



Optimizing the sensitivity of detection of respiratory syncytial virus infections in longitudinal studies using the combination of weekly sample testing and biannual serology

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Abstract

Cohort studies are often challenged by incomplete adherence to sampling regimens, limiting the full capture of disease burden. We describe the detection of respiratory syncytial virus (RSV) infections achieved in a birth cohort using a combination of weekly nasal sample testing and serology.

The PREVAIL Cohort followed 245 maternal-child dyads from birth to age 18–24 months. Weekly mid-turbinate nasal swabs were tested for RSV using real-time polymerase chain reaction (RT-qPCR). Serum was tested for RSV pre-fusion F IgG and IgA antibody at age 6 weeks and biannually from 6–24 months. Mixed effects classification and regression trees (CART) identified antibody thresholds consistent with a RT-qPCR-identified RSV infection using a subset of participants having 90% weekly sample adherence ($n=53$, 21%). Resulting thresholds were applied to participants with either 70% of weekly samples or serum at age 18–24 months ($n=194$, 79%). Incidence rates were compared using Fisher's exact test.

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CART identified a \log_{10} change in $\text{IgG} > 0.32$ or $\text{IgA} > 0.20$ as indicative of an RSV infection. Comparing RT-qPCR-only to a combination of RT-qPCR and serology, RSV cumulative incidence (49% vs 75%, $p < 0.001$) and incidence rate (0.33 vs 0.71 infections/child-year, $p < 0.001$) increased; these rates did not differ from those calculated in those with 90% sample adherence.

Keywords

Cohort studies; decision trees; incidence rate; infectious disease epidemiology; misclassification bias; selection bias; attrition bias; epidemiologic methods

Introduction

Cohort studies can provide powerful insights into the natural history of acute infections through longitudinal testing and follow-up^{1,2}. However, complete ascertainment of infections depends on participant adherence to frequent sample collection, which is highly variable^{1,3–5}. Restricting analyses to participants with high sample adherence optimizes infection detection^{3,4,6}, but reduces sample size and may differentially exclude participants, resulting in potential selection bias^{1,7}. Reducing participant burden by collecting samples only when symptomatic may increase participation but limits the ability to detect asymptomatic infections, underestimating incidence^{8,9}.

Serological assays in cohort studies provide another approach to detecting infections and are less reliant on frequent sampling. However, most serologic assays are validated to identify seropositivity, defined as having ever been infected, requiring further evaluation to detect repeat infections and calculate incidence rates¹⁰. Furthermore, serological detections alone cannot date an infection, and therefore restrict the ability to assess symptomatology, healthcare utilization, or time-varying factors associated with disease⁸.

The lack of consistent methods to determine RSV incidence results in heterogeneity and lack of comparability across studies^{8,11–14}. Furthermore, identifying mild and asymptomatic infections is essential to understanding the natural history of immune development, necessary to assess and optimize vaccine effectiveness^{9,15}. Here, we describe an integrated method to identify incident RSV infections using a combination of real-time polymerase chain reaction (RT-qPCR)-tested nasal swabs and biannual serologic collection in a two-year birth cohort. We compare detection sensitivity by methodologic decision points and evaluate the differences by methods in RSV incidence, statistical power, and selection and misclassification biases.

Methods

Study cohort

The Pediatric Respiratory and Enteric Viral Acquisition and Immunogenesis Longitudinal (PREVAIL) Cohort is a two-year birth cohort conducted in Greater Cincinnati, Ohio from April 2017–July 2020. Institutional review board approvals were obtained from the US Centers for Disease Control and Prevention, Cincinnati Children's Hospital Medical Center, and enrolling birth hospitals. Full study methods have been published². Briefly,

prenatal enrollment included mothers who were 34 weeks gestation, 18 years of age, and expecting a healthy, singleton child. Children were followed from birth until age two, including weekly symptom surveillance *via* text message, weekly nasal swab and stool sample submission, and regular in-person study visits and blood draws.

RSV detection

Caregivers collected mid-turbinate nasal samples each week using flocked swabs (Copan Diagnostics, Inc.), placed into vials containing BD Universal Viral Transport medium (Becton, Dickinson, and Co., Franklin Lakes, NJ) and delivered to the study laboratory within 48 hours of collection. Swabs were tested for respiratory pathogens, including RSV A and B, using the Luminex multiplex RT-qPCR NxTAG Respiratory Pathogen Panel assay¹⁶. Detections were considered part of a continuous RSV infection if the same subtype was detected 30 days apart⁸. RT-qPCR-detected infections were dated using the date of the first RT-qPCR-positive.

Serum samples were collected using a standardized protocol at birth and at in-clinic study visits at ages 6 weeks and 6, 12, 18, and 24 months. RSV pre-fusion F IgA and IgG antibodies were measured using the MesoScale Discovery Respiratory Panel 1 multiplex serology assay (MesoScale Diagnostics, Rockville MD) according to the manufacturer's instructions, calculated in arbitrary units (AU) per mL using standard curves with manufacturer provided calibrators. Concentrations below or above the level of quantification (LOQ) were imputed as half the lower LOQ or the upper LOQ, respectively.

Definition of adherence levels

To replicate study populations for different analytic designs, we identified subsets using varying weekly sample adherence levels as inclusion criteria. It is important to note that each of these rules is applied to the prior subset, so the subsets are nested, and adherence level represents proportion of weekly samples submitted over the course of the entire study (Figure 1). Participants were considered evaluable for infectious outcomes if they participated in the study 18 months and submitted either 70% of weekly samples or provided a serum sample at 18 months of age. For studies reliant on RT-qPCR only, we subsetted evaluable participants who were 70% weekly sample adherent. For analysis requiring minimal gaps in submissions, we subsetted evaluable participants who submitted 90% of weekly samples.

Identification of thresholds

Using the 90% adherent subset, we applied a mixed-effects Classification and Regression Tree (CART) analysis to generate detection thresholds in serum antibody consistent with having an incident RSV infection during the interim between blood draws (study interval). CART models consider all possible combinations and levels of variables to recursively split data, selecting thresholds in the available predictors that result in the most accurate terminal nodes¹⁷. Study intervals were identified as positive or not positive for RSV based on the presence of a RT-qPCR-identified RSV infection within the study interval. Due to the time required to mount a detectable antibody response after an infection, study intervals with a RT-qPCR positive within four weeks of the blood draw were identified as positive

during the subsequent study interval. Using the change in antibody levels between blood draws, three possible predictors were included in the CART models; 1.) change in \log_{10} concentration, 2.) fold-change in concentration, and 3.) a binary indicator of 4-fold change in concentration. A fourth predictor, \log_{10} concentration at the time of the blood draw, was also included. CART models for each isotype were constructed without restriction to the number of splits or application of variable weights, first for each predictor separately to identify method-specific thresholds, then in combination to identify the most accurate method.

