

Journal Pre-proof

Anti-PEG IgE In Anaphylaxis Associated with Polyethylene Glycol

Zhao-Hua Zhou, PhD, Cosby A. Stone, MD, MPH, Baruch Jakubovic, MD, Elizabeth J. Phillips, MD, Gordon Sussman, MD, JuMe Park, MS, Uyen Hoang, MS, Susan L. Kirshner, PhD, Robert Levin, MD, Steven Kozlowski, MD



PII: S2213-2198(20)31231-9

DOI: <https://doi.org/10.1016/j.jaip.2020.11.011>

Reference: JAIP 3254

To appear in: *The Journal of Allergy and Clinical Immunology: In Practice*

Received Date: 8 September 2020

Revised Date: 29 October 2020

Accepted Date: 1 November 2020

Please cite this article as: Zhou ZH, Stone CA, Jakubovic B, Phillips EJ, Sussman G, Park J, Hoang U, Kirshner SL, Levin R, Kozlowski S, Anti-PEG IgE In Anaphylaxis Associated with Polyethylene Glycol, *The Journal of Allergy and Clinical Immunology: In Practice* (2020), doi: <https://doi.org/10.1016/j.jaip.2020.11.011>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology

Anti-PEG IgE In Anaphylaxis Associated with Polyethylene Glycol

Zhao-Hua Zhou, PhD^{1*}, Cosby A. Stone, MD, MPH², Baruch Jakubovic, MD³, Elizabeth J. Phillips, MD², Gordon Sussman, MD⁴, JuMe Park, MS¹, Uyen Hoang, MS¹, Susan L Kirshner, PhD¹, Robert Levin, MD⁵ and Steven Kozlowski, MD^{1*}

¹Office of Biotechnology Products, Office of Pharmaceutical Quality, Center for Drug Evaluation and Research, Food and Drug Administration, USA;

²Vanderbilt University Medical Center, Nashville, Tennessee, USA;

³Department of Medicine, Sunnybrook Health Sciences Centre, Toronto, ON, Canada;

⁴Division of Clinical Immunology & Allergy, St. Michael's Hospital, Department of Medicine, University of Toronto, Toronto, ON, Canada;

⁵Office of Surveillance and Epidemiology, Center for Drug Evaluation and Research, Food and Drug Administration, USA

*Address correspondence to Dr. Zhao-Hua Zhou and Dr. Steven Kozlowski, Office of Biotechnology Products, OPQ, CDER, Food and Drug Administration, White Oak Building 71 Room 2230, 10903 New Hampshire Ave., Silver Spring, MD 20993

Tel: 301-796-2046; Fax: 301-796-9743; E-mails: Zhaohua.Zhou@fda.hhs.gov; Steven.Kozlowski@fda.hhs.gov

Running Title: Anti-PEG IgE in Anaphylaxis

Word Count: 976

Competing Interests statement

The authors declare no conflict of interest with this paper

Gordon Sussman declares the following interests:

Advisory board member: Novartis, Aralez, CSL Behring, Sanofi.

Received grant or honorarium: Novartis, Aralez, Pediapharm, GSK, Genentech, DBV technologies, Aimmune, CSL Behring, Astrazeneca, Stallergenes, Merck, Pfizer, Dyax, Biocryst, Greencross, Kendrion, Shire, Leopharma, Regeneron, mdBriefCase.

Currently participating or have participated in clinical trial (PI): Novartis, GSK, Genentech, DBV technologies, Aimmune, CSL Behring, Astrazeneca, Stallergenes, Merck, Pfizer, Dyax, Biocryst, Greencross, Kendrion, Leo Pharma, Regeneron, Sanofi, Blueprint, ALK, Amgen, Clianza.

Elizabeth J. Phillips has received royalties from Uptodate, consultancy fees from Biocyst and Janssen and has a patent for HLA-A*32:01 for vancomycin hypersensitivity testing and is co-director of IIID Pty Ltd that has a patent for HLA-B*57:01 testing for abacavir hypersensitivity. EJP received funding from the National Institutes of Health (P50GM115305, R01HG010863, R01AI152183, R21AI139021, U01AI154659) and the National Health and Research Council of Australia.

Clinical Implications

The dual cytometric beads assay described in this report demonstrates that IgE Type 1 hypersensitivity is a mechanism of PEG associated anaphylaxis. This new detection method could be a useful tool both for clinical practice and in screening for at-risk subjects.

Polyethylene glycol (PEG) has been used in a wide range of medical and pharmaceutical products as an active ingredient or excipient. In addition, an increasing number of PEG-modified (PEGylated) therapeutic proteins and drugs are being developed and approved for marketing¹. The covalent attachment of PEG to a drug or therapeutic protein increases hydrodynamic size and can increase half-life.

