The Journal of Infectious Diseases

MAJOR ARTICLE

Evaluation of Immunoglobulin A Enzyme Immunoassays to Detect Primary Respiratory Syncytial Virus Infection in Infants and Young Children

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Background. Respiratory syncytial virus (RSV) is a leading cause of acute lower respiratory infections in children <2 years of age. Prior infection in a child is usually determined by RSV antibodies; however, in young children, persisting maternal immunoglobulin G antibodies can incorrectly indicate past RSV infection. We developed and evaluated 4 immunoglobulin A (IgA) antibody enzyme immunoassays (EIAs) with the RSV F, subgroup G (Ga or Gb proteins) or RSV lysate antigens to distinguish infection induced from persisting maternal RSV antibodies.

Methods. We tested the EIAs against 62 cord blood specimens (group A), 39 plasma specimens from infants not exposed to an RSV season (group B), 102 plasma specimens from infants with a documented RSV infection (group C), and 124 plasma specimens from infants exposed to their first RSV season but without a documented RSV infection (group D).

Results. Among the 2 negative control groups, no group A specimens and 1 of the group B specimens were positive in all 4 IgA EIAs, giving a specificity of 100% and 97%, respectively. The sensitivity of the F, Ga, Gb, and Lysate IgA EIAs were 88%, 31%, 26%, and 61%, respectively, for group C specimens. Forty-four percent of the 124 specimens in group D were positive in the RSV-F IgA EIA.

Conclusions. The RSV-F protein IgA EIA exhibited a high level of sensitivity and specificity for detecting previous RSV infections in the presence of maternal antibodies and can help in RSV clinical trials and epidemiologic studies in young children.

Keywords. respiratory syncytial virus; IgA EIA; assay sensitivity and specificity; infants.

Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infections with as much as 33 million acute respiratory infections, 30% of pneumonia cases, and significant mortality worldwide in young children [1–3]. In the United States alone, RSV is associated with an estimated 100–500 deaths and, in a recent estimate, approximately 58 000 hospitalizations annually in children <5 years of age [4–6].

Three vaccines have recently been licensed for RSV prevention, including 2 for adults >60 years of age [7, 8] and 1 for pregnant individuals during 32–36 weeks of pregnancy, to protect young infants [7, 9, 10]. A long-acting monoclonal antibody to prevent RSV disease in infants <8 months, entering their first RSV season, and for infants and children at high risk for severe RSV disease entering their second RSV season has also been licensed [11]; the previously licensed antibody, palivizumab, was only recommended for use in young children at high risk from RSV infection. However, neither an RSV vaccine for young children nor a highly effective antiviral drug to treat RSV is available. Important to both epidemiological studies and clinical trials of RSV in young children is to know if a child has been previously infected with RSV and is already immunologically primed. In young children, past infection is usually determined by presence of RSV antibodies; however, persisting maternal or exogenously administered antibodies can lead to mislabeling an RSV-naive child as previously infected. Since little serum immunoglobulin A (IgA) is transferred from mother to infant [12, 13], its presence should indicate

Received 03 July 2024; editorial decision 11 October 2024; published online 22 November 2024

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The Journal of Infectious Diseases®

https://doi.org/10.1093/infdis/jiae514

infection-induced antibodies. Thus, pathogen-specific IgA antibodies may help to correctly identify an infected infant despite persisting maternally derived or exogenous immunoglobulin G (IgG) antibodies.

In this study, we developed and evaluated 4 IgA antibody enzyme immunoassays (EIAs) for their ability to detect previous RSV infection in young children. We evaluated these EIAs in 4 groups of plasma specimens—that is, cord blood, blood from young children never exposed to an RSV season (presumably RSV naive), blood from children with an earlier RSV positive illness, and blood from children exposed to a single RSV season but not diagnosed with RSV infection. Our results suggest that the RSV IgA EIA can correctly identify previous RSV infections in infants despite the presence of maternal antibody.

MATERIALS AND METHODS

Collection of Plasma Specimen

Plasma specimens and clinical and demographic data were collected between July 2015 and September 2018 under an Emory institutional review board–approved protocol after informed consent was obtained as previously reported [14]. To evaluate the assays, 4 groups of specimens were tested. Group A consisted of plasma from cord blood collected at the time of birth, if birth occurred >4 months after the last RSV season but before the onset of the next RSV season at Emory University Midtown Hospital, Atlanta, Georgia. Group B consisted of plasma from healthy infants seen at Egleston Emergency Department, Hughes Spalding Hospital Primary Care, or admitted to Egleston Hospital in Atlanta, Georgia, who were born after the RSV season and blood collected before the next RSV season. These children were not exposed to an RSV season and were assumed to be RSV naive. Group C consisted of plasma from infants with an RSV polymerase chain reaction (PCR)–positive respiratory illness when seen at Egleston Emergency Department or admitted to Egleston Hospital or Scottish Rite Hospital in Atlanta, Georgia, during their first RSV season. Blood specimens for this group were collected ≥6 months after their RSV respiratory illness, but before the next RSV season. Group D consisted of plasma from healthy children who had experienced their first RSV season without a documented RSV-positive illness and had blood collected when seen in the Egleston Emergency Department or admitted to Egleston Hospital for other reasons >4 months after the end of their first RSV season and before the start of the next RSV season.