After applying the selected method's thresholds, serologic detections associated with a positive study interval were considered confirmatory of the RT-qPCR-positive, while serologic detections in a not-positive study interval were considered new incident infections. Investigations, including timing of the serum sample relative to the RT-qPCR positive, weekly sample adherence, and reported respiratory symptoms during the study interval, were conducted for all unconfirmed RT-qPCR-identified infections and new incident infections. Once detection thresholds were established in 90% adherent participants, they were applied to all evaluable participants.

Statistical analysis

Fisher's exact test or Kruskal-Wallis were used to compare socio-demographic factors among the adherence-defined subsets. Cumulative incidence in each subset was calculated for infections detected using RT-qPCR only and by combining RT-qPCR and serology. To calculate incidence rates, we calculated weeks-at-risk by multiplying the number of infections by four weeks, as RSV detections within 30 days were considered part of a continuous infection, then subtracting the resulting number from the total weeks of follow-up. Incidence rates were reported per child year. Fisher's Exact test with Holms corrections was used to compare proportions of infections identified by RT-qPCR and by serology, as well as cumulative incidence and incidence rate for each subset.

To evaluate the effect of study design criteria on power, we calculated detectable effect size at 80% power for a chi-squared test for each of our three adherence subsets. To examine the impact on misclassification and selection biases, we compared the demographic factors between those RSV-infected vs not RSV-infected in our evaluable subset using the RT-qPCR-only method, serology-only method, and the combined method using Kruskal-Wallis for continuous and Fisher's Exact Test for categorical data. All analyses were conducted using the R Environment for Statistical Computing (version 4.2.3).

Results

PREVAIL enrolled 245 participants; 194 (79%) were defined as evaluable, 101 (41%) were 70% adherent, and 53 (22%) were 90% adherent (Figure 2). Each increase in adherence criteria resulted in a smaller subset with greater proportions of subjects who identified as White race, higher income, privately insured, higher education level and who breastfed for longer durations (Table 1). Nearly half of evaluable participants were included due to providing a blood sample at 18 or 24 months. These participants were more likely to identify as Black race, lower education level, publicly insured and family income <\$50,000/

year compared to those with higher weekly sample adherence. The evaluable subset was demographically similar to the Cincinnati metropolitan area¹⁸.

Identification of RSV thresholds in the 90% adherent participants

RT-qPCR identified 54 RSV infections (36 first, 18 repeat infections) occurring in 68% of children ($n=36$) with an incidence rate of 0.56 infections/child-year (Figure 3). While the CART-identified thresholds (Table S1) for the three predictors related to change in antibody had high percent agreement with RT-qPCR, the threshold selected for \log_{10} concentration resulted in a high number of serial positives, overestimating incident infections. Seropositivity comparisons with the validated ROC curves were determined using IgA only, as all children were seropositive by IgG between birth and 6 months of age. All methods had high percent agreement with the validated ROC curves in terms of detecting seropositivity.

In the combined model, CART identified a single variable and threshold per isotype, \log_{10} increases of >0.202 AU or >0.32 AU, for IgA and IgG, respectively, from the previous blood draw as the most accurate predictor of an incident RSV infection, hereafter referred to concentration change. Using the concentration change thresholds, we identified 21 previously undetected RSV infections in 17 children, including 4 infections in 3 children without a detectable RT-qPCR-identified infection (Figure 4, participants 5, 11, 22). Most ($n=19$, 90%) of these new infections were identified using IgA; 33% ($n=7$) were identified using IgA only, including both new infections in the first 6 months of life. Nearly half of new infections ($n=10$, 48%) were detected following a study interval with very high weekly sample adherence (mean $98.0\% \pm 2.8$), while 52% ($n=11$) were detected following study intervals of lower adherence (mean $64.7\%, \pm 14.6$). Nearly all children (15/17, 88%) reported respiratory symptoms by text survey during the study interval prior to the serologic positive.

Our thresholds confirmed 47/54 (87%) RT-qPCR-identified infections. Of the seven unconfirmed (Figure 4) two children did not have a blood draw after the RT-qPCR-positive (participants 4 and 30) and five children (participants 6, 10, 12, 13, 21) did not report any respiratory symptoms during the interval. However, given the high specificity of the Lumindex NxTAG RPP (1, 95%CI 0.97–1), these infections were retained for incidence calculations. After combining RT-qPCR- and concentration change-identified infections, we identified 77 infections in 39 children, resulting in 77% cumulative incidence and 0.75 infections/child-year (Figure 3).

Application to all evaluable participants

RT-qPCR detected 124 RSV infections (96 first, 28 repeat infections) in 96/194 (49%) children, with an incidence rate of 0.33 infections/child-year (Figure 3). Concentration change identified 133 new infections, including 85 infections in 49 children who previously did not have an RT-qPCR-detected infection (Table 2); 51/133 (38%) were identified using IgA only, 3/133 (2%) were identified using IgG only, and 79/133 (60%) met both criteria. IgA only identified 85% (11/13) of new infections in the first year of life and 39% (30/76) of repeat infections. A comparison of results by each detection method can be found in Table S2.

Of the 124 RT-qPCR-identified infections, 109 (88%) were confirmed serologically; nearly half (7/15, 47%) of the unconfirmed RT-qPCR infections occurred in the first 6 months of life and 53% (8/15) did not report symptoms. When combining RT-qPCR and serology, 259 infections were identified in 145 children, increasing cumulative incidence to 75% by two years of age ($p<0.001$), and incidence rate to 0.71 infections/child-year ($p<0.001$).

Comparison of incidence, power achieved, and bias by protocol adherence and method

Using RT-qPCR-identified infections, cumulative incidence at 2 years of age (68%, 65%, 49%, $p=0.02$) and incidence rate (0.56, 0.47, 0.33, $p=0.003$) varied among the 90% adherent, 70% adherent, and the evaluable group, respectively. (Figure 3). Using our integrated approach, no differences remained in cumulative incidence (77%, 73%, 75%, $p=0.68$) or incidence rate (0.75, 0.72, 0.71, $p=0.20$).

