

Letters

Detection of allergen component–specific immunoglobulin E with the luciferase immunoprecipitation systems immunoassay

Current laboratory methods to determine the presence of circulating allergen-specific immunoglobulin E (IgE) involve the use of crude biological extracts or purified recombinant allergens.¹ Such extracts differ in composition because of difficulties in obtaining pure, raw materials and/or differences in processing,^{1,2} and recombinant allergens are difficult to obtain in biologically relevant, conformationally active forms.² These limitations can lead to variable results in assays.¹ In addition, challenges arise in patients undergoing evaluation for multiple allergens with restrictions on blood draw volume, such as children or individuals with anemia or reduced cardiac output. These constraints indicate a need for an efficient and sensitive assay using small sample volumes and recombinant proteins. The luciferase immunoprecipitation systems (LIPS) immunoassay is an effective method of detection of auto-antibodies and antibodies against infectious agents with high sensitivity and specificity.³ Advantages of this method compared with other assays for antibody detection include fast turnaround time, the use of recombinant antigens without elaborate purification schemes, and ease of optimization.³ Here, we demonstrate the power of synthetic biology coupled with the LIPS immunoassay as a novel, rapid method to detect allergen-specific IgE in low-volume samples.

Serum samples from healthy volunteers ($n = 76$), helminth-infected adults ($n = 26$), and children with peanut allergy ($n = 12$) were screened for IgE to cat using ImmunoCAP (ThermoFisher Scientific, Waltham, Massachusetts). Cat IgE levels ranged from 0 to more than 100 kU/L. Renilla luciferase–Fel d 1 fusion protein constructs were generated by GenScript Biotech (Piscataway, New Jersey). These constructs were transfected into mammalian 293-F cells that allowed for greater likelihood of proper protein folding and glycosylation. LIPS immunoassays were performed as previously described.³ Briefly, 5 μ L of patient serum was combined with 1 million light units (LU; optimized for best signal-to-noise ratio) of Renilla luciferase–Fel d 1 protein extracts in a 96-well microtiter plate and incubated for 10 minutes. Resulting IgE–fusion protein complexes were immunoprecipitated in a 96-well filter plate using anti-IgE beads. After washes to remove unbound fusion proteins, LU were measured on a Berthold LB 960 Centro luminometer (Berthold Technologies, Bad Wildbad, Germany) using a coelenterazine substrate mixture. All samples were run in duplicate. Assays were completed in less than 1 hour. All statistical analyses were performed using Prism software, version 6.0 (GraphPad Software, San Diego, California).

For Fel d 1 LIPS, the signal-to-noise ratio differed significantly among cat IgE–negative (<0.35 kU/mL, $n = 74$) and cat IgE–positive samples (>0.35 kU/mL, $n = 40$; $P < .01$) and encompassed a broad dynamic range (Fig 1, top panel). LIPS signal correlated with cat IgE levels ($r_s = 0.5577$, $P < .001$). Receiver operating characteristic analysis gave an area under the curve (AUC) of 0.7887 ($P < .001$). A threshold of 244 LU/ μ L distinguished individuals with a negative test result for cat IgE from those with a positive test result for cat IgE, with 83% sensitivity and 68% specificity.

In the subset of samples for which Fel d 1 IgE levels were available, the LIPS signal-to-noise ratio differed significantly among Fel d 1 IgE–negative ($n = 40$) and Fel d 1 IgE–positive samples ($n = 20$; Fig 1, bottom panel; $P < .01$). Fel d 1 LIPS signal correlated more closely with Fel d 1 IgE levels ($r_s = 0.6005$, $P < .001$). Receiver operating characteristic analysis gave an AUC of 0.8813 ($P < .001$). A threshold of 395.3 LU/ μ L distinguished individuals with a positive test result from those with a negative test result, with 85% sensitivity and 75% specificity.