Production and Purification of Antigen

Lysate Antigen

Lysate antigen was prepared by combining lysate from cells infected with RSV A2 (VR-1540, American Type Culture Collection [ATCC]) and B1 (VR-1400, ATCC) strains, representing subgroups A and B, respectively. The viruses were grown in HEp-2 cells (ATCC) using Dulbecco's modified Eagle medium (Thermo Fisher Scientific) supplemented with 5% fetal bovine serum at a multiplicity of infection of 0.1 50% tissue culture infectious dose ($TCID_{50}$) per cell as previously described [14]. In brief, cells were harvested at 3–4+ cytopathic effect and centrifuged at 3000*g* for 20 minutes, and the pellet was lysed through sonication. Next, the supernatant and lysed cell pellet were combined, and final preparation was clarified by centrifugation. Halt protease inhibitor (Thermo Fisher Scientific) was added to the preparations, and aliquots were prepared and stored at −80°C. HEp-2 cells, not infected with the virus, were subjected to the same treatment to serve as control for the EIA.

F, Ga, and Gb Antigens

The F, Ga, and Gb antigens were produced by expressing the human codon optimized secreted portion of wild-type RSV-F (from the A2 strain) or Ga/Gb (G protein from the A2 or B1 strains, respectively) genes tagged with 6× histidine at the carboxyl terminus as previously described [14]. The proteins were purified through affinity chromatography using a nickel-sepharose column per manufacturer's instructions. In brief, following equilibration, the beads were added to the filtered media containing the secreted protein, and the tube was rotated overnight at 4°C. The protein-bound beads were added back gently to the column, allowed to settle, and the column was washed with 5× column volumes of wash buffer (20 mM sodium phosphate, 0.5 M NaCl, 10 mM imidazole, pH 7.4). The protein was eluted in 1 mL fractions using elution buffer (20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4). Purified protein was dialyzed overnight at 4°C using phosphate-buffered saline (PBS), pH 7.4, and presence of the F and Ga/Gb proteins was confirmed through Western blotting using motavizumab (MedImmune LLC) and human mAb 3D3 (provided by Trellis Bioscience LLC), respectively.

IgA EIAs

IgA EIAs were performed using the RSV expressed F protein (F), Ga protein (Ga), Gb protein (Gb), and lysate preparations (Lysate) analogous to the previously reported IgG EIAs [14]. All steps in the EIA were performed at room temperature. The wash buffer used was PBS + 0.05% Tween-20. RSV F, Ga or Gb, and glyceraldehyde 3-phosphate dehydrogenase (control) antigens were diluted in PBS to a final concentration of 1 μg/mL. RSV A and B lysates were combined, each at a dilution of 1:50, while HEp-2 lysate (control for Lysate EIA) was diluted at a ratio of 1:25 in PBS. One hundred microliters of the appropriate antigen/control solution was coated onto a 96-well microtiter plate and incubated at 4°C overnight. The plate was then washed twice using PBS and blocked for 2 hours using 200 μL blocking buffer (0.33% gelatin, 0.33% casein, and 0.33% dry milk dissolved in PBS). Following incubation, the

plate was washed twice with PBS, and 100 μL of plasma (diluted at a ratio of 1:100 in blocking buffer + 0.15% Tween-20 [BB-T]) was added to the plates. Cord blood specimens (group A) were diluted at a ratio of 1:33.33 in BB-T as they were previously diluted at 1:3 in PBS. The plate was incubated at room temperature for 1.5 hours and washed 5 times in wash buffer, and 100 μL of goat anti-human IgA (Jackson Immunoresearch), diluted 1:2000 in BB-T, was added to all wells and the plate was incubated for 1 hour. The plate was then washed 5 times with wash buffer and the reaction was developed with o-phenylenediamine dihydrochloride as substrate for 0.5 hour. Finally, the reaction was stopped using 4N sulfuric acid and the absorbance was read at 490 nm.