In comparing power using chi-squared tests by subset (Figure 5), 80% power was achieved with an effect size 39% for the 90% adherent, 28% for the 70% adherent, and >20% for all eligible participants.

When we compared those who did and did not have a RT-qPCR-identified RSV infection (Table 3), differences by race, insurance type, marital status, maternal education, and family income were detected. However, after adding serologically-identified infections, no differences remained in these factors.

Using RT-qPCR-only, 49/194 (25%) of evaluable children were misclassified as never having an RSV infection by age two. Furthermore, 25 RT-qPCR-identified infections were misclassified as first infections; 19 of these were reclassified as second and 6 were reclassified as third after adding concentration change-identified infections.

Sensitivity analysis

Unconfirmed RT-qPCR-positives were removed from incidence rate calculations for each adherence subset (90% adherent, 70% adherent, evaluable). The adjusted incidence rates (0.71, 0.65, 0.66, respectively) did not differ from each-other ($p=0.20$) or from the previous estimates ($p=0.22$).

Discussion

The PREVAIL Cohort accurately and more completely identified RSV infections by age two by integrating RT-qPCR-tested nasal swabs with biannual blood draws assayed for both RSV IgG and IgA antibodies. We demonstrate that this combined method resulted in more robust RSV incidence estimates while simultaneously increasing power and reducing selection and misclassification biases. In addition, this method allows comparisons across detection methods, including those that rely on RT-qPCR-only and serology-only procedures to detect RSV infections.

Our reported cumulative incidence and incidence rates (Figure 3) are consistent with findings from recent international cohorts employing a range of methods. The Australian ORChID Cohort reported an RSV incidence rate of 0.46 (95%CI 0.37–0.58) infections/

child-year using weekly RT-qPCR detections from birth to age two¹⁴. Nyiro *et al* reported RSV seropositivity of 83% by age two in all children admitted to a rural Kenyan hospital using crude ELISA assays¹³, while Kutsaya *et al* reported 68% seropositivity by age two in healthy Finnish children by IgG enzyme immunoassay¹². While the heterogeneity of methods across these studies makes direct comparisons difficult, our findings are consistent with each, underscoring the value of our integrated approach to detections.

The transplacental transfer of IgG combined with its durability leads to challenges in identifying early-life and repeat RSV infections when only IgG is measured^{8,12,13}. For example, Kutsaya *et al* detected no discernable rise in IgG in five symptomatic, PCR-confirmed RSV infections occurring prior to nine months of age and concluded that they underestimated incidence rates due the attenuated rise in IgG associated with repeat infection¹². Nyiro *et al* reported that 100% of study children were seropositive by IgG at 1 month of age and estimated the average duration of maternally-acquired antibodies to be 4.7 months¹³. Our use of CART to identify thresholds in IgA and IgG rather than a standard 4-fold rise in IgG better enabled detection of these early and repeat infections; 98% of all serologically-detected infections were detected using IgA, with 38% ($n=51$) using IgA only including 85% of new infections between birth-12 months, 33% of new infections in the second year of life and 39% of repeat infections (Table 2). Removing these infections would result in a significantly decreased incidence rate of 0.56 infections/child year ($p=0.01$) in the evaluable group. We should note that our blood collections were twice-yearly; detection of IgA F antigen declines over time¹⁹, making the frequency of blood sampling an important decision point in assay selection.

Combining RT-qPCR- and serology-detected infections eliminated differences in cumulative incidence and incidence rates by adherence-defined subsets, allowing for the inclusion of nearly 80% of enrolled participants and increasing power. In addition, the reduction in selection bias associated with socio-demographic factors using our integrated approach may provide a more robust and generalizable estimation of disease burden^{1,3,5,6}. Finally, the reduction in misclassifications, particularly in order of infection, is instrumental for understanding the natural history of immune response, necessary to estimate and optimize vaccine effectiveness⁹.

It is important to note that many caregivers may hesitate to participate in a pediatric study with regular blood draws, which could result in volunteer bias at enrollment²⁰. PREVAIL screened and approached 1206 mothers in the third trimester of pregnancy for potential enrollment; 941 (78%) met eligibility but declined to enroll². Those who consented to enroll in the study did not differ racially from those who declined². Most of those who declined did not provide a specific reason ($n=843$, 90%), but it is probable that some were hesitant about the blood draws. However, only 14% ($n=14$) who provided a reason cited the blood draws; most ($n=56$, 57%) had delivered prior to contact and 29% ($n=28$) cited the time commitment. Finally, participants who met our evaluation criteria by providing a blood sample improved the representativeness of our study (Table 1). We concluded that blood draws were not a significant source of bias in our study enrollment.

Although incidence calculations using serology-only were nearly identical to those combining RT-qPCR and serology, serologically-identified infections cannot be precisely dated, hindering examinations of severity or symptomatology of infections. Such examinations are important for RSV, as reinfections are common throughout life^{12,13,21,22}, with rapidly waning immunity following exposure. Effectiveness studies of two RSV immunization strategies report strong protection against severe illness within the first six months of receipt, followed by waning protection^{23–25}. Gauging effectiveness of immunization to prevent or reduce severity of symptoms requires precise dating of the immunization event, infection, and symptom onset, while accurate incidence calculations and characterization of the natural history of immune development requires identifying all infections, including those that are mild or asymptomatic. Our integrated method provides data for both purposes.

Our study is not without limitations. While the 90% adherent group rarely skipped submissions in consecutive weeks, a weekly sample protocol can miss short, transient infections. Furthermore, weekly sample adherence was calculated over the study duration, but some participants experienced periods of lower adherence, including half of those with serologically-identified new infections. As these participants provided the training data for the CART models, missed infections may have resulted in a misclassification of study intervals as ‘not positive’ and thereby reduced the sensitivity of the detections. It is also possible that many serology-only detected infections, especially in the high adherence group, represent transient exposures that provided a boosting effect rather than true RSV infections. However, the presence of respiratory symptoms occurring in 88% of the intervals with a new detection and the consistency of our incidence calculations with other studies^{12–14} supports our inclusion of these infections in our calculations.

