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Allergens

Measurement of German cockroach allergens and their isoforms in allergen extracts with mass spectrometry

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Abstract

Background: Allergen extracts are the primary tool for diagnosis and treatment of allergic diseases. In the United States, most allergen extracts are non-standardized. More sophisticated analytical approaches are needed to characterize these products and enable manufacturers and regulators to better determine potency.

Objective: To expand the multiple reaction monitoring (MRM) assay for an in-depth characterization of German cockroach (GCr; *Blattella germanica*) allergen extracts.

Methods: We applied advanced liquid chromatography (LC) and mass spectrometry (MS) techniques including MRM. The expanded LC/MRM-MS method was optimized to measure known GCr allergens and their isoforms/variants in commercial extracts and environmental samples. We performed isoform-specific allergen measurements in multiple extracts from four commercial sources and extracts prepared using environmental samples from urban homes. To investigate causes of heterogeneity, we examined over 30 extraction process variables.

Results: Evaluation of the commercial extracts confirmed the variability of production lots and commercial sources. Commonly used defatting and extraction protocols yielded extracts with comparable allergen profiles and content. However, the identity and quality of source materials was a major contributor to variability. In comparing commercial GCr extracts to environmental samples, relative quantities of Bla g 1, Bla g 2, Bla g 3, Bla g 4 and Bla g 11 were similar, while Bla g 5, Bla g 6, Bla g 7 and Bla g 8 were present in the environmental samples but largely absent for the commercial extracts.

Conclusions and Clinical Relevance: LC/MRM-MS can be used to measure all known GCr allergens in commercial allergen extracts and environmental samples. Significant differences exist between allergen profiles of commercial extracts and the profiles of environmental samples from dwellings. This analytical platform can serve as a template to achieve better product characterization of similarly complex products.

KEYWORDS

allergens and epitopes, regulatory aspects, venom and insect allergy

1 | INTRODUCTION

Management and therapy of allergic diseases include accurate diagnosis, allergen avoidance, pharmacotherapy and, when appropriate, allergen-specific immunotherapy (AIT).¹ Both diagnosis and AIT require the availability of high-quality species-specific allergen extracts. In the United States, the quality and consistency of commercial allergen extracts are regulated in two ways. For most allergen extracts (non-standardized), manufacturing consistency is the only assurance of a consistent product, as the units used to label these products are not directly associated with extract potency.² For the remaining allergen extracts (standardized products), biological potency is confirmed on a lot-by-lot basis and reported in assay-specific units. Potency may be based on total protein, specific allergen content or overall allergenicity. Notably, none of these measures provide a complete content profile of these complex natural products, even though several non-allergen components (beta-glucans, proteases) are known to affect allergic and T-cell responses *in vivo*.^{3,4}

German cockroach (GCr; *Blattella germanica*) is an indoor pest and important source of allergens associated with asthma and allergic rhinoconjunctivitis.⁵ Commercial allergen extracts for GCr are non-standardized, and several studies have confirmed low biological potency and a lack of consistency among products manufactured by different companies.^{6,7} Further, although 10 GCr allergens have been identified (www.allergen.org), none of them is immunodominant.^{8,9}

The FDA identified GCr allergen extracts as an appropriate standardization target in 2001.¹⁰ To achieve this goal, its efforts to measure the allergen content of these products have included ELISA,⁶ ID₅₀EAL,^{11,12} a multiplex monoclonal antibody-based approach¹³ and physicochemical characterization.¹⁴ The advantages of the last approach include increased sensitivity, accuracy, precision and linearity. In addition, the use of a non-immunologic approach allows the measurement of non-allergen components that may alter biological potency.

Previously, we reported the development of a multiplex assay for five GCr allergens (Bla g 1-Bla g 5) based on MRM technology.¹⁴ The current work describes the development and optimization of a new MRM method for 10 additional GCr allergens, including isoforms of known allergens and vitellogenin. We applied this comprehensive MRM method to detect and quantify all GCr allergens in various background matrices including in commercial extracts and environmental samples. We also applied the method to evaluate the effect of extraction conditions and source materials on the heterogeneity of GCr extracts.

2 | METHODS

2.1 | Materials

Glycerinated German and American cockroach extracts were purchased from ALK-Abelló, Allermed Laboratories, Stallergenes-Greer and Jubilant HollisterStier (Table S1 in the Online Data Repository).

These extracts presumably include all life stages of GCr, including nymphs, adult males, adult females and egg cases, as well as faeces and exuviae. Sequencing grade trypsin was obtained from Promega. Reference peptides (Table 1) were chemically synthesized in isotopically labelled forms (Thermo Fisher Scientific). All solvents were LC-MS grade (Fisher Scientific). Reagents for defatting and extraction were from Sigma.

2.2 | Source materials for extraction

Frozen whole-body GCrs were from Carolina Biological Supply Company (Burlington, NC), Stallergenes-Greer and North Carolina State University (NSCU). Gender-separated GCr faeces were from NSCU, and GCr egg cases were from Stallergenes-Greer. Faeces were obtained separately from adult males and adult females; they were not contaminated with shed cuticle from moulting nymphs (commonly referred to as "frass") or egg case remains. For analysis of environmental samples, settled dust was collected from homes of asthma patients in Baltimore using Mitest dust collectors (Indoor Biotechnologies), sieved and stored at -30°C until extraction.¹⁵ All source materials were received on dry ice and stored at -80°C. Protein determinations were made by bicinchoninic acid (BCA) assay.

Multiple reaction monitoring method development was previously described.¹⁴ Peptides were selected for each identified allergen on the basis of sequence uniqueness, ionizability and suitability for chromatography. Selected peptides were chemically synthesized as isotopically tagged peptides, where the carbon and nitrogen of N-terminal residues (R or K) were substituted for heavier isotopes (C-13 and N-15) so that fragments generated from isotopically tagged references can be distinguished from fragment ions of native counterparts. These labelled peptides were employed extensively for optimization of fragmentation parameters such as cone voltage, collision energy and dwell time, followed by selection of best representative fragment ions for quantification and qualification. The chromatographic separation was also optimized to achieve best-resolved peaks with the least interference.

Multiple reaction monitoring method was evaluated for precision, linearity and comparability to orthogonal methods. In addition, we used MRM to detect and quantify allergens in commercial extracts and extracts prepared from environmental samples. Because these extracts have components unsuitable for direct MRM analysis, buffer exchange was performed using 3 kDa MWCO spin filter (Millipore Sigma) by repeated washing with 50 mM ammonium bicarbonate (ABC). Allergen loss was estimated at 10%-15% (data not shown) using rBla g 2, rBla g 4 and rBla g 5 (Indoor Biotechnologies).

2.3 | Proteomic mapping using liquid chromatography-high-resolution mass spectrometry

We subjected 29 commercial extracts and 13 laboratory-made extracts to reduction by dithiothreitol (10 mM DTT; 60°C for 30 min),

TABLE 1 Reference peptides and MRM parameters

Allergen	Isoforms and variants	Peptide sequence	Retention time (min)	Cone voltage	Quantifier 1		Quantifier 2	
					[Precursor (M + H) ⁺ ion (M + H) ⁺]	Collision energy	[Precursor (M + H) ⁺⁺ , Fragment ion (M + H) ⁺]	Collision energy
Bla g 1	Bla g 1.0101	ALFNEK	9.81	30	[361.3, 390.5], [365.3, 398.5]	18	[361.3, 537.4], [365.3, 545.4]	12
		IIELIR	12.67	18	[378.7, 530.5], [383.7, 540.5]	12	[378.7, 643.6], [383.7, 653.6]	16
		SPEFQSIVETLK	14.76	13	[689.3, 589.3], [693.3, 597.3]	24	[689.3, 789.5], [693.3, 797.5]	24
Bla g 2	Bla g 2.0101	AIIVGPK	9.88	36	[349.2, 400.2], [353.2, 408.2]	14	[349.2, 513.4], [353.2, 521.4]	10
Bla g 3	UniProt ID: DOVNY6	EELTFDGVK	11.73	30	[519.1, 565.4], [523.1, 573.4]	14	[519.1, 666.4], [523.1, 674.4]	16
		FVEIDR	9.85	18	[389.5, 403.2], [394.5, 413.3]	14	[389.5, 532.3], [394.5, 542.3]	12
		LEDVPNVDIR	12.08	28	[585.3, 713.6], [590.3, 723.6]	18	[585.3, 927.4], [590.3, 937.4]	18
Bla g 3.0101		NYFVEIDR	12.37	30	[528.3, 631.6], [533.3, 641.5]	16	[528.3, 778.7], [533.3, 788.7]	16
		AIIEDLK	9.07	20	[409.2, 615.5], [413.2, 623.5]	16	[409.2, 633.5], [413.2, 641.5]	12
		TPVLEIDGK	11.49	18	[486.3, 435.9], [490.3, 443.9]	18	[486.3, 674.5], [490.3, 682.5]	20
Bla g 5	Bla g 5.0101	VLGLPAIK	13.77	16	[405.7, 428.4], [409.7, 436.4]	18	[405.7, 598.6], [409.7, 606.3]	12
		EGNGYITTNVLR	12.02	44	[668.7, 703.4], [673.7, 713.4]	22	[668.7, 816.6], [673.7, 826.6]	22
		FLVEEDAEAMQQELR	14.98	26	[904.4, 804.5], [909.4, 814.6]	28	[904.4, 875.4], [909.4, 885.5]	26
Bla g 6.0201	Bla g 6.0201	EGNGYITTAFLR	7.06	14	[647.2, 660.7], [652.2, 670.7]	22	[647.2, 773.6], [652.2, 783.6]	22
		SGSISTNMVEEILR	15.78	30	[768.3, 758.4], [773.3, 768.4]	26	[768.3, 1191.7], [773.3, 1201.7]	26
		TLEELIDEVDADK	12.41	30	[745.3, 791.4], [749.3, 799.4]	24	[745.3, 903.4], [749.3, 911.4]	24
Bla g 7	Bla g 7.0101	LAEASQAADER	13.28	25	[688.8, 706.3], [692.8, 716.3]	20	[688.8, 777.4], [692.8, 787.4]	20
		IQLLEEDLR	8.37	38	[629.2, 903.5], [634.2, 915.3]	20	[629.2, 1016.6], [634.2, 1026.6]	20
		SLEVSEEK	7.2	18	[460.6, 591.34], [464.6, 599.4]	14	[460.6, 720.4], [464.6, 728.4]	14
Bla g 8	Bla g 8.0101	TFEDDGK	12.35	24	[406.1, 545.3], [410.1, 553.2]	16	[406.1, 563.3], [410.1, 571.3]	12
		EAFQLMDADK	11.45	16	[584.1, 575.3], [588.1, 583.3]	12	[584.1, 692.3], [588.1, 700.3]	12
		GSNVFSMFSQK	7.56	25	[616.1, 640.3], [620.1, 583.3]	14	[616.1, 727.4], [620.1, 735.4]	14
Bla g 9	Bla g 9.0101	VPFSHDDR	7.05	30	[486.7, 629.3], [491.7, 639.3]	25	[486.7, 776.4], [491.7, 786.4]	25
Bla g 11	Bla g 11.0101	TVTVGSDGK	11.02	36	[431.9, 463.3], [435.9, 471.3]	18	[431.9, 663.4], [435.9, 671.4]	10
		DIGDAFR	12.3	25	[396.9, 393.2], [401.9, 403.3]	18	[396.9, 565.3], [401.9, 575.3]	10
		NVGDSLVSLSLVK	12.3	25	[691.7, 884.5], [695.7, 610.3]	20	[691.7, 1169.7], [695.7, 1177.7]	20

alkylation by iodoacetamide (50 mM IAA, 60°C for 30 min) and quenching (40 mM DTT; 60°C for 30 min). After an overnight trypsin digestion and trypsin inactivation, Rapigest was hydrolysed and the supernatant was concentrated in a vacuum concentrator. The digest was reconstituted in 0.1% formic acid and analysed by liquid chromatography-high-resolution mass spectrometry (LC/HRMS).

2.4 | LC/HRMS parameters

Extract digests (1–2 µg total protein) were loaded on a pre-column and separated on the UltiMate 3000RSLC nano system equipped with nano pump NCS-3000 and autosampler WPS-3000TPL (Thermo Scientific Dionex). The EASY-Spray analytical column (Acclaim PepMap: C18, 2 µm, 75 µm i.d., 75 cm long) was connected to the LC system with a Thermo Scientific Dionex nanoViper fingertight fitting. Column temperature was maintained at 40°C during all experiments. Injection, sample loading, column equilibration and autosampler wash conditions were kept consistent between the gradient durations and column lengths; flow rate during the gradient was 300 nL/min.

An Orbitrap Fusion Lumos MS was used for peptide MS/MS analysis. Survey scans of peptide precursors were performed at 350–1500 m/z at 120K FWHM resolution with a 4×10^5 ion count target and a maximum injection time of 50 ms. The instrument was set to run with 3 s cycles for survey and MS/MS scans. After a survey scan, tandem MS was performed on the most abundant precursors with charge states from 2 to 6 and intensity $> 5 \times 10^3$ by isolating them in the quadrupole. Collision-induced dissociation at constant 35% collision energy was used for fragmentation, and the resulting fragments were detected using the rapid scan rate in the ion trap. The automatic gain control target for MS/MS was set to 10^4 and the maximum injection time limited to 35 ms.

Raw data were processed using Bionic software version 2.1.0.80, and MS/MS spectra were searched against *Blattella germanica* database (NCBI, 30 192 entry). The search rule included trypsin-specific peptides with up to two missed cleavages, and carbamidomethylation (+57.021 Da) of cysteine residues was set as a fixed modification. Oxidation of methionine residues (+15.9949 Da), acetylation of the protein N-terminus (+42.0106 Da) and deamidation of asparagine and glutamine (+0.984 Da) were treated as variable modifications. Precursor mass tolerance was 20 ppm, and product ions were searched at 0.5 Da tolerances.