These results indicate that LIPS immunoassays can quantify component-specific IgE in small volumes of human serum. Although our sample cohort was relatively small and heterogeneous, the sensitivity of the LIPS assay in identifying individuals with a positive test result for cat IgE was similar to that reported for aeroallergen skin prick testing; however, specificity was lower. Sensitivity and specificity for distinguishing individuals who were positive for Fel d 1 IgE were similar to those for skin prick testing.⁴ We did not have information regarding skin prick sensitivity to cat or clinical symptoms after cat exposure for our cohort; the efficacy of LIPS would likely be increased if our analysis had been able to include these parameters. Future studies will use well-defined clinical cohorts.

For Fel d 1, the LU correlated with cat IgE and Fel d 1 IgE levels by ImmunoCAP. This finding is consistent with studies that found that Fel d 1 is a reliable marker for cat sensitization with high sensitivity and specificity for clinical reactivity to cat.⁵ For patients with a positive test result for cat IgE with low LIPS signal, it is possible that their IgE response is specific for a different Fel d component; samples with LIPS signal-to-noise ratios in the 25th percentile had Fel d 1 IgE levels less than 0.75, including negative results for 57%. Most other aeroallergens do not have a component with the same dominance over others that Fel d 1 has in cat²; thus, using a single component to determine sensitivity may not be clinically useful. However, LIPS is a high-throughput system for which responses to multiple target antigens can be performed simultaneously,⁶ and components can be combined in a single assay to determine allergen sensitivity more accurately. Indeed, we have piloted detection of IgE specific for 5 peanut components (data not shown).

Disclosures: The authors have no conflicts of interest to report.

Funding: This study was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

<https://doi.org/10.1016/j.anai.2020.04.014>

1081-1206/Published by Elsevier Inc. on behalf of the American College of Allergy, Asthma & Immunology.

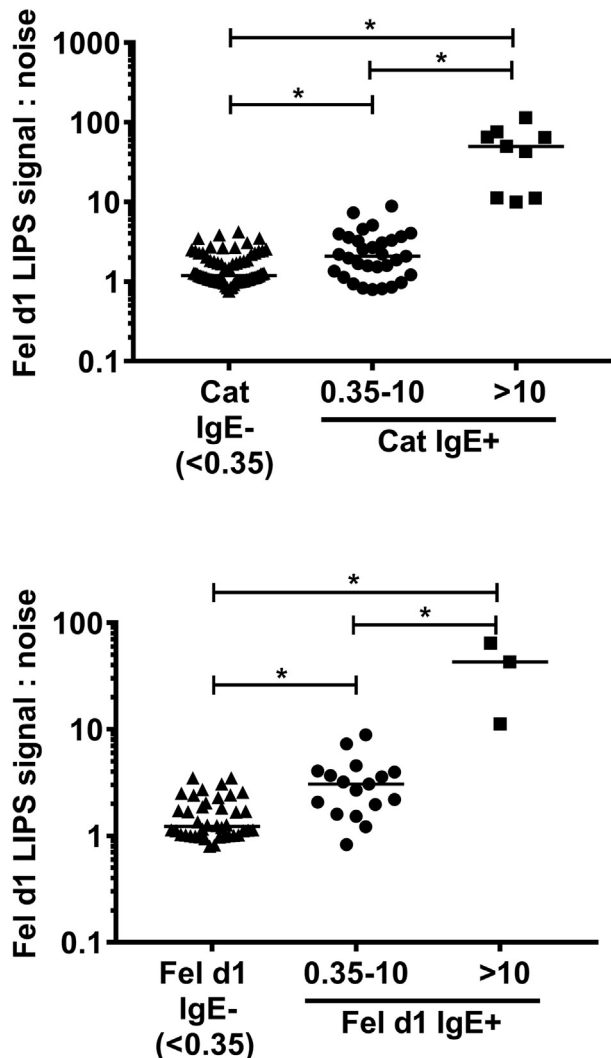


Figure 1. The LIPS assay detects Fel d 1–specific IgE. Top: Fel d 1 LIPS signal-to-noise ratio in individuals with a negative ($n = 74$) and positive test result ($n = 40$) for cat IgE. Bottom: Fel d 1 LIPS signal-to-noise ratio in individuals with a negative ($n = 40$) and positive ($n = 20$) test result for Fel d 1 IgE. Significant differences were found among all groups (Kruskal-Wallis test) and between all pairs (Mann-Whitney test). * $P < .01$. IgE, immunoglobulin E; LIPS, luciferase immunoprecipitation systems.