Statistical Analysis

Descriptive statistics were summarized using medians and interquartile ranges (IQRs) for continuous variables and frequencies and percentages for categorical variables. The mean absorbance of the RSV antigen-coated wells (P) minus mean absorbance of the control antigen-coated wells (N) was used for analysis of IgA antibody results. Group B specimens were utilized to calculate the cut-off value (mean [P-N] plus 3 standard deviations [SDs]) for the EIAs. To compare P-N value differences among group C specimens between age groups, time postinfection groups, and sex, Kruskal-Wallis tests were utilized for >2 group comparison, followed by Bonferroni correction for pairwise differences; Mann-Whitney *U* tests were used for 2 group comparison when appropriate. Multiple linear regressions were performed to assess the combined effect of age group and time postinfection group on P-N values. To assess the correlation between RSV-F IgA and RSV-F IgG EIAs, Spearman rank correlation coefficient was calculated. A *P* value of <.05 was considered significant. All statistical analyses were performed using IBM SPSS (version 28.0.0) and R and RStudio software (version 4.3.1).

RESULTS

We tested 62 group A specimens (cord blood), 39 group B specimens (specimens from infants not exposed to an RSV season including 18 males and 21 females), 102 group C specimens (specimens from RSV-positive infants including 63 males and 39 females), and 124 group D specimens (specimens from infants exposed to their first RSV season, but without a documented RSV infection, including 73 males and 51 females) for the presence of IgA antibodies against RSV.

Of the 39 specimens in group B, we used all but 1 to determine the cut-off values. This specimen had a much higher P-N value than the other group B specimens, that is, 0.230, 0.086, 0.516, and 0.181 for F, Ga, Gb, and Lysate EIAs, respectively, as illustrated in Figure 1. We hypothesize that this

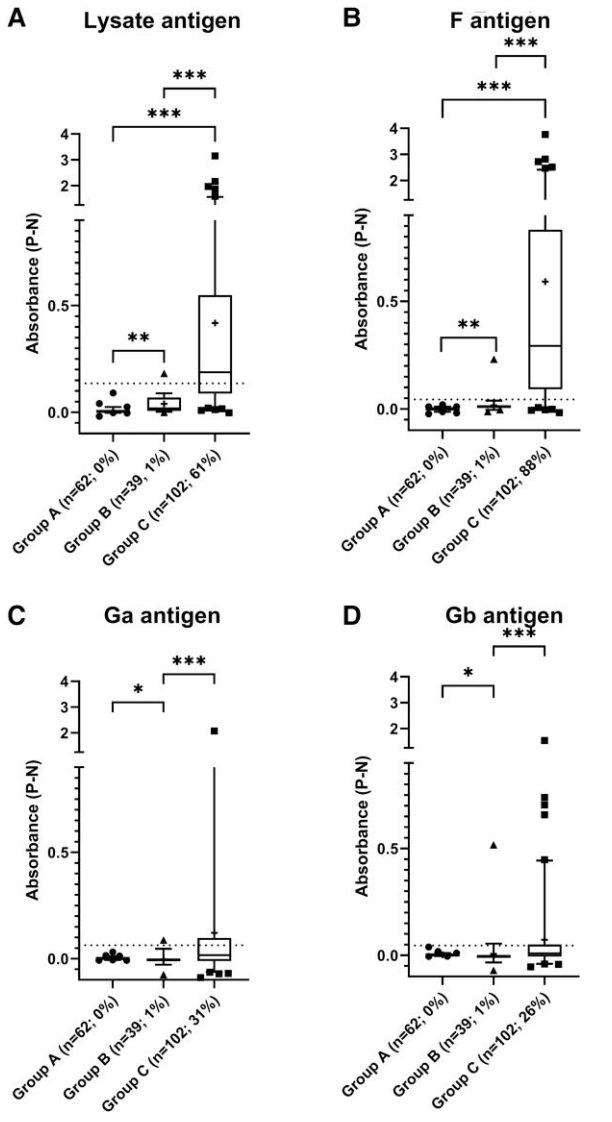

Figure 1. *A–D*, Immunoglobulin A (IgA) antibody signal obtained in the cord blood (group A), respiratory syncytial virus (RSV)–naive (group B), and convalescent RSV-positive (group C) groups when tested against different RSV antigens. Data are represented as a box-and-whisker plot with a 95% confidence interval. After blocking, specimens from each group were added to the wells coated with Lysate antigen (*A*), F antigen (*B*), Ga antigen (*C*), or Gb antigen (*D*) followed by goat anti-human IgA secondary antibody. O-phenylenediamine dihydrochloride was added to all the wells and absorbance was measured at 490 nm. Signal was calculated as mean absorbance of antigen-coated wells minus mean absorbance of control wells (P-N). Lysate antigen was diluted at 1:50, while F, Ga, and Gb were coated at a concentration of 1 μg/mL. The dotted lines indicate the cut-off values calculated for each assay: 0.044, 0.063, 0.045, and 0.135 for the F, Ga, Gb, and Lysate enzyme immunoassays, respectively. The specimens include 62 group A specimens, 39 group B specimens, and 102 group C specimens. The numbers in parentheses following the group label indicate the number of specimens in each group and the percentage of specimens above the cut-off value for each group in the respective assays. Kruskal-Wallis test, along with pairwise comparison and Bonferroni correction, was used to determine the difference between groups, with $P < .05$ being considered significant. *$P < .05$, **$P < .01$, ***$P < .001$.

