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Viral Respiratory Infections in Preterm Infants during and after Hospitalization

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Objective To determine the burden of viral respiratory infections in preterm infants both during and subsequent to neonatal intensive care unit (NICU) hospitalization and to compare this with term infants living in the community.

Study design From March 2013 through March 2015, we enrolled 189 newborns (96 term and 93 preterm) into a prospective, longitudinal study obtaining nose/throat swabs within 7 days of birth, weekly while hospitalized and then monthly to 4 months after hospital discharge. Taqman array cards were used to identify 16 viral respiratory pathogens by real-time polymerase chain reaction. Demographic, clinical, and laboratory data were gathered from electronic medical records, and parent interview while hospitalized with interval histories collected at monthly visits. The hospital course of all preterm infants who underwent late-onset sepsis evaluations was reviewed.

Results Over 119 weeks, we collected 618 nose/throat swabs from at risk preterm infants in our level IV regional NICU. Only 4 infants had viral respiratory infections, all less than 28 weeks gestation at birth. Two infants were symptomatic with the infections recognized by the clinical team. The daily risk of acquiring a respiratory viral infection in preterm infants in the NICU was significantly lower than in the full term cohort living in the community. Once discharged from the hospital, viral respiratory infections were common in all infants.

Conclusions Viral respiratory infections are infrequent in a NICU with strict infection prevention strategies and do not appear to cause unrecognized illness. Both preterm and term infants living in the community quickly acquire respiratory viral infections. (J Pediatr 2017;182:53-8).

Almost 4 million babies are born in the US each year with approximately 12% of those births occurring prematurely.¹ Preterm infants suffer significant respiratory morbidity because of lung immaturity at birth, especially those born before 32 weeks gestation. The more severe cases are diagnosed with bronchopulmonary dysplasia (BPD) based on oxygen requirement near term corrected gestational age. However, infants born at less than 32 weeks who do not develop BPD and those born moderate to late preterm, from 32 to <37 weeks gestation, also have an increased prevalence of respiratory symptoms and rehospitalization because of respiratory problems during their first year of life as well as a greater degree of respiratory symptoms at preschool age.²,³

Viral respiratory infections contribute to poor respiratory outcomes and are the most common pathogens identified in children under the age of 18 years hospitalized for community-acquired pneumonia.⁴ In addition to well-documented outbreaks, a prior surveillance study suggested a high burden of on-going respiratory viral infections in preterm infants born at less than 32 weeks gestation while they are still hospitalized in the neonatal intensive care unit (NICU).⁵ NICU infections with human rhinovirus also have been described in both extremely and moderately preterm infants and postulated as a cause of significant respiratory morbidity.⁶ A recent report identified respiratory viral infections in a number of clinically significant systemic illnesses in the NICU population and suggested that testing for viral respiratory pathogens may be helpful in the diagnostic evaluation of infants developing signs of sepsis after the first 72 hours of age (late-onset sepsis).⁷

We sought to determine the full extent of viral respiratory infections in the extremely to moderately preterm population in the NICU and during the first 4 months following discharge from the hospital. This study is part of a larger study of infant immune system development and respiratory function (Prematurity, Respiratory outcomes, Immune System and Microbiome study or PRISM) that is part of the Respiratory Pathogens Research Center at the University of Rochester. We hypothesized that the risk of respiratory viral infections in preterm babies in the NICU was significantly lower than term infants residing in the community. Secondarily, we hypothesized that the rate of respiratory viral infections in preterm infants would rise to match the term infants’ rate of infection once they were discharged from the NICU.

| BPD   | Bronchopulmonary dysplasia |
|-------|------------------------------|
| HRV   | Human rhinovirus            |
| NICU  | Neonatal intensive care unit|
| RSV   | Respiratory syncytial virus |
| TAC   | Taqman array card          |

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Term (≥37⁰/⁷ weeks gestation) and preterm (<36 weeks gestation) neonates born at the University of Rochester Medical Center, Rochester, New York were eligible for enrollment. Exclusion criteria included abnormalities of the airway or chest wall, neuromuscular or cardiac disorders (not including patent ductus arteriosus or isolated atrial septal defect), congenital malformations or genetic disorders known to impact immune system development or respiratory function, maternal HIV infection, nonviability, or lack of ability to speak and read English. In addition, term infants were not eligible if they were admitted to the NICU for any period of time, and preterm infants born at 36⁰/⁷ weeks through 36⁰/⁷ weeks gestation were excluded because they were not routinely admitted to the NICU. Parents were approached within 24-72 hours of birth and all newborns were enrolled by 7 days of life. The Research Subjects Review Board of the University of Rochester approved the study and all parents provided informed consent.

**Study Protocol**

At the initial visit, information was obtained regarding the birth history of the child and the maternal medical history including medication use or medical problems during pregnancy. Parents also self-reported family demographic information. Nose and throat swabs were obtained from each newborn on study day 1 and then weekly during hospitalization, monthly following discharge until 12 months corrected gestational age, and again at 3 years of age. Results of research testing were not shared with the clinical team. Samples through 4 months after discharge are included in this report. In addition, families were reminded at each visit to notify the study team if a child developed respiratory symptoms that reached a score of ≥3 on the Childhood Origins of Asthma or “COAST” score. When a respiratory illness was identified, a study visit was completed as soon as possible. At all visits following hospitalization, parents provided the child’s interval medical history.

In addition to our prospective, active surveillance, we reviewed the charts of all enrolled preterm infants who underwent a late onset sepsis evaluation (>72 hours after birth) to determine if the illness episode was associated with a viral respiratory infection.

**NICU Environment**

During the study period, the University of Rochester Medical Center NICU was a regional level IV, 60-bed unit organized into nine 6-8 bed “rooms” opening into a common corridor with 4 negative pressure isolation rooms. Patients with suspected or proven viral illness were isolated promptly before a definitive diagnosis was made. Visitor restrictions were in place from mid-December to mid-March limiting visitors to 4 for each infant with no visitors permitted under the age of 14 years. Influenza vaccination or surgical mask use was required of staff each winter and strongly encouraged for family members. Sibling visits were allowed outside the winter months but required review by a NICU nurse to obtain an updated immunization history and review of symptoms. At all times, visitors were asked to refrain from entering the NICU if they had symptoms of a respiratory illness.

Hand hygiene for staff included hand sanitizing and gloves for all patient contact. All patients were assigned a stethoscope and infants less than approximately 34 weeks gestation at birth were cared for in incubators until able to maintain temperatures in <27°C beds. Palivizumab was not administered to hospitalized infants.

**Specimens**

Separate flocked swabs (Copan, FLOQSwabs catalog no. 525CS01; Copan, Murrieta, California) were used to obtain samples from the nares and oropharynx/tonsillar region using a tongue depressor. Specimens were immediately combined in 3 mL of universal transport media (Cat no. 330CHL; Quidel [formerly Diagnostic Hybrids], Athens, Ohio), shaken, placed on ice, and transported to the laboratory.

**Real-Time Polymerase Chain Reaction**

Total nucleic acid was extracted using 200 μL of universal transport media with the QIAamp Viral RNA Mini-Kit on a QIAcube (Qiagen, Valencia, California) with a final volume of 75 μL. TaqMan array card (TAC) technology was used on the ViiA7 instrument (Life Technologies, Carlsbad, California) as previously described, with primer and probe modifications as outlined (Table 1; available at www.jpeds.com). Targets included influenza A and B, respiratory syncytial virus (RSV), parainfluenza virus 1, 2, and 3, human rhinovirus (HRV), enterovirus, adenovirus, coronavirus 1 through 4 (229, NL63, OC43, and HKU1, respectively), human metapneumovirus, human bocavirus, and human parechovirus.

**Statistical Analyses**

Groups were compared by 2-sample t-test for continuous variables and χ² test for categorical variables. Corresponding non-parametric version of Wilcoxon rank sum test and Fisher exact test were used for confirmation. Survival analysis was applied to study the infection-free curves of preterm babies during NICU hospitalization vs term babies in the community, and of both cohorts in the community, controlling for other covariates. For the NICU vs community comparison, time to first infection was calculated as the interval between birth date and infection date for the first infection for preterm babies and discharge date and infection date for term babies. For the comparison of both cohorts in the community, time to first infection was the interval between discharge date and infection date. Time to repeat infection was the interval between previous and current infection dates. Log-rank test and Kaplan-Meier nonparametric estimation of infection-free probability curves were used to compare days with infection between groups (eg, cohort [preterm vs term], sex [female vs male], and others). Further, the intensity model using the model-based covariance estimate and coupled with stepwise variable selection was used to explore the effect of demographics and to account for within-subject correlation. All statistical analyses were conducted using v 9.4 of the SAS System for Windows (SAS Institute Inc, Cary, North Carolina).
**Table II. Characteristics of the study population**

| Cohorts | PT | | Term | | | Total | | | P value |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Delivery mode | | | | | | | | | | |
| Cesarean | 67 | 72.0 | 41 | 42.7 | 108 | <.001 |
| Vaginal | 26 | 28.0 | 55 | 57.3 | 81 | |
| Multiples | | | | | | | | | | |
| Singles | 54 | 58.1 | 94 | 97.9 | 148 | <.001 |
| Multiples | 39 | 41.9 | 2 | 2.1 | 41 | |
| Sex | | | | | | | | | | |
| Female | 46 | 49.5 | 37 | 38.5 | 83 | .13 |
| Male | 47 | 50.5 | 59 | 61.5 | 106 | |
| Race | | | | | | | | | | |
| White | 50 | 53.8 | 54 | 56.3 | 104 | .10 |
| Black/AA | 33 | 35.5 | 23 | 24.0 | 56 | |
| More than 1 race/others/unknown or not reported | 10 | 10.8 | 19 | 19.8 | 29 | |
| Ethnicity | | | | | | | | | | |
| Hispanic/Latino | 10 | 10.8 | 19 | 19.8 | 29 | .05 |
| Not Hispanic/Latino | 82 | 88.2 | 72 | 75.0 | 154 | |
| Unknown or not stated | 1 | 1.1 | 5 | 5.2 | 6 | |

AA, African American; PT, preterm.

From March 2013 through March 2015, we approached 539 full term and 297 preterm eligible families and enrolled 93 preterm and 96 term infants. Of the preterm cohort, the largest numbers of subjects were between 23.1 and 25.6 weeks gestation and 30.1 and 31.9 weeks gestation with the remainder fairly equally divided between the remaining 2-week blocks (Table II). The term and preterm cohorts were generally well matched although, as expected, there were significantly more preterm infants born by cesarean delivery than term infants and more multiple births among the preterm cohort (Table II).

**Respiratory Sample Testing**

Because of variability in the length of hospitalization, the number of specimens from each infant ranged from 1 to 18 with a total of 618 NICU samples that were fairly evenly distributed over all 4 seasons and represented 119 weeks at risk (Figure 1; available at www.jpeds.com). Eighty-nine of 96 term infants contributed 1 nose/throat swab during the birth hospitalization.

Postdischarge, we obtained a total of 489 samples (range of 1-6 per subject) during the first 4 months following hospitalization with 235 samples contributed by the preterm group. These samples were also fairly equally divided over all 4 seasons (Figure 1).

**Respiratory Infections**

Four infants with viral respiratory infections were identified in the NICU during the 119 weeks at risk. All 4 were less than 28 weeks gestation at birth and had been in the NICU an average of 11 weeks (Table III). Two infants were ill with respiratory symptoms within 48 hours of the weekly sampling. Log-rank test and Kaplan-Meier curve estimators suggest that the risk of acquiring a respiratory viral infection in preterm infants in the NICU was significantly lower than in the term cohort living in the community, and the risk was not associated with mode of delivery, multiple birth, or sex (Figure 2, A). These findings were confirmed in the intensity model with only a younger age as measured by the corrected gestational age significantly increasing the daily infection rate (hazard ratio 0.951, *P* = .002) when delivery mode, multiple births, sex, race, and ethnicity were included in the model.

Thirty-one preterm infants (33%) had late-onset sepsis evaluations in the NICU. Six infants had testing for respiratory viruses concomitant with routine bacterial cultures for the evaluation of sepsis, and 2 had viruses identified in the clinical laboratory via molecular methods that also were identified in weekly research samples as noted above (RSV, coronavirus 4) (Table III). The first infant had symptoms including sneezing, progressive congestion, and cough, and the clinical team suspected a viral respiratory infection. RSV was identified in the clinical laboratory and by weekly study sampling obtained the following day. The second infant had tachypnea and tachycardia and the following day had an elevated temperature to 38.1°C. Coronavirus 4 was identified by the clinical laboratory and in the study sample the following day. The third infant underwent a sepsis evaluation for bacterial infection 9 days before hRV was identified in a study sample. Worsening respiratory function prompted the sepsis investigation that included a tracheal aspirate for bacterial culture and Mycoplasma culture but no viral diagnostic studies were performed and the symptoms were attributed to evolving BPD when all routine cultures were negative. A weekly study specimen obtained 4 days before the sepsis evaluation was negative for respiratory pathogens. At the time of hRV identification,
no specific symptoms were noted in the infant. The fourth infant had influenza B identified in a weekly sample, had not undergone a sepsis evaluation in the prior 3 months, and was clinically asymptomatic. Thus, only 2 of 71 (2.8%) sepsis evaluations identified a viral respiratory infection, and both infants had symptoms suggestive of the diagnosis.

Following hospitalization, a majority of infants acquired a viral respiratory infection in the subsequent 4 months of life (Table IV; available at www.jpeds.com). Seventy-one percent of term babies were infected within 4 months with 27% acquiring a viral respiratory infection in the first 2 months of life. Preterm infants had a slightly higher rate of infection, with 37% acquiring at least 1 infection in the 2 months after discharge. However, the difference in the likelihood of acquiring at least 1 respiratory viral infection in the first 4 months between the 2 groups while living in the community was not significant ($P = .39$). Further, the log-rank test suggested no difference in the infection-free probability curves between the 2 groups after hospital discharge (Figure 2, B). The immediate respiratory viral infection rate after hospital discharge was not associated with mode of delivery, multiple birth, or sex by the marginal analyses. Although hRV was the predominant virus detected in both groups, 12 different viral species were identified in infants in the community (Table V; available at www.jpeds.com).

The number of sick visits for respiratory symptoms was not different between the 2 groups of infants living in the community. Fourteen percent of preterm infants had 1-2 sick visits in the first 4 months following discharge, compared with 17% of term infants ($P = .66$), (Table VI; available at www.jpeds.com).

We prospectively evaluated a large group of preterm and term newborns for viral respiratory infections from birth through hospital discharge followed by the first 4 months in the community and found a very low rate (4%) of viral respiratory infections in our NICU environment. This is in contrast to the findings of Bennett et al$^5$ who followed 50 preterm infants with biweekly sampling for 1 year and noted a viral respiratory infection in 52%. Our NICU infection rate was significantly lower than both the rate in term infants living in the community and in preterm infants once discharged from the hospital. Other variables that were associated with preterm birth were not associated with the risk of acquiring a viral respiratory infection while still being cared for in the NICU suggesting that the location of care was the key factor responsible for this finding. Our data support the conclusion that it is possible to limit the frequency of respiratory viral infections in premature infants in the NICU.

Our NICU employs standard infection prevention strategies including hand hygiene and gloves for all patient contact with visitor restrictions during the winter months and

![Figure 2. A, Kaplan-Meier curve estimators and log-rank test for preterm NICU samples compared with term home samples, B, Preterm home samples compared with term home samples.](image)

**Discussion**

We prospectively evaluated a large group of preterm and term newborns for viral respiratory infections from birth through hospital discharge followed by the first 4 months in the community and found a very low rate (4%) of viral respiratory infections in our NICU environment. This is in contrast to the findings of Bennett et al$^5$ who followed 50 preterm infants with biweekly sampling for 1 year and noted a viral respiratory infection in 52%. Our NICU infection rate was significantly lower than both the rate in term infants living in the community and in preterm infants once discharged from the hospital. Other variables that were associated with preterm birth were not associated with the risk of acquiring a viral respiratory infection while still being cared for in the NICU suggesting that the location of care was the key factor responsible for this finding. Our data support the conclusion that it is possible to limit the frequency of respiratory viral infections in premature infants in the NICU.

![Table VII. Hazard ratio estimates from the intensity model of days to virus infection in the first 4 months after hospital discharge](table)

| Descriptions               | Hazard ratio | Lower  | Upper  | $P$ value |
|---------------------------|--------------|--------|--------|-----------|
| Corrected gestational age | 0.582        | 0.535  | 0.632  | <.0001    |
| Multiples vs singles      | 0.368        | 0.246  | 0.549  | <.0001    |
| Female vs male            | 0.616        | 0.431  | 0.880  | .036      |
| Black/AA vs White         | 0.522        | 0.344  | 0.792  | .034      |
exclusion of staff and visitors with respiratory symptoms throughout the year. These measures are similar to those reported by Homaira et al in their prospective surveillance study of nosocomial RSV infection where a similar low rate of infection was detected. Although Bennett et al reported that all staff performed an extended hand and arm scrub on arrival to their units, with gloves used for all patient contact there is no information given on hand hygiene before and after patient care or visitation practices so it is difficult to compare practices between the centers.

During the 24 months of this study our unit was arranged in multipatient rooms and since that time, we have moved to a new facility with all single patient rooms. Although not yet formally evaluated, we speculate that many families visit more frequently and stay for more extended periods when there are single patient rooms such that our low infection rate may have been due to inadvertent limitations on family visitation in the previous physical space.

Our data are consistent with those of Ronchi et al who found that hospitalized infants with respiratory viral infections were likely to have symptoms of congestion and rhinorrhea and be tested based on clinical suspicion. We did not find substantial undetected respiratory viral infections associated with non-specific concerns for sepsis in our NICU but instead that infants with respiratory infections had suggestive symptoms. Because only 2.8% of sepsis evaluations in the study population were associated with viral detection by surveillance sampling, including viral investigation routinely with sepsis evaluations will have very low yield in this NICU.

The acquisition of a viral respiratory infection in the NICU setting has been linked with a longer length of hospital stay as well as markers of more significant lung disease of prematurity. In this regard, it is interesting to note the lower rate of chronic lung disease in our NICU very low birth weight population from 2006 to 2014 (17.1%) than comparable units of nosocomial RSV infection where a similar low rate of infection was detected. Although Bennett et al reported that all NICU, and infection rates appear to vary substantially between different centers based upon limited prior reports.

Another potential limitation is the frequency of sample collection. We obtained nose and throat samples from our population once weekly while in the NICU; this may have led to a decreased detection rate. Previous studies have shown that respiratory samples obtained from the nasal turbinates with a flocked swab have similar sensitivity to nasopharyngeal aspirates and that adding a throat swab to a nasal swab improves the detection of respiratory viruses. In addition, viral identification by polymerase chain reaction is highly sensitive, and the TAC platform has been shown to have at least equivalent detection of viral nucleic acid as other commercially available detection systems. Prior studies also have identified extended periods of shedding of respiratory viruses (27 days), especially in younger age cohorts, suggesting that our sampling should have been sufficient to identify infections in our NICU population.

Once discharged from the hospital, both preterm and term infants acquired viral respiratory infections at a similar rate and reported an equivalent number of symptomatic illnesses. Male sex, white race, and younger age were associated with an increased daily risk of acquiring an infection. Because our study design focuses on the first 6 months of life, it is difficult to compare our results with other studies. However, respiratory infection rates have been reported to be higher in younger infants than in children over the age of 12 months, with male sex a risk factor for acquiring hRV infection. White race and young age also have been associated with severity of bronchiolitis suggesting that our findings are consistent with prior research.

The strengths of this study include the prospective, longitudinal design with repeated sampling of a large number of preterm and term infants. In addition, the study spanned all 4 seasons of the year and included infants while hospitalized and also while living in the community, both when well and ill with respiratory symptoms.

Our study has limitations. First, our center is a regional referral center creating some difficulties for enrollment into long-term prospective studies and limiting the percentage of subjects we were able to enroll. In addition, this study included only 1 NICU, and infection rates appear to vary substantially between different centers based upon limited prior reports.

We are indebted to all of the children and families that participated in this study. We thank the University of Rochester Medical Center Obstetrics and NICU Nursing Teams for subject recruitment, sample collection, data management, and coordination: Elizabeth Werner, Gerry Loftus, Tanya Scalise, Dee Mauffett, Amy Murphy, Lisa Denmark, Heidi Hueck, Jennifer Carnahan, Kenneth Schnabel, Lynne Shelley, Sara Misra, Claire Wyman, and Jennifer Dutra.
Figure 1. Sampling seasons by cohort and location. *PT*, preterm.
### Table I. Primers and probes used in TAC detection system

| Pathogens                  | Forward           | Final conc. | Reverse           | Final conc. | Key                  | Probe                        | Final conc. | Authors          | Year |
|----------------------------|-------------------|-------------|-------------------|-------------|----------------------|------------------------------|-------------|------------------|------|
| Influenza A                | GAC CRA TCC TGT CAC TCG TGA C | 800 nM      | AGG GCA TTY TGG ACA AAG ACG GTA A | 800 nM      | FAM-TGC AGT CCT GCG TCA CTG GGC ACB-BHQ1 | 200 nM CDC 11 Biosearch Technologies 12 | 2009 |
| Influenza B                | TCC TCA AYT CAC TCT TCG AGC G | 800 nM      | CGG TGC TCT TGA CCA AAT TGG | 800 nM      | FAM-CCA ATT CGA GCT GAA ACT GCG GTG-BHQ1 | 200 nM CDC 11 Biosearch Technologies 12 | 2009 |
| RSV                       | GGC AAA TAT GGA AAG ATA GTA A | 500 nM      | TCT TTT TGG AGG ATA TGG TAV TAY TGA ACA A | 250 nM      | FAM-CTG TAT AGT TGC CCT GCT GAA GCT-BHQ1 | 50 nM Fry et al 13 Kodian et al 14 | 2010 |
| PIV 1                     | AGA AGT CTT CAAG YTT CTT AAT TCR TAT | 500 nM      | TCG TCA CCT AAG TAT TAR TTY TGA GTT | 750 nM      | FAM-ATA GCC CAA AGA AGA TTT GTG TGC AGA CTA TCG CA A | 50 nM Weinberg et al 15 | 2013 |
| PIV 2                     | GCA TTT CCA AAT TCC ACG AGT ATG A | 750 nM      | ACC TCC TGT TSG AGT AGC TAC GAC TCA A | 750 nM      | FAM-CCA TTT ACC TTT AA AGT ATG GAA TAA ACG GCA GTA AGA | 50 nM Kodian et al 14 | 2011 |
| PIV 3                     | TGG YTC AAT CTC ACG ACG AAT ATG G | 750 nM      | TAC CCG AGA AAT ATT ATG TCG | 500 nM      | FAM-CCG RTC TGT TGG TGG ACC AGG GAT ATG TCA CAA A | 200 nM Kodian et al 14 | 2011 |
| hRV                       | CY, A GCC TGC GTG NY | 1000 nM     | GAA ACA AGG GCA ACC AAA GTA | 1000 nM      | FAM-CCG GCC GGC TCC TAG AGG YGG C-BHQ1 | 100 nM Harvey et al 16 | 2016 |
| EV                        | GGT GCC TGC GTG GGC | 1000 nM     | GAA AGG AGC AGG ACC AAA GTA | 1000 nM      | FAM-TCC GCC GCC TTA GYG YGG C-BHQ1 | 100 nM Harvey et al 16 | 2016 |
| ADV                       | GAC GATG GTC TTA CAT CAC CAT C | 500 nM      | GAC GGC TGG GTG TTT CTA AAT CT | 500 nM      | FAM-CTG AGC AGA CCC GGG GGT ATC AGG TAC TCC CAA-BHQ1 | 100 nM Heim et al 17 Kodian et al 14 | 2003 |
| Coronavirus 1 (229E)       | CAG TCA AAT GGG CTG ATG CA | 750 nM      | AAA GGG CTA TAA AGA GAA TAA GGT ATT CT | 500 nM      | FAM-CCC TGA CCA CGG CCT TGT GTC TCA-BHQ1 | 50 nM Dare et al 18 | 2007 |
| Coronavirus 2 (NI63)       | GAC CAA AGC ACT GAA TAA CAT TCT CC | 250 nM      | ACC TAA TAA GCG CCT TCT TCC TAG ACC C | 250 nM      | FAM-AAC AGG CT T T CC ACG AGT TTT CCT CAAG CTG AG | 50 nM Dare et al 18 | 2007 |
| Coronavirus 3 (OC43)       | CGA TGA GGC TAT TCC GAC TAG GT | 500 nM      | CCT TCG TGA CCT TACC AAT GTA ATG TA | 750 nM      | FAM-CCG GCC GGC TCC ACC ACT TCC C-BHQ1 | 50 nM Dare et al 18 | 2007 |
| Coronavirus 4 (HKU1)       | CCT TGC GAA TGA ATG TGT C | 100 nM      | TGT TCA CAC TGC TGC TAG CAC TAC | 750 nM      | FAM-TCT GTG GGC GTC TGG ATG TAG TTA ACC GTG-BHQ1 | 50 nM Dare et al 18 | 2007 |
| RNP3                      | GTA AWA GTG AGG GCT GGA AAG | 600 nM      | TGT TTG GCC TCA TGC TGT AAT AAA GGA | 600 nM      | FAM-CC GCA GTC TTC GTC ACC ACT CCC TAT TCC C-BHQ1 | 200 nM Weinberg et al 19 | 2013 |
| GAPDH                     | Life Technologies |            |                   |             |                      |                              |             |                   |      |
| hMPV                      | CA TGA TCT GCC TGC TGA YCT RAA | 600 nM      | ACT GCC GCA CAA CAT TAA GRA A | 600 nM      | FAM-TGG CYY TGA SGT CCT TCT GTA TAT AAG GAA GTA A | 100 nM Kodian et al 18 | 2011 |
| hBoV                      | TGG ACA CAA CCG YTA GGT TCT TAA | 500 nM      | CGG TCC GCC CCA ACA TAC | 500 nM      | FAM-CCA GGA TGT GGT GAA ACC AAA-BHQ1 | 100 nM Lu et al 20 | 2006 |
| hPeV                      | GTA AWA SWY GCC TCT GGG GCC AAA G | 500 nM      | GCC TCC WGR TCA CCT YAG TAA GAG T | 500 nM      | FAM-CCT RYG GGT ATG TGC TCG WGG GCA TCC TCC-BHQ1 | 200 nM Nix et al 21 Kodian et al 14 | 2011 |
| H influenzae              | ATG GCC GGA ACA TCA ATG A | 300 nM      | ACG CAT AGG AGG GAA AGT GTT | 300 nM      | FAM-CCG TAA TTG GAA GAT ATG MGB | 100 nM Meyer et al 22 | 2012 |
| S pneumoniae              | AGC TGA CAA TGC AGA TGA A | 500 nM      | TGG TCG TTT ATT CCT ACA A | 500 nM      | FAM-CTG CCA AAA CGC TGG TAA CAG GGG A | 100 nM Carvalho et al 23 | 2007 |
| M pneumoniae              | TTT GGT AGC TTA CCA GGG ATA A | 500 nM      | GGT CGG CAA GAA TAA ATA AGA | 500 nM      | FAM-TGT AGC AGA CCC CAG AAG GGT-BHQ1 | 100 nM Winchell et al 24 | 2008 |
| C pneumoniae              | GGG CTA AAG AGG GTC TGG TTG | 500 nM      | AGA CCT TGT TCC AGT AGG TGT TCC T | 500 nM      | FAM-CCG TTA CAG ACA GAG SCC GCT GGC B-BHQ1 | 100 nM Mitchell et al 25 | 2009 |
| M hominis                 | TCA CTA CAG GCA GTT TT TCG ACG A | 300 nM      | TGT TCA ATT AGG GGC ATG GTT | 300 nM      | FAM-CCA AAT ATG TTA ATC TAT GCT GTG ATG-BHQ1 | 200 nM Kodani et al 14 | 2011 |
| RecA                      | CATACAGAAGGTGCTGGTTGG | 500 nM      | CTATAGGATTTAAGTGGTGACATAC | 500 nM      | FAM-CCG AAT ATG TTA ATC TAT GCT GTG ATG-BHQ1 | 200 nM Kodani et al 14 | 2011 |
| B pertussis (target I)    | CAA GCC CGA AGG CTT CAT | 300 nM      | GAG TGC TAG GTG TGG GGC TAA | 300 nM      | FAM-CAG TGC GCC TGT GGT GAC TGG B-BHQ1 | 300 nM Tatti et al 26 | 2008 |
| Bordetella pertussis (target II) | CCG CAG CTC GAT CCA | 700 nM      | GAT AGC GCG TCA ACC AGT | 700 nM      | FAM-AAT AGG TCG AAT CTA AGG GCA A-BHQ1 | 300 nM Tatti et al 26 | 2008 |

**ADV**, adenovirus; **B pertussis**, Bordetella pertussis; **C pneumoniae**, Chlamydia pneumoniae; **D concentra.**, EV, enterovirus; **GAPDH**, Glyceraldehyde 3-phosphate dehydrogenase; **H influenzae**, Haemophilus influenzae; **hBoV**, human bocavirus; **hMPV**, human metapneumovirus; **hPeV**, human parvovirus; **M hominis**, Mycoplasma hominis; **M pneumoniae**, Mycoplasma pneumoniae; **P**, parainfluenza virus; **RNP3**, Human RNP3; **S pneumoniae**, Streptococcus pneumoniae.

Underlining and boldface indicate a locked nucleic acid (Exiqon, Woburn, Massachusetts). Quotation marks around a letter indicate an internal quencher.

*§FAM 3′BHQ1*

5′-FAM 3′BHQ1.}

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**RAW TEXT END**
### Table IV. Total number of viral respiratory infections in the first 4 months after hospital discharge by cohort

| Total number of positive infection | 0   | 1   | 2   | 3   | 4   | Total |
|-----------------------------------|-----|-----|-----|-----|-----|-------|
|                                    | n % | n % | n % | n % | n % | n %   |
| PT: home                          | 27  | 35.5| 25  | 32.9| 17  | 22.4  | 7     | 9.2  | .   | 76  |
| Term: home                        | 22  | 29.0| 32  | 42.1| 17  | 22.4  | 3     | 4.0  | 2   | 2.6 | 76  |
| Total                             | 49  | 32.2| 57  | 37.5| 34  | 22.4  | 10    | 6.6  | 2   | 1.3 | 152 |

*PT*, preterm.  
*P* value = .39 for test of same rate of ever infection between 2 cohorts at home.  
Full-term cohort, *n* = 76 because of study attrition.  
Preterm cohort, *N* = 76 due to death, transfer to outside hospital, continuing hospitalization.

### Table V. Viruses causing infection after hospital discharge

|                   | PT | Term | Total |
|-------------------|----|------|-------|
|                   | n  | n    | n     |
| Adenovirus        | 2  | 2    | 4     |
| Bocavirus         | 1  | 2    | 3     |
| Coronavirus 1     | 0  | 1    | 1     |
| Coronavirus 2     | 7  | 2    | 9     |
| Coronavirus 3     | 2  | 1    | 3     |
| Coronavirus 4     | 5  | 0    | 5     |
| Enterovirus       | 8  | 4    | 12    |
| Parainfluenza 3   | 2  | 1    | 3     |
| Parechovirus      | 1  | 0    | 1     |
| RSV               | 3  | 7    | 10    |
| Rhinovirus        | 49 | 62   | 111   |
| Metapneumovirus   | 0  | 1    | 1     |

### Table VI. Number of illness visits in first 4 months after hospital discharge by cohort

| Total number of illness visits | 0   | 1   | 2   | Total |
|-------------------------------|-----|-----|-----|-------|
|                               | n   | %   | n   | %    | n   |
| PT                            | 65  | 85.5| 10  | 13.2 | 1   | 1.3 | 76  |
| Term                          | 63  | 82.9| 11  | 14.5 | 2   | 2.6 | 76  |
| Total                         | 128 | 84.2| 21  | 13.8 | 3   | 2.0 | 152 |

*P* value = .66 for test of same rate of ever sick visit between 2 cohorts at home.  
Full-term cohort, *N* = 76 because of study attrition.  
*PT* cohort, *N* = 76 because of a death, transfer to outside hospital, continuing hospitalization.