

# Journal Pre-proof

Diagnostic Testing for Galactose-alpha-1,3-galactose (Alpha-gal), United States, 2010–2018

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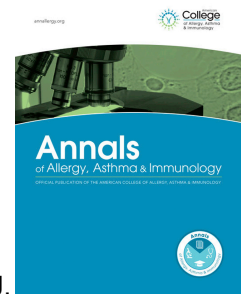
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**Title:** Diagnostic Testing for Galactose-alpha-1,3-galactose (Alpha-gal), United States, 2010–2018

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**Statement of Authors' Contributions:** A.M. Binder and P.A. Armstrong designed the study and the analytic strategy. A.M. Binder carried out analyses and drafted the article. S.P. Commins facilitated data sharing and assisted with drafting the article. P.A. Armstrong supervised the study and writing. A.M. Binder, S.P. Commins, M.L. Altrich, T. Wachs, B.J. Biggerstaff, C.B. Beard, L.R. Petersen, G.K. Kersh, and P.A. Armstrong reviewed and interpreted the data and revised the manuscript critically for important intellectual content.

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**Background:** Alpha-gal syndrome (AGS) is an emerging immunoglobulin E (IgE) mediated allergy to galactose-alpha-1,3-galactose (alpha-gal). The geographic distribution and burden of AGS in the United States is unknown.

**Objective:** To characterize alpha-gal IgE testing patterns and describes trends and distribution during 2010–2018 in the United States.

**Methods:** This retrospective analysis included all persons tested for alpha-gal IgE antibodies by Viracor-IBT Laboratories (Lee's Summit, MO), the primary site of testing in the United States. Data included age and sex of person tested, specimen state of origin, collection date, and result value; persons with at least one positive test ( $\geq 0.1$  kU/L) were compared to negatives. Proportions tested and with positive test results were calculated using U.S. Census population estimates.

**Results:** Overall, 122,068 specimens from 105,674 persons were tested for alpha-gal IgE during July 1, 2010–December 31, 2018. Nearly one-third (34,256, 32.4%) had at least one positive result. The number of persons testing positive increased 6-fold from 1,110 in 2011 to 7,798 in 2018. Of those testing positive, mean [SD] age was 46.9 [19.8] years; males were more likely to test positive than females (43.3% vs 26.0%). Arkansas, Virginia, Kentucky, Oklahoma, and Missouri had the highest number of persons who were tested and had a positive result per 100,000 population.

**Conclusion:** More than 34,000 persons, most presumably symptomatic, have tested positive for IgE antibodies to alpha-gal, suggesting AGS is an increasingly recognized public health problem. The geographic distribution of persons who tested positive is consistent with exposure to *Amblyomma americanum* ticks.

# 1 Main text (2,784)

## 2 Introduction

3 Alpha-gal syndrome (AGS) is a type-1, immunoglobulin E (IgE)-mediated allergy to the  
4 oligosaccharide galactose-alpha-1,3-galactose (alpha-gal), present in non-primate mammals.<sup>1,2</sup>  
5 AGS is characterized by a delayed onset allergic reaction following ingestion of mammalian  
6 meat (e.g., beef, pork, lamb) or its derivatives, and a positive serum IgE result to alpha-gal ( $\geq 0.1$   
7 kU/L).<sup>3</sup> While time from ingestion to manifestation of symptoms is typically 3–6 hours, route of  
8 exposure and co-factors (e.g., exercise, alcohol) can influence onset. Alpha-gal is also present in  
9 many non-food sources, including certain pharmaceuticals (e.g., cetuximab, heparin),  
10 xenotransplants (e.g., porcine heart valve) and vaccines containing gelatin or grown in  
11 mammalian-based culture media [e.g., rabies, MMR (measles, mumps and rubella)]. Delivery by  
12 intramuscular or intravenous injection allows for faster absorption, and symptoms can occur  
13 within minutes.<sup>2</sup> The spectrum of AGS clinical presentation is broad, ranging from urticaria to  
14 angioedema and life-threatening anaphylaxis.<sup>4</sup> A subset of patients report only gastrointestinal  
15 symptoms, which can manifest as nausea and emesis, or debilitating abdominal pain. While  
16 alpha-gal IgE values are not directly correlated with severity of symptoms, higher levels are  
17 associated with increased likelihood of clinical manifestations.<sup>5,6</sup> The mainstay of symptom  
18 management is the use of antihistamines together with an avoidance diet, eliminating all  
19 mammalian products, including dairy and other derivatives.<sup>4</sup> Typically, patients are able to  
20 reintroduce meat and mammalian products into their diet over time.

21 Since 2007, AGS has been reported worldwide, including in North America, Australia, Europe,  
22 and Asia.<sup>7</sup> Various tick species have been implicated in the development of AGS, and IgE  
23 sensitization is strongly correlated with history of tick bites.<sup>8</sup> Nonetheless, the exact causal

relationship and risk factors associated with development of AGS are still unknown. Most patients tolerate mammalian products for years or decades prior to onset of a reaction, suggesting an inciting event.<sup>4</sup> In the United States, most cases have been reported from the Southeast, where *Amblyomma americanum* (lone star tick) is most prevalent.<sup>9</sup> A limited number of studies performed in the United States have shown sensitization rates to alpha-gal among individuals with symptoms of esophageal dysfunction of 22% in North Carolina,<sup>10</sup> among asymptomatic individuals of 20.8% in Tennessee,<sup>11</sup> 6.1% in regions in northern California, and <1% in the northeast.<sup>11</sup> Worldwide, sensitization rates range from 8.1% in Spain and 5.5% in Denmark, to <1% in northern Sweden. Certain exposure groups may experience higher sensitization rates; a study revealed 35% of people in a cohort of forest workers in Germany tested positive for alpha-gal IgE.<sup>8,12,13</sup> While proportion of sensitized individuals in a region does not establish burden of AGS, the correlation between elevated IgE to alpha-gal and AGS suggests that at least a portion of these individuals will be symptomatic.

The true incidence and geographic distribution of AGS in the United States and globally remains unknown. Yet, the information is critical for risk stratification and to increase provider awareness facilitating prompt and correct diagnosis. A study showed that nearly 10% of patients with a diagnosis of idiopathic anaphylaxis were found to have AGS on further evaluation, suggesting underdiagnosis is occurring.<sup>14</sup> AGS is neither reportable at the state level nor nationally notifiable in the United States, and so current understanding is based on limited case reports. Viracor-IBT Laboratories (Lee's Summit, MO) developed the first FDA-approved alpha-gal IgE testing commercially in 2010 and remains the primary source of AGS testing.

This study uses Viracor testing data to describe the extent and patterns of testing and those resulting positive for alpha-gal IgE in the United States. Since individuals in this cohort were

tested by healthcare providers, the majority likely had clinical characteristics suggestive of AGS to prompt testing. Though severity of symptoms is not directly correlated with IgE values, an IgE level  $\geq 0.54$  is highly suggestive of AGS as demonstrated in an allergy clinic setting in Europe.<sup>6</sup> We use these data to estimate the possible magnitude of AGS as a public health threat. We also demonstrate the geographic distribution of positive alpha-gal IgE test results in the United States.

## Methods

### *Study design*

We performed a retrospective analysis of specimens submitted to Viracor for alpha-gal IgE testing during July 1, 2010–December 31, 2018. The Viracor galactose-alpha-1,3-galactose IgE test is a solid phase immunoassay using galactose-alpha-1-3-galactose as the bound antigen. It detects and quantifies IgE antibodies specific to alpha-gal in serum specimens. Results are provided in kilounits of IgE per liter, with  $\geq 0.1$  kU/L as the limit of detection (<https://www.viracor-eurofins.com>). Unique identifiers were used by Viracor to distinguish multiple specimens arising from the same patient; only de-identified data were provided to CDC. Data analyzed included age and sex of person tested, specimen state of origin, collection date and alpha-gal IgE level.