2.5 | Preparation of allergen extracts

Frozen GCr bodies (nymphs and adult males and females) or egg cases were rinsed to remove debris and faeces, transferred to a disposable grinding chamber containing a small amount of dry ice and ground using an IKA Tube Mill control (twice, 30 seconds each, 25 000 rpm). Ground material was transferred to a 30-mL round-bottom glass tube and defatted at room temperature with at least

5 volumes of solvent (Table S2 in the Online Data Repository), for 8 cycles, 1 hour per cycle, on a shaking platform (300 rpm). Solvent was discarded, and defatted material was air-dried. Defatted powder was suspended in 20 volumes of extraction buffer (Table S2 in the Online Data Repository) in a 30-mL glass tube, agitated at 4°C for 72 hours on a shaker platform (750 rpm) and centrifuged at 800g for 5 minutes. The supernatant was transferred to 1.5-mL microcentrifuge tubes and spun at 18000g for 30 min. Supernatants were collected and stored at –20°C, either in the extraction buffer alone or with glycerol added to 50% (v/v).

GCr faeces (adult male or adult female) was pulverized in the presence of dry ice, as above, and defatted in at least 4 volumes of diethyl ether/ethyl acetate, 1:2 v/v, 4 times, 1.5 h each, at room temperature. Dried defatted powder was suspended in ABC at 4°C for 72 h with constant shaking. The extract was separated from the pellet by centrifugation. Extract was stored at –20°C, either in the extract buffer alone or with glycerol (50%, v/v) and phenol (0.4% w/v).

Environmental dust samples were extracted at 4°C (1:20, w/v, in PBS with 0.1% Tween20). After centrifugation, the supernatant was sterile-filtered and stored at –30°C.

For all MRM analyses, PBS was exchanged for ABC. Samples were subjected to overnight trypsinization (ratio 1:1); digestion was stopped by acidification and dried. Digests were reconstituted in 0.1% formic acid; the appropriate reference peptide cocktail was added, and LC/MRM-MS analysis was performed.

3 | RESULTS

3.1 | Multiple reaction monitoring mass (MRM) method development

3.1.1 | Isoform- or variant-specific allergen quantification

Most of the GCr allergens have sequence variants^{16,17} but it is unknown whether these protein variants are represented in allergen extracts. Because our MRM method aimed to include all known allergens and their variants, we first analysed allergen extracts for protein composition and sequence diversity using high-resolution LC/MS (Orbitrap Fusion). From 42 allergenic extracts obtained from the US market or prepared by us, we identified 539 proteins and sequence variants (Table S3 in the Online Data Repository). Based on the proteomic map, most of the predicted allergen variants were undetectable in the analysed samples. Our analysis identified four Bla g 4 variants, three Bla g 2 variants and two variants each for Bla g 1 and Bla g 3. The variants of Bla g 1 and Bla g 3 were identifiable in all examined extracts, while the variants of Bla g 2 and Bla g 4 were detected in only a subset of samples. Bla g 6 was a minor component by mass in many of the analysed extracts, a finding that was confirmed using a LC-MRM/MS approach.

The allergens and variants for which the MRM method was developed are summarized in Table 1. The criteria for peptide selection

were previously described¹⁴; the main criterion is the uniqueness of the peptide sequence to the given variant. Those allergens or sequence variants confirmed to be frequently detectable were represented by at least one unique peptide. In total, 26 peptides representing all recognized GCr allergens (www.allergen.org) were identified and their isotopically tagged analogues were synthetically prepared for LC gradient and MRM method development and optimization.

The optimized MRM parameters are depicted in Table 1. We first established a calibration curve by spiking isotopically labelled analogue (SI) into digested extract at various concentrations. This approach differed from the one we used previously, which required synthetic native peptide (sNP) for analysis. We confirmed that the modified method is suitable and has comparable performance to our previous method.¹⁴ Linearity was confirmed by spiking various concentrations of SI into GCr digests and plotting the MRM response against SI concentration. The MRM method was linear over the range 1–1000 fmol/μL with $R^2 > 0.97$ (representative data in Figure S1 in the Online Data Repository, panels A–C). Quantitation accuracy was confirmed by simultaneous quantification of recombinant allergens using the modified and unmodified MRM methods (data not shown). We also tested the effect of SI quantity on accuracy by spiking lower (10 fmol) and higher (100 fmol) concentrations of SI in GCr digests. Accuracy is robust within the linearity range (summarized in Figure S1 panels D, E, and F for Bla g 1.01, Bla g 2.01 and Bla g 5.01, respectively). Therefore, for this work, calibration was performed by a single point at 50 fmol/μL SI for each peptide. For Bla g 3 and Bla g 4, where the content of endogenous allergens is high, a fivefold dilution of GCr digest was prepared prior to spiking with SI.

3.1.2 | Evaluation of the LC-MRM/MS method

The methods evaluation for precision, accuracy and other pertinent parameters has been discussed.¹⁴ Only additional evaluations are covered here.

Performance compared to orthogonal method

We compared Bla g 1 and Bla g 2 content measured by MRM to the content measured by commercially available ELISA (Indoor Biotechnologies) in parallel experiments on 20 different commercial GCr extracts. We found a strong correlation ($R > 0.9$) between LC/MRM-MS and ELISA, although there appears a systematic bias towards lower concentration of LC/MRM-MS over ELISA (Figure S2 in the Online Data Repository), as has been previously observed.¹⁸

Use of LC/MRM-MS method to detect and quantify allergens in different matrices

Commercial extracts: We examined 21 GCr allergen extract lots and 8 lots containing both GCr and American cockroach (Table S1 in the Online Data Repository). Expired extracts > 72 months were included to assess the performance of LC-MRM/MS on degraded samples. For the allergen content analysis, an equal total protein mass of each extract was run through the LC-MRM/MS protocol. Individual allergen content was determined, and the concentration in the original extract was calculated in ng/mL (Table 2). As expected, Bla g 1, Bla g 2 and Bla g 3, which are neither stage-specific nor sex-specific, are most abundant. Bla g 4, an adult male-specific allergen, also occurs in high abundance, suggesting that adult males were well-represented in these extracts. Bla g 3 and Bla g 4 account for 55%–85% of the allergen mass in these extracts, while Bla g 5 accounts for less than 0.5% of the content. Specific allergen content varies considerably among extracts from various sources (Table 2, and Figure S3 in the Online Data Repository). Finally, it appears that GCr allergens, as measured by this method, are stable under normal methods of storage (see, eg Table 2, Bla g 1 and Bla g 2 contents of manufacturer 2/lots 5, 6 and 7, among others). These MRM quantification results are largely confirmed by ELISA (data not shown).

Detection and measurement of allergens in environmental samples: LC-MRM/MS was optimized and verified to measure allergens in GCr aqueous extracts. We then determined the applicability of the method to measure allergens in environmental dust samples. Dust samples from 24 households (16 confirmed cockroach-infested and

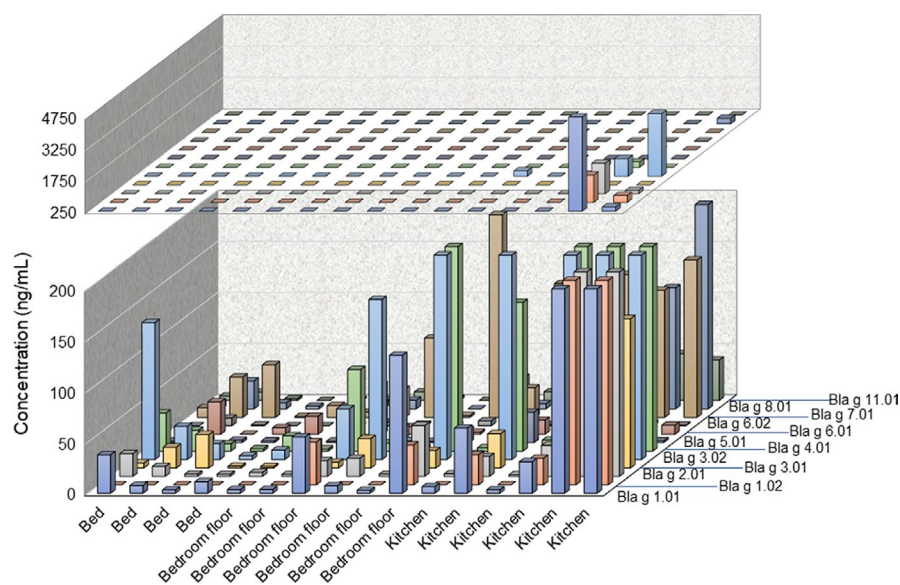


FIGURE 1 Application of the MRM method for detection and quantification of allergens in settled dusts of selected homes. Note different scale in top panel

TABLE 2 Quantification of detectable allergens and their isoforms in cockroach allergenic products obtained from four commercial sources (ng/mL)

Product #	Product type	Strength (w/v)	Age at MRM assay day	Bla g 1.0101	Bla g 1.0201	Bla g 2.0101	Bla g 3 UniProt ID: D0VNY6	Bla g 3.0101
Manufacturer 1								
1	GCr	1:10	25	5380.1	643.3	2222.3	7680.7	43 603.5
2	GCr	1:10	17	4931.3	493.0	2062.2	6288.6	35 792.7
3	GCr	1:10	69	8670.0	1059.8	1504.1	4115.2	14 941.1
4	GCr	1:10	60	9310.7	1088.6	1475.1	4157.4	16 746.2
5	GCr	1:10	207	3949.8	473.5	2545.2	8702.1	47 787.0
6	Mix	1:10	25	3343.0	210.6	1106.7	4727.0	33 336.9
7	Mix	1:10	195	3269.2	356.3	757.3	5216.5	26 905.7
Manufacturer 2								
1	GCr	1:20	33	6998.1	675.6	1347.9	4094.3	15 224.0
2	GCr	1:20	78	10 543.8	934.1	1147.0	6185.1	22 551.4
3	GCr	1:20	80	6216.0	601.4	1484.3	5973.7	35 896.3
4	GCr	1:20	80	6025.0	538.8	1457.3	4525.3	19 081.8
5	GCr	1:20	157	3055.5	274.3	1454.1	2259.8	7258.1
6	GCr	1:20	16	5832.6	524.2	1907.3	3536.2	10 947.2
7	GCr	1:20	16	6319.9	698.9	1950.6	3315.5	10 589.3
8	Mix	1:20	31	2598.7	176.3	493.1	2605.6	34 999.5
9	Mix	1:20	154	1677.8	183.4	509.0	1926.5	9646.5
Manufacturer 3								
1	GCr	1:10	35	30 044.5	2548.1	14 211.2	27 218.7	199 862.8
2	GCr	1:10	93	25 893.4	2142.7	12 473.1	28 017.1	166 254.9
3	GCr	1:10	167	31 598.5	3153.7	7847.0	10 382.4	51 301.5
4	Mix	1:10	66	9556.5	543.8	3438.9	10 505.2	49 654.5
5	Mix	1:10	129	15 635.0	1782.6	5039.4	8667.2	60 580.7
Manufacturer 4								
1	GCr	1:20	43	6933.9	307.6	5676.2	4603.1	23 518.7
2	GCr	1:20	63	5483.1	291.7	6105.1	4040.4	43 521.4
3	GCr	1:20	99	5096.9	205.1	4668.1	3839.7	52 029.7
4	GCr	1:20	150	2383.7	314.1	857.6	2386.0	40 008.0
5	GCr	1:20	27	5789.5	260.7	4490.6	5542.4	27 831.8
6	GCr	1:20	15	6181.3	296.5	5026.3	4040.8	16 100.7
7	Mix	1:20	42	3140.9	186.5	1736.5	1930.8	8687.5
8	Mix	1:20	150	750.7	96.8	492.3	1542.4	17 737.1

8 with no detectable Bla g 1 using ELISA) were obtained, extracted and subjected to LC-MRM/MS. Consistent with previous findings,¹⁹⁻²² highest levels of GCr allergens are found in kitchens, but allergens are also detected in bed and bedroom samples (Figure 1). The mean allergen profile in the environmental samples is compared with the mean profiles from commercial extracts in Table 3. Relative quantities of Bla g 1, Bla g 2, Bla g 3, Bla g 4 and Bla g 11 are similar, while Bla g 5, Bla g 6, Bla g 7 and Bla g 8 are present in the environmental samples but largely absent for the commercial extracts.