The LIPS immunoassay offers a sensitive and efficient method to qualitatively and quantitatively determine an individual's sensitization patterns to major components of allergens, using substantially less serum than is typically used for a single allergen-specific IgE. This finding is important in vulnerable populations with limitations on blood collection volumes, who require evaluation for multiple allergens and allergen components. LIPS could have major utility in food allergy, for which component-resolved diagnostics are applied in determining likelihood of clinical reactivity.⁷ With increasing interest in the development of component-resolved immunotherapy, one can also envision LIPS as part of the process

in creating customizable immunotherapy based on an individual's sensitization profile.

The Immuno-Solid Phase Allergen Chip is also capable of simultaneously measuring IgE to multiple allergens and components using a small volume of serum.⁸ However, LIPS is able to provide quantitative and semiquantitative results over a dynamic range that covers several logs. In addition, LIPS may be used to determine levels of other antibody isotypes, such as IgG4 or IgA, specific to a particular allergen or allergen component, simply by changing the isotype specificity of the antibody used to precipitate antibody-protein complexes.⁹ Furthermore, the LIPS assay may be modified to evaluate the quantification of components themselves,¹⁰ which could be used to determine component levels in foods, environmental samples, and allergen extracts.

In summary, because of its sensitivity, specimen conservation, and rapidity, the LIPS immunoassay is a powerful and malleable tool with numerous potential uses in allergy research, diagnostics, and treatment.

Adora A. Lin, MD, PhD*

Natalia S. Perez, BS*

Pamela A. Frischmeyer-Guerrero, MD, PhD†

Thomas B. Nutman, MD*

*Helminth Immunology Section

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

National Institutes of Health

Bethesda, Maryland

†Food Allergy Research Unit

Laboratory of Allergic Diseases

National Institute of Allergy and Infectious Diseases

National Institutes of Health

Bethesda, Maryland

aalin@childrensnational.org

References

- Hamilton RG. Clinical laboratory assessment of immediate-type hypersensitivity. *J Allergy Clin Immunol*. 2010;125(2 Suppl 2):S284–S296.
- Cromwell O, Hafner D, Nandy A. Recombinant allergens for specific immunotherapy. *J Allergy Clin Immunol*. 2011;127(4):865–872.
- Burbelo PD, Ching KH, Klimavicz CM, Iadarola MJ. Antibody profiling by Luciferase Immunoprecipitation Systems (LIPS). *J Vis Exp*. 2009;32:1549.
- Nevis IF, Binkley K, Kabali C. Diagnostic accuracy of skin-prick testing for allergic rhinitis: a systematic review and meta-analysis. *Allergy Asthma Clin Immunol*. 2016;12:20.
- Grönlund H, Saarne T, Gafvelin G, van Hage M. The major cat allergen, Fel d 1, in diagnosis and therapy. *Int Arch Allergy Immunol*. 2010;151(4):265–274.
- Burbelo PD, Leahy HP, Iadarola MJ, Nutman TB. A four-antigen mixture for rapid assessment of *Onchocerca volvulus* infection. *PLoS Negl Trop Dis*. 2009;3(5):e438.
- Santos AF, Brough HA. Making the most of in vitro tests to diagnose food allergy. *J Allergy Clin Immunol Pract*. 2017;5(2):237–248.
- Gadisseur R, Chapelle JP, Cavalier E. A new tool in the field of in-vitro diagnosis of allergy: preliminary results in the comparison of ImmunoCAP® 250 with the ImmunoCAP® ISAC. *Clin Chem Lab Med*. 2011;49(2):277–280.
- Burbelo PD, Ramanathan R, Klion AD, Iadarola MJ, Nutman TB. Rapid, novel, specific, high-throughput assay for diagnosis of *Loa* infection. *J Clin Microbiol*. 2008;46(7):2298–2304.
- Drame PM, Meng Z, Bennuru S, Herrick JA, Veenstra TD, Nutman TB. Identification and validation of *Loa* microfilaria-specific biomarkers: a rational design approach using proteomics and novel immunoassays. *mBio*. 2016;7(1):e02132-15.