Despite the benefit, PEG and PEGylated products are not free of risk and immune-mediated adverse events are of concern. There are an increasing number of PEG-associated anaphylaxis case reports², many of which demonstrate clinical cross-reactivity with polysorbates³. Serious allergic reactions have been seen in patients that received PEG-L-asparaginase⁴, PEGylated IFN- α ⁵, and PEGylated G-CSF (pegfilgrastim), pegvaliase-pqpz and peginesatide⁶. In some cases of PEG-associated reactions, an immediate skin test response suggests IgE-mediated type 1 hypersensitivity reactions; However, a reliable specific assay to sensitively detect specific pre-existing anti-PEG IgE was not available to evaluate these events.

The majority of reported adverse events occurred upon an apparent first exposure to a parenteral version of a specific-PEG-containing product⁶ suggesting previous sensitization to PEG. Data on sensitization to PEG in samples reflective of a broader population would be of value.

We developed a Dual Cytometric Bead Assay (DCBA) for anti-PEG IgG, IgM and IgE in patient sera. Target beads and control beads were generated, incubated with samples

and washed as described in Online Repository. Anti-human IgE-PE, anti-human IgGFC-PE or anti-human-IgM-V450 were added and analyzed by flow cytometry after washing. Single bead populations were gated by FSC-SSC. Target beads and control beads were separated by APC fluorescence intensity. PE fluorescence was compared between target and control beads. Flow cytometry data were analyzed with FlowJo software (FlowJo, LLC); More than 1000 signal events were collected per sample. Plasma or serum samples from cases and controls were evaluated with pegloticase beads and normal individuals were evaluated with peginesatide beads. Positive sera were serially diluted to determine antibody titers and specificity was confirmed by competition with free PEG.

We used the DCBA to test anti-PEG antibodies in anaphylactic patients and controls. The exposures of anaphylaxis patients and controls are described in Online Repository (Table E1). Details of clinical symptoms and skin tests of some of the anaphylaxis patients have been published in case reports ^{2,7}. Plasma or serum samples from cases and controls were collected at various time points after the last episode or exposure, blinded and sent to the FDA lab for anti-PEG IgE testing. In addition to the clinical samples, serum or plasma samples from ~2000 individuals with or without known disease background were purchased from BioIVT (Westbury, NY) and Equitech Enterprises, Inc. (Kerrville, TX). There was no information on previous exposure to PEG or allergic reaction to PEG.

Biospecimens were collected under an IRB approved protocol and/or with patient consent as indicated in previously published case reports. De-identified case and control samples from Vanderbilt University were collected under Vanderbilt University #150754, and #131836. The FDA lab evaluated remnant de-identified biospecimens and the FDA IRB made a “not human subject research” determination.

To determine whether PEG and PEGylated drug-associated anaphylaxis is due to specific IgE-mediated type 1 hypersensitivity, we tested serum samples from patients with documented PEG-associated anaphylaxis for specific anti-PEG IgE. We obtained nine patient samples from two clinical units. The samples included cases of PEG-associated anaphylaxis and controls from PEG exposed patients without associated allergic symptoms^{2, 7, 8}. The sources of PEG exposure included a visualization agent for echocardiograms, PEG 3350 in colonoscopy preparations and as an excipient, and a PEG 8000 lubricating gel. The samples for cases and controls were blinded for testing using the DCBA assay.

As summarized in Table 1, all the anaphylaxis case samples and none of the control samples were clearly positive for anti-PEG IgE. Samples from anaphylaxis cases also had high titers of anti-PEG IgG. Although subjects positive for anti-PEG IgE also had high anti-PEG IgG titers, the reverse was not true. Except for one case (Case PEG9) with an anti-PEG IgM titer of less than 10, all other cases were anti-PEG IgM negative. An example titration for a positive sample (PEG1) is shown in figure 1. The bell-shaped binding curve indicates inhibition of binding at very low dilutions. A control sample PEG3

had a marginal anti-PEG IgE signal with a difference between target beads and control beads well below assay variability (Online Repository Figure E1). The PEG1 positive signal was fully inhibited by free PEG (50ug/mL), demonstrating specificity.

PEG- associated anaphylaxis cases often occurred upon a “first exposure” to PEG or PEGylated drugs. To look for pre-existing anti-PEG antibodies, we screened normal serum samples for anti-PEG IgG, IgM and IgE. We found 5 to 9% of 1721 tested serum samples were positive for anti-PEG IgG and 3 to 6% of 948 were positive for anti-PEG IgM. This range is based on two screening thresholds (i.e., 60% and 100% signals increase of target beads from control beads). We also found two out of 2091 samples positive for anti-PEG IgE. All the antibodies tested were specific for PEG, as demonstrated by free PEG inhibition.