3.1.3 | High-resolution LC/MS to explore level of heterogeneity in GCr extracts

We performed an in-depth proteomic evaluation using high-resolution LC/MS and identified 539 proteins from all commercial extracts included in this study (Table S3 in the Online Data Repository), but each extract has < 250 proteins identified. Of the total proteomic mass, 10%-15% is identifiable allergens (Bla g 1-Bla g 11). Several other proteins that bind IgE, such as aldolase, enolase, heat shock protein 70 kDa, triosephosphate isomerase and vitellogenin,²³ are

Bla g 4.0101	Bla g 5.0101	Bla g 6.0101	Bla g 6.0201	Bla g 6.0301	Bla g 7.0101	Bla g 8.0101	Bla g 9.0101	Bla g 11.0101	Vitellogenin
14 611.2	875.3	13.2	2.4	44.0	23.7	4.7	2.0	4621.3	336.7
14 937.7	959.7	13.0	0.9	505.4	22.8	1.4	2.2	3183.1	1080.5
18 901.4	601.7	4.3	0.8	467.2	5.3	3.2	2.5	2245.6	70.2
21 989.5	353.1	7.6	0.6	644.7	11.7	2.8	1.8	2302.0	116.9
16 808.3	178.5	4.7	1.8	813.9	11.5	3.9	1.6	4562.0	251.8
9465.5	350.2	3.6	1.4	124.1	16.4	99.9	0.9	3340.5	1955.0
5513.9	267.4	2.5	1.1	42.6	11.4	65.3	1.1	2567.7	21.0
8210.5	11.0	8.9	1.9	524.3	667.9	34.4	40 558.3	1060.8	61.5
4338.6	23.3	29.0	1.4	1150.9	292.6	33.1	0.9	2009.1	59.5
9294.4	47.5	18.0	1.9	7470.1	995.5	34.9	2.4	3917.9	119.8
8880.1	46.7	14.4	0.7	497.5	559.1	1.7	1.1	1720.7	160.6
9244.7	11.5	5.1	0.7	1904.9	507.1	1.2	0.2	993.8	160.7
11 603.0	36.7	11.0	1.1	443.6	879.9	4.2	0.6	1635.6	266.3
12 149.9	34.9	5.0	0.4	1543.0	377.0	2.0	1.1	1359.7	4289.4
5864.5	55.8	4.1	1.3	737.5	331.0	80.7	0.1	1269.3	26.4
7585.7	70.3	4.3	0.8	1314.2	18.4	52.3	7353.5	1337.7	184.3
62 473.5	695.4	12.5	0.8	158.7	27.4	3.5	2.8	33 589.4	4432.9
63 498.4	353.5	10.7	0.8	181.1	15.4	3.5	3.2	29 860.7	3466.6
26 031.6	51.2	7.2	0.5	49.5	14.0	5.3	8.2	22 448.1	784.4
9805.4	27.1	5.7	0.5	20.3	23.9	106.3	1.9	10 553.0	767.9
14 985.2	49.9	6.4	1.5	578.4	21.0	142.3	5.4	14 537.2	645.5
36 161.9	59.7	5.6	1.9	42.9	146.6	6.7	1.7	5506.9	3561.0
63 894.9	730.3	2.1	2.9	182.7	646.9	29.9	1.7	3638.2	2213.7
52 996.3	413.3	9.9	0.5	106.5	911.5	24.2	2.5	5120.3	1492.8
3699.3	6.8	2.3	0.4	69.9	1530.8	3.6	0.3	2283.2	312.6
49 934.2	286.5	5.8	1.8	54.8	60.6	12.5	1.3	3695.9	4539.2
53 557.5	123.5	7.7	0.5	68.7	55.2	4.9	2.1	2502.9	2146.0
11 923.8	46.9	2.6	1.7	49.6	12.3	491.0	0.5	1861.4	550.0
2547.4	110.1	0.9	0.6	46.6	20.0	51.9	0.2	1375.2	1138.1

abundant in commercial extracts. When these are considered, the proportion of overall IgE-binding protein in these extracts increases to 45%-50% of the total proteomic mass.

Relative protein quantification was performed on the commercial GCr extracts. This was achieved using a label-free approach based on normalized total ion intensity attributed to a given protein in an extract digest. An equimolar amount of synthetic bovine catalase was spiked to each extract digest to normalize variability during analysis. A heatmap was constructed using the 366 (out of 539 total) proteins most frequently detected in GCr extracts.

Differences between extracts are readily apparent (Figure 2A). For example, products from manufacturer 1 have more proteins shown in the upper left quadrant of the heatmap (such as phosphoglycerate mutase, aminopeptidase, chemosensory protein, hexamerin, transferrin and various uncharacterized proteins), while manufacturer 4 has more proteins in the lower right quadrant (such as cathepsin D, lysosomal alpha-galactosidase, apolipoprotein, superoxide dismutase and cytosol aminopeptidase). Gene ontology shows that most constituents of these extracts are enzymes or binding proteins (Figure 2B).

TABLE 3 Allergen profiles in GCr commercial extracts and in extracts prepared from environmental samples. Proportion is expressed as per cent of total allergenic mass detected. The per cent shown is the mean of determinations on several lots for each commercial extract and for samples from several homes for each environmental source, as indicated on the table

Allergen isoform/ variant	Manufacturer 1	Manufacturer 2	Manufacturer 3	Manufacturer 4	Bed	Bedroom	kitchen
N	7	9	5	4	4	6	6
Bla g 1.0101	6.9	13.1	8.0	6.2	14.0	9.1	13.2
Bla g 1.0201	0.8	1.3	0.7	0.3	ND	8.0	7.7
Bla g 2.0101	2.9	3.8	3.8	5.0	5.4	5.4	5.8
Blag 3 UniProt ID: DOVNY6	9.3	7.5	7.3	4.9	10.9	5.8	2.1
Bla g 3.0101	52.8	24.5	53.3	22.5	22.8	32.6	30.9
Bla g 4.0101	19.8	23.4	16.7	54.0	9.3	14.8	13.8
Bla g 5.0101	1.2	0.06	0.2	0.2	2.3	2.8	1.9
Bla g 6.0101	0.02	0.02	0	0.01	17.3	10.7	4.9
Bla g 6.0201	0	0	0	0	2.1	0.7	0.7
Bla g 7.0101	0.03	1.4	0.01	0.06	16.7	14.3	12.3
Bla g 8.0101	0	0.02	0	0.01	7.2	4.6	11.8
Bla g 11.0101	5.2	3.0	9.0	3.2	2.3	1.5	2.9

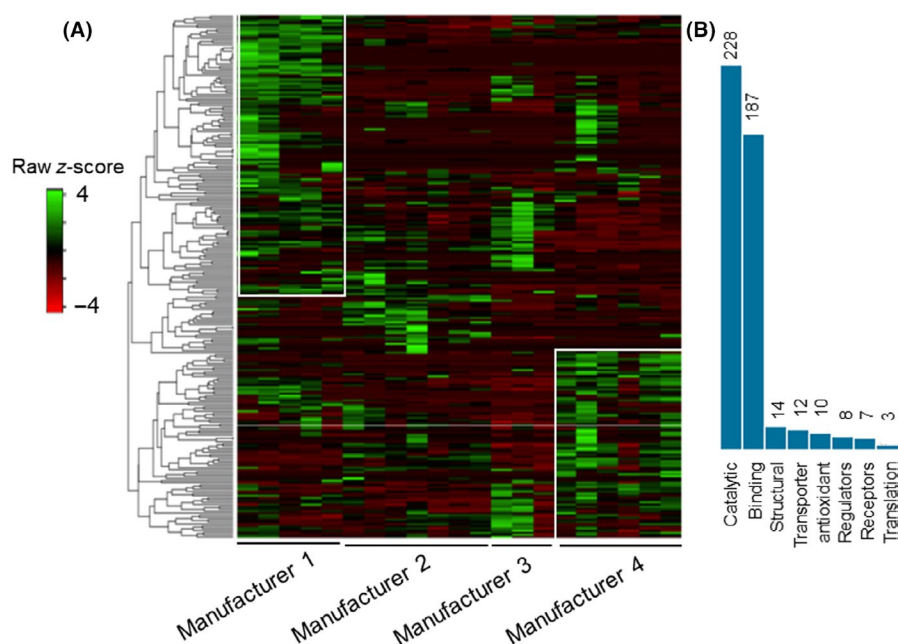


FIGURE 2 Proteomic map of typical GCr allergenic extracts. Panel A: Composition of various lots of GCr extracts from four commercial sources. Panel B: Functional ontology of the total proteome ($n = 539$ proteins) recovered from commercial extracts

3.1.4 | Compositional heterogeneity in GCr extracts

The effects of extraction conditions

Most, but not all, GCr source material extraction protocols begin with defatting steps.²³⁻²⁹ We evaluated the impact of defatting and extraction choices on the extractable allergen quantity and profile. We subjected pulverized frozen GCr whole bodies to various combinations of 3 defatting solvents and 10 extraction buffers (Table S2 in the Online Data Repository). The total extractable proteins under these conditions are summarized in Table S4 in the Online Data Repository. An

allergen-specific evaluation was performed using LC/MRM-MS, and the results are provided in Table E5. Further, Bla g 1 and Bla g 2 contents were assayed by specific ELISA for each of the extracts (Table S6 in the Online Data Repository). No single combination of extraction conditions was optimal across all of the tested parameters: total protein, MRM profile, specific allergen yield or completeness of extraction. Overall, differences in the resulting extracts were minor. Because of the yield and compatibility with downstream LC/MS analysis, we chose diethyl ether/ethyl acetate defatting solvent and 50 mM ABC buffer extraction for further assessment of the role of source materials in allergen extract composition.

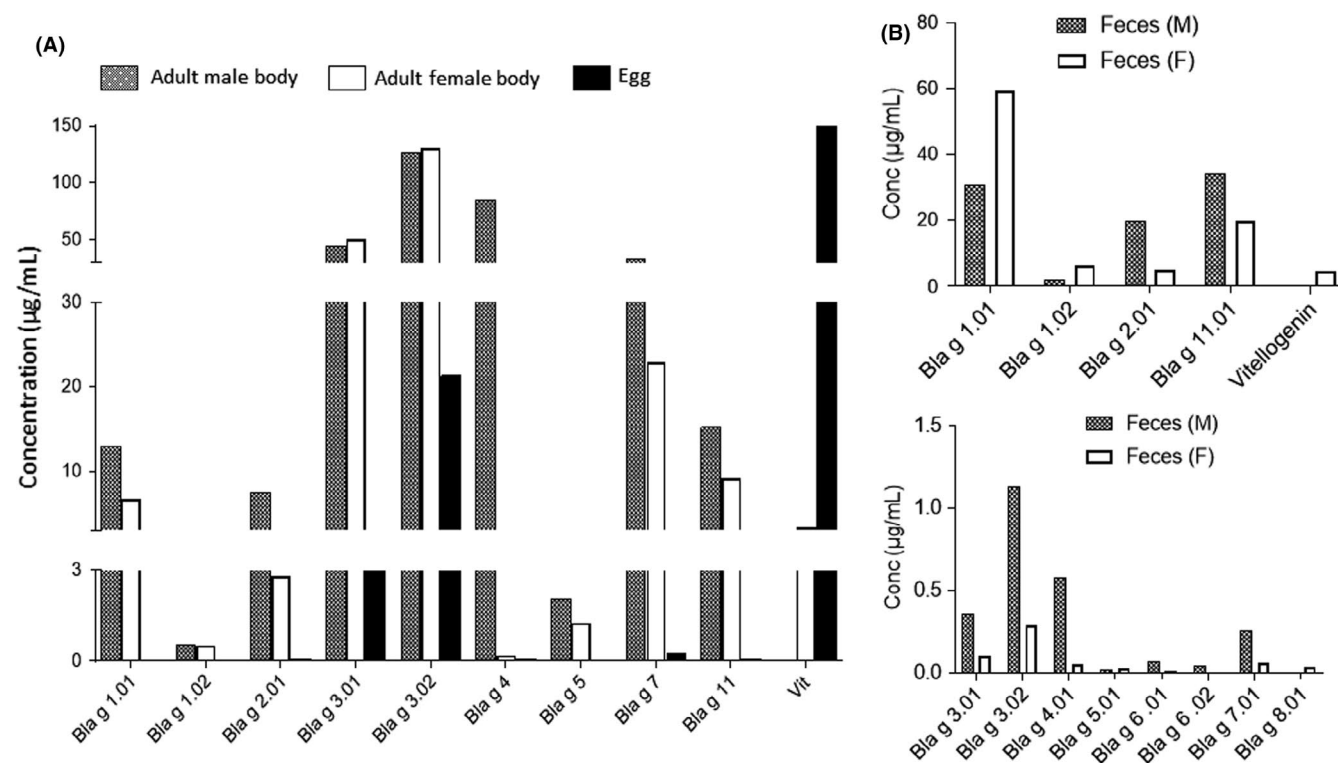


FIGURE 3 Impact of source materials used for extraction on the allergen content and profile of final product. Panel A compares the allergen profiles from whole-body extract based on gender of insects. The cockroaches were grown and treated the same way in the same location, and for comparison, the allergen content of female egg cases was included. Panel B: Comparison of excreted allergens from male and female German cockroach through faeces. Concentration is expressed in µg/mL. M and F: Adult male and adult female, respectively

The impact of source material used for extraction

We examined the effects of source materials on GCr extract allergen content using high-resolution LC/MS and LC/MRM-MS. For these analyses, we prepared extracts from whole bodies of GCr adults sorted by gender or from different providers; faeces from adult female and adult male GCr; and GCr eggs. While modest enrichment in male whole bodies over female was noted for several allergens, Bla g 4, a lipocalin previously localized to the male reproductive organs,³⁰ appears only in extracts from male GCr, and vitellogenin, an egg storage protein,³¹ appears only in extracts from female GCr and eggs (Figure 3A). Faecal extracts had similar content differences, with Bla g 4 and vitellogenin found exclusively in faeces from adult male and female GCr, respectively (Figure 3B). Comparing the overall allergen levels, content in extracts from faeces was lower than levels in whole-body extracts, except for Bla g 1.01 and Bla g 11.

4 | DISCUSSION

Sensitization to GCr allergens causes asthma and rhinitis, which are significant public health burdens. Accurate skin test diagnosis and effective AIT for GCr allergy depend on the availability of well-characterized and consistent allergen extracts. The use of natural aqueous allergen extracts, as opposed to recombinant or synthetic

allergens or allergen fragments, can be highly effective, safe and cost-effective,³² but consistent manufacture will be enhanced by better methods of extract characterization. GCr extracts contain multiple allergens, and no one or two allergens are immunodominant.⁶ In prior work, we have demonstrated that some GCr allergens can be measured using a multiplex monoclonal antibody-based immunoassay¹³ and by MRM.¹⁴ We now expand on our prior work by optimizing LC/MRM-MS to detect and quantify all known GCr allergens and their sequence variants.

Our previously described LC/MRM-MS protocol is expanded with minor modifications to be applicable to all known GCr allergens, and the performance of the modified assay continues to have excellent linearity, accuracy and precision.¹⁴ Hence, properly designed and optimized, investigators can use LC/MRM-MS to detect and quantify allergens with precision and consistency because the identification and quantification are based on unique identifier sequences of the target allergens. Although the initial investment in analytical equipment and software is high, reagents are relatively inexpensive and easy to obtain, and development time is short compared to antibody-based assays. In our hands, LC/MRM-MS could be utilized on complex allergen mixes in a variety of matrices, without noticeable loss in performance characteristics.