### *Outcomes*

Persons tested more than once were considered positive (sensitized to alpha-gal) if any alpha-gal IgE test result was  $\geq 0.1$  kU/L, and negative if all specimens tested were  $< 0.1$  kU/L. When a person was tested more than once, the date and state of origin of first positive result was used. The first test result was the referent for negative persons.

To account for differences in population size in state and regional analyses, the number of persons who tested positive for IgE antibodies to alpha-gal per year was divided by the respective population estimates from the U.S. Census Bureau.<sup>15</sup> Annual estimates were then averaged to provide the overall estimate. Similar calculations for age and sex used U.S. Census population estimates within each strata.<sup>16</sup> Proportions of persons testing positive annually, seasonally, by U.S. Census region, age, and sex were also calculated and evaluated for trends. Percentiles were calculated based on number of persons positive per 100,000 and mapped to show regional trends (Figure 3). Seasonal data used month of specimen collection and were defined as March–May (spring), June–August (summer), September–November (fall), and December–February (winter). The mean number of tests performed were determined for all persons and by result. The geometric mean and data-derived quartile ranges of the first positive value were calculated for all persons testing positive. While  $\geq 0.1$  kU/L is the limit of detection for Viracor alpha-gal IgE tests, a cut-off of  $\geq 0.35$  kU/L is often reported in the literature, reflective of the limit of detection of other tests. We also calculated the proportions of tests resulting in values ranging from 0.10–0.34, and  $\geq 0.35$ .<sup>3</sup>

### ***Statistical analysis***

Descriptive statistics, risk ratios (RRs), corresponding 95% confidence intervals (95% CIs), and *P* values were calculated for each variable to assess association with positive results. Summaries of continuous variables are expressed as means (standard deviations [SDs]), and summaries of categorical variables are expressed as proportions. Testing began in July 2010, providing only a partial year of data; these data are reported, but excluded from analyses. Data from the first complete year (2011) was chosen as the reference group for RR. The geometric mean and corresponding 95% CI of first positive test result was calculated by log-transforming the original

values. Differences were compared using Pearson  $\chi^2$  test for nominal variables, Cochran-Armitage tests for trend for ordinal variables, and Student's t-tests with unequal variances for continuous variables, as appropriate. Trends were analyzed using simple linear regression models; regression statistics (slopes of best fit lines, and corresponding 95% confidence intervals and p-values) are reported. Two-sided statistical tests were considered significant at a  $P < 0.05$ . Analyses were done using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina).

### **Ethics**

The study was reviewed by the CDC Human Research Protection Office and deemed research not involving human subjects; approval from an institutional review board was not required.

## **Results**

### **Demographics**

Overall, 122,068 specimens from 105,674 persons were tested for alpha-gal IgE antibodies during July 1, 2010–December 31, 2018; 34,256 persons (32.4%) had at least one positive result (Table 1). The mean (SD) age of persons tested was 42.2 (19.9) years. Persons with a positive result were older on average than persons with only negative results (mean [SD]: 46.9 [19.8] vs. 39.3 [19.6] years;  $p < 0.001$ ). Persons  $\geq 70$  years were most likely to test positive, and 0–9 was the age group least likely to test positive. Females were more likely to be tested, but males were more often positive (Table 1).

The number testing positive per 100,000 population rose for both males and females as age increased; at 70 years there was a decline (Figure 1). There was a steep rise from ages 0–9 to 10–19 years, particularly for males (Figure 1). Both males and females aged 60–69 years had the highest number positive compared to all other age groups (2.1 and 1.9 per 100,000, respectively);

the lowest number positive for both sexes was among 0–9 years (0.4 for males and 0.2 for females, per 100,000).

### ***Annual and seasonal trends***

The number of persons tested annually rose from 2,330 in 2011 to 26,148 in 2018, and those testing positive per year also rose from 1,110 in 2011 to a high of 7,798 in 2018. The proportion of persons testing positive decreased from 47.6% (1,110) in 2011 to 29.8% (7,798) in 2018, with a low of 28.5% (5,077) in 2016 (line of best fit: slope =  $-2.6\%$  per year; 95% CI:  $-3.8, -1.3$ ;  $p=0.003$ ) (Figure 2A). The number of newly positive persons averaged 1.2 per 100,000 population per year, and substantially increased during the study period from 0.4 in 2011 to 2.4 in 2018 (line of best fit: slope =  $0.3$ ; 95% CI:  $0.2, 0.3$ ;  $p<0.001$ ) (Figure 2B).

Seasonal patterns showed testing was performed most frequently in the fall (29.2%) and least often in the spring (21.5%) (Table 1). Persons were more likely to test positive in the summer and fall compared to the spring (RR: 1.2; 95% CI:  $1.2, 1.3$ ;  $p=0.002$ ).

### ***Geographic distribution***

Data on state of specimen origin was provided for 45,834 (43.4%) persons; regionally, most specimens (30,955; 67.5%) originated from the South U.S. Census region, where the percent testing positive was highest (10,766; 34.8%), and the fewest (831; 1.8%) originated from the West, where the percent positive was lowest (6.5%) (RR: 5.4; 95% CI:  $4.1, 6.9$ ,  $p<0.0001$ ). Although fewer specimens originated from the Northeast (5,328; 11.6%) and Midwest (8,717; 19.0%), the percent positive in these regions (31.1% and 30.0%) was similar to the South.

In total, 15,091 persons with known state of origin tested positive during 2010–2018 (Figure 3). Arkansas, Virginia, Kentucky, Oklahoma, and Missouri had the highest number of positive persons per 100,000 population ( $\geq 2.35$ ). States with the lowest number were Idaho, New Mexico, and Arizona (0 to  $<0.01$  per 100,000). Hawaii was the only state without any persons tested during the study period (Supplemental Table 1).

### ***Laboratory characteristics***

Most persons (95,603; 90.5%) had only one test performed, and 26,177 (27.4%) of those were positive (Table 1). Persons with at least one positive result were tested more often than those with negative results (mean [SD]: 1.4 [1.0] vs. 1.0 [0.2] tests;  $p<0.001$ ). Among persons with a positive result, the geometric mean of the first positive value was 2.6 kU/L (95% CI: 2.5, 2.6); data-derived quartile ranges were: Q1 [0.1– $<0.5$ ], Q2 [0.5– $<2.4$ ], Q3 [2.4– $<13$ ], Q4  $\geq 13$ . Most (27,209, 79.4%) persons testing positive had an alpha-gal IgE value of  $\geq 0.35$  on the first positive test.

### **Discussion**

Our data show both a remarkable utilization of testing as well as a dramatic increase in capture of persons sensitized to alpha-gal. During 2010–2018, more than 105,000 persons were tested for alpha-gal IgE, the number of tests performed annually increased more than ten-fold, and the annual number of persons testing positive increased six-fold. The increase in testing volume was accompanied initially by a steep decline in the proportion of persons testing positive, which stabilized around 30% from 2015 to 2018. A likely explanation for this level of increased testing is provider awareness and increased access to diagnostics. However, the high proportion of positive test results throughout the study period suggests that physicians continue to have a high

156 degree of clinical suspicion and diagnostic acumen when ordering diagnostic testing for alpha-  
157 gal IgE.