specimen was from an infant with an undetected off-season infection. The cut-off values, mean (P-N) plus 3 SDs, were calculated to be 0.044, 0.063, 0.045, and 0.135 for the F, Ga, Gb, and Lysate IgA EIAs, respectively (Supplementary Table 1); a value above the cut-off was considered positive for RSV IgA antibody. With this cut-off value, all group A specimens were negative by the 4 EIAs, giving a specificity of 100% for this group (Figure 1 and Table 1). In contrast, 1 of the group B specimens, as noted above, was positive, giving a specificity of 97.43% for all the EIAs. The median P-N for the F EIA, Ga EIA, Gb EIA, and Lysate EIA was 0.000 (IQR, −0.002 to 0.002), 0.001 (IQR, −0.001–0.003), 0.001 (IQR, −0.001 to 0.003), and 0.003 (IQR, 0.001–0.006) for group A specimens, and 0.009 (IQR, 0.005–0.017), −0.004 (IQR, −0.012 to −0.001), −0.006 (IQR, −0.013 to −0.001), and 0.018 (IQR, 0.006–0.069) for group B specimens, respectively (Table 1). Interestingly, the P-N values for group A were significantly lower than for group B specimens for all 4 EIAs (Figure 1). Of the 102 group C specimens, 90 were found to be positive with the F IgA EIA, 32 with the Ga IgA EIA, 27 with the Gb IgA EIA, and 62 with the Lysate IgA, giving a sensitivity of 88%, 31%, 26%, and 61%, respectively, for these IgA EIAs (Table 1). The combined sensitivity of the Ga and Gb IgA EIAs was 50%. All specimens that were positive for the Lysate, Ga, and Gb EIAs were positive for the F EIA, giving no increase in sensitivity for combination of all 4 assays. The median P-N value for the RSV IgA-positive specimens in group C was 0.384 (IQR, 0.152–0.956) for the F EIA, 0.224 (IQR, 0.150–0.747) for the Ga EIA, 0.098 (IQR, 0.065–0.418) for the Gb EIA, and 0.385 (IQR, 0.227–0.838) for the Lysate EIA. As the sensitivity of the F and Lysate EIAs was significantly higher than that of the G EIAs, we limited additional analysis of group C specimens to the F and Lysate EIAs.

Table 1. Immunoglobulin A Assay Comparison Across Different Groups

Group	EIA	P-N Values, Median (IQR)	No. Positive
A (n = 62)	Lysate IgA	0.003 (0.001–0.006)	0 (0%)
	F IgA	0.000 (−0.002 to 0.002)	0 (0%)
	Ga IgA	0.001 (−0.001 to 0.003)	0 (0%)
	Gb IgA	0.001 (−0.001 to 0.003)	0 (0%)
B (n = 39)	Lysate IgA	0.018 (0.006–0.069)	1 (3%)
	F IgA	0.009 (0.005–0.017)	1 (3%)
	Ga IgA	−0.004 (−0.012 to −0.001)	1 (3%)
	Gb IgA	−0.006 (−0.013 to −0.001)	1 (3%)
C (n = 102)	Lysate IgA	0.385 (0.227–0.838)[a]	62 (61%)
	F IgA	0.384 (0.152–0.956)[a]	90 (88%)
	Ga IgA	0.224 (0.150–0.747)[a]	32 (31%)
	Gb IgA	0.098 (0.065–0.418)[a]	27 (26%)
D (n = 124)	F IgA	0.461 (0.159–1.498)[a]	54 (44%)
	F IgG	9839 (5685.2–13 187.6)[b] [14]	64 (52%)

Abbreviations: EIA, enzyme immunoassay; F, F protein antigen; Ga, subgroup A G protein antigen; Gb, subgroup B G protein antigen; IgA, immunoglobulin A; IgG, immunoglobulin G; IQR, interquartile range; P-N, absorbance for RSV antigen - absorbance for control antigen.

[a]Median and IQR were calculated only for the positive specimen in groups C and D.

[b]F IgG EIA level was estimated as titers (95% confidence interval).