One possible limitation of this approach—common to all physicochemical methods—is that it may lead to an overestimation of

allergen content, due to the detection of non-allergenic fragments and epitopes. When orthogonal testing is available, correlation with existing immunoassays is excellent, but ongoing validation will be necessary as this technique is applied to allergen extracts going forward.

Our work also indicates that LC/MRM-MS method can be complemented with high-resolution LC/MS to enhance product characterization beyond known allergens. Using the two techniques, we studied the proteomic compositions of various extracts from commercial sources as well as laboratory-made extracts and analysed the extent of heterogeneity among them, with special focus on factors that influence extract composition. Our analysis of three different defatting solvents and 10 different extraction protocols indicated that the methods are roughly comparable, although overall and allergen-specific yields varied. Source material selection had a much greater impact on extract composition than defatting and extraction protocols. Conventional whole-body GCr allergen source materials used by US manufacturers contain male and female insects, egg cases and faeces. Our analysis confirms⁷ that the specific allergen content of a GCr allergen extract will depend on the relative proportions of these specific components. Extracts derived from source materials rich in faeces will contain more Bla g 1 and Bla g 11, whereas extracts from adult male whole bodies and adult female whole bodies/eggs will contain more Bla g 4 and vitellogenin, respectively. Furthermore, extracts derived from purified components can be expected to be much less diverse in their allergen content than extracts from crude whole bodies and excreta.

One further application of the LC/MRM-MS method in this study was the detection and evaluation of the GCr allergen profiles in selected homes of asthma patients around Baltimore. The profile of GCr allergens in the indoor environment is different from the distribution observed in extracts derived from mixed whole GCr bodies. Our study confirms that only some of the allergens in commercial GCr extracts reflect the GCr allergens to which urban asthma patients are exposed every day in their GCr-infested homes.

GCr infestations are difficult to control and especially difficult to eradicate in homes and schools,^{15,33} and studies to improve GCr pest control are of critical public health importance. In addition, existing GCr allergen extracts are neither potent enough nor consistent enough to support their use as diagnostic and therapeutic agents for GCr-induced allergic disease¹¹; aggressive research efforts to develop better GCr diagnostics and immunotherapeutics will be important to address this problem. As an analytical tool, LC/MRM-MS can be used to monitor and guide the results of both improved interventions in the environment and in allergen extract manufacture, towards improved data-guided solutions to GCr-induced asthma and allergic disease.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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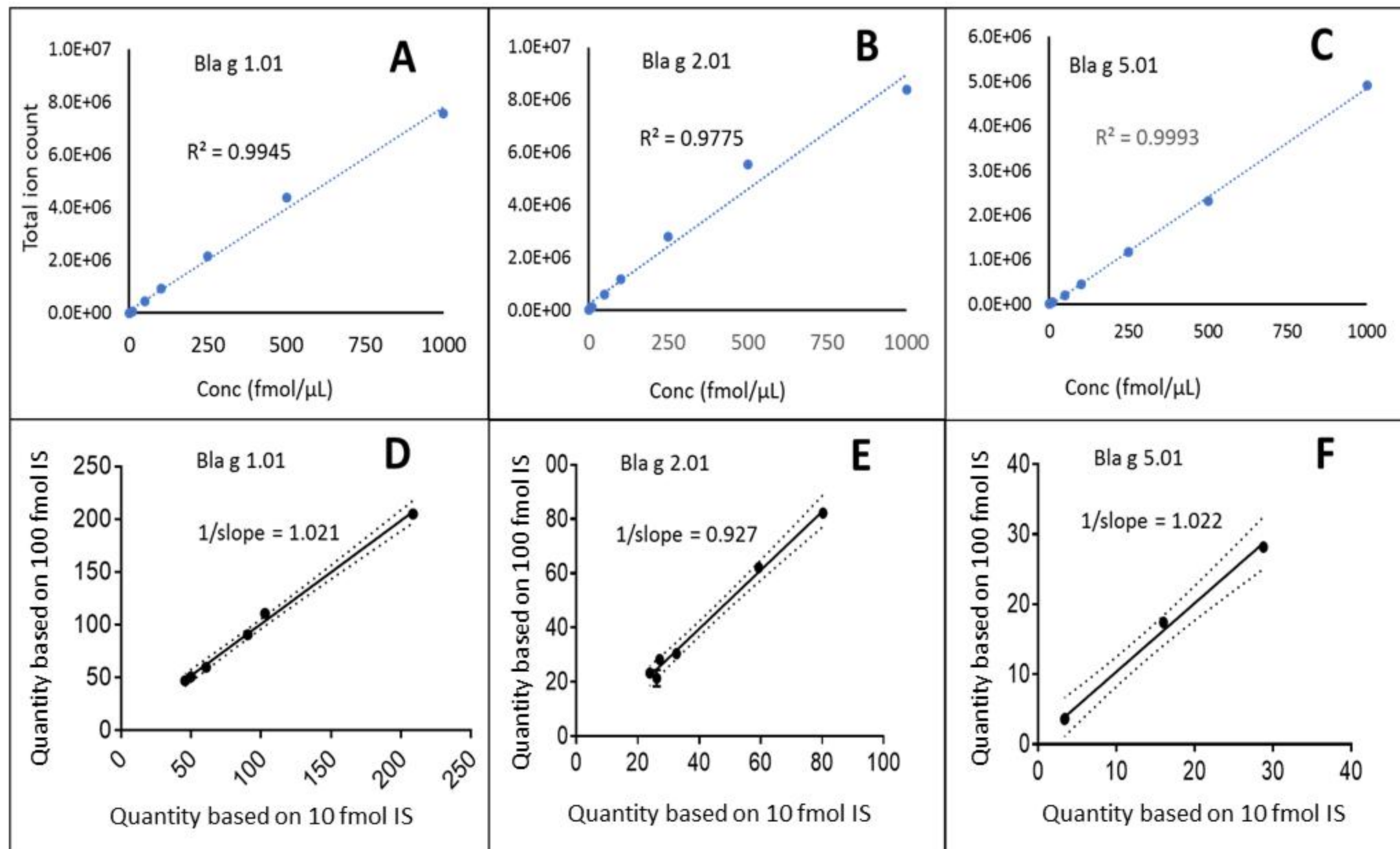
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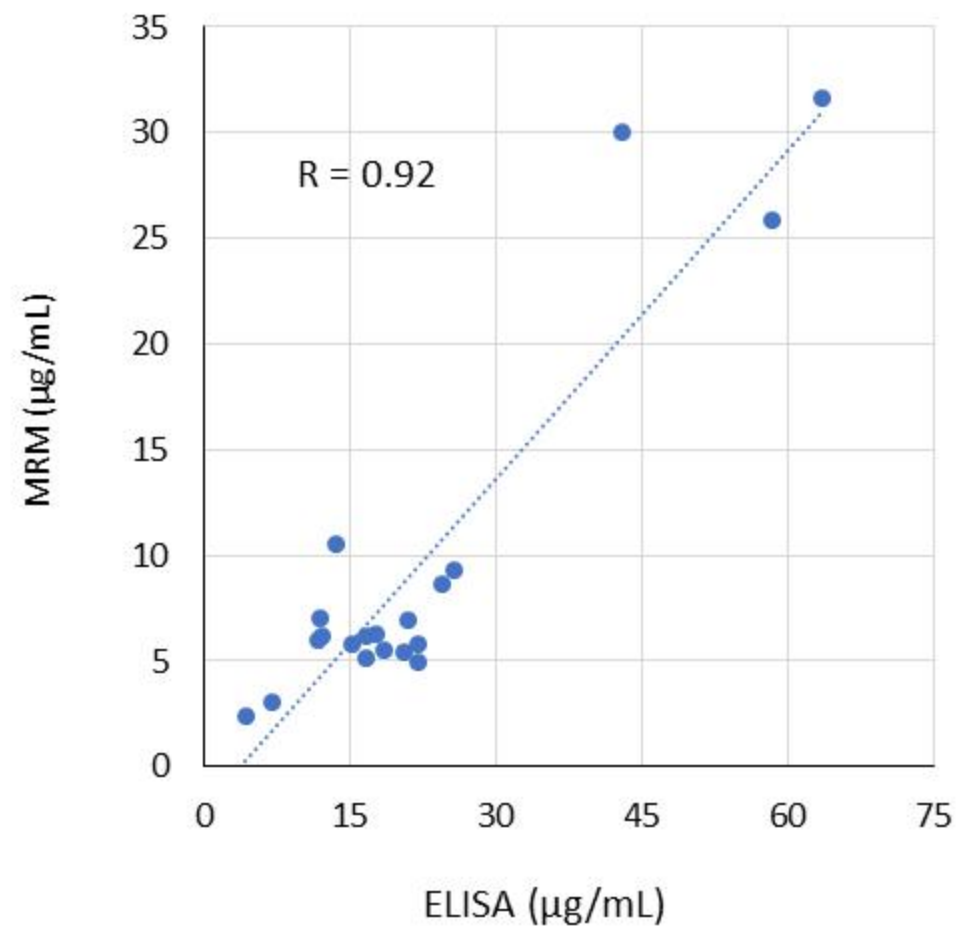
SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

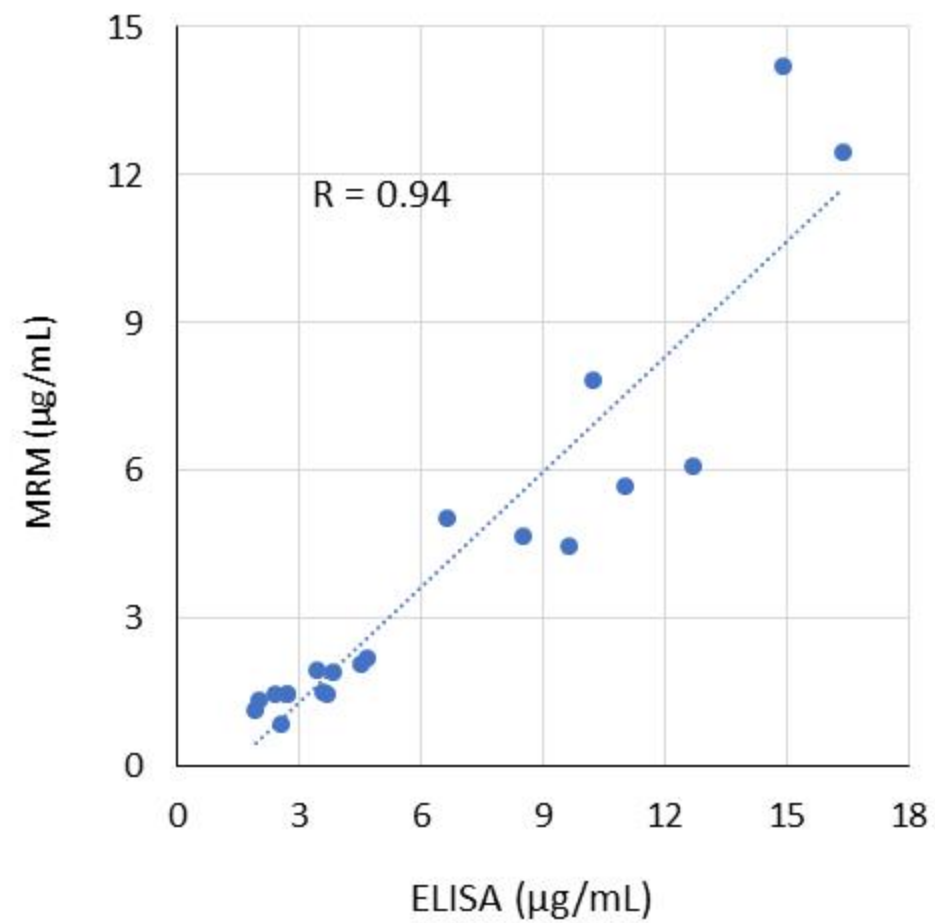
How to cite this article: Mindaye ST, David NA, Zarkesh Esfahani SA, et al. Measurement of German cockroach allergens and their isoforms in allergen extracts with mass spectrometry. *Clin Exp Allergy*. 2020;50:741-751. <https://doi.org/10.1111/cea.13604>