158 We identified 34,256 persons whose samples were positive for IgE antibodies to alpha-gal.  
159 Presumably persons tested had clinical characteristics suggestive of AGS to prompt testing and  
160 nearly three-quarters (72.5%) of persons who tested positive had IgE levels  $\geq 0.54$ , a value shown  
161 to be highly predictive of AGS in an allergy clinic setting in Europe.<sup>6</sup> These results suggest the  
162 number of persons with AGS is an order of magnitude larger than the hundreds to thousands of  
163 cases previously reported in the literature in the United States.<sup>9</sup> Increased clinical awareness and  
164 access to diagnostics is likely responsible for much of this increased detection. However, this  
165 increase may also stem from increasing sensitization of the population presumably through tick  
166 exposure, or by an increase in the likelihood that sensitized persons develop AGS.

167 Exposure to lone star ticks has been strongly implicated in the development of AGS, but a causal  
168 relationship has not been confirmed.<sup>9</sup> Our understanding of the geographic distribution of AGS  
169 in the United States is based on small case series<sup>9</sup>, and to date, nearly all cases have been  
170 reported from the known geographic distribution of the lone star tick.<sup>17</sup> Similarly, our study  
171 showed that most tests were submitted from the South U.S. Census region and the highest  
172 numbers of persons testing positive relative to state population were in states highly endemic for  
173 *Ehrlichia chaffeensis* and *E. ewingii* ehrlichiosis, also transmitted by the lone star tick.

174 Oklahoma, Arkansas, Missouri, Kentucky, and Virginia had the highest number of persons  
175 testing positive for alpha-gal IgE per 100,000 population. Arkansas, Missouri, Virginia and  
176 Oklahoma also have the highest incidence rates for *E.chaffeensis* ehrlichiosis.<sup>18,19</sup>

177 While most samples originated from the South U.S. census region, a high proportion of persons  
178 whose samples originated from the Midwest and Northeast tested positive (30–31%). In states

such as Minnesota and New Hampshire, the lone star tick has only rarely been observed, but these states have large, established populations of the black-legged tick, *Ixodes scapularis*.<sup>20</sup> Recent studies have implicated *I. scapularis* in the development of AGS, and our data show individuals sensitized to alpha-gal in areas within the geographic distribution of *I. scapularis*.<sup>21</sup> American dog ticks (*Dermacentor variabilis*) are also widely distributed in the United States and are found in states such as North Dakota and in northern Minnesota.<sup>17</sup> This may reflect travel to areas where *A. americanum* is present, or a role of tick species other than *A. americanum* in the development of AGS in the United States. Other *Ixodes* species have been linked to the development of AGS in other parts of the world, with *I. ricinus* the primary species implicated in Europe, and *I. holocyclus* recently joined by *I. australiensis* associated with the condition in Australia.<sup>22,23</sup> However, the most prominent geographic patterns in our study further solidify the primary role of *A. americanum* in the development of AGS.

The number of persons testing positive increased with age, which may reflect cumulative exposure to ticks, or an increased likelihood of development of AGS with age among previously sensitized persons. Although positive tests were equally distributed by gender, more females were tested with a lower proportion of positive test results. The reason for this testing pattern is unknown. A higher proportion of persons whose samples were obtained in the summer and fall tested positive, which would be consistent with a condition induced by tick bite as ticks are more prevalent during these months. Nevertheless, seasonal variation in the proportion positive was not substantial, likely due to variability in the timing between symptom onset and testing.

This study is limited by the lack of clinical information. Furthermore, the geographic data were only available for less than half of the specimens and reflected the specimen state of origin, not necessarily where the exposure occurred or where the condition was acquired. Differential

awareness among healthcare providers may have led to geographic variation in testing practices and thus identification of symptomatic persons sensitized to alpha-gal. Lastly, it is possible that some persons could have been counted twice because of missing data elements needed to determine if an individual was unique. We think this represents an infrequent occurrence and does not significantly impact findings. It is also possible additional testing is performed in laboratories using their own assays, and so these data may be an underestimate.

Our data demonstrate that the number of people tested for alpha-gal IgE in the United States has been rapidly increasing since the test became available. The number of people with positive alpha-gal IgE test results at levels consistent with clinical AGS, suggests that AGS is an increasingly recognized public health problem. While trends in seasonality and geographic distribution provide further evidence of an association with *A. americanum* exposure, persons testing positive outside of areas endemic for this tick raise the possibility that other tick species contribute to the development of AGS in the United States. Awareness among healthcare providers of the possibility of AGS in their patients, even in regions not endemic for the lone star tick, is critical for accurate and timely diagnosis.

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**Table 1. Characteristics of persons tested for alpha-gal IgE by result — United States, 2010–2018**

|                                                     | <b>Total<br/>(n=105,674)</b> | <b>Positive<sup>†</sup><br/>(n=34,256)</b> | <b>Negative<sup>‡</sup><br/>(n=71,418)</b> | <b>RR (95% CI)</b> | <b>P value<sup>§</sup></b> |
|-----------------------------------------------------|------------------------------|--------------------------------------------|--------------------------------------------|--------------------|----------------------------|
| <b>Age (years), mean (SD)</b>                       | 42.2 (19.9)                  | 46.9 (19.8)                                | 39.9 (19.6)                                | 1.1 (1.1–1.1)      | <0.0001                    |
| <b>Age category (years), n (%)</b>                  |                              |                                            |                                            |                    |                            |
| 0–9                                                 | 5,357                        | 1,127 (21.0)                               | 4,230 (79.0)                               | reference          | <0.0001                    |
| 10–19                                               | 12,411                       | 3,372 (27.2)                               | 9,039 (72.8)                               | 1.3 (1.2–1.4)      |                            |
| 20–29                                               | 12,680                       | 2,932 (23.1)                               | 9,748 (76.9)                               | 1.1 (1.0–1.2)      |                            |
| 30–39                                               | 16,169                       | 4,037 (25.0)                               | 12,132 (75.0)                              | 1.2 (1.1–1.3)      |                            |
| 40–49                                               | 17,986                       | 5,527 (30.7)                               | 12,459 (69.3)                              | 1.5 (1.4–1.5)      |                            |
| 50–59                                               | 17,672                       | 6,839 (38.7)                               | 10,833 (61.3)                              | 1.8 (1.7–1.9)      |                            |
| 60–69                                               | 14,427                       | 6,322 (43.8)                               | 8,105 (56.2)                               | 2.1 (2.0–2.2)      |                            |
| 70+                                                 | 8,969                        | 4,098 (45.7)                               | 4,871 (54.3)                               | 2.2 (2.1–2.3)      |                            |
| <b>Sex, n (%)</b>                                   |                              |                                            |                                            |                    |                            |
| Male                                                | 38,983                       | 16,881 (43.3)                              | 22,102 (56.7)                              | 1.7 (1.6–1.7)      | <0.0001                    |
| Female                                              | 65,904                       | 17,118 (26.0)                              | 48,786 (74.0)                              | reference          |                            |
| <b>Year, n (%)</b>                                  |                              |                                            |                                            |                    |                            |
| 2010 <sup>¶</sup>                                   | 372                          | 209 (56.2)                                 | 163 (43.8)                                 | –                  | <0.0001                    |
| 2011                                                | 2,330                        | 1,110 (47.6)                               | 1,220 (52.4)                               | reference          |                            |
| 2012                                                | 4,412                        | 1,952 (44.2)                               | 2,460 (55.8)                               | 0.9 (0.9–1.0)      |                            |
| 2013                                                | 7,200                        | 2,846 (39.5)                               | 4,354 (60.5)                               | 0.8 (0.8–0.9)      |                            |
| 2014                                                | 11,571                       | 4,295 (37.1)                               | 7,276 (62.9)                               | 0.8 (0.7–0.8)      |                            |
| 2015                                                | 14,766                       | 4,722 (32.0)                               | 10,044 (68.0)                              | 0.7 (0.6–0.7)      |                            |
| 2016                                                | 17,795                       | 5,077 (28.5)                               | 12,718 (71.5)                              | 0.6 (0.6–0.6)      |                            |
| 2017                                                | 21,080                       | 6,247 (29.6)                               | 14,833 (70.4)                              | 0.6 (0.6–0.7)      |                            |
| 2018                                                | 26,148                       | 7,798 (29.8)                               | 18,350 (70.2)                              | 0.6 (0.6–0.7)      |                            |
| <b>Season<sup>††</sup>, n (%)</b>                   |                              |                                            |                                            |                    |                            |
| March–May                                           | 22,771                       | 6,477 (28.4)                               | 16,294 (71.6)                              | reference          | 0.0022                     |
| June–August                                         | 28,987                       | 10,120 (34.9)                              | 18,867 (65.1)                              | 1.2 (1.2–1.3)      |                            |
| September–November                                  | 30,850                       | 10,766 (34.9)                              | 20,074 (65.1)                              | 1.2 (1.2–1.3)      |                            |
| December–February                                   | 23,066                       | 6,883 (29.8)                               | 16,183 (70.2)                              | 1.0 (1.0–1.1)      |                            |
| <b>U.S. Census region, n (%)</b>                    |                              |                                            |                                            |                    |                            |
| West                                                | 831                          | 54 (6.5)                                   | 777 (93.5)                                 | reference          | <0.0001                    |
| Northeast                                           | 5,328                        | 1,658 (31.1)                               | 3,670 (68.9)                               | 4.8 (3.7–6.2)      |                            |
| Midwest                                             | 8,717                        | 2,613 (30.0)                               | 6,104 (70.0)                               | 4.6 (3.6–6.0)      |                            |
| South                                               | 30,955                       | 10,766 (34.8)                              | 20,189 (65.2)                              | 5.4 (4.1–6.9)      |                            |
| Unknown                                             | 59,840                       | 19,165 (32.0)                              | 40,678 (68.0)                              | 4.9 (3.8–6.4)      |                            |
| <b>No. specimens per person<sup>‡‡</sup>, n (%)</b> |                              |                                            |                                            |                    |                            |
| Mean (SD; range)                                    | 1.2 (0.6; 1–33)              | 1.4 (1.0; 1–33)                            | 1.0 (0.2; 1–5)                             | –                  | <0.0001                    |
| 1                                                   | 95,603                       | 26,177 (27.4)                              | 69,426 (72.6)                              | reference          | <0.0001                    |
| 2–3                                                 | 8,545                        | 6,571 (76.9)                               | 1,974 (23.1)                               | 2.8 (2.8–2.9)      |                            |
| 4–5                                                 | 1,204                        | 1,186 (98.5)                               | 18 (1.5)                                   | 3.6 (3.5–3.6)      |                            |
| 6+                                                  | 322                          | 322 (100)                                  | 0 (0)                                      | 3.7 (3.6–3.7)      |                            |