A review of the literature for anti-PEG antibody detection notes that most if not all assays, including commercial kits, are flawed and lack specificity⁹. This was addressed by the use of a dual cytometric beads assay (DCBA) for the detection of anti-PEG antibodies, including anti-PEG IgE. Samples for PEG-associated anaphylaxis cases were positive for anti-PEG IgE confirming the long-considered hypothesis of IgE-mediated type 1 hypersensitivity. We also demonstrated pre-existing antibodies to PEG in normal sera, a potential explanation for reactivity on first known exposure. Pre-existing anti-PEG in normal sera suggest a broad exposure to PEG in daily life. DCBA methods may be of significant value in verifying the diagnosis of suspected IgE-

mediated PEG hypersensitivity and in identifying previously sensitized patients at risk for anaphylaxis with PEG products.

Acknowledgements

We thank Tao Wang and Tao Xie for helpful discussions and comments. We also thank Chunlin Gao, Sujata Bupp and Hanxia Huang for technical support. We also owe thanks to CDER Pharmaceutical Quality Working Group (PWG) on OMONTYS particularly David Frucht, Jack Ragheb and Lucinda Buhse for support of initial peginesatide investigation. This work was supported by FDA intramural research programs including CDER Regulatory Science and Review Enhancement (RSR) funding and OPQ Center of Excellence (COE) funding. The views expressed in this manuscript represent the opinions of the authors, and do not necessarily represent the official views of the FDA.

Authorship Contributions

ZHZ and SK conceived the project, ZHZ developed the method, ZHZ, JMP and UH did the experiments, CAS, BJ, EJP and GS consented patients and collected the patient samples, ZHZ and SK wrote the manuscript, all the authors reviewed and interpreted the data and revised the manuscript.

References

1. Duncan R, Veronese FM. PEGylated protein conjugates: A new class of therapeutics for 21st century; 2009.
2. Stone CA, Jr., Liu Y, Relling MV, Krantz MS, Pratt AL, Abreo A, et al. Immediate Hypersensitivity to Polyethylene Glycols and Polysorbates: More Common Than We Have Recognized. *J Allergy Clin Immunol Pract* 2019; 7:1533-40 e8.
3. Wenande E, Garvey LH. Immediate-type hypersensitivity to polyethylene glycols: a review. *Clin Exp Allergy* 2016; 46:907-22.
4. Abbott LS, Zakova M, Shaikh F, Shewaramani N, Punnett A, Dupuis LL. Allergic Reactions Associated with Intravenous Versus Intramuscular Pegaspargase: A Retrospective Chart Review. *Paediatr Drugs* 2015; 17:315-21.
5. Sakatani A, Doi Y, Matsuda T, Sasai Y, Nishida N, Sakamoto M, et al. Protracted anaphylaxis developed after peginterferon alpha-2a administration for chronic hepatitis C. *World J Gastroenterol* 2015; 21:2826-9.
6. Bennett CL, Jacob S, Hymes J, Usvyat LA, Maddux FW. Anaphylaxis and hypotension after administration of peginesatide. *N Engl J Med* 2014; 370:2055-6.
7. Jakubovic BD, Saperia C, Sussman GL. Anaphylaxis following a transvaginal ultrasound. *Allergy Asthma Clin Immunol* 2016; 12:3.
8. Krantz MS, Liu Y, Phillips EJ, Stone CA, Jr. Anaphylaxis to PEGylated liposomal echocardiogram contrast in a patient with IgE-mediated macrogol allergy. *J Allergy Clin Immunol Pract* 2020.
9. Schellekens H, Hennink WE, Brinks V. The immunogenicity of polyethylene glycol: facts and fiction. *Pharm Res* 2013; 30:1729-34.

Fig. 1 Detection of Anti-PEG IgE and IgG antibodies in Patients that experienced Anaphylaxis to PEG

Anaphylaxis case sample, PEG1, is positive for both anti-PEG IgE and IgG with bell-shaped titration curves. Histograms of anti-PEG IgE signals for PEG1 are shown (blue for target beads and red for control beads); The signal was inhibited by free PEG.

Table 1. Anti-PEG IgE and Anti-PEG IgG in Sera from Cases and Controls of PEG-associated Anaphylaxis.