Bla g 1.01



Bla g 2.01



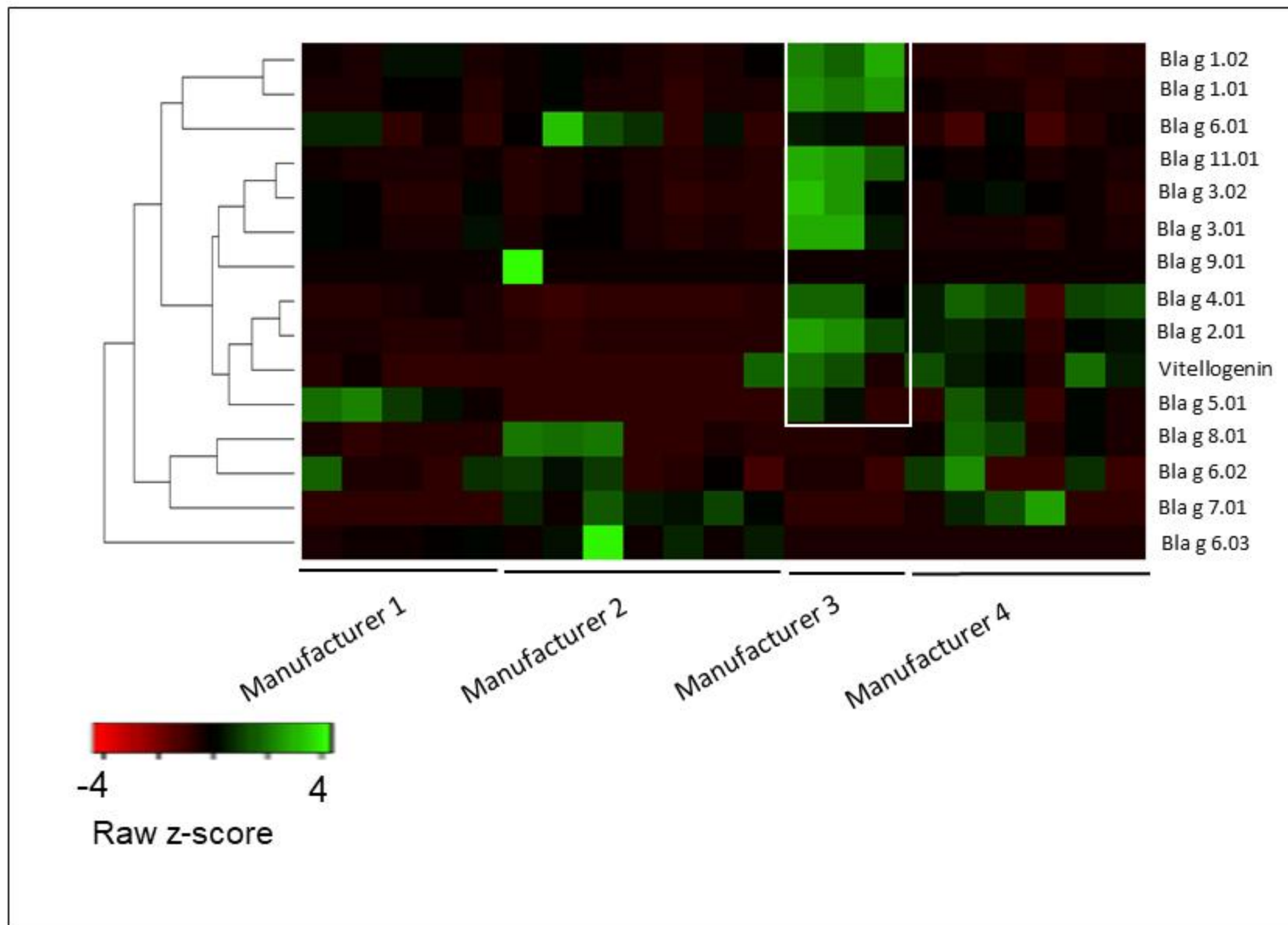


Table S1: Allergen extracts obtained from four commercial sources for this study

Manufacturer	Composition	Extraction w/v	Age (months)	Manufacturer	Composition	Extraction w/v	Age (months)
1	GCr	1:10	25	1	GCr	1:20	33
2	GCr	1:10	17	2	GCr	1:20	78
3	GCr	1:10	69	3	GCr	1:20	80
4	GCr	1:10	60	4	GCr	1:20	80
5	GCr	1:10	*	5	GCr	1:20	157
6	Mix	1:10	25	6	GCr	1:20	16
7	Mix	1:10	*	7	GCr	1:20	16
				8	Mix	1:20	31
				9	Mix	1:20	154
Manufacturer 3				Manufacturer 4			
1	GCr	1:10	35	1	GCr	1:20	43
2	GCr	1:10	93	2	GCr	1:20	63
3	GCr	1:10	167	3	GCr	1:20	99
4	Mix	1:10	66	4	GCr	1:20	150
5	Mix	1:10	129	5	GCr	1:20	27
				6	GCr	1:20	15
				7	Mix	1:20	42
				8	Mix	1:20	150

GCr: German cockroach extract

Mix: a mix of GCr and American cockroach extract

* Bulk manufacturing date not available

Table S2: Description of defatting and extraction reagents used to study the effect of extraction condition on efficiency of allergen extraction

Defatting solvents	Extraction buffer
Diethyl ether	Phosphate buffer saline (pH 7.4)
Diethyl ether and ethyl acetate (1:1)	10 mM ammonium acetate buffer (pH 8.5)
Acetone	Coca's solution (0.5% NaCl, 0.25% NaHCO ₃ , 0.4% phenol)
	50 mM Tris buffer (pH 8)
	50 mM ammonium bicarbonate (pH 8)
	Phosphate buffer saline (pH 7.5), glycerol (50%, v/v)
	Ammonium acetate buffer (pH 8.5), glycerol (50%, v/v)
	Coca's solution, glycerol (50%, v/v)
	50 mM Tris buffer (pH 8.0), glycerol (50%, v/v)
	50 mM ammonium bicarbonate (pH 8), glycerol (50%, v/v)

Table S3: Proteins identified in 42 allergen extracts using high resolution LC/MS

1	>CAA06379.2 vitellogenin [Blattella germanica]
2	>PSN31594.1 hypothetical protein C0J52_22464 [Blattella germanica]
3	>ACY40651.1 allergen Bla g 3 isoform 2 precursor, partial [Blattella germanica]
4	>PSN58157.1 Hexamerin [Blattella germanica]
5	>PSN32258.1 Heat shock 70 kDa protein 12A [Blattella germanica]
6	>PSN45416.1 hypothetical protein C0J52_20841 [Blattella germanica]
7	>ACY40650.1 allergen Bla g 3 isoform 1 precursor, partial [Blattella germanica]
8	>PSN45914.1 hypothetical protein C0J52_24204 [Blattella germanica]
9	>PSN45839.1 hypothetical protein C0J52_05510, partial [Blattella germanica]
10	>CEO43686.1 Hexamerin, partial [Blattella germanica]
11	>PSN29671.1 hypothetical protein C0J52_27980 [Blattella germanica]
12	>ABC96322.1 enolase [Blattella germanica]
13	>PSN39208.1 hypothetical protein C0J52_22281 [Blattella germanica]
14	>PSN56263.1 Alpha-actinin, partial [Blattella germanica]
15	>PSN48368.1 hypothetical protein C0J52_16187 [Blattella germanica]
16	>PSN46736.1 Apolipoprotein [Blattella germanica]
17	>PSN50992.1 Transferrin [Blattella germanica]
18	>AMA98183.1 chemosensory protein [Blattella germanica]
19	>PSN37209.1 Titin, partial [Blattella germanica]
20	>ABI63547.1 triosephosphate isomerase [Blattella germanica]
21	>AAR39433.1 ubiquitin, partial [Blattella germanica]
22	>CAA60869.1 peptidyl-prolyl cis-trans isomerase [Blattella germanica]
23	>PSN46075.1 Guanine deaminase [Blattella germanica]
24	>PSN49158.1 Ferritin subunit [Blattella germanica]
25	>CEO43691.1 Transferrin, partial [Blattella germanica]
26	>PSN54931.1 Glyceraldehyde-3-phosphate dehydrogenase [Blattella germanica]
27	>AAA86744.1 aspartic protease precursor [Blattella germanica]
28	>PSN47917.1 hypothetical protein C0J52_03363 [Blattella germanica]
29	>PSN34221.1 hypothetical protein C0J52_13127 [Blattella germanica]
30	>ACF53837.1 Bla g 4 isoallergen 2 [Blattella germanica]
31	>PSN39804.1 hypothetical protein C0J52_13623, partial [Blattella germanica]
32	>PSN56278.1 Aspartic protease Bla g 2 [Blattella germanica]
33	>PSN49157.1 hypothetical protein C0J52_10136 [Blattella germanica]
34	>PSN39277.1 Cytochrome b5 [Blattella germanica]
35	>PSN41806.1 Beta-ureidopropionase [Blattella germanica]
36	>PSN48207.1 hypothetical protein C0J52_03611 [Blattella germanica]
37	>PSN46786.1 hypothetical protein C0J52_19140 [Blattella germanica]
38	>PSN55088.1 Catalase [Blattella germanica]
39	>PSN30152.1 hypothetical protein C0J52_23236, partial [Blattella germanica]
40	>AEV23881.1 glutathione S transferase class sigma [Blattella germanica]
41	>PSN40292.1 putative aminopeptidase W07G4.4, partial [Blattella germanica]
42	>PSN48989.1 hypothetical protein C0J52_03865 [Blattella germanica]
43	>PSN42022.1 Phosphoglycerate mutase 1 [Blattella germanica]
44	>PSN52222.1 Maltase 1 [Blattella germanica]
45	>PSN36667.1 Allergen Cr-PI [Blattella germanica]
46	>CEO43694.1 Nucleoside diphosphate kinase, partial [Blattella germanica]
47	>PSN39308.1 hypothetical protein C0J52_15574 [Blattella germanica]
48	>PSN44181.1 hypothetical protein C0J52_20710, partial [Blattella germanica]
49	>SIW59360.1 Farnesoic acid methyltransferase crustacean-type [Blattella germanica]
50	>PSN54723.1 Calreticulin [Blattella germanica]
51	>AII81929.1 phenol oxidase [Blattella germanica]
52	>PSN45834.1 hypothetical protein C0J52_05494, partial [Blattella germanica]
53	>PSN38006.1 hypothetical protein C0J52_13516 [Blattella germanica]
54	>PSN36205.1 hypothetical protein C0J52_07289 [Blattella germanica]
55	>PSN30334.1 OV-16 antigen [Blattella germanica]
56	>PSN47606.1 Aldose reductase [Blattella germanica]
57	>PSN40829.1 14-3-3 protein zeta [Blattella germanica]
58	>PSN56224.1 Filamin-A [Blattella germanica]
59	>PSN56074.1 Thioredoxin-2 [Blattella germanica]
60	>PSN51340.1 Fatty acid-binding protein [Blattella germanica]
61	>PSN31887.1 hypothetical protein C0J52_20822 [Blattella germanica]

62 >PSN48537.1 Protein disulfide-isomerase [Blattella germanica]
63 >PSN32014.1 hypothetical protein C0J52_18082 [Blattella germanica]
64 >PSN48675.1 Dystonin [Blattella germanica]
65 >PSN46991.1 hypothetical protein C0J52_17349 [Blattella germanica]
66 >PSN38838.1 Aminoacylase-1 [Blattella germanica]
67 >CEO43698.1 Apolipoprotein, partial [Blattella germanica]
68 >PSN39247.1 3-oxoacyl-[acyl-carrier-protein] reductase FabG [Blattella germanica]
69 >AMA98182.1 chemosensory protein [Blattella germanica]
70 >PSN45348.1 hypothetical protein C0J52_06533 [Blattella germanica]
71 >PSN57195.1 hypothetical protein C0J52_01465 [Blattella germanica]
72 >PSN41826.1 hypothetical protein C0J52_07852 [Blattella germanica]
73 >PSN43923.1 hypothetical protein C0J52_03667 [Blattella germanica]
74 >PSN41753.1 hypothetical protein C0J52_08860 [Blattella germanica]
75 >PSN57811.1 hypothetical protein C0J52_04364 [Blattella germanica]
76 >PSN57097.1 Glucose dehydrogenase [FAD, quinone] [Blattella germanica]
77 >PSN58037.1 Dipeptidyl peptidase 1 [Blattella germanica]
78 >PSN56222.1 Filamin-A [Blattella germanica]
79 >PSN55970.1 hypothetical protein C0J52_02128 [Blattella germanica]
80 >PSN39518.1 hypothetical protein C0J52_24345 [Blattella germanica]
81 >AAD13531.1 major allergen Bla g 1.02, partial [Blattella germanica]
82 >PSN31172.1 Arginine kinase [Blattella germanica]
83 >PSN47365.1 Protein disulfide-isomerase A3 [Blattella germanica]
84 >PSN57511.1 Alpha-N-acetylgalactosaminidase [Blattella germanica]
85 >PSN40902.1 hypothetical protein C0J52_18726 [Blattella germanica]
86 >PSN44772.1 Cytosol aminopeptidase, partial [Blattella germanica]
87 >PSN53206.1 hypothetical protein C0J52_04508 [Blattella germanica]
88 >PSN56726.1 14-3-3 protein epsilon [Blattella germanica]
89 >PSN55861.1 hypothetical protein C0J52_02748 [Blattella germanica]
90 >SIW61628.1 Kettin, partial [Blattella germanica]
91 >PSN57375.1 Adenosine deaminase [Blattella germanica]
92 >PSN30559.1 hypothetical protein C0J52_23206 [Blattella germanica]
93 >PSN50589.1 hypothetical protein C0J52_18356 [Blattella germanica]
94 >PSN38126.1 Selenium-binding protein 1-A [Blattella germanica]
95 >PSN52188.1 Calmodulin-like protein 4 [Blattella germanica]
96 >PSN37364.1 Band 7 protein [Blattella germanica]
97 >PSN30366.1 Beta-1 [Blattella germanica]
98 >PSN52174.1 Titin [Blattella germanica]
99 >PSN36039.1 Aspartate aminotransferase [Blattella germanica]
100 >PSN42604.1 hypothetical protein C0J52_19716 [Blattella germanica]
101 >PSN36814.1 Aspartate aminotransferase [Blattella germanica]
102 >PSN41387.1 Angiotensin-converting enzyme [Blattella germanica]
103 >PSN54801.1 hypothetical protein C0J52_02008 [Blattella germanica]
104 >PSN48054.1 hypothetical protein C0J52_09116 [Blattella germanica]
105 >PSN56208.1 hypothetical protein C0J52_01400 [Blattella germanica]
106 >PSN31161.1 RNA polymerase II transcriptional coactivator [Blattella germanica]
107 >PSN47193.1 Sucrose-6-phosphate hydrolase [Blattella germanica]
108 >PSN54924.1 Glyceraldehyde-3-phosphate dehydrogenase [Blattella germanica]
109 >PSN42044.1 hypothetical protein C0J52_22058 [Blattella germanica]
110 >PSN44593.1 Aspartic protease Bla g 2 [Blattella germanica]
111 >PSN49362.1 Transitional endoplasmic reticulum ATPase TER94 [Blattella germanica]
112 >PSN47187.1 hypothetical protein C0J52_07877 [Blattella germanica]
113 >PSN43518.1 hypothetical protein C0J52_21202, partial [Blattella germanica]
114 >PSN32218.1 Chymotrypsin-2 [Blattella germanica]
115 >PSN52130.1 hypothetical protein C0J52_04322 [Blattella germanica]
116 >PSN55402.1 putative hydroxypyruvate isomerase [Blattella germanica]
117 >PSN57924.1 hypothetical protein C0J52_00237 [Blattella germanica]
118 >PSN56433.1 Actin-5C [Blattella germanica]
119 >CDO39381.1 aspartate aminotransferase mitochondrial like, partial [Blattella germanica]
120 >PSN38101.1 Dynein light chain 1 [Blattella germanica]
121 >PSN42806.1 hypothetical protein C0J52_12679 [Blattella germanica]
122 >PSN48800.1 Collagen alpha-1(IV) chain [Blattella germanica]
123 >PSN57780.1 hypothetical protein C0J52_07266 [Blattella germanica]