Abbreviations: RR = risk ratio; CI = confidence interval; SD = standard deviation; IgE = immunoglobulin E

Referent dates for analysis are the date of first positive test (for persons with at least one positive test result) and of first test (for persons with all negative test results).

<sup>†</sup>Persons with at least one alpha-gal IgE test result  $\geq 0.1$  kU/L were considered positive.

<sup>‡</sup>Persons with all alpha-gal IgE test results  $< 0.1$  kU/L were considered negative.

<sup>§</sup>*P* values were calculated to identify significant differences in trends (season, year, age category, no. specimens per person) or interactions (U.S. Census region, sex) between those testing positive and negative;  $p < 0.05$  indicates statistical significance.

<sup>¶</sup>Risk ratio and 95% CI not calculated for 2010 since data represents persons tested during July 2010–December 2010.

<sup>††</sup>Seasons are defined as Spring (March–May), Summer (June–August), Fall (September–November) and Winter (December–February).

<sup>‡‡</sup>Data are based on 122,068 specimens collected and tested for alpha-gal IgE from 105,674 persons during 2010–2018, of which 47,706 specimens (39.1%) were positive.

**Table 1. Characteristics of persons tested for alpha-gal IgE by result — United States, 2010–2018**

|                                                     | <b>Total<br/>(n=105,674)</b> | <b>Positive<sup>†</sup><br/>(n=34,256)</b> | <b>Negative<sup>‡</sup><br/>(n=71,418)</b> | <b>RR (95% CI)</b> | <b>P value<sup>§</sup></b> |
|-----------------------------------------------------|------------------------------|--------------------------------------------|--------------------------------------------|--------------------|----------------------------|
| <b>Age (years), mean (SD)</b>                       | 42.2 (19.9)                  | 46.9 (19.8)                                | 39.9 (19.6)                                | 1.1 (1.1–1.1)      | <0.0001                    |
| <b>Age category (years), n (%)</b>                  |                              |                                            |                                            |                    |                            |
| 0–9                                                 | 5,357                        | 1,127 (21.0)                               | 4,230 (79.0)                               | reference          | <0.0001                    |
| 10–19                                               | 12,411                       | 3,372 (27.2)                               | 9,039 (72.8)                               | 1.3 (1.2–1.4)      |                            |
| 20–29                                               | 12,680                       | 2,932 (23.1)                               | 9,748 (76.9)                               | 1.1 (1.0–1.2)      |                            |
| 30–39                                               | 16,169                       | 4,037 (25.0)                               | 12,132 (75.0)                              | 1.2 (1.1–1.3)      |                            |
| 40–49                                               | 17,986                       | 5,527 (30.7)                               | 12,459 (69.3)                              | 1.5 (1.4–1.5)      |                            |
| 50–59                                               | 17,672                       | 6,839 (38.7)                               | 10,833 (61.3)                              | 1.8 (1.7–1.9)      |                            |
| 60–69                                               | 14,427                       | 6,322 (43.8)                               | 8,105 (56.2)                               | 2.1 (2.0–2.2)      |                            |
| 70+                                                 | 8,969                        | 4,098 (45.7)                               | 4,871 (54.3)                               | 2.2 (2.1–2.3)      |                            |
| <b>Sex, n (%)</b>                                   |                              |                                            |                                            |                    |                            |
| Male                                                | 38,983                       | 16,881 (43.3)                              | 22,102 (56.7)                              | 1.7 (1.6–1.7)      | <0.0001                    |
| Female                                              | 65,904                       | 17,118 (26.0)                              | 48,786 (74.0)                              | reference          |                            |
| <b>Year, n (%)</b>                                  |                              |                                            |                                            |                    |                            |
| 2010 <sup>¶</sup>                                   | 372                          | 209 (56.2)                                 | 163 (43.8)                                 | –                  | <0.0001                    |
| 2011                                                | 2,330                        | 1,110 (47.6)                               | 1,220 (52.4)                               | reference          |                            |
| 2012                                                | 4,412                        | 1,952 (44.2)                               | 2,460 (55.8)                               | 0.9 (0.9–1.0)      |                            |
| 2013                                                | 7,200                        | 2,846 (39.5)                               | 4,354 (60.5)                               | 0.8 (0.8–0.9)      |                            |
| 2014                                                | 11,571                       | 4,295 (37.1)                               | 7,276 (62.9)                               | 0.8 (0.7–0.8)      |                            |
| 2015                                                | 14,766                       | 4,722 (32.0)                               | 10,044 (68.0)                              | 0.7 (0.6–0.7)      |                            |
| 2016                                                | 17,795                       | 5,077 (28.5)                               | 12,718 (71.5)                              | 0.6 (0.6–0.6)      |                            |
| 2017                                                | 21,080                       | 6,247 (29.6)                               | 14,833 (70.4)                              | 0.6 (0.6–0.7)      |                            |
| 2018                                                | 26,148                       | 7,798 (29.8)                               | 18,350 (70.2)                              | 0.6 (0.6–0.7)      |                            |
| <b>Season<sup>††</sup>, n (%)</b>                   |                              |                                            |                                            |                    |                            |
| March–May                                           | 22,771                       | 6,477 (28.4)                               | 16,294 (71.6)                              | reference          | 0.0022                     |
| June–August                                         | 28,987                       | 10,120 (34.9)                              | 18,867 (65.1)                              | 1.2 (1.2–1.3)      |                            |
| September–November                                  | 30,850                       | 10,766 (34.9)                              | 20,074 (65.1)                              | 1.2 (1.2–1.3)      |                            |
| December–February                                   | 23,066                       | 6,883 (29.8)                               | 16,183 (70.2)                              | 1.0 (1.0–1.1)      |                            |
| <b>U.S. Census region, n (%)</b>                    |                              |                                            |                                            |                    |                            |
| West                                                | 831                          | 54 (6.5)                                   | 777 (93.5)                                 | reference          | <0.0001                    |
| Northeast                                           | 5,328                        | 1,658 (31.1)                               | 3,670 (68.9)                               | 4.8 (3.7–6.2)      |                            |
| Midwest                                             | 8,717                        | 2,613 (30.0)                               | 6,104 (70.0)                               | 4.6 (3.6–6.0)      |                            |
| South                                               | 30,955                       | 10,766 (34.8)                              | 20,189 (65.2)                              | 5.4 (4.1–6.9)      |                            |
| Unknown                                             | 59,840                       | 19,165 (32.0)                              | 40,678 (68.0)                              | 4.9 (3.8–6.4)      |                            |
| <b>No. specimens per person<sup>‡‡</sup>, n (%)</b> |                              |                                            |                                            |                    |                            |
| Mean (SD; range)                                    | 1.2 (0.6; 1–33)              | 1.4 (1.0; 1–33)                            | 1.0 (0.2; 1–5)                             | –                  | <0.0001                    |
| 1                                                   | 95,603                       | 26,177 (27.4)                              | 69,426 (72.6)                              | reference          | <0.0001                    |
| 2–3                                                 | 8,545                        | 6,571 (76.9)                               | 1,974 (23.1)                               | 2.8 (2.8–2.9)      |                            |
| 4–5                                                 | 1,204                        | 1,186 (98.5)                               | 18 (1.5)                                   | 3.6 (3.5–3.6)      |                            |
| 6+                                                  | 322                          | 322 (100)                                  | 0 (0)                                      | 3.7 (3.6–3.7)      |                            |