	Clinic	Anti-PEG IgE			Anti-PEG IgG		
Lab ID		Positivity (Max MFI)	Titration	Inhibition	Positivity (Max MFI)	Titration	Inhibition
PEG1	Case	+++ (4,855)	>512	100%	++ (154,969)	>16,384	100%
PEG2	Case	+ (1,076)	>32	100%	++ (109,079)	>8,192	100%
PEG3	Control	+/- (295)	>4	ND	+ (39,826)	>2,048	100%
PEG4	Control	- (-86)		ND	- (130)	1	ND
PEG5	Control	- (0)		ND	- (4,603)	>1	ND
PEG6	Case	++ (493)	>90	100%	+++ (40,419)	>10,000	100%
PEG7	Case	++ (291)	>100	100%	+++ (78,647)	>10,000	100%
PEG8	Case	++ (1,800)	>90	100%	+ (29,494)	>2,500	100%
PEG9	Case	+ (4,058)	>30	100%	++ (160,690)	>6,000	100%

186 Note: Number of “+” was assigned based upon the titer., for IgE a titer >30 is +, >90 is
187 ++, >512 is +++; for IgG a titer >2000 is +, >6000 is ++, >10000 is +++. Max MFI is the
188 maximum difference of target and control beads. Titer is the dilution where target-control
189 bead MFI becomes flat. “ND”, not done.

Fig. E1

Control PEG3

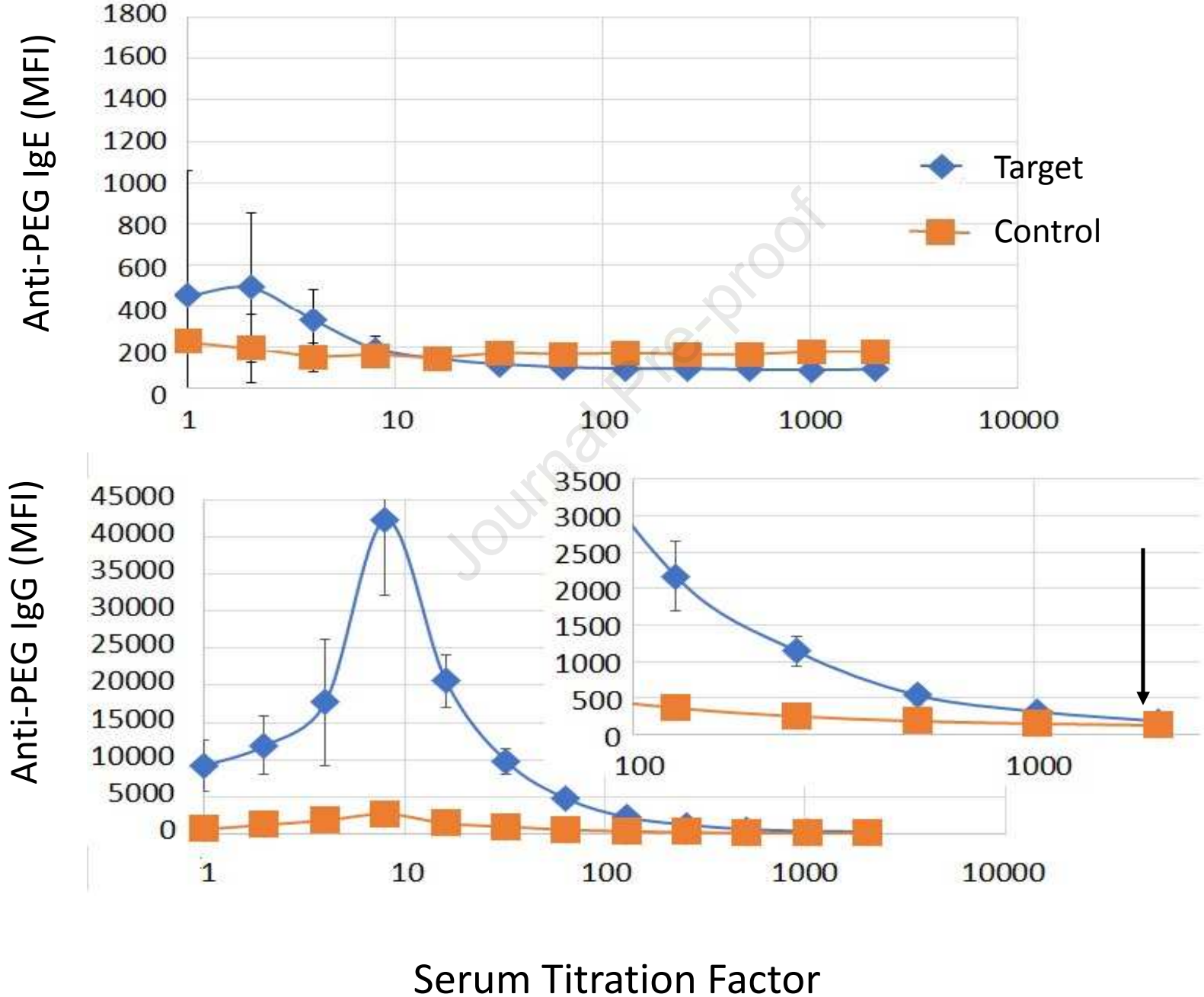
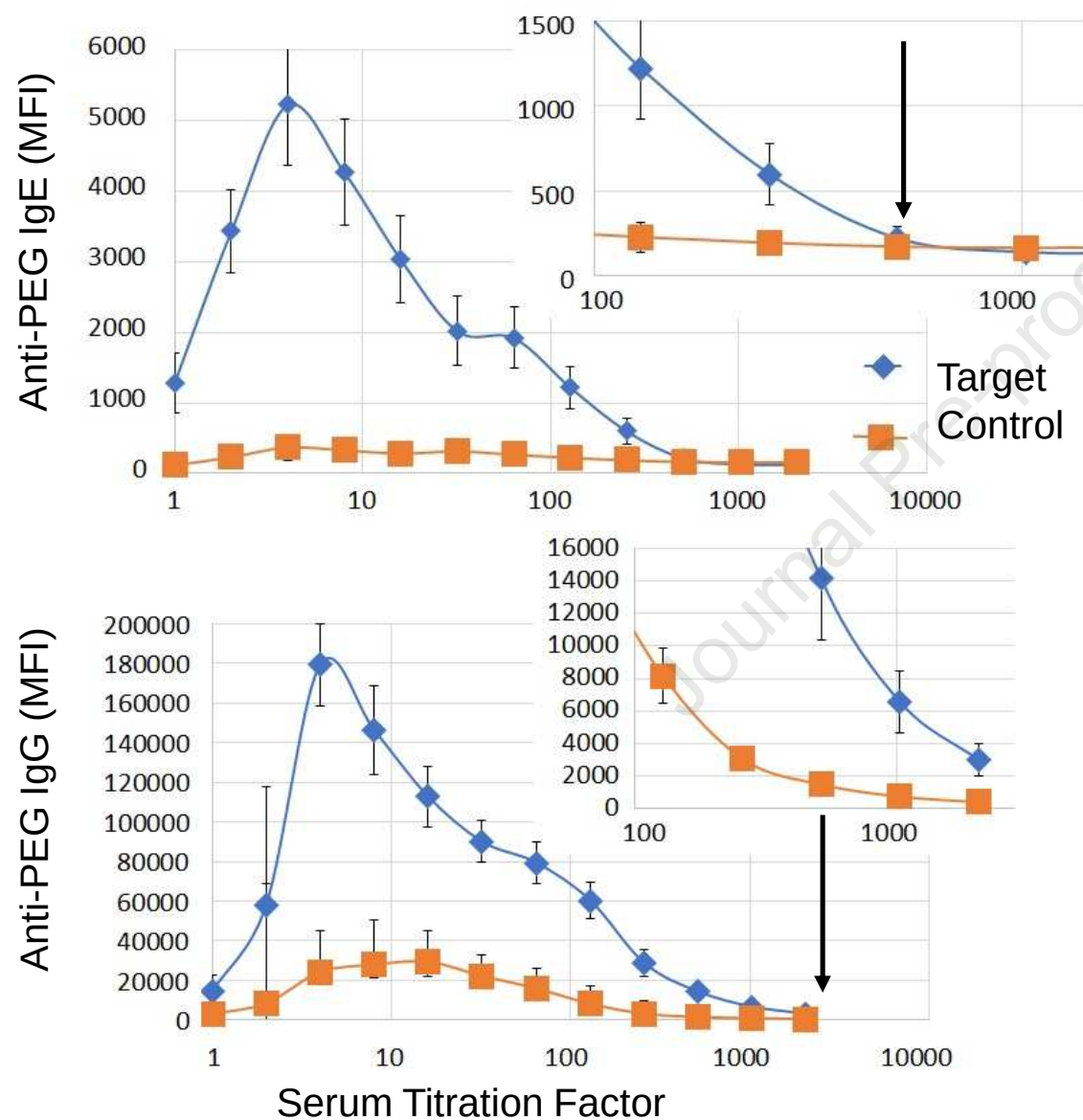


Fig. 1**Anti-PEG IgE & IgG Titration Assay****Specificity Assay**