124 >AAA87851.1 allergen Bla g 4, partial [Blattella germanica]
125 >PSN50577.1 AMME syndrome protein 1 [Blattella germanica]
126 >CEO43692.1 15-hydroxyprostaglandin dehydrogenase [NAD+], partial [Blattella germanica]
127 >PSN31543.1 hypothetical protein COJ52_20922 [Blattella germanica]
128 >PSN33081.1 Adenylyl cyclase-associated protein 1 [Blattella germanica]
129 >PSN43659.1 hypothetical protein COJ52_11984 [Blattella germanica]
130 >PSN30832.1 Glucose dehydrogenase [FAD, quinone] [Blattella germanica]
131 >AII81931.1 trehalase [Blattella germanica]
132 >PSN48793.1 hypothetical protein COJ52_07039 [Blattella germanica]
133 >PSN41352.1 hypothetical protein COJ52_10389 [Blattella germanica]
134 >PSN50451.1 Lysosomal alpha-mannosidase [Blattella germanica]
135 >PSN36617.1 Trypsin-1, partial [Blattella germanica]
136 >PSN44773.1 hypothetical protein COJ52_05590, partial [Blattella germanica]
137 >PSN30279.1 Heat shock 70 kDa protein cognate 3, partial [Blattella germanica]
138 >PSN45802.1 Programmed cell death protein 6 [Blattella germanica]
139 >PSN53786.1 Superoxide dismutase [Cu-Zn] [Blattella germanica]
140 >PSN36674.1 Beta-glucuronidase [Blattella germanica]
141 >PSN58413.1 hypothetical protein COJ52_02790 [Blattella germanica]
142 >PSN39398.1 hypothetical protein COJ52_16579 [Blattella germanica]
143 >PSN57770.1 hypothetical protein COJ52_07257 [Blattella germanica]
144 >PSN36796.1 Calmodulin [Blattella germanica]
145 >PSN54048.1 Maltase A1 [Blattella germanica]
146 >PSN33246.1 39S ribosomal protein L40 [Blattella germanica]
147 >PSN43219.1 hypothetical protein COJ52_17256 [Blattella germanica]
148 >PSN58153.1 Allergen Cr-PI [Blattella germanica]
149 >PSN55974.1 Serine protease inhibitor 88Ea [Blattella germanica]
150 >PSN44634.1 Superoxide dismutase [Cu-Zn], partial [Blattella germanica]
151 >PSN53079.1 Peroxiredoxin 1 [Blattella germanica]
152 >PSN36678.1 hypothetical protein COJ52_16276 [Blattella germanica]
153 >PSN58403.1 Heat shock 70 kDa protein cognate 4 [Blattella germanica]
154 >PSN52022.1 Plastin-2, partial [Blattella germanica]
155 >PSN56866.1 Lysosomal alpha-glucosidase [Blattella germanica]
156 >PSN49652.1 hypothetical protein COJ52_14793 [Blattella germanica]
157 >PSN57967.1 FK506-binding protein 2B [Blattella germanica]
158 >PSN52172.1 Histone H4 [Blattella germanica]
159 >PSN42961.1 60S acidic ribosomal protein P2-beta [Blattella germanica]
160 >PSN44146.1 hypothetical protein COJ52_15662 [Blattella germanica]
161 >PSN49586.1 hypothetical protein COJ52_09739 [Blattella germanica]
162 >PSN40607.1 Elongation factor 1-gamma [Blattella germanica]
163 >PSN45629.1 hypothetical protein COJ52_16991 [Blattella germanica]
164 >PSN36075.1 Glucosidase 2 subunit beta [Blattella germanica]
165 >PSN46260.1 Peroxiredoxin-4 [Blattella germanica]
166 >PSN39266.1 hypothetical protein COJ52_18443 [Blattella germanica]
167 >CEO43690.1 cathepsin D, partial [Blattella germanica]
168 >PSN41331.1 hypothetical protein COJ52_20909 [Blattella germanica]
169 >PSN44982.1 hypothetical protein COJ52_05477 [Blattella germanica]
170 >PSN39881.1 putative phospholipid hydroperoxide glutathione peroxidase [Blattella germanica]
171 >PSN36053.1 Tubulin alpha-1 chain [Blattella germanica]
172 >PSN48819.1 putative aminopeptidase NPEPL1 [Blattella germanica]
173 >CEO43689.1 twitchin, partial [Blattella germanica]
174 >PSN48957.1 hypothetical protein COJ52_11366, partial [Blattella germanica]
175 >PSN46643.1 Moesin/ezrin/radixin 1 [Blattella germanica]
176 >PSN33241.1 Glucose dehydrogenase [FAD, quinone] [Blattella germanica]
177 >PSN50099.1 hypothetical protein COJ52_04710, partial [Blattella germanica]
178 >PSN42202.1 Bifunctional glutamate/proline--tRNA ligase [Blattella germanica]
179 >PSN41836.1 Profilin [Blattella germanica]
180 >PSN39288.1 Glucose dehydrogenase [FAD, quinone] [Blattella germanica]
181 >PSN37020.1 Chitotriosidase-1 [Blattella germanica]
182 >PSN41699.1 Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex [Blattella germanica]
183 >PSN32854.1 Rho GDP-dissociation inhibitor 1 [Blattella germanica]
184 >PSN31291.1 Hydroxyacylglutathione hydrolase [Blattella germanica]
185 >PSN43664.1 hypothetical protein COJ52_11981 [Blattella germanica]

186 >PSN34235.1 Kynurenine--oxoglutarate transaminase 3 [Blattella germanica]
187 >PSN38492.1 hypothetical protein C0J52_15865, partial [Blattella germanica]
188 >PSN47718.1 hypothetical protein C0J52_04599, partial [Blattella germanica]
189 >PSN54166.1 Glucose-6-phosphate isomerase [Blattella germanica]
190 >ACF53836.1 Bla g 4 isoallergen 1 [Blattella germanica]
191 >PSN32074.1 Uroporphyrinogen decarboxylase [Blattella germanica]
192 >PSN36192.1 Peptidyl-prolyl cis-trans isomerase B [Blattella germanica]
193 >PSN51245.1 Peptidyl-prolyl cis-trans isomerase B [Blattella germanica]
194 >PSN48715.1 hypothetical protein C0J52_08782 [Blattella germanica]
195 >PSN46074.1 Guanine deaminase [Blattella germanica]
196 >PSN54919.1 Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex [Blattella germanica]
197 >PSN45174.1 hypothetical protein C0J52_03943, partial [Blattella germanica]
198 >PSN52721.1 Beta-1 [Blattella germanica]
199 >PSN43501.1 Angiotensin-converting enzyme [Blattella germanica]
200 >PSN38570.1 hypothetical protein C0J52_19766 [Blattella germanica]
201 >PSN45641.1 hypothetical protein C0J52_12342 [Blattella germanica]
202 >PSN57168.1 hypothetical protein C0J52_31472 [Blattella germanica]
203 >PSN36047.1 Lactoylglutathione lyase [Blattella germanica]
204 >PSN32808.1 hypothetical protein C0J52_22968 [Blattella germanica]
205 >PSN34309.1 Protein 5NUC, partial [Blattella germanica]
206 >PSN41394.1 hypothetical protein C0J52_20339 [Blattella germanica]
207 >PSN50721.1 Actin-5C [Blattella germanica]
208 >PSN57245.1 Transaldolase [Blattella germanica]
209 >PSN39361.1 hypothetical protein C0J52_13583 [Blattella germanica]
210 >PSN37305.1 hypothetical protein C0J52_20813 [Blattella germanica]
211 >ACJ37390.1 Bla g 4 allergen variant 1, partial [Blattella germanica]
212 >PSN47532.1 hypothetical protein C0J52_02248 [Blattella germanica]
213 >PSN52813.1 Alcohol dehydrogenase [NADP(+)] [Blattella germanica]
214 >PSN40498.1 Nitrilase and fragile histidine triad fusion protein NitFhit [Blattella germanica]
215 >PSN58234.1 hypothetical protein C0J52_00152 [Blattella germanica]
216 >PSN48042.1 hypothetical protein C0J52_09102 [Blattella germanica]
217 >PSN31025.1 hypothetical protein C0J52_26736 [Blattella germanica]
218 >PSN30899.1 Proteasome subunit alpha type-7-like protein [Blattella germanica]
219 >PSN48063.1 hypothetical protein C0J52_03430 [Blattella germanica]
220 >PSN50402.1 hypothetical protein C0J52_08570 [Blattella germanica]
221 >PSN50375.1 putative ferric-chelate reductase 1 [Blattella germanica]
222 >PSN34784.1 hypothetical protein C0J52_06204 [Blattella germanica]
223 >AAZ78212.1 trypsin [Blattella germanica]
224 >PSN43432.1 hypothetical protein C0J52_02637 [Blattella germanica]
225 >PSN34238.1 Inosine-5'-monophosphate dehydrogenase 1 [Blattella germanica]
226 >PSN56235.1 Spectrin beta chain [Blattella germanica]
227 >PSN29161.1 hypothetical protein C0J52_27958 [Blattella germanica]
228 >PSN42849.1 hypothetical protein C0J52_16421 [Blattella germanica]
229 >PSN37397.1 Ribosomal protein S6 kinase alpha-5, partial [Blattella germanica]
230 >PSN29438.1 hypothetical protein C0J52_27345, partial [Blattella germanica]
231 >PSN50460.1 Peptidyl-prolyl cis-trans isomerase [Blattella germanica]
232 >CDF66123.1 aspartate aminotransferase, partial [Blattella germanica]
233 >PSN50530.1 hypothetical protein C0J52_03525 [Blattella germanica]
234 >PSN42603.1 Beta-agarase AgaB34 [Blattella germanica]
235 >PSN47598.1 Tubulin alpha-1 chain [Blattella germanica]
236 >AJO53282.1 chymotrypsin-like protein [Blattella germanica]
237 >PSN32632.1 hypothetical protein C0J52_26170 [Blattella germanica]
238 >PSN37507.1 malate dehydrogenase [Blattella germanica]
239 >PSN42183.1 Lysine-specific demethylase 8 [Blattella germanica]
240 >PSN42120.1 Gem-associated protein 5 [Blattella germanica]
241 >PSN50763.1 hypothetical protein C0J52_05936 [Blattella germanica]
242 >AAY23288.1 1,4-alpha-D-glucan glucanohydrolase precursor [Blattella germanica]
243 >PSN55445.1 hypothetical protein C0J52_05704, partial [Blattella germanica]
244 >PSN41543.1 Maltase 1 [Blattella germanica]
245 >PSN30040.1 hypothetical protein C0J52_23587 [Blattella germanica]
246 >PSN49650.1 hypothetical protein C0J52_14795 [Blattella germanica]
247 >PSN50240.1 Thioredoxin reductase 1 [Blattella germanica]