Our study also has several important strengths. Using CART to identify thresholds using highly adherent children rather than relying on a standardized 4-fold rise increased our detections, while applying both IgA and IgG thresholds allowed for the detection of early and repeat infections. While our sample size was modest, our method allowed us to maximize power by including participants with lower weekly sample adherence. The identification of serology-only infections during periods of high sample adherence suggests that this method may also address detection sensitivity deficits due to the use of a mid-turbinate nasal swab or sample quality due to parental collection. Finally, our method reduced potential socio-demographic biases associated with limiting analysis to highly-adherent participants, resulting in greater study robustness and power.

Conclusions

Using CART analysis to identify serologic thresholds consistent with RSV infection integrated with weekly RT-qPCR-detections allowed the full capture of RSV infections, facilitated improved accuracy of disease burden estimates, and provided a better understanding of RSV immunologic events in infancy and early childhood. While larger studies are needed to establish generalizable serology thresholds for RSV infection, use of IgG and IgA serology combined with weekly nasal swab testing is a way for cohort studies to maximize power and reduce biases in RSV detections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments2:

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Data Availability Statement:

Data and statistical code available upon approved emailed request

References

1. Howe LD, Tilling K, Galobardes B, Lawlor DA. Loss to follow-up in cohort studies: bias in estimates of socioeconomic inequalities. *Epidemiology*. Jan 2013;24(1):1–9. doi:10.1097/EDE.0b013e31827623b1 [PubMed: 23211345]
2. Morrow AL, Staat MA, DeFranco EA, et al. Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis in US Mothers and Children Aged 0–2: PREVAIL Cohort Study. *JMIR Res Protoc*. Feb 12 2021;10(2):e22222. doi:10.2196/22222 [PubMed: 33576746]
3. Accorsi EK, Qiu X, Rumpler E, et al. How to detect and reduce potential sources of biases in studies of SARS-CoV-2 and COVID-19. *Eur J Epidemiol*. Feb 2021;36(2):179–196. doi:10.1007/s10654-021-00727-7 [PubMed: 33634345]
4. Teague S, Youssef GJ, Macdonald JA, et al. Retention strategies in longitudinal cohort studies: a systematic review and meta-analysis. *BMC Med Res Methodol*. Nov 26 2018;18(1):151. doi:10.1186/s12874-018-0586-7 [PubMed: 30477443]
5. Agampodi S, Tadesse BT, Sahastrabuddhe S, Excler JL, Kim JH. Biases in COVID-19 vaccine effectiveness studies using cohort design. *Front Med (Lausanne)*. 2024;11:1474045. doi:10.3389/fmed.2024.1474045 [PubMed: 39540039]
6. Stockwell T, Zhao J, Clay J, et al. Why Do Only Some Cohort Studies Find Health Benefits From Low-Volume Alcohol Use? A Systematic Review and Meta-Analysis of Study Characteristics That May Bias Mortality Risk Estimates. *J Stud Alcohol Drugs*. Jul 2024;85(4):441–452. doi:10.15288/jsad.23-00283 [PubMed: 38289182]
7. Hellfritsch M, Pottegård A, Haastrup SB, Rasmussen L, Grove EL. Cohort selection in register-based studies of direct oral anticoagulant users with atrial fibrillation: An inevitable trade-off between selection bias and misclassification. *Article. Basic & Clinical Pharmacology & Toxicology*. 2020;127(1):3–5. doi:10.1111/bcpt.13423
8. Teoh Z, Conrey S, McNeal M, et al. Burden of Respiratory Viruses in Children Less Than 2 Years Old in a Community-based Longitudinal US Birth Cohort. *Clin Infect Dis*. Sep 18 2023;77(6):901–909. doi:10.1093/cid/ciad289 [PubMed: 37157868]

9. Aguiar M, Van-Dierdonck JB, Mar J, Stollenwerk N. The role of mild and asymptomatic infections on COVID-19 vaccines performance: A modeling study. *J Adv Res.* Jul 2022;39:157–166. doi:10.1016/j.jare.2021.10.012 [PubMed: 35777906]
10. MedlinePlus. Antibody Serology Tests. National Library of Medicine. Updated 9/30/2024. Accessed 2/20, 2025. <https://medlineplus.gov/lab-tests/antibody-serology-tests/#:~:text=Antibody%20serology%20tests%20are%20not,past%20infection%20or%20a%20vaccination.>
11. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child.* Jun 1986;140(6):543–6. doi:10.1001/archpedi.1986.02140200053026 [PubMed: 3706232]
12. Kutsaya A, Teros-Jaakkola T, Kakkola L, et al. Prospective clinical and serological follow-up in early childhood reveals a high rate of subclinical RSV infection and a relatively high reinfection rate within the first 3 years of life. *Epidemiol Infect.* Jun 2016;144(8):1622–33. doi:10.1017/s0950268815003143 [PubMed: 26732801]
13. Nyiro JU, Kombe IK, Sande CJ, et al. Defining the vaccination window for respiratory syncytial virus (RSV) using age-seroprevalence data for children in Kilifi, Kenya. *PLoS One.* 2017;12(5):e0177803. doi:10.1371/journal.pone.0177803 [PubMed: 28531224]
14. Takashima MD, Grimwood K, Sly PD, et al. Epidemiology of respiratory syncytial virus in a community birth cohort of infants in the first 2 years of life. *Eur J Pediatr.* Jul 2021;180(7):2125–2135. doi:10.1007/s00431-021-03998-0 [PubMed: 33634335]
15. Velázquez FR, Matson DO, Calva JJ, et al. Rotavirus infection in infants as protection against subsequent infections. *The New England journal of medicine.* Oct 3 1996;335(14):1022–1028. doi:10.1056/NEJM199610033351404 [doi] [PubMed: 8793926]
16. Tang Y-W, Gonsalves S, Sun JY, et al. Clinical Evaluation of the Luminex NxTAG Respiratory Pathogen Panel. *Journal of clinical microbiology.* American Society for Microbiology 2016;54(7):1912–1914. doi:10.1128/JCM.00482-16 [PubMed: 27122378]
17. Fokkema M, Smits N, Zeileis A, Hothorn T, Kelderman H. Detecting treatment-subgroup interactions in clustered data with generalized linear mixed-effects model trees. *Behav Res Methods.* Oct 2018;50(5):2016–2034. doi:10.3758/s13428-017-0971-x [PubMed: 29071652]
18. U. S. Census Bureau. Quick Facts, Hamilton County, OH. Accessed Web Page, 2025. <https://www.census.gov/quickfacts/hamiltoncountyohio>
19. Sankaranarayanan R, Ha B, Sun H, et al. Evaluation of Immunoglobulin A Enzyme Immunoassays to Detect Primary Respiratory Syncytial Virus Infection in Infants and Young Children. *J Infect Dis.* Apr 15 2025;231(4):1060–1068. doi:10.1093/infdis/jiae514 [PubMed: 39575588]
20. Jordan S, Watkins A, Storey M, et al. Volunteer Bias in Recruitment, Retention, and Blood Sample Donation in a Randomised Controlled Trial Involving Mothers and Their Children at Six Months and Two Years: A Longitudinal Analysis. *PLOS ONE.* 2013;8(7):e67912. doi:10.1371/journal.pone.0067912 [PubMed: 23874465]
21. Lambert L, Sagfors AM, Openshaw PJ, Culley FJ. Immunity to RSV in Early-Life. *Front Immunol.* 2014;5:466. doi:10.3389/fimmu.2014.00466 [PubMed: 25324843]
22. Li Y, Wang X, Blau DM, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. *Lancet.* May 28 2022;399(10340):2047–2064. doi:10.1016/s0140-6736(22)00478-0 [PubMed: 35598608]
23. Moline HL, Toepfer AP, Tannis A, et al. Respiratory Syncytial Virus Disease Burden and Nirsevimab Effectiveness in Young Children From 2023–2024. *JAMA Pediatr.* Feb 1 2025;179(2):179–187. doi:10.1001/jamapediatrics.2024.5572 [PubMed: 39652359]
24. Principi N, Perrone S, Esposito S. Challenges and Limitations of Current RSV Prevention Strategies in Infants and Young Children: A Narrative Review. *Vaccines (Basel).* Jul 1 2025;13(7)doi:10.3390/vaccines13070717
25. Sevaldal ATK, Hurley S, Bartlett AW, Rawlinson W, Walker GJ. Systematic Review of the Efficacy and Safety of RSV-Specific Monoclonal Antibodies and Antivirals in Development. *Rev Med Virol.* Sep 2024;34(5):e2576. doi:10.1002/rmv.2576 [PubMed: 39209729]