Abbreviations: RR = risk ratio; CI = confidence interval; SD = standard deviation; IgE = immunoglobulin E

Referent dates for analysis are the date of first positive test (for persons with at least one positive test result) and of first test (for persons with all negative test results).

<sup>†</sup>Persons with at least one alpha-gal IgE test result  $\geq 0.1$  kU/L were considered positive.

<sup>‡</sup>Persons with all alpha-gal IgE test results  $< 0.1$  kU/L were considered negative.

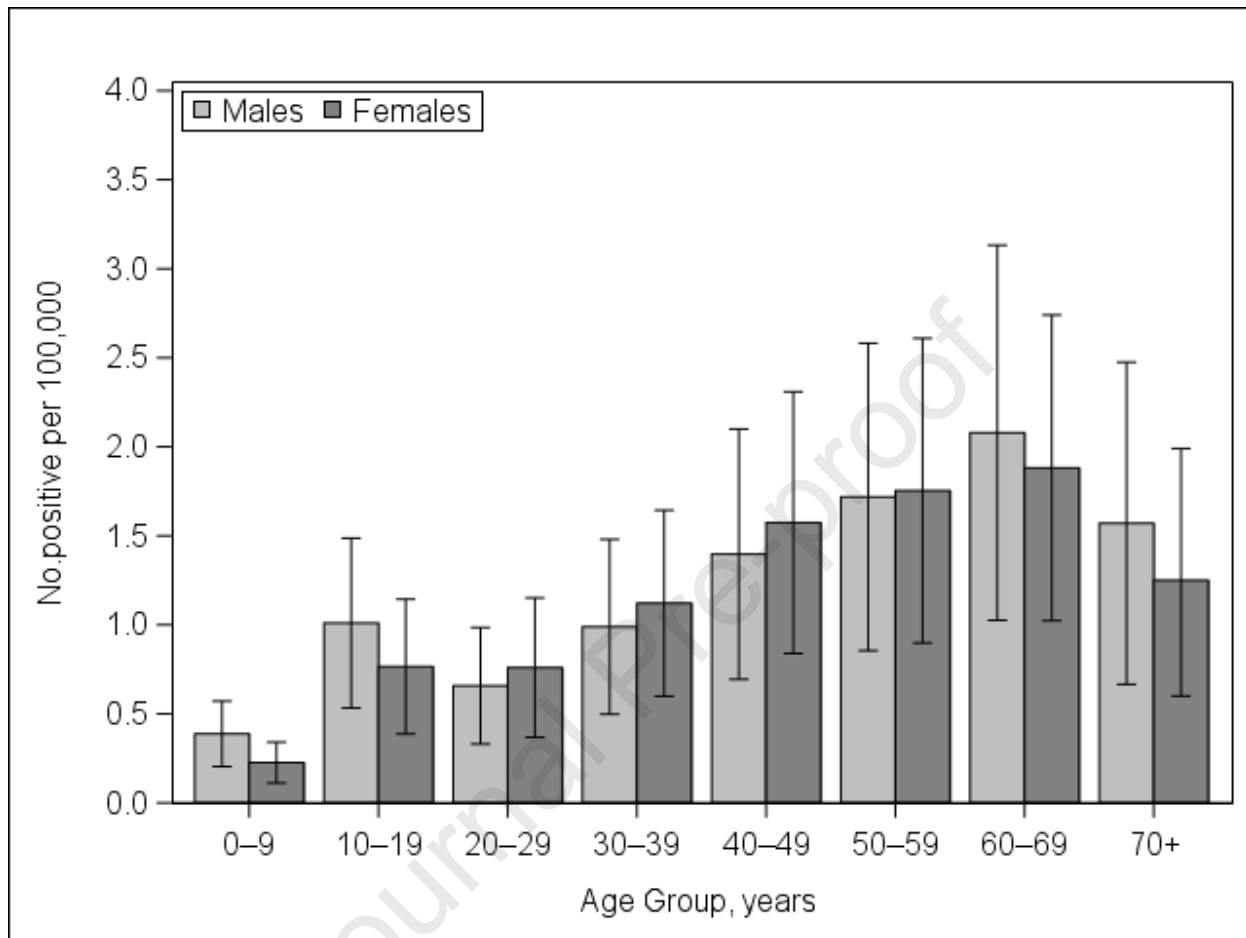
<sup>§</sup>P values were calculated to identify significant differences in trends (season, year, age category, no. specimens per person) or interactions (U.S. Census region, sex) between those testing positive and negative;  $p < 0.05$  indicates statistical significance.

<sup>¶</sup>Risk ratio and 95% CI not calculated for 2010 since data represents persons tested during July 2010–December 2010.

<sup>††</sup>Seasons are defined as Spring (March–May), Summer (June–August), Fall (September–November) and Winter (December–February).