We evaluated the effect of age at time of infection, days between infection and blood draw, and sex, on IgA EIA P-N values (as proxy for amount of antibody). To assess the effect of age at infection, we compared 5 age groups of 60-day intervals from 0 days to >240 days old. Figures 2*A* and 2*B* illustrate the increase in median P-N values observed with increasing age of the child at time of infection for both the F and Lysate EIAs. Children 0–60 days of age, at the time of infection, had the lowest level of IgA antibodies (median P-N, 0.111 [IQR, 0.053–0.270] for F and 0.103 [IQR, 0.039–0.174] for Lysate) while children >180 days of age at time of infection had the highest levels of IgA in their plasma (median P-N, 0.984 [IQR, 0.513–1.993] for F and 0.722 [IQR, 0.282–1.168] for Lysate). The difference between the IgA levels between the 0–60 days age group and >180 days age group was statistically significant for both EIAs (Figures 2*A* and 2*B*). The difference between these age groups remained significant after adjusting for the effect of days between infection and blood draw in the multivariable models (all $P < .001$; Supplementary Tables 2 and 3).

Next, we compared 180–210 days, 211–240 days, and >240 days between the RSV-positive illness and blood draw. As shown in Figures 3*A* and 3*B*, there was a trend for decreasing P-N values with longer time between the illness and blood collection, but this difference did not achieve statistical significance. The trend remained when adjusting for age at infection (Supplementary Tables 2 and 3). There were no significant differences observed in IgA levels between males and females in group C (data not shown).

Finally, we tested group D specimens for IgA antibodies against the F antigen (the most sensitive IgA EIA), to determine its potential to aid in identifying past undiagnosed infections. Of the 124 specimens in this group, 54 (44%) specimens tested positive for RSV IgA antibodies (Table 1). One specimen that was RSV-F and Lysate IgG negative was RSV-F IgA positive, suggesting an early infection missed with both IgG EIAs. We then focused on the 64 group D specimens that were positive by the F IgG EIA, suggestive of having had an RSV infection, as previously reported [14]. Of these 64 specimens, 10 had IgG titers low enough (considering the age at blood draw) to be residual maternal antibodies. Two specimens for which age and IgG titer were consistent with maternal antibody were IgA EIA positive. Fifty-one of the 54 (94%) children presumably RSV-infected, based on F IgG positivity, were RSV-F IgA EIA positive. The median P-N for the IgA-positive group D specimens was 0.461 (IQR, 0.159–1.498).

DISCUSSION

In this study, we described 4 IgA antibody EIAs with good specificity and varying sensitivity for detecting prior RSV infection in young children. Our results indicate that one of the IgA

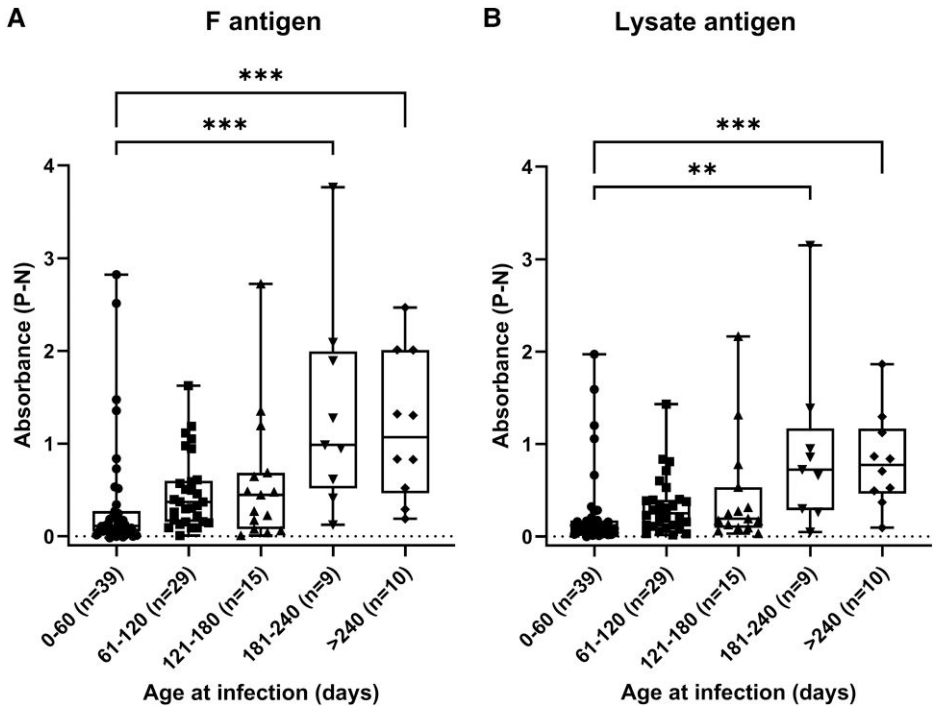

Figure 2. Effect of age at infection on signal obtained in group C specimens. Results obtained from the F antigen (*A*) and Lysate antigen (*B*) immunoglobulin A (IgA) enzyme immunoassays were used to analyze the effect of age at infection on IgA levels in plasma for group C specimens. In this group, there were 39 specimens from infants aged 0–60 d, 29 specimens from infants aged 61–120 d, 15 specimens from infants aged 121–180 d, 9 specimens from infants aged 181–240, and 10 specimens from infants aged >240 d at the time of infection. Graph is represented as a box-and-whisker plot, with each point representing the signal generated from a specimen and whiskers indicating the maximum and minimum (mean absorbance of antigen-coated wells minus mean absorbance of control wells [P-N]) values observed for each group. Kruskal-Wallis test, along with pairwise comparison and Bonferroni correction, was used to determine the difference between groups, with $P < .05$ being considered significant. $^{**}P < .01$, $^{***}P < .001$.