248 >PSN44657.1 hypothetical protein COJ52_15734 [Blattella germanica]
249 >PSN29226.1 hypothetical protein COJ52_28096 [Blattella germanica]
250 >PSN42797.1 hypothetical protein COJ52_20536 [Blattella germanica]
251 >PSN41054.1 Facilitated trehalose transporter Tret1 [Blattella germanica]
252 >PSN48381.1 Epidermal growth factor receptor [Blattella germanica]
253 >ABI63548.1 receptor for activated protein kinase C-like [Blattella germanica]
254 >PSN48724.1 hypothetical protein COJ52_08796 [Blattella germanica]
255 >PSN37610.1 hypothetical protein COJ52_20081 [Blattella germanica]
256 >PSN44203.1 Sideroflexin-1 [Blattella germanica]
257 >PSN38922.1 Integrator complex subunit 6-A [Blattella germanica]
258 >PSN58434.1 hypothetical protein COJ52_02826 [Blattella germanica]
259 >PSN52601.1 Glucose dehydrogenase [FAD, quinone] [Blattella germanica]
260 >PSN53758.1 Methionine adenosyltransferase 2 subunit beta [Blattella germanica]
261 >PSN57011.1 Puromycin-sensitive aminopeptidase [Blattella germanica]
262 >PSN44404.1 Protein CutA [Blattella germanica]
263 >AMA98162.1 chemosensory protein [Blattella germanica]
264 >PSN53862.1 hypothetical protein COJ52_09951 [Blattella germanica]
265 >PSN32360.1 Alpha-N-acetylglucosaminidase [Blattella germanica]
266 >PSN36575.1 hypothetical protein COJ52_09132, partial [Blattella germanica]
267 >PSN41366.1 hypothetical protein COJ52_19117 [Blattella germanica]
268 >PSN29572.1 hypothetical protein COJ52_27534, partial [Blattella germanica]
269 >PSN35060.1 E3 ubiquitin-protein ligase [Blattella germanica]
270 >PSN37919.1 hypothetical protein COJ52_12242 [Blattella germanica]
271 >CEG62429.1 Epidermal growth factor receptor [Blattella germanica]
272 >PSN56858.1 hypothetical protein COJ52_08291 [Blattella germanica]
273 >PSN40134.1 Aldehyde dehydrogenase X, partial [Blattella germanica]
274 >PSN46056.1 Laminin subunit alpha [Blattella germanica]
275 >PSN30375.1 hypothetical protein COJ52_25315 [Blattella germanica]
276 >ABP35603.1 Bla g 2 allergen variant [Blattella germanica]
277 >PSN56279.1 hypothetical protein COJ52_00363 [Blattella germanica]
278 >PSN37290.1 putative phosphoserine aminotransferase [Blattella germanica]
279 >PSN54217.1 Dynein heavy chain 8 [Blattella germanica]
280 >PSN48620.1 hypothetical protein COJ52_24274 [Blattella germanica]
281 >PSN31602.1 Guanine nucleotide-binding protein subunit beta-like protein [Blattella germanica]
282 >PSN43928.1 hypothetical protein COJ52_03778 [Blattella germanica]
283 >PSN34375.1 hypothetical protein COJ52_07823 [Blattella germanica]
284 >PSN56987.1 Phosphotriesterase-related protein [Blattella germanica]
285 >PSN50414.1 hypothetical protein COJ52_08569 [Blattella germanica]
286 >PSN45044.1 putative small nuclear ribonucleoprotein G [Blattella germanica]
287 >PSN39640.1 hypothetical protein COJ52_23415 [Blattella germanica]
288 >PSN40091.1 hypothetical protein COJ52_13739 [Blattella germanica]
289 >PSN36887.1 Triosephosphate isomerase [Blattella germanica]
290 >PSN34475.1 Glucose dehydrogenase [FAD, quinone] [Blattella germanica]
291 >PSN35673.1 Tubulin alpha-1 chain [Blattella germanica]
292 >PSN36518.1 hypothetical protein COJ52_25431 [Blattella germanica]
293 >PSN42420.1 hypothetical protein COJ52_11735 [Blattella germanica]
294 >PSN36166.1 hypothetical protein COJ52_16997 [Blattella germanica]
295 >PSN30008.1 hypothetical protein COJ52_27210 [Blattella germanica]
296 >PSN34951.1 Maltase A1 [Blattella germanica]
297 >PSN40966.1 hypothetical protein COJ52_22716 [Blattella germanica]
298 >CDO39390.1 phosphoserine transaminase [Blattella germanica]
299 >PSN46269.1 hypothetical protein COJ52_16640 [Blattella germanica]
300 >PSN31732.1 DNA/RNA-binding protein KIN17 [Blattella germanica]
301 >PSN51659.1 hypothetical protein COJ52_09611 [Blattella germanica]
302 >PSN58482.1 hypothetical protein COJ52_00792, partial [Blattella germanica]
303 >PSN46857.1 hypothetical protein COJ52_13682 [Blattella germanica]
304 >PSN34035.1 hypothetical protein COJ52_17493 [Blattella germanica]
305 >PSN36430.1 hypothetical protein COJ52_11673 [Blattella germanica]
306 >PSN34122.1 Myosin heavy chain [Blattella germanica]
307 >AAF63726.1 beta-1,4-glucanase, partial [Blattella germanica]
308 >PSN31199.1 hypothetical protein COJ52_22102 [Blattella germanica]
309 >PSN35890.1 Adenylosuccinate synthetase [Blattella germanica]

310 >PSN30139.1 Protein diaphanous [Blattella germanica]
311 >PSN45591.1 hypothetical protein COJ52_15074 [Blattella germanica]
312 >PSN57378.1 hypothetical protein COJ52_02903 [Blattella germanica]
313 >PSN33158.1 Pre-mRNA-processing factor 6 [Blattella germanica]
314 >PSN45335.1 hypothetical protein COJ52_18707 [Blattella germanica]
315 >PSN40757.1 hypothetical protein COJ52_13703 [Blattella germanica]
316 >ACA79909.1 beta-actin, partial [Blattella germanica]
317 >PSN49616.1 Ionotropic receptor 546 [Blattella germanica]
318 >PSN35460.1 hypothetical protein COJ52_06634 [Blattella germanica]
319 >PSN48069.1 Reticulon-1-A [Blattella germanica]
320 >PSN32294.1 MICOS complex subunit Mic60 [Blattella germanica]
321 >PSN39000.1 Phosphoglycerate mutase 2 [Blattella germanica]
322 >AEV23883.1 arginine kinase [Blattella germanica]
323 >PSN48270.1 Kinesin-like protein unc-104, partial [Blattella germanica]
324 >PSN48149.1 hypothetical protein COJ52_08719 [Blattella germanica]
325 >PSN43497.1 DNA polymerase subunit gamma-1 [Blattella germanica]
326 >PSN56741.1 Prolactin regulatory element-binding protein [Blattella germanica]
327 >PSN31834.1 hypothetical protein COJ52_20871, partial [Blattella germanica]
328 >PSN37645.1 hypothetical protein COJ52_24500 [Blattella germanica]
329 >ACM24358.1 arginine kinase [Blattella germanica]
330 >PSN44954.1 ADP,ATP carrier protein 1 [Blattella germanica]
331 >PSN41509.1 hypothetical protein COJ52_07224 [Blattella germanica]
332 >PSN48663.1 hypothetical protein COJ52_04766 [Blattella germanica]
333 >PSN52679.1 hypothetical protein COJ52_06019 [Blattella germanica]
334 >PSN42494.1 Leucine--tRNA ligase [Blattella germanica]
335 >PSN39824.1 hypothetical protein COJ52_19185 [Blattella germanica]
336 >PSN55715.1 hypothetical protein COJ52_09002 [Blattella germanica]
337 >PSN52265.1 hypothetical protein COJ52_08332 [Blattella germanica]
338 >PSN48832.1 hypothetical protein COJ52_05121 [Blattella germanica]
339 >PSN52578.1 hypothetical protein COJ52_13085, partial [Blattella germanica]
340 >PSN32178.1 Protein numb [Blattella germanica]
341 >PSN51242.1 hypothetical protein COJ52_10556 [Blattella germanica]
342 >PSN43216.1 hypothetical protein COJ52_17262, partial [Blattella germanica]
343 >PSN49243.1 Protein SEC13 [Blattella germanica]
344 >PSN55420.1 hypothetical protein COJ52_11092 [Blattella germanica]
345 >PSN36815.1 Aspartate aminotransferase [Blattella germanica]
346 >PSN56689.1 hypothetical protein COJ52_00004 [Blattella germanica]
347 >PSN55967.1 hypothetical protein COJ52_02147 [Blattella germanica]
348 >PSN38811.1 Beta-hexosaminidase subunit beta [Blattella germanica]
349 >PSN49312.1 hypothetical protein COJ52_09419, partial [Blattella germanica]
350 >PSN57588.1 Translationally-controlled tumor protein [Blattella germanica]
351 >PSN55285.1 hypothetical protein COJ52_01781 [Blattella germanica]
352 >PSN32135.1 Carbonic anhydrase 13 [Blattella germanica]
353 >PSN46297.1 Beta-lactamase-like protein 2 [Blattella germanica]
354 >PSN49293.1 hypothetical protein COJ52_09404 [Blattella germanica]
355 >PSN54479.1 hypothetical protein COJ52_05556, partial [Blattella germanica]
356 >PSN33797.1 Soma ferritin [Blattella germanica]
357 >PSN56478.1 Malectin-A [Blattella germanica]
358 >PSN30895.1 Chitinase-like protein EN03, partial [Blattella germanica]
359 >PSN48665.1 Plectin [Blattella germanica]
360 >PSN29897.1 hypothetical protein COJ52_28002 [Blattella germanica]
361 >PSN48762.1 hypothetical protein COJ52_05187 [Blattella germanica]
362 >PSN32444.1 Rab GDP dissociation inhibitor alpha [Blattella germanica]
363 >PSN33117.1 Serine/threonine-protein phosphatase CPPED1 [Blattella germanica]
364 >PSN31537.1 hypothetical protein COJ52_26408, partial [Blattella germanica]
365 >PSN45987.1 hypothetical protein COJ52_09758 [Blattella germanica]
366 >PSN40830.1 14-3-3 protein zeta, partial [Blattella germanica]
367 >PSN50515.1 hypothetical protein COJ52_14364 [Blattella germanica]
368 >PSN32814.1 hypothetical protein COJ52_14170 [Blattella germanica]
369 >PSN54313.1 hypothetical protein COJ52_11058, partial [Blattella germanica]
370 >PSN43064.1 Neuroglian [Blattella germanica]
371 >CEO43687.1 Paramyosin, partial [Blattella germanica]

372 >PSN52874.1 Lamin-C [Blattella germanica]
373 >PSN34550.1 hypothetical protein COJ52_24353 [Blattella germanica]
374 >PSN43845.1 hypothetical protein COJ52_11887 [Blattella germanica]
375 >PSN50354.1 Peroxiredoxin-5 [Blattella germanica]
376 >PSN54651.1 Peptidyl-prolyl cis-trans isomerase FKBP4 [Blattella germanica]
377 >PSN51401.1 hypothetical protein COJ52_04462 [Blattella germanica]
378 >PSN30013.1 Protein NipSnap, partial [Blattella germanica]
379 >PSN41608.1 Allatostatin [Blattella germanica]
380 >PSN54007.1 hypothetical protein COJ52_10169 [Blattella germanica]
381 >PSN48205.1 hypothetical protein COJ52_03613 [Blattella germanica]
382 >PSN49779.1 hypothetical protein COJ52_01388 [Blattella germanica]
383 >PSN44412.1 hypothetical protein COJ52_05740 [Blattella germanica]
384 >PSN44497.1 60S ribosomal protein L10 [Blattella germanica]
385 >PSN44272.1 Sodium/calcium exchanger 3 [Blattella germanica]
386 >PSN45244.1 hypothetical protein COJ52_07001 [Blattella germanica]
387 >PSN37307.1 hypothetical protein COJ52_20811 [Blattella germanica]
388 >PSN45619.1 Histone H4, partial [Blattella germanica]
389 >PSN36676.1 Venom carboxylesterase-6 [Blattella germanica]
390 >CEO43697.1 Heat shock 70 kDa protein cognate, partial [Blattella germanica]
391 >AAF72534.1 tropomyosin [Blattella germanica]
392 >AAF88140.1 actin, partial [Blattella germanica]
393 >PSN50017.1 hypothetical protein COJ52_03296 [Blattella germanica]
394 >PSN52173.1 hypothetical protein COJ52_06445 [Blattella germanica]
395 >PSN37625.1 hypothetical protein COJ52_25083 [Blattella germanica]
396 >PSN32053.1 Histone H2A [Blattella germanica]
397 >PSN39418.1 hypothetical protein COJ52_22771 [Blattella germanica]
398 >PSN33705.1 hypothetical protein COJ52_26702 [Blattella germanica]
399 >PSN36943.1 Protein C10 [Blattella germanica]
400 >PSN30423.1 hypothetical protein COJ52_22611, partial [Blattella germanica]
401 >PSN57098.1 Glucose dehydrogenase [FAD, quinone] [Blattella germanica]
402 >PSN53076.1 hypothetical protein COJ52_09160 [Blattella germanica]
403 >PSN43507.1 hypothetical protein COJ52_13414 [Blattella germanica]
404 >PSN50798.1 Xanthine dehydrogenase/oxidase [Blattella germanica]
405 >PSN57772.1 hypothetical protein COJ52_07259 [Blattella germanica]
406 >PSN51158.1 actin [Blattella germanica]
407 >PSN57174.1 putative tRNA (guanine(26)-N(2))-dimethyltransferase [Blattella germanica]
408 >PSN34673.1 hypothetical protein COJ52_22482 [Blattella germanica]
409 >PSN52415.1 hypothetical protein COJ52_03160, partial [Blattella germanica]
410 >PSN34188.1 hypothetical protein COJ52_23020 [Blattella germanica]
411 >PSN57920.1 hypothetical protein COJ52_00233 [Blattella germanica]
412 >PSN33846.1 hypothetical protein COJ52_24244 [Blattella germanica]
413 >PSN36442.1 hypothetical protein COJ52_16300 [Blattella germanica]
414 >PSN36080.1 Casein kinase II subunit alpha [Blattella germanica]
415 >PSN42851.1 hypothetical protein COJ52_16424, partial [Blattella germanica]
416 >PSN58344.1 hypothetical protein COJ52_11577 [Blattella germanica]
417 >PSN54162.1 hypothetical protein COJ52_06845 [Blattella germanica]
418 >PSN37269.1 AN1-type zinc finger protein 2B [Blattella germanica]
419 >PSN38393.1 hypothetical protein COJ52_18749 [Blattella germanica]
420 >PSN55058.1 General transcription factor IIH subunit 4 [Blattella germanica]
421 >PSN52273.1 hypothetical protein COJ52_08318 [Blattella germanica]
422 >PSN56752.1 Dynein heavy chain 7 [Blattella germanica]
423 >PSN41548.1 Catalase [Blattella germanica]
424 >PSN31880.1 hypothetical protein COJ52_19690 [Blattella germanica]
425 >PSN37164.1 Cytochrome P450 4C1 [Blattella germanica]
426 >PSN44643.1 hypothetical protein COJ52_07729 [Blattella germanica]
427 >PSN54559.1 Vacuolar protein sorting-associated protein VTA1 [Blattella germanica]
428 >PSN36228.1 hypothetical protein COJ52_22803 [Blattella germanica]
429 >PSN31557.1 Bleomycin hydrolase [Blattella germanica]
430 >PSN36673.1 hypothetical protein COJ52_16277 [Blattella germanica]
431 >PSN31308.1 hypothetical protein COJ52_14633 [Blattella germanica]
432 >PSN45880.1 Prolyl endopeptidase [Blattella germanica]
433 >PSN51627.1 hypothetical protein COJ52_09440 [Blattella germanica]