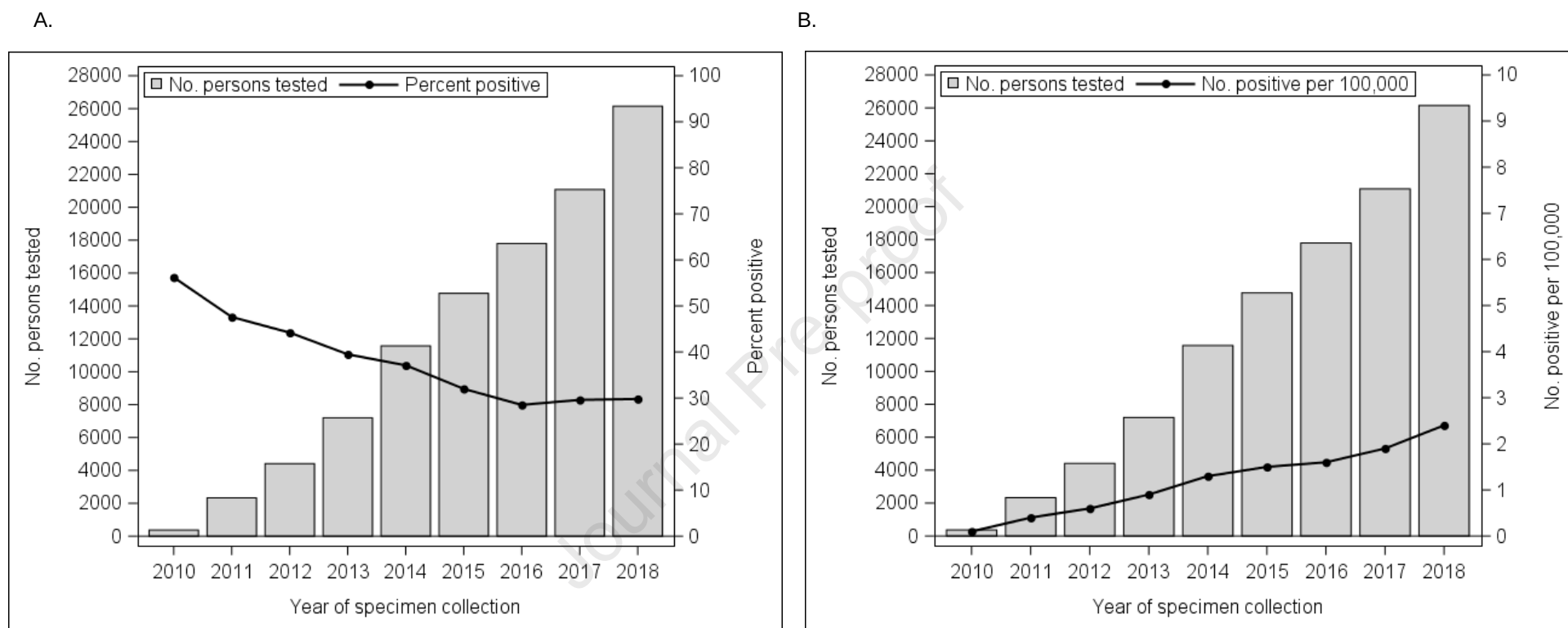
<sup>‡‡</sup>Data are based on 122,068 specimens collected and tested for alpha-gal IgE from 105,674 persons during 2010–2018, of which 47,706 specimens (39.1%) were positive.

**Figure 1. Number of persons positive per 100,000 population by age group and sex — United States, 2010–2018 (N = 33,999)**



No. positive per 100,000 was calculated using the average U.S. Census population by age and sex during the study period. Mean estimates for each age group among males and females are represented by the clustered bars with 95% confidence intervals included for each mean estimate.

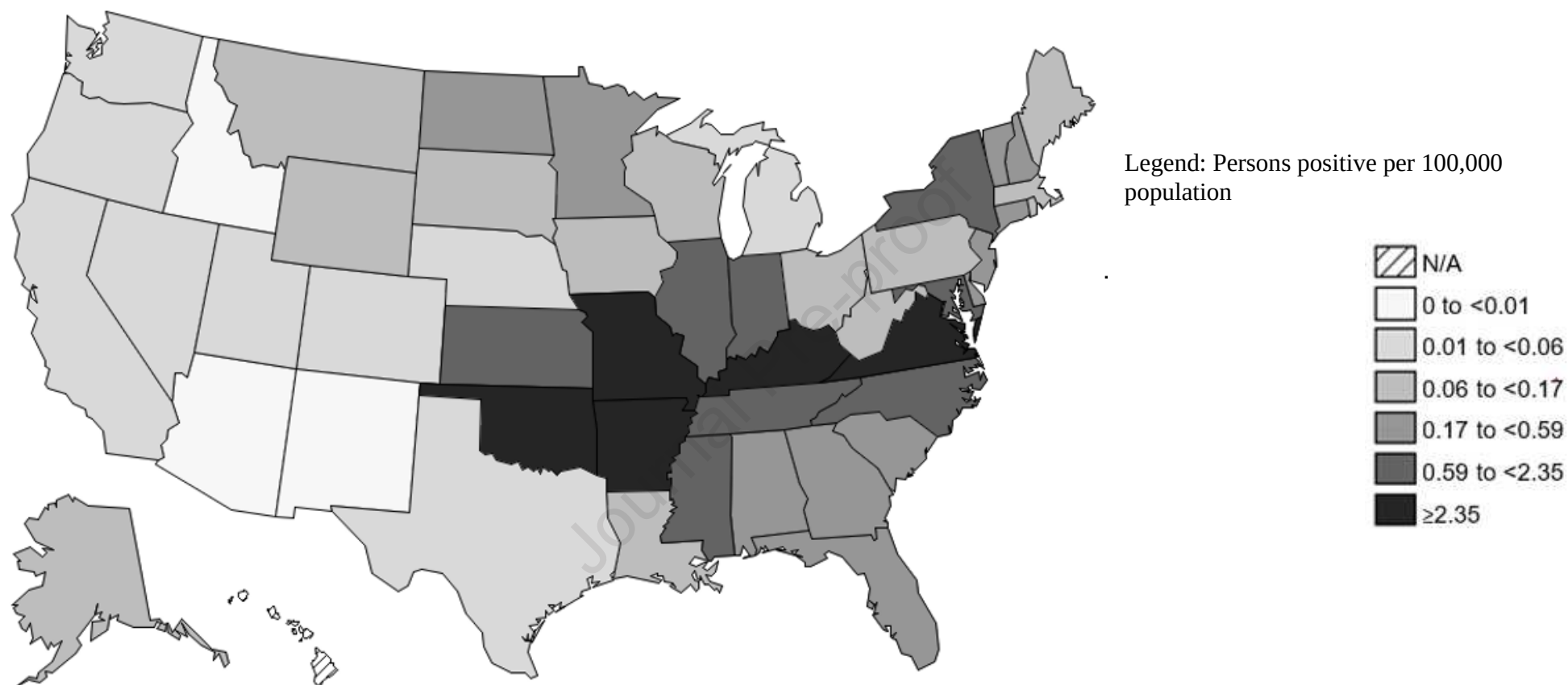
**Figure 2. Number of persons tested for alpha-gal IgE and A) percent positive<sup>a</sup> annually, B) number positive per 100,000<sup>b</sup> population annually — United States, 2010–2018**



<sup>a</sup>Line of best fit: slope= -2.6; 95% CI: -3.8, -1.3;  $p=0.003$ .

<sup>b</sup>Line of best fit: slope=0.3; 95% CI: 0.2, 0.3;  $p<0.001$ .