EIAs, F, has good sensitivity and specificity for identifying infants previously infected with RSV despite the presence of maternal IgG antibodies. The specificity exhibited by these IgA EIAs is consistent with the low transfer rate of IgA antibodies from mother to infant (less than 1/10 to 1/1000 of that found in mother's serum), presumably by passive diffusion [12, 13, 15, 16]; other studies have demonstrated lack of RSV IgA antibodies in cord blood [17, 18]. In our study, none of the group A (cord blood) specimens and one of the group B (children not exposed to an RSV season) specimens were positive for IgA antibodies, giving a specificity of 100% and 97%, respectively, for these 2 negative control groups. The one group B specimen positive for RSV IgA antibodies may be from an infant with an out-of-season infection. Interestingly, the mean P-N values for these 2 negative control specimen groups, A and B, were significantly different. We suspect that this difference, group B being significantly higher than group A for IgA EIAs, arises from the increase in total IgA associated with the infant's postdelivery production of IgA antibody resulting from exposure to microbes and other antigens [19, 20]. For this reason, we chose group B specimens as most comparable to group C and D specimens and best for determining the cut-off value for this study. The IgA EIA results for the 102 specimens from RSV PCR-positive children, group C, demonstrated good sensitivity of 88% for the F IgA EIA, 61% for the Lysate IgA EIA, and 50% for one or the other G IgA EIAs. Among the group C specimens, the RSV IgA antibody levels were higher in children who were older at the time of their infection. This finding is consistent with other studies that show younger children have a dampened RSV antibody response to infection [21, 22]. In group C, one RSV-F IgA-positive specimen was negative for the RSV-F IgG antibodies but positive with a titer of 484 in the Lysate IgG EIA as previously reported [14]. This specimen was from an infant who was infected at 36 days of age with blood collected 300 days postinfection. We speculate that, in this child, the F IgG antibodies waned faster than the F IgA antibodies, leaving them undetectable while the F IgA antibodies remained detectable. Among the 124 specimens in group D, children exposed to an RSV season but without a diagnosed infection, 44% were positive for anti-RSV-F IgA antibodies. We used RSV-F IgG antibody titer and age at blood draw as previously reported [14], along with the RSV-F IgA signal, to identify specimens for which the RSV-F IgG results would be indicative of infection and not residual maternal antibody. Among the 64 RSV-F IgG antibody-positive specimens, 10 were collected at an age and a low enough titer (<1200) that