Key messages

- We sought to develop a method to maximize RSV infection detection to optimize estimation of disease burden in longitudinal studies, which are prone to incomplete protocol adherence to weekly sample collection.
- Using a combination of weekly sample submissions and regular IgG and IgA serology, we identified incident RSV infections in participants with lower weekly sample submissions.
- The combination of weekly samples and periodic serology can be used to increase power, reduce selection bias, and improve study compatibility in infectious disease cohort studies.

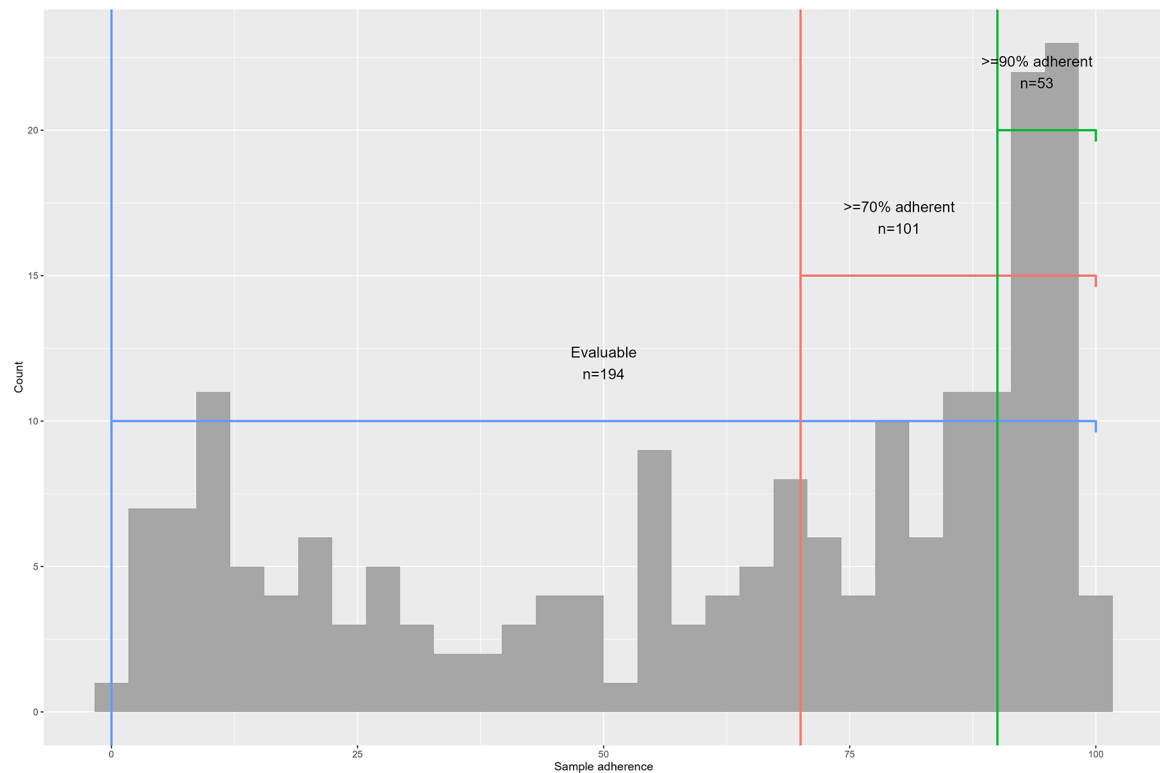


Figure 1.

Distribution of weekly sample adherence by subset inclusion criteria. Participants in the PREVAIL Cohort were considered evaluable for infectious disease outcomes if completed 18 months of the study and submitted either 70% of weekly samples or provided a serum sample 18 months of age (blue bracket). To reduce misclassification bias in studies requiring more consistent sample submission, analysis was restricted to evaluable participants who were 70% weekly sample adherent (coral bracket). When identifying training data for predictive modeling, participants who were 90% weekly sample adherent were included, reducing time between missed samples (green bracket).

