research in Human Subjects Committee to be exempt from IRB review under 45 CFR 46.101 (b) (4).

## Conflict of Interest Statement

The authors declare no conflicts of interest.


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## Author Contributions

All authors (C.H., A.B., J.S., C.S., C.B., K.S., M.B.S., R.G.H., and J.E.S.) contributed to the planning, design, implementation, and interpretation of the experimental plan and the preparation of the manuscript.

## Data Availability Statement

All relevant data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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## References


1 Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med. 1997;336(19):1356–63.
2 Rabito FA, Carlson JC, He H, Werthmann D, Schal C. A single intervention for cockroach control reduces cockroach exposure and asthma morbidity in children. J Allergy Clin Immunol. 2017;140(2):565–70.
3 Grant TL, McCormack MC, Peng RD, Keet CA, Rule AM, Davis MF, et al. Comprehensive home environmental intervention did not reduce allergen concentrations or controller medication requirements among children in Baltimore. J Asthma. 2023;60(3):625–34.
4 Chuang JG, Su SN, Chiang BL, Lee HJ, Chow LP. Proteome mining for novel IgE-binding proteins from the German cockroach (*Blattella germanica*) and allergen profiling of patients. Proteomics. 2010;10(21):3854–67.
5 Jeong KY, Jeong KJ, Yi MH, Lee H, Hong CS, Yong TS. Allergenicity of sigma and delta class glutathione S-transferases from the German cockroach. Int Arch Allergy Immunol. 2009;148(1):59–64.
6 Pomes A, Schulten V, Glesner J, da Silva Antunes R, Sutherland A, Bacharier LB, et al. IgE and T Cell reactivity to a comprehensive panel of cockroach allergens in relation to disease. Front Immunol. 2020;11:621700.
7 Mindaye ST, David NA, Esfahani SAZ, Schal C, Matsui EC, Ronald RL, et al. Measurement of German cockroach (GCr) allergens and their isoforms in allergen extracts with mass spectrometry. Clin Exp Allergy. 2020;50(6):741–51.
8 Tufail M, Lee JM, Hatakeyama M, Oishi K, Takeda M. Cloning of vitellogenin cDNA of the American cockroach, *Periplaneta americana* (Dictyoptera), and its structural and expression analyses. Arch Insect Biochem Physiol. 2000;45(1):37–46.
9 Hatakeyama M, Sawa M, Oishi K. Ovarian development and vitellogenesis in the sawfly, *Athalia rosae* ruficornis jakovlev (hymenoptera, tenthredinidae). Invertebr Reprod Dev. 1990;17(3):237–45.
10 Schmidt-Hieltjes Y, Teodorowicz M, Jansen A, den Hartog G, Elfvering-Berendsen L, de Jong NW, et al. An alternative inhibition method for determining cross-reactive allergens. Clin Chem Lab Med. 2017;55(2):248–53.
11 Gretch DR, Suter M, Stinski MF. The use of biotinylated monoclonal antibodies and streptavidin affinity chromatography to isolate herpesvirus hydrophobic proteins or glycoproteins. Anal Biochem. 1987;163(1):270–7.
12 Sander I, Kespohl S, Merget R, Goldscheid N, Degens PO, Bruning T, et al. A new method to bind allergens for the measurement of specific IgE antibodies. Int Arch Allergy Immunol. 2005;136(1):39–44.
13 Hamilton RG, Hemmer W, Nopp A, Kleine-Tebbe J. Advances in IgE testing for diagnosis of allergic disease. J Allergy Clin Immunol Pract. 2020;8(8):2495–504.
14 Eggleston PA, Arruda LK. Ecology and elimination of cockroaches and allergens in the home. J Allergy Clin Immunol. 2001;107(3 Suppl):S422–9.
15 Wood RA, Togias A, Wildfire J, Visness CM, Matsui EC, Gruchalla R, et al. Development of cockroach immunotherapy by the inner-city asthma consortium. J Allergy Clin Immunol. 2014;133(3):846–52.e6.
16 Pomes A, Arruda LK. Cockroach allergy: understanding complex immune responses to develop novel therapies. Mol Immunol. 2023;156:157–69.
17 Birrueta G, Frazier A, Pomes A, Glesner J, Filep S, Schal C, et al. Variability in German Cockroach extract composition greatly impacts T cell potency in cockroach-allergic donors. Front Immunol. 2019;10:313.
18 Glesner J, Filep S, Vailes LD, Wunschmann S, Chapman MD, Birrueta G, et al. Allergen content in German cockroach extracts and sensitization profiles to a new expanded set of cockroach allergens determine in vitro extract potency for IgE reactivity. J Allergy Clin Immunol. 2019;143(4):1474–81.e8.
19 Tufail M, Takeda M. Molecular characteristics of insect vitellogenins. J Insect Physiol. 2008;54(12):1447–58.
20 Khurana T, Collison M, Chew F, Jeong K, Slater J. Allergen specificity of 3 scFv antibodies developed for a multiplex assay of *Blattella germanica* extract potency. J Allergy Clin Immunol. 2012;129(2):Ab89.
21 Somers J, Rosen B, Schal C, Khurana T, Mindaye S, Slater J. Purification and initial studies on vitellogenin, an allergen in German cockroach. J Allergy Clin Immunol. 2021;147(2):Ab141.
22 Strader M, Blank A, Somers J, Schal C. High quality purification of German cockroach vitellin/vitellogenin from whole body acetone defatted powder. J Allergy Clin Immunol. 2022;149(2):Ab218.
23 Khurana T, Collison M, Chew FT, Slater JE. Bla g 3: a novel allergen of German cockroach identified using cockroach-specific avian single-chain variable fragment antibody. Ann Allergy Asthma Immunol. 2014;112(2):140–5.e1.