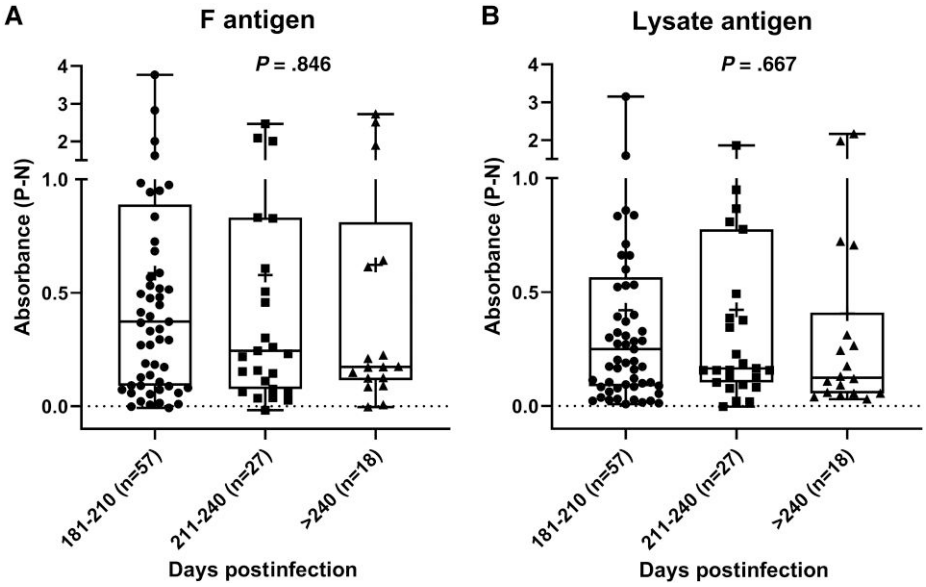

Figure 3. Effect of time postinfection on immunoglobulin A (IgA) levels in group C specimens. Results obtained from the F antigen (*A*) and Lysate IgA enzyme immunoassay (*B*) were used to analyze the effect of time between RSV infection and blood draw on IgA levels in plasma for group C specimens. In this group, there were 57 specimens collected between 181 and 210 d, 27 specimens collected between 211 and 240 d, and 18 specimens collected >240 d after initial infection. Graph is represented as a box-and-whisker plot, with each point representing the signal generated from a specimen and the whiskers indicating the maximum and minimum (mean absorbance of antigen-coated wells minus mean absorbance of control wells [P-N]) values observed for specimen in each group. Kruskal-Wallis test, along with pairwise comparison and Bonferroni correction, was used to determine the difference between groups, with $P < .05$ being considered significant.

might represent residual maternal antibody. Two of these 10 specimens were F IgA positive, suggesting that these specimens were from RSV-infected children, and the IgG positivity from the other 8 specimens was likely due to maternal antibodies. The sensitivity of the F IgA EIA between group C (88%) and IgG-positive group D (53/64 [83%]) specimens was not significantly different ($P = .338$). When we analyzed the correlation between the IgG titer and the IgA signal for each specimen, there was a strong correlation observed between the levels of anti-F IgG and IgA in the cohort tested ($\rho = 0.84$ [95% confidence interval, .78–.88]; Figure 4) and the IgA-negative specimens had a median IgG titer of 696 compared to 12 015 for the IgA-positive specimens. It is important to note that this study used the wild-type secreted form of F protein (and not the pre-fusion form) for the IgA EIA. It would be interesting to compare the sensitivities for both forms of F antigen and this is an avenue we are currently exploring. Additionally, all IgA EIAs in this study were done using plasma specimens; when testing the RSV-F IgA EIA using serum and plasma specimens simultaneously collected from 5 adults, the P-N values were similar for both types of specimens (data not shown).

The lower sensitivity of the G protein IgA EIAs is consistent with other reports noting a higher titer of both IgG and IgA F compared to G antibodies [17, 23, 24]. The lower rate of positivity for G compared to F IgG antibodies in the group C specimens was previously reported [14]. The lower sensitivity of the Lysate IgA EIA compared to the F IgA EIA is surprising since, as previously reported, the Lysate IgG EIA had equal sensitivity to the F IgG EIA [14]. One possibility for this difference is that Lysate antigen mix includes a greater percentage of proteins that induce IgG than those that induce IgA antibodies. Another possibility is that the higher background levels with the Lysate IgA EIA obscures the lower level of IgA antibodies, but not the higher level of IgG antibodies. Nonetheless, both the F and Lysate IgA EIAs had good sensitivity relative to previously reported IgA EIAs [25, 26].

Analysis of demographic data indicated that the age at infection affected the magnitude of the IgA response as indicated by the absorbance reading. The lower absorbance reading in the youngest children may result from residual maternal antibody inhibiting the response or the immaturity of the young child's immune system limiting capacity to respond [19]. The presence of maternal antibodies at the time of infection suppressing the antibody response to RSV has been described previously [27, 28]. We also noted some decrease in IgA levels with increasing time between infection and blood draw. Interestingly, this decrease was relatively small and not significant. This relatively slow rate of decrease in F IgA levels with persistence to 300 days after infection indicates that IgA antibodies are more likely to be helpful in diagnosing primary infections in young children. By a child's second RSV season, F IgA antibodies may be indicative of a previous infection that could have occurred during the child's first or second RSV season. A study with serial serum