434 >PSN42178.1 hypothetical protein C0J52_09855 [Blattella germanica]
435 >ABC68516.1 alpha-amylase [Blattella germanica]
436 >PSN51984.1 hypothetical protein C0J52_18905 [Blattella germanica]
437 >PSN58422.1 hypothetical protein C0J52_02807 [Blattella germanica]
438 >PSN31582.1 Protein-lysine methyltransferase METTL21D [Blattella germanica]
439 >PSN36766.1 hypothetical protein C0J52_26091 [Blattella germanica]
440 >PSN45284.1 hypothetical protein C0J52_17537 [Blattella germanica]
441 >PSN45750.1 Gamma-secretase subunit Aph-1 [Blattella germanica]
442 >PSN40786.1 Aldose 1-epimerase [Blattella germanica]
443 >ABP04043.1 Bla g 4 allergen, partial [Blattella germanica]
444 >PSN46859.1 hypothetical protein C0J52_13684 [Blattella germanica]
445 >PSN48988.1 hypothetical protein C0J52_03864 [Blattella germanica]
446 >PSN39326.1 DNA-directed RNA polymerase II subunit RPB2 [Blattella germanica]
447 >PSN42313.1 hypothetical protein C0J52_11149 [Blattella germanica]
448 >PSN35217.1 hypothetical protein C0J52_16889 [Blattella germanica]
449 >PSN57644.1 hypothetical protein C0J52_05414 [Blattella germanica]
450 >PSN55490.1 hypothetical protein C0J52_02575 [Blattella germanica]
451 >PSN35927.1 putative asparagine--tRNA ligase [Blattella germanica]
452 >PSN35065.1 hypothetical protein C0J52_25925 [Blattella germanica]
453 >PSN39633.1 hypothetical protein C0J52_13798 [Blattella germanica]
454 >PSN53837.1 hypothetical protein C0J52_09944 [Blattella germanica]
455 >PSN33003.1 hypothetical protein C0J52_27785 [Blattella germanica]
456 >PSN41005.1 Gamma-glutamyltranspeptidase 1 [Blattella germanica]
457 >PSN40348.1 Spectrin alpha chain [Blattella germanica]
458 >PSN31886.1 hypothetical protein C0J52_20823 [Blattella germanica]
459 >PSN38670.1 Lysosomal alpha-mannosidase [Blattella germanica]
460 >PSN53578.1 hypothetical protein C0J52_00966 [Blattella germanica]
461 >PSN58291.1 hypothetical protein C0J52_00163 [Blattella germanica]
462 >PSN50444.1 hypothetical protein C0J52_04941 [Blattella germanica]
463 >PSN55713.1 Low-density lipoprotein receptor-related protein 2 [Blattella germanica]
464 >PSN32070.1 hypothetical protein C0J52_16221 [Blattella germanica]
465 >PSN41590.1 hypothetical protein C0J52_17616 [Blattella germanica]
466 >ABC86902.1 arginine kinase [Blattella germanica]
467 >PSN52989.1 Talin-1 [Blattella germanica]
468 >PSN32807.1 hypothetical protein C0J52_22967 [Blattella germanica]
469 >PSN36937.1 hypothetical protein C0J52_20058 [Blattella germanica]
470 >PSN29794.1 Tropomyosin [Blattella germanica]
471 >PSN35314.1 Calcium-transporting ATPase sarcoplasmic/endoplasmic reticulum type [Blattella germanica]
472 >PSN31849.1 Cytochrome c-2 [Blattella germanica]
473 >AEV23880.1 glutathione S transferase class delta [Blattella germanica]
474 >PSN39395.1 Homogentisate 1 [Blattella germanica]
475 >PSN58298.1 Fructose-1 [Blattella germanica]
476 >PSN51556.1 Ejaculatory bulb-specific protein 3 [Blattella germanica]
477 >PSN53366.1 Elongation factor 2 [Blattella germanica]
478 >PSN46753.1 hypothetical protein C0J52_21806 [Blattella germanica]
479 >PSN44042.1 putative citrate synthase 1 [Blattella germanica]
480 >PSN45161.1 hypothetical protein C0J52_03933 [Blattella germanica]
481 >PSN46335.1 6-pyruvoyl tetrahydrobiopterin synthase [Blattella germanica]
482 >PSN39082.1 Glucosamine-6-phosphate isomerase [Blattella germanica]
483 >PSN42836.1 hypothetical protein C0J52_14390, partial [Blattella germanica]
484 >PSN42458.1 hypothetical protein C0J52_18913, partial [Blattella germanica]
485 >PSN31665.1 hypothetical protein C0J52_27498 [Blattella germanica]
486 >PSN42539.1 Venom carboxylesterase-6 [Blattella germanica]
487 >PSN36117.1 Dipeptidyl peptidase 3 [Blattella germanica]
488 >PSN57157.1 hypothetical protein C0J52_01496 [Blattella germanica]
489 >AAB72147.1 allergen Bla g 5, partial [Blattella germanica]
490 >PSN47809.1 hypothetical protein C0J52_08172 [Blattella germanica]
491 >PSN34396.1 hypothetical protein C0J52_15169 [Blattella germanica]
492 >PSN39855.1 hypothetical protein C0J52_21000 [Blattella germanica]
493 >PSN52685.1 Acetyl-CoA carboxylase [Blattella germanica]
494 >PSN49542.1 V-type proton ATPase subunit H [Blattella germanica]
495 >PSN57945.1 Paramyosin [Blattella germanica]

496 >PSN46227.1 hypothetical protein C0J52_11395 [Blattella germanica]
497 >PSN38290.1 Collagen alpha-1(XI) chain [Blattella germanica]
498 >PSN31995.1 Cytosol aminopeptidase [Blattella germanica]
499 >PSN38882.1 hypothetical protein C0J52_18971 [Blattella germanica]
500 >PSN42376.1 hypothetical protein C0J52_08490 [Blattella germanica]
501 >PSN37549.1 hypothetical protein C0J52_12557 [Blattella germanica]
502 >PSN56364.1 hypothetical protein C0J52_03755 [Blattella germanica]
503 >PSN57822.1 hypothetical protein C0J52_04396 [Blattella germanica]
504 >pdb|2NR6|B Chain B, Aspartic Protease Bla G 2
505 >PSN29168.1 hypothetical protein C0J52_28311, partial [Blattella germanica]
506 >PSN34053.1 Pterin-4-alpha-carbinolamine dehydratase [Blattella germanica]
507 >CEO43693.1 thaumatin-like protein, partial [Blattella germanica]
508 >PSN44461.1 hypothetical protein C0J52_10963 [Blattella germanica]
509 >PSN45114.1 hypothetical protein C0J52_18783 [Blattella germanica]
510 >PSN58279.1 Myosin light chain alkali [Blattella germanica]
511 >PSN46958.1 Tropomyosin [Blattella germanica]
512 >PSN52303.1 Acyl-CoA-binding protein [Blattella germanica]
513 >PSN53202.1 Cathepsin L [Blattella germanica]
514 >PSN56676.1 putative serine carboxypeptidase CPVL [Blattella germanica]
515 >PSN43549.1 hypothetical protein C0J52_03693 [Blattella germanica]
516 >PSN37766.1 Neprilysin-2 [Blattella germanica]
517 >PSN38551.1 hypothetical protein C0J52_14812 [Blattella germanica]
518 >PSN36624.1 hypothetical protein C0J52_10451 [Blattella germanica]
519 >PSN49174.1 hypothetical protein C0J52_10148 [Blattella germanica]
520 >PSN38036.1 Tubulin alpha-1D chain [Blattella germanica]
521 >PSN38258.1 hypothetical protein C0J52_17442 [Blattella germanica]
522 >PSN52559.1 hypothetical protein C0J52_08952 [Blattella germanica]
523 >PSN48668.1 Ionotropic receptor 768 [Blattella germanica]
524 >PSN46841.1 hypothetical protein C0J52_09572, partial [Blattella germanica]
525 >PSN52313.1 Enolase [Blattella germanica]
526 >PSN49711.1 hypothetical protein C0J52_16337 [Blattella germanica]
527 >PSN32005.1 Myrosinase 1 [Blattella germanica]
528 >AAD13530.2 major allergen Bla g 1.0101, partial [Blattella germanica]
529 >PSN43902.1 hypothetical protein C0J52_03679 [Blattella germanica]
530 >PSN49497.1 Laminin subunit alpha-1, partial [Blattella germanica]
531 >PSN29892.1 hypothetical protein C0J52_26834 [Blattella germanica]
532 >PSN51955.1 Octopamine receptor beta-1R [Blattella germanica]
533 >PSN42899.1 Myosin-IB [Blattella germanica]
534 >PSN58171.1 hypothetical protein C0J52_00971 [Blattella germanica]
535 >PSN45627.1 putative cytochrome P450 301a1 [Blattella germanica]
536 >PSN47364.1 hypothetical protein C0J52_06568 [Blattella germanica]
537 >PSN54767.1 hypothetical protein C0J52_01975 [Blattella germanica]
538 >PSN33946.1 hypothetical protein C0J52_19348 [Blattella germanica]
539 >PSN44602.1 CDK5 regulatory subunit-associated protein 1 [Blattella germanica]

Table S4: Total protein concentrations of extracts prepared from the same source material at 1:20 (w/v) using various combinations of defatting and extraction conditions. Concentration is expressed in $\mu\text{g/mL}$

Extraction buffer	Defatting reagent					
	Acetone		Ether		Mixture	
	Round 1	Round 2	Round 1	Round 2	Round 1	Round 2
PBS	11394	5548	8911	7164	5653	1714
Ammonium acetate	16103	4262	6994	4981	6011	1881
Coca's solution	14557	3860	18665	2010	11582	3143
Tris	16686	9364	14736	10114	7703	9731
ABC	7194	1614	7544	1514	9311	1831
PBS with glycerol	17369	5748	11053	2548	7103	1181
Ammonium acetate with glycerol	15694	3348	15136	5881	8144	4764
Coca solution with glycerol	12648	5727	5465	227	5282	3060
Tris with glycerol	27103	9281	14969	6781	8186	5864
ABC with glycerol	21711	1914	19444	8564	12528	6331

Table S5: Concentration of representative five GCr allergens in lab made extracts prepared using various combination of defatting and extraction conditions. This compares the relative extraction efficiency of conditions under investigation. Concentration is determined using MRM, results in fmol/ μ L.

Extraction buffer	Defatting agent	Non-glycerinated extraction buffer							Glycerinated extraction buffer					
		Bla g 1.01	Bla g 1.02	Bla g 2.01	Bla g 3	Bla g 4.01	Bla g 5.01		Bla g 1.01	Bla g 1.02	Bla g 2.01	Bla g 3	Bla g 4.01	Bla g 5.01
PBS	Acetone	39.8	8.3	21.3	67.4	31	2.5		30.5	6.9	17.5	47.3	26.6	4
	Diethyl ether	50.6	9.5	24.4	55.4	39.2	0.8		29.5	6.2	16.8	39.9	34.1	0.9
	Diethyl ether: ethyl acetate (1:1)	25	5.3	20.1	59.9	21.7	1.4		16.5	4.8	15.6	44.8	20	1.4
Ammonium acetate	Acetone	40.1	8.2	20.4	54.3	28.2	2.8		26.7	6.3	15.4	40.9	24.9	3
	Diethyl ether	33.1	6.6	13.9	22.6	15.4	1.5		26.3	6.3	19.2	29.5	26.3	0.9
	Diethyl ether: ethyl acetate (1:1)	28.7	7.1	22	60.7	18.3	0.9		15.8	5	18.7	53.6	17.2	1.4
Coca's solution	Acetone	30.6	6.7	8.9	38.8	13.5	0.9		36.3	7.7	14.9	53.1	28.2	3.1
	Diethyl ether	42.3	7.7	12.7	41	17.2	1		38.2	7.7	22.3	54.4	36.1	1
	Diethyl ether: ethyl acetate (1:1)	27.7	6.1	16.4	52.1	13.1	1.4		24.2	6	16.5	52.4	17.5	1.4
Tris	Acetone	35.7	7.7	14.9	52	20.2	2.3		38.7	8.6	21.3	53	32.9	4.2
	Diethyl ether	27.9	5.5	10.9	31.6	13.8	1		24.8	5.7	12.7	31.8	20.8	0.9
	Diethyl ether: ethyl acetate (1:1)	25.3	4.9	15	45.9	17.1	1.4		21.3	5.3	17.2	47.1	18.4	1.4
Ammonium bicarbonate	Acetone	37.9	7.9	16.8	54	21.4	2.3		30	7.1	16.2	45.4	24.8	4.2
	Diethyl ether	43.8	8.5	18.8	56.2	27.1	0.8		18.3	4.3	8.7	23.5	15	0.8
	Diethyl ether: ethyl acetate (1:1)	37.3	7.9	22.4	66.2	23	1.4		23.7	5.5	16.4	48.3	19.8	1.4

Table S6: Concentration of Bla g 1 and Bla g 2 in lab-made extracts prepared using various combinations of defatting and extraction conditions. Concentration is determined using a commercial ELISA.

Extraction buffer	Defatting reagent					
	Acetone		Diethyl ether		Diethyl ether: ethyl acetate (1:1)	
	Bla g 1 (mg/mL)	Bla g 2 (µg/mL)	Bla g 1 (mg/mL)	Bla g 2 (µg/mL)	Bla g 1 (mg/mL)	Bla g 2 (µg/mL)
PBS	42.9	15.7	32.2	12.4	38	22.3
PBS with glycerol	31.6	15.7	63.3	11.8	22.9	24
Ammonium acetate	44.2	15.5	42.9	14.4	53.3	21.4
Ammonium acetate with glycerol	39.3	12.1	100.3	15.4	33.5	19
Coca's solution	53.5	19.2	63	25.7	58.6	22.9
Coca's solution with glycerol	43.2	12.5	162.5	15.7	50	14.8
Tris	52.8	22.6	48	23.8	59.9	20.2
Tris with glycerol	41.7	13.9	65.2	13.2	41.6	15.7
Ammonium bicarbonate	61.8	19.9	58.7	33.2	87	36.9
Ammonium bicarbonate with glycerol	49	16.8	72.2	13.6	41.8	14.8