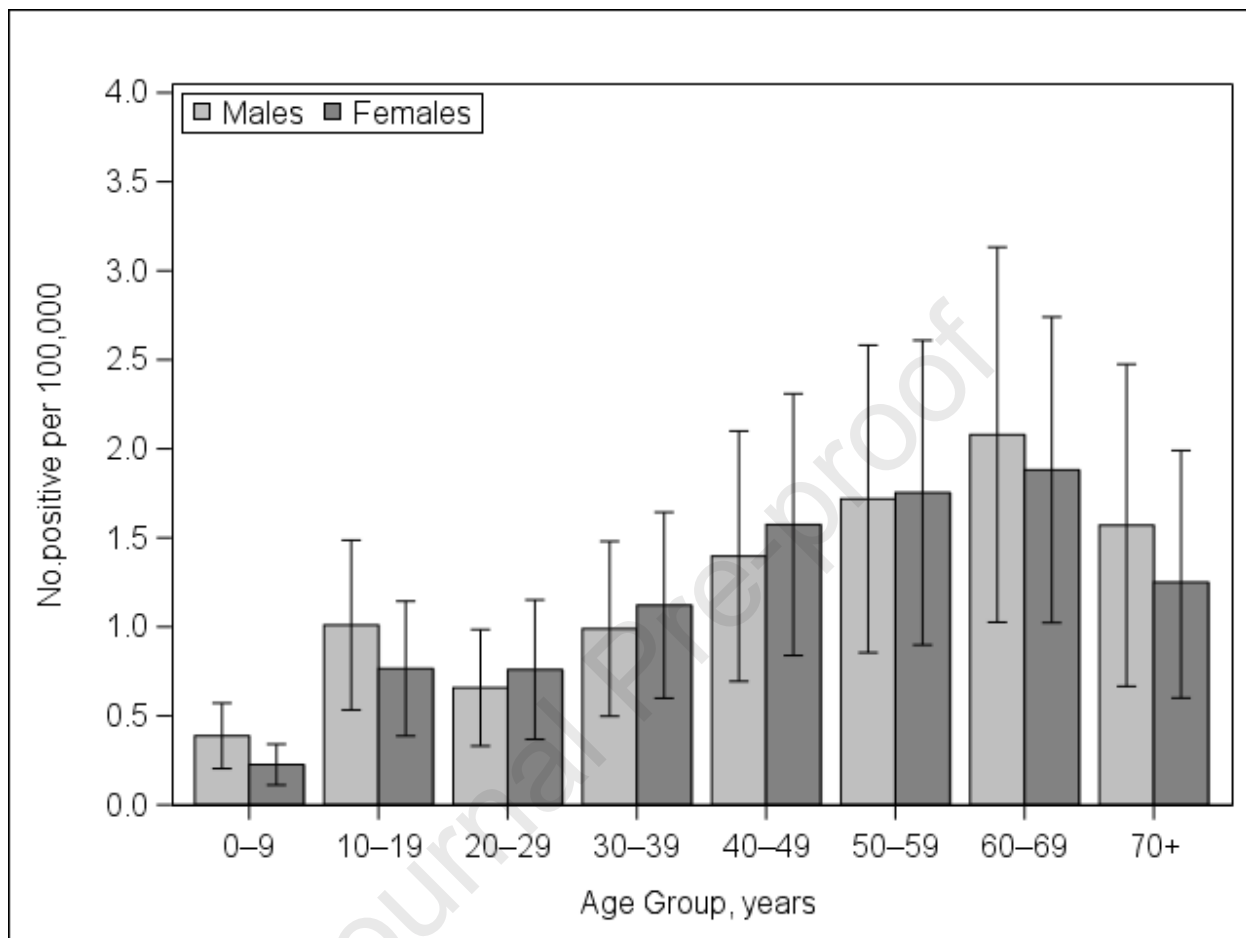
**Figure 3. Geographic distribution of alpha-gal IgE positive persons per 100,000 population by state — United States, 2010–2018 (N = 15,091)**



Data are based on the state of origin of the first specimen testing positive for 15,091 persons. No. of persons tested and no. positive per 100,000 was calculated using the average U.S. Census population by state during the study period. Legend categories created using percentile distribution of data.

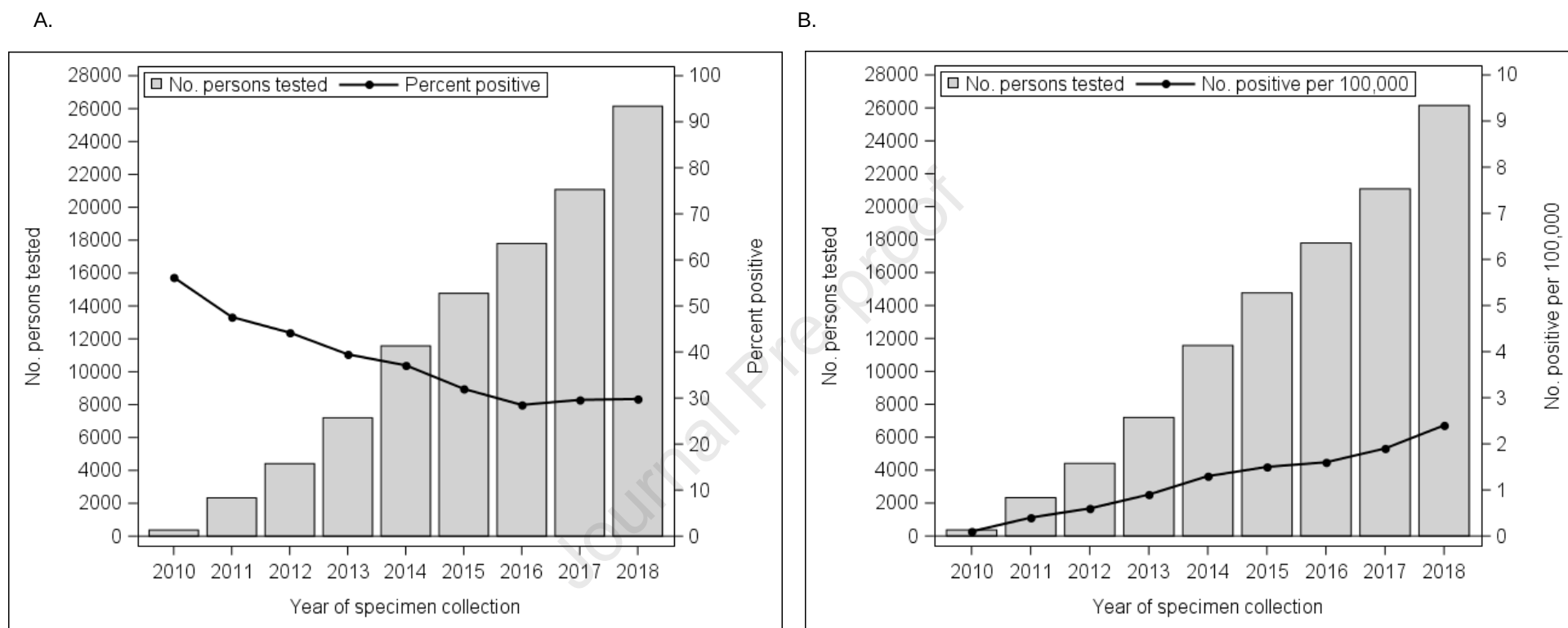
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**Figure 1. Number of persons positive per 100,000 population by age group and sex — United States, 2010–2018 (N = 33,999)**



No. positive per 100,000 was calculated using the average U.S. Census population by age and sex during the study period. Mean estimates for each age group among males and females are represented by the clustered bars with 95% confidence intervals included for each mean estimate.