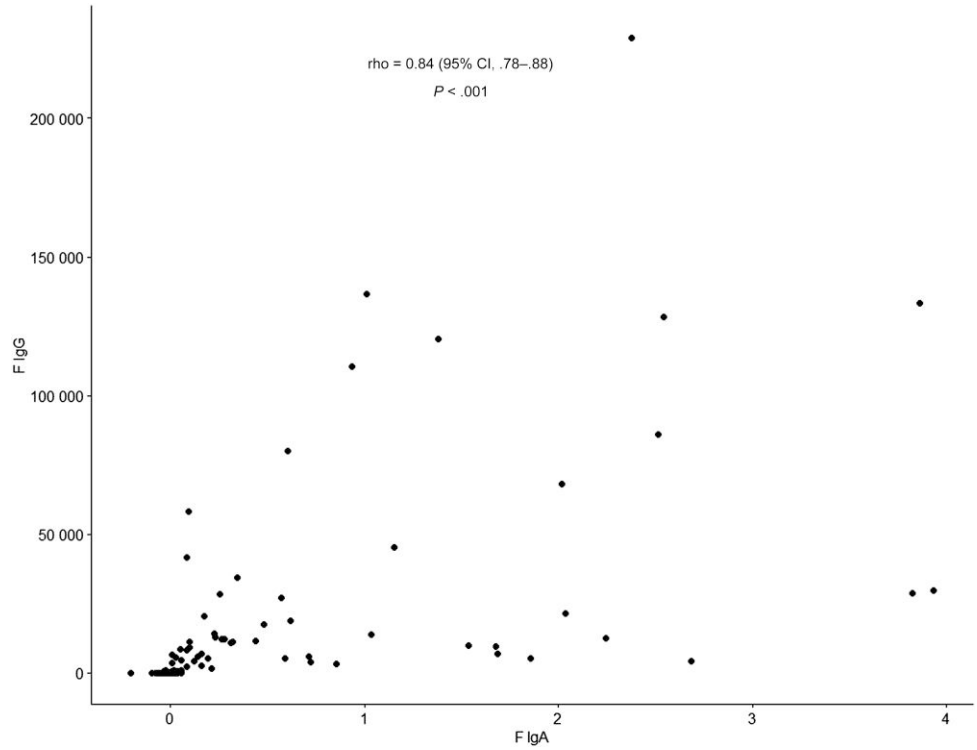

Figure 4. Spearman correlation analysis between immunoglobulin A levels and immunoglobulin G (IgG) titers for group D specimens. The IgG antibody titer was estimated from absorbance values from an enzyme immunoassay with respiratory syncytial virus F antigen using standard serum [14]. Abbreviations: CI, confidence interval; IgA, immunoglobulin A; IgG, immunoglobulin G.

or plasma specimens that include acute or semi-acute specimens is needed to accurately determine the kinetics of the RSV IgA antibody response.

In conclusion, the RSV IgA EIA could be an effective tool, in infants, to determine the immune status of the child and identify previous RSV infections in the presence of either persisting maternal antibodies or other exogenous IgG antibodies. The RSV-F protein IgA EIA exhibited a high level of sensitivity and specificity for identifying who was previously RSV infected and can add value to studies of RSV prevention and disease.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. R. S.: Conceptualization, formal analysis, data curation, investigation, methodology, validation, writing–original draft. B. H.: Conceptualization, data curation, investigation, methodology, validation, writing–review & editing. H. S., I. Y., J. Y., C. Ko., and C. Ka.: Investigation, methodology, writing–review & editing. K. L.: Formal analysis, investigation, methodology, writing–review & editing. S. J.: Data curation, investigation, methodology, validation, writing–review & editing. L. H.: Investigation, project administration, supervision, writing–review & editing. C. M.: Formal analysis, investigation, methodology, writing–review & editing. T. G.: Data curation, investigation, project administration, supervision, writing–review & editing. K. S.: Investigation, methodology, supervision, project administration, writing–review & editing. C. A. R.: Investigation, methodology, supervision, writing–review & editing. E. J. A.: Investigation, methodology, supervision, writing–review & editing, conceptualization, funding acquisition, project administration, resources. L. J. A.: Conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, supervision, validation, writing–review & editing.

Financial support. This study was funded through the NIAID NIH Vaccine and Treatment Evaluation Units awarded to Emory University (HHSN272201300018I). The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institutes of Health.

Potential conflicts of interest. L. J. A. has done paid consultancies on RSV vaccines for AstraZeneca, Bavarian Nordic, GSK, and Janssen and on influenza virus vaccines for Pfizer. His laboratory is currently receiving funding through Emory University from Pfizer for RSV surveillance and maternal infant studies and from Advac, Sciogen, and Vernagen for RSV vaccine-related studies. L. J. A. is co-inventor on several Centers for Disease Control and Prevention (CDC) or Emory patents or patent filings on the RSV G protein and its CX3C chemokine motif relative to immune therapy and vaccine development and a patent filing for use of RSV platform VLPs, virus-like particles, with the F and G proteins for vaccines. E. J. A. has consulted for Pfizer, Sanofi Pasteur, GSK, Janssen, Moderna, and Medscape, and his institution receives funds to conduct clinical research unrelated to this manuscript from MedImmune, Regeneron, PaxVax, Pfizer, GSK, Merck, Sanofi Pasteur, Janssen, and Micron. He serves on a safety monitoring board for Kentucky BioProcessing, and Sanofi Pasteur. He serves on a data adjudication board for WCG and ACI Clinical. His institution has also received funding from the National Institutes of Health (NIH) to conduct clinical trials of COVID-19 vaccines. C. A. R. has received institutional research support from Pfizer, BioFire, GSK plc, Janssen Pharmaceuticals, MedImmune, Micron Technology, ModernaTX, Merck & Co, Inc, Novavax, PaxVax, Regeneron, Sanofi Pasteur, CDC, and NIH. She is co-inventor of patented RSV vaccine technology that has been licensed to Meissa Vaccines. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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