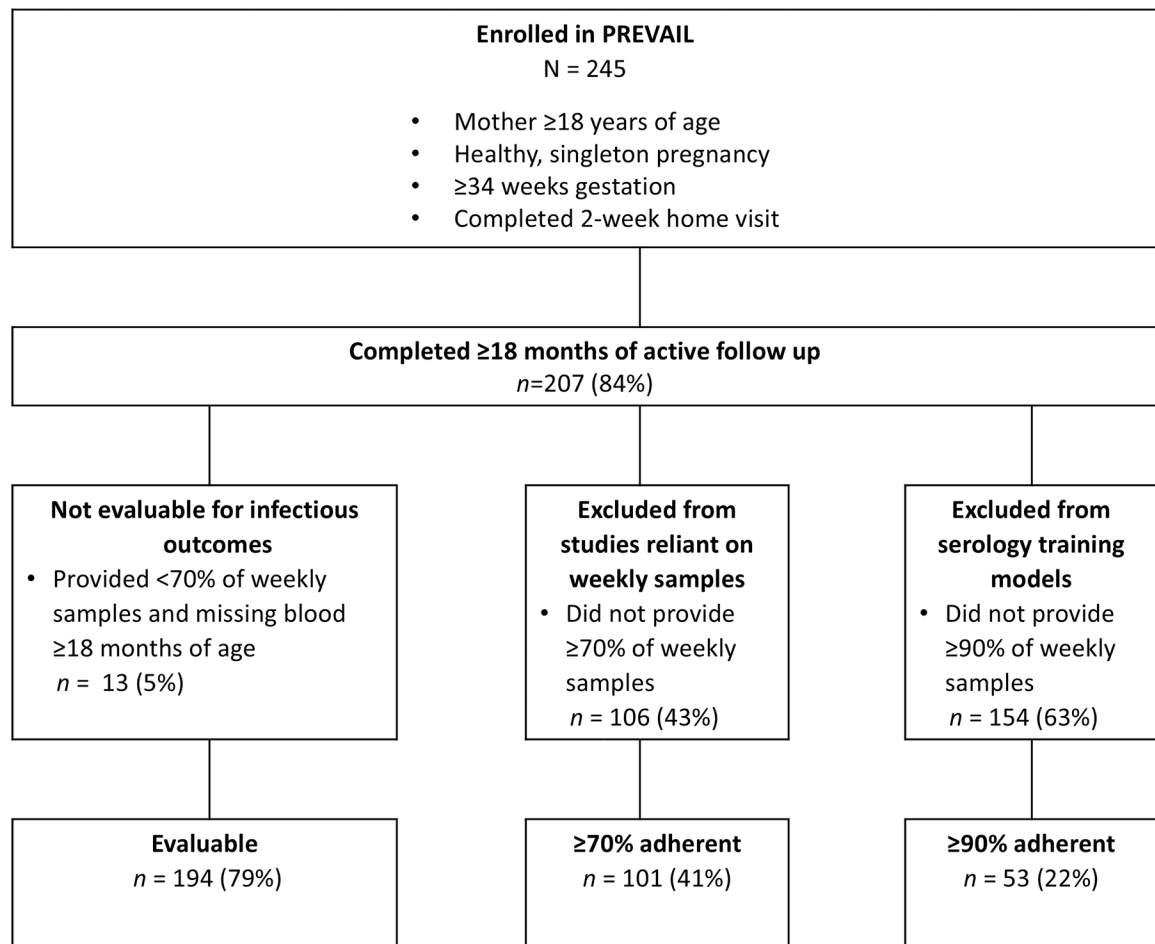
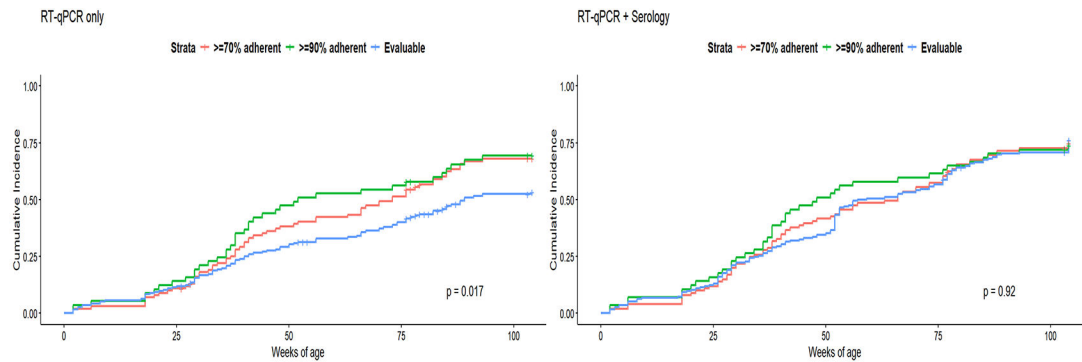


Figure 2.
STROBE flowchart: Strengthening the Reporting in Observational studies in Epidemiology (STROBE) flowchart indicating the inclusion and exclusion criteria for each subset.



Subset inclusion criteria	Weeks of follow up	Infections Identified by PCR	Cumulative incidence by PCR	Incidence rate by PCR	Infections identified by serology ¹	Cumulative incidence combined	Incidence rate combined ²
≥90% adherent (n=53)	5512	54 (70%)	36 (68%)	0.56	21 (28%)	41 (77%)	0.75
≥70% adherent (n=101)	10,504	91 (66%)	66 (65%)	0.47	46 (34%)	74 (73%)	0.72
Evaluable (n=194)	20,111	124 (48%)	96 (49%)	0.33	135 (52%)	145 (75%)	0.71
<i>P</i> value ³							
≥90% vs ≥70%		0.44	0.48	0.48	0.44	1.0	1.0
≥90% vs evaluable	--	<0.001	0.01	0.01	<0.001	1.0	1.0
≥70% vs evaluable		<0.001	0.02	0.02	<0.001	1.0	1.0