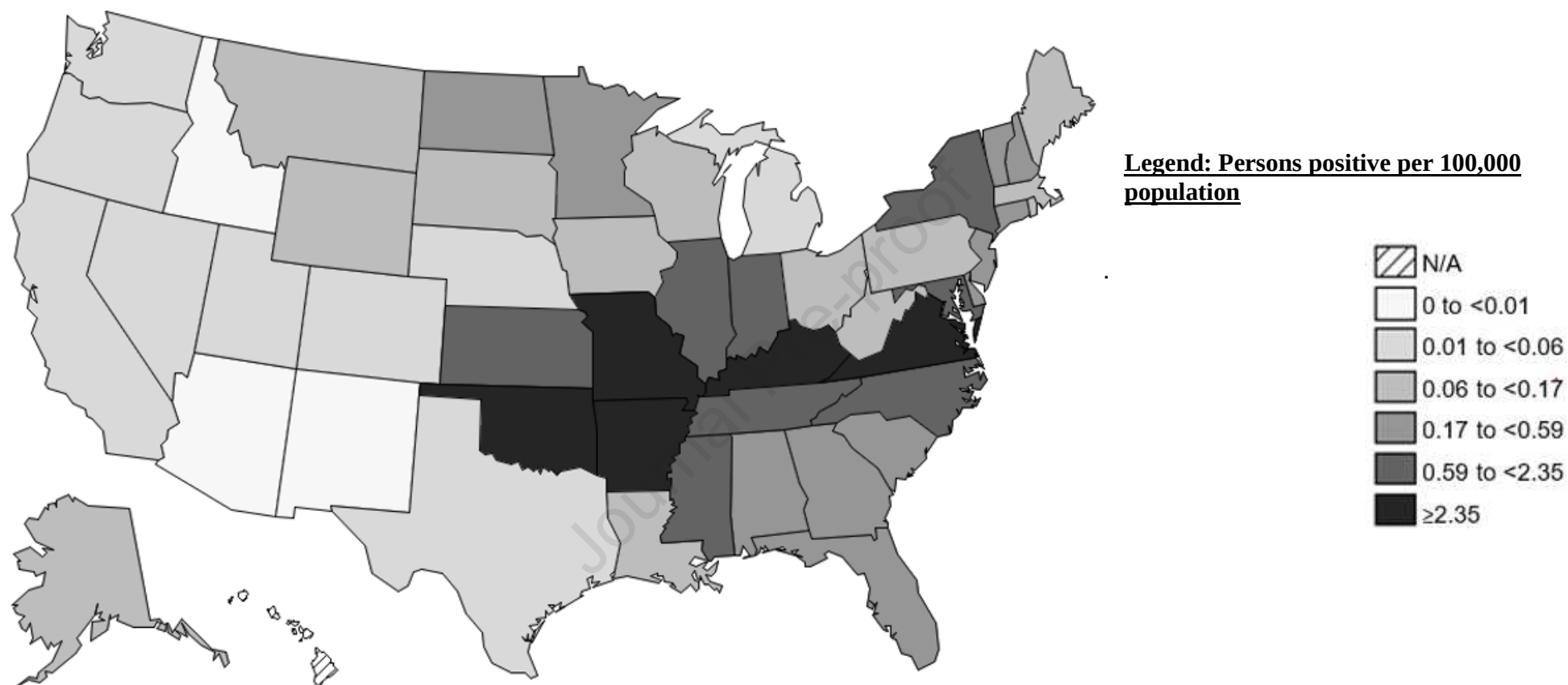
**Figure 2. Number of persons tested for alpha-gal IgE and A) percent positive<sup>a</sup> annually, B) number positive per 100,000<sup>b</sup> population annually — United States, 2010–2018**



<sup>a</sup>Line of best fit: slope= -2.6; 95% CI: -3.8, -1.3;  $p=0.003$ .

<sup>b</sup>Line of best fit: slope=0.3; 95% CI: 0.2, 0.3;  $p<0.001$ .

**Figure 3. Geographic distribution of alpha-gal IgE positive persons per 100,000 population by state — United States, 2010–2018 (N = 15,091)**



Data are based on the state of origin of the first specimen testing positive for 15,091 persons. No. of persons tested and no. positive per 100,000 was calculated using the average U.S. Census population by state during the study period. Legend categories created using percentile distribution of data.

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**Supplemental table 1: Number persons tested for alpha-gal IgE and number positive per 100,000 population by state of origin — United States, 2010–2018**

| State                | No. persons tested | No. persons positive | No. persons positive per 100,000 population |
|----------------------|--------------------|----------------------|---------------------------------------------|
| Alabama              | 857                | 222                  | 0.57                                        |
| Alaska               | 15                 | 2                    | 0.07                                        |
| Arizona              | 15                 | 1                    | 0.00                                        |
| Arkansas             | 2,215              | 1,128                | 4.70                                        |
| California           | 330                | 17                   | 0.01                                        |
| Colorado             | 153                | 15                   | 0.05                                        |
| Connecticut          | 244                | 41                   | 0.19                                        |
| Delaware             | 46                 | 21                   | 0.44                                        |
| District of Columbia | 104                | 10                   | 0.29                                        |
| Florida              | 1,444              | 209                  | 0.20                                        |
| Georgia              | 1,300              | 352                  | 0.42                                        |
| Hawaii               | 0                  | 0                    | N/A                                         |
| Idaho                | 9                  | 0                    | 0.00                                        |
| Illinois             | 1,326              | 456                  | 0.59                                        |
| Indiana              | 1,122              | 330                  | 0.83                                        |
| Iowa                 | 147                | 14                   | 0.09                                        |
| Kansas               | 1,155              | 232                  | 1.14                                        |
| Kentucky             | 2,375              | 1,093                | 3.07                                        |
| Louisiana            | 196                | 24                   | 0.10                                        |
| Maine                | 34                 | 7                    | 0.13                                        |
| Maryland             | 2,519              | 831                  | 1.73                                        |
| Massachusetts        | 1,004              | 73                   | 0.15                                        |
| Michigan             | 206                | 7                    | 0.01                                        |
| Minnesota            | 925                | 91                   | 0.27                                        |
| Mississippi          | 1,077              | 393                  | 2.19                                        |
| Missouri             | 3,289              | 1,380                | 2.51                                        |
| Montana              | 57                 | 4                    | 0.06                                        |
| Nebraska             | 24                 | 2                    | 0.02                                        |
| Nevada               | 23                 | 1                    | 0.01                                        |
| New Hampshire        | 175                | 21                   | 0.31                                        |
| New Jersey           | 666                | 155                  | 0.25                                        |
| New Mexico           | 12                 | 0                    | 0.00                                        |
| New York             | 2,659              | 1,305                | 0.73                                        |
| North Carolina       | 3,760              | 1,218                | 1.48                                        |
| North Dakota         | 95                 | 5                    | 0.22                                        |
| Ohio                 | 173                | 57                   | 0.08                                        |
| Oklahoma             | 1,942              | 628                  | 2.66                                        |
| Oregon               | 68                 | 2                    | 0.01                                        |
| Pennsylvania         | 368                | 43                   | 0.06                                        |
| Rhode Island         | 107                | 7                    | 0.13                                        |
| South Carolina       | 654                | 113                  | 0.28                                        |
| South Dakota         | 19                 | 4                    | 0.11                                        |
| Tennessee            | 3,436              | 1,153                | 1.91                                        |
| Texas                | 605                | 54                   | 0.03                                        |
| Utah                 | 62                 | 5                    | 0.03                                        |
| Vermont              | 71                 | 6                    | 0.24                                        |
| Virginia             | 8,261              | 3,303                | 4.35                                        |
| Washington           | 78                 | 6                    | 0.01                                        |
| West Virginia        | 164                | 14                   | 0.15                                        |

|           |     |    |      |
|-----------|-----|----|------|
| Wisconsin | 236 | 35 | 0.12 |
| Wyoming   | 9   | 1  | 0.06 |

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