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Viral Respiratory Infections in Preterm Infants during and after Hospitalization

Mary T. Caserta, MD[1], Hongmei Yang, PhD[2], Steven R. Gill, PhD[3], Jeanne Holden-Wiltse, MPH[2], and Gloria Pryhuber, MD[1,4]

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	RSV	Respiratory syncytial virus
hRV	Human rhinovirus	TAC	Taqman array card
NICU	Neonatal intensive care unit		

From the [1]Department of Pediatrics; [2]Department of Biostatistics and Computational Biology; [3]Department of Microbiology and Immunology; and [4]Department of Environmental Medicine, University of Rochester Medical Center, Rochester, NY

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Methods

Term (≥$37^{0/7}$ 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 $36^{0/7}$ weeks through $36^{6/7}$ 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.[8-10] 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 I**; available at www.jpeds.com).[11-28] 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 χ^2 test for categorical variables. Corresponding nonparametric 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[29] 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

	PT		Term		Total	
	n	%	n	%	n	*P* value
Cohorts						
Term	.	.	96	100.00	96	
PT 23-25 6/7 wk	21	22.6	.	.	21	
PT 26-27 6/7 wk	11	11.8	.	.	11	
PT 28-29 6/7 wk	10	10.8	.	.	10	
PT 30-31 6/7 wk	21	22.6	.	.	21	
PT 32-33 6/7 wk	14	15.1	.	.	14	
PT 34-35 6/7 wk	16	17.2	.	.	16	
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.

Results

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 71 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,

Table III. Characteristics of infants who acquired a viral respiratory infection during the NICU hospitalization

Gestational ages (wk) at birth	Gestational ages (wk) at infection	Age (d) at infection	Sex	Race	Virus
$26^{6/7}$	$39^{2/7}$	88	Female	Black/AA	RSV
$23^{6/7}$	$26^{6/7}$	22	Female	Black/AA	Rhinovirus
$24^{5/7}$	$39^{6/7}$	107	Female	Black/AA	Coronavirus 4
$23^{1/7}$	$37^{3/7}$	101	Male	More than 1 race/others/unknown or not reported	Influenza B

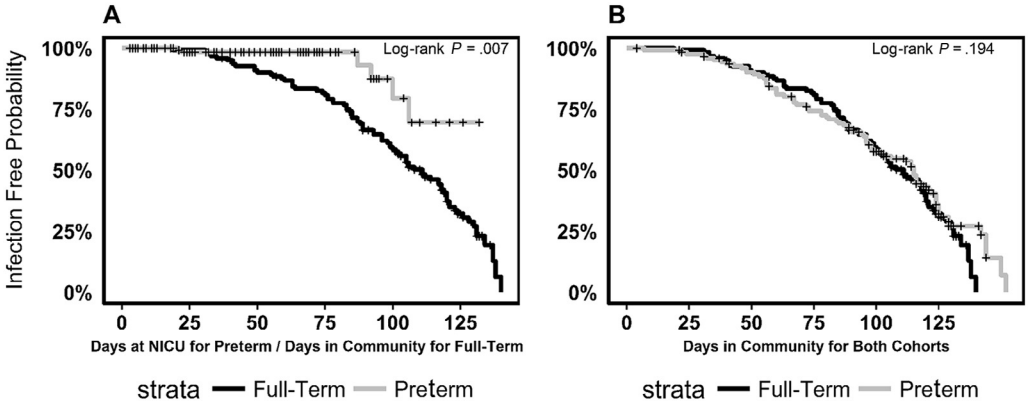

Figure 2. **A,** Kaplan-Meier curve estimators and log-rank test for preterm NICU samples compared with term home samples, **B,** Preterm home samples compared with term home samples.

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).

The intensity model was applied to determine the factors associated with the time to acquisition of a viral respiratory infection once discharged from the hospital and included age as measured by corrected gestational age, delivery mode, multiple birth, sex, race, and ethnicity, with days to infection as the outcome. The model fitting after variable selection procedures showed that the daily infection rate for all infants following hospital discharge was higher for younger infants as measured by a smaller corrected gestational age (range for the preterm cohort was 38.4-64.3; full term cohort range was 40.1-59.7). Singleton births, boys, and white race were associated with a higher daily infection rate when other covariates were held constant (**Table VII**).

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.

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

Table VII. Hazard ratio estimates from the intensity model of days to virus infection in the first 4 months after hospital discharge

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[30] in their prospective surveillance study of nosocomial RSV infection where a similar low rate of infection was detected. Although Bennett et al[5] 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[7] 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 nonspecific 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.[5] 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 that belong to the Vermont Oxford Network (2006-2014, 25.4%) with a risk adjusted observed to expected average of -12% (data available from authors upon request).

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.[31] White race and young age also have been associated with severity of bronchiolitis suggesting that our findings are consistent with prior research.[32]

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.

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.[33,34] 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.[16] Prior studies also have identified extended periods of shedding of respiratory viruses (≥7 days), especially in younger age cohorts, suggesting that our sampling should have been sufficient to identify infections in our NICU population.[5,35-39] Our pre- and postdischarge TAC platform and sampling techniques were identical and readily detected viral infections in both term and preterm infants after discharge, supporting the study design. A further limitation is that we did not obtain respiratory samples specifically at the time of suspected sepsis while the preterm infants were in the NICU, and our sampling schedule differed between hospitalized infants and those living in the community. Nevertheless, our data suggest that weekly sampling was sufficient to identify both symptomatic and asymptomatic infections in hospitalized preterm infants. Once living in the community, the monthly sampling schedule likely missed asymptomatic infections in both preterm and term infants. However, as the schedule was the same between these 2 groups and we were comparing the infection rates between them, we do not believe this limitation substantially changes our results. ■

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Reprint requests: Mary T. Caserta, MD, Division of Pediatric Infectious Diseases, University of Rochester Medical Center, 601 Elmwood Ave, Box 690, Rochester, NY 14642. E-mail: mary_caserta@urmc.rochester.edu

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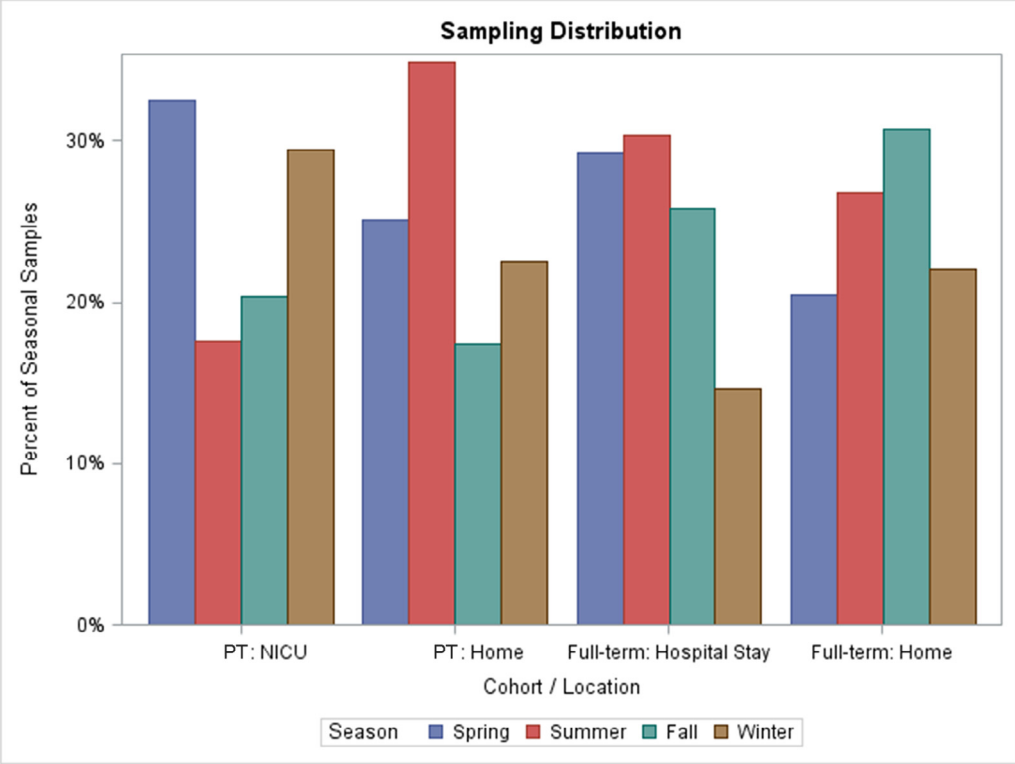

Figure 1. Sampling seasons by cohort and location. *PT*, preterm.

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 CTC TGA C	800 nM	AGG GCA TTY TGG ACA AAK CGT CTA	800 nM	#	FAM-TGC AGT CCT CGC TCA CTG GGC ACG-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 GCA GCT GAA ACT GCG GTG-BHQ1	200 nM	CDC[11] Biosearch Technologies[12]	2009
RSV	GGC AAA TAT GGA AAC ATA CGT GAA	500 nM	TCT TTT TCT AGG ACA TTG TAY TGA ACA G	250 nM	*	FAM-CTG TGT ATG TGG AGC CTT CGT GAA GCT-BHQ1	50 nM	Fry et al [13] Kodani et al[14]	2010 2011
PIV 1	ACA AGT TGT CAA YGT CTT AAT TCR TAT	500 nM	TCG GCA CCT AAG TAR TTY TGA GTT	500 nM	†	FAM-ATA GGC CAA AGA "T"TG TTG TCG AGA CTA TTC CAA	50 nM	Weinberg et al[15]	2013
PIV 2	GCA TTT CCA ATC TAC AGG ACT ATG A	750 nM	ACC TCC TGG TAT AGC AGT GAC TGA AC	750 nM	†	FAM-CCA TTT ACC "T"AA GTG ATG GAA TCA ATC GCA AA	50 nM	Kodani et al[14]	2011
PIV 3	TGG YTC AAT CTC AAC AAC AAG ATT TAA G	750 nM	TAC CCG AGA AAT ATT ATT TTG CC	500 nM	†	FAM-CCC RTC TG"T" TGG ACC AGG GAT ATA CTA CAA A