¹ Infections identified by serology were previously not identified by PCR

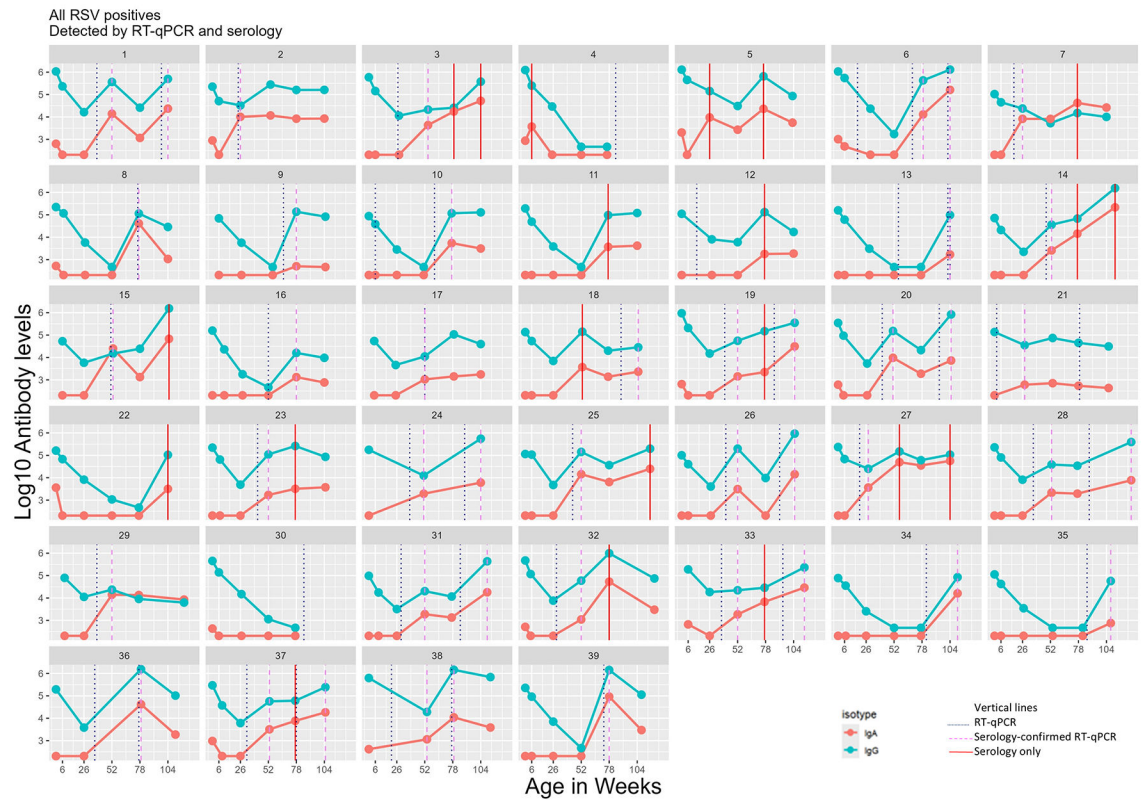
² Incidence rate calculated as infections per child year:

- Total number of weeks of follow up = sum(weeks active)
- Weeks at risk: (total weeks of follow up - weeks not at risk (infections * 4 weeks))
- (total infections/weeks at risk) * 52

³ *p* values were determined using a pairwise Fisher's exact test with Holms corrections

Figure 3.

Comparison of incidence by adherence level and detection method. Cumulative incidence comparisons by subset adherence level demonstrating the attenuation of differences between adherence levels in cumulative incidence and incidence density when using a combination of real-time polymerase chain reaction (RT-qPCR) and serological RSV detections. *P*-values in the plots represent differences by log-likelihood.

**Figure 4.**

RSV detections in highly-adherent PREVAIL children.

PREVAIL: Pediatric Respiratory and Enteric Viral Acquisition and Immunogenesis
Longitudinal Cohort

RSV: Respiratory Syncytial Virus

RT-qPCR: Real-time polymerase chain reaction

Line graphs of IgA (coral) and IgG (aqua) antibodies at each study interval, with vertical lines indicating RT-qPCR-identified infections (dark blue), serology confirmation of the RT-qPCR-identified infections (dotted lilac), and serology-only identified infections (solid red). Participants were 90% weekly sample adherent over the course of the 2-year PREVAIL Cohort.

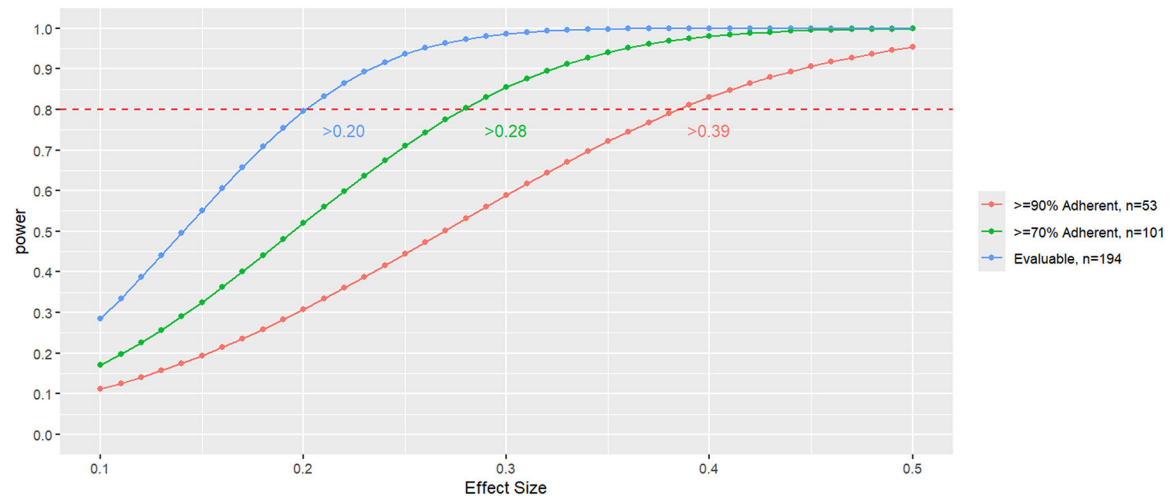


Figure 5.

Power achieved by effect size for each of the adherence criteria. The dotted red horizontal line represents the point at which 80% power is achieved. The figure labels represent the effect size detectable at 80% power for each adherence level

Table 1:

Demographics by subset inclusion criteria

			Evaluable N=194			
				70% adherent N=101		
	Enrolled N = 245 ¹	Non-evaluable/ excluded from analysis n = 51 ¹	<70% weekly samples, but serum 18M n = 93 ¹	70% weekly samples <90% n = 48 ¹	90% weekly samples n = 53 ¹	p-value
Nasal swab adherence	61 (22, 88)	29 (13, 54)	29 (11, 51)	80 (74, 87)	94 (91, 95)	<0.001
Maternal age	29.6 (25.7, 33.2)	26.1 (23.2, 30.3)	28.7 (25.2, 31.3)	31.6 (28.3, 35.0)	33.0 (30.0, 34.6)	<0.001
Race						<0.001
Black	107 (44%)	25 (49%)	65 (70%)	11 (23%)	6 (11%)	
White/Other	138 (56%)	26 (51%)	28 (30%)	37 (77%)	47 (89%)	
Hispanic Ethnicity	6 (2.4%)	0 (0%)	1 (1.1%)	2 (4.2%)	3 (6%)	0.2
Insurance type						<0.001
Private	106 (43%)	13 (25%)	15 (16%)	30 (63%)	48 (91%)	
Public	139 (57%)	38 (75%)	78 (84%)	18 (38%)	5 (9.4%)	
Marital status						<0.001
Married/Partner	163 (67%)	29 (57%)	47 (51%)	40 (83%)	47 (89%)	
Single	82 (33%)	22 (43%)	46 (49%)	8 (17%)	6 (11%)	
Maternal Education						<0.001
High school or less	115 (47%)	34 (67%)	63 (68%)	13 (27%)	5 (9.4%)	
2+ years post-secondary	130 (53%)	17 (33%)	30 (32%)	35 (73%)	48 (91%)	
Family Income						<0.001
<\$25k	88 (36%)	27 (53%)	49 (53%)	10 (21%)	2 (3.8%)	
\$25k-\$50k	49 (20%)	13 (25%)	26 (28%)	7 (15%)	3 (5.7%)	
>\$50k	108 (44%)	11 (22%)	18 (19%)	31 (65%)	48 (91%)	
Infant sex						0.2
Female	127 (52%)	31 (61%)	50 (54%)	24 (50%)	22 (42%)	
Male	118 (48%)	20 (39%)	43 (46%)	24 (50%)	31 (58%)	
Delivery mode						0.14
C Section	94 (38%)	16 (31%)	30 (32%)	23 (48%)	25 (47%)	
Vaginal	151 (62%)	35 (69%)	63 (68%)	25 (52%)	28 (53%)	
Birthweight (kg)	3.29 (2.95, 3.53)	3.27 (2.87, 3.46)	3.22 (2.83, 3.50)	3.32 (3.09, 3.62)	3.37 (3.07, 3.61)	0.2
Initiated breastfeeding	212 (87%)	41 (80%)	75 (81%)	45 (94%)	51 (96%)	0.023
Days of breastfeeding	83 (16, 312)	46 (9, 138)	33 (2, 123)	227 (47, 430)	310 (122, 428)	<0.001

¹Median (Q1, Q3); n (%)²Participants in the evaluable subset (n=194) participated in PREVAIL at least 18 months and contributed a serum sample at 18 months of age or 70% of weekly samples.

³Highly adherent participants were defined as evaluable participants with either 70% ($n=101$) or 90% ($n=53$) weekly sample adherence

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Table 2:

New serologic RSV detections by detection method in 194 PREVAIL children

		All new concentration change-identified infections N=133	Method used to identify infection		
			IgA only n=51 (38%)	IgG only n=3 (2%)	Both n=79 (60%)
Age category	< 6 months	2 (2%)	2 (4%)	0 (0%)	0 (0%)
	6–11 months	11 (8%)	9 (18%)	0 (0%)	2 (3%)
	12–17 months	59 (44%)	19 (37%)	2 (67%)	38 (48%)
	18–24 months	61 (46%)	21 (41%)	1 (33%)	39 (49%)
Infection number	First	57 (43%)	21 (41%)	0 (0%)	36 (46%)
	Second	61 (46%)	28 (55%)	2 (66%)	31 (39%)
	Third	15 (11%)	2 (4%)	1 (33%)	12 (15%)

Mixed effects classification and regression tree (CART) analysis was used to identify thresholds of positivity using pre-fusion F IgA and IgG assays from serum collected at 6 weeks and 6, 12, 18, and 24 months of age. CART-derived thresholds of ≥ 2.02 change in log10 concentration of IgA or a ≥ 0.32 change in log10 concentration of IgG were applied to PREVAIL participants who participated in the study at least 18 months and were either 70% adherent in weekly sample submission or provided a serum sample at 18 or 24 months of age.

Table 3: Comparison of RSV infections by demographics using RT-qPCR-only and a combination of RT-qPCR- and serology-identified infections among all evaluable participants.

	Variable	Evaluable N = 194 ¹	Positive by RT-qPCR n = 96 (49%) ¹	p ²	Positive by serology n = 143 (74%) ¹	p ²	Positive combined n = 145 (75%) ¹	p ²
Maternal age		30.2 (26.9, 33.7)	30.6 (28.4, 34.3)	0.06	30.2 (27.0, 33.7)	0.9	30.3 (27.2, 33.7)	0.8
Race	Black	82 (42%)	27 (28%)	<0.001	60 (42%)	0.9	60 (41%)	0.5
	White/Other	112 (58%)	69 (72%)		83 (58%)		85 (59%)	
Hispanic	Yes	6 (3%)	4 (4%)	0.4	5 (4%)	>0.9	5 (3%)	>0.9
	No	188 (97%)	92 (96%)		138 (96%)		140 (97%)	
Insurance	Private	93 (48%)	59 (61%)	<0.001	65 (45%)	0.2	67 (46%)	0.4
	Public	101 (52%)	37 (39%)		78 (55%)		78 (54%)	
Marital status	Married/Partner	134 (69%)	75 (78%)	0.007	99 (69%)	>0.9	101 (70%)	0.8
	Single	60 (31%)	21 (22%)		44 (31%)		44 (30%)	
Maternal education	High school or less	81 (42%)	24 (25%)	<0.001	58 (41%)	0.6	58 (40%)	0.4
	2 years post-secondary	113 (58%)	72 (75%)		85 (59%)		87 (60%)	
Family Income	<\$25,000	61 (31%)	19 (20%)	<0.001	43 (30%)	0.5	44 (30%)	0.4
	\$25,000–\$50,000	36 (19%)	13 (14%)		32 (22%)		30 (21%)	
	>\$50,000	97 (50%)	64 (67%)		68 (48%)		71 (49%)	
Infant sex	Female	96 (49%)	46 (48%)	0.7	76 (53%)	0.3	76 (52%)	0.2
	Male	98 (51%)	50 (52%)		67 (47%)		69 (48%)	

¹Median (Q1, Q3); n (%)
²Compared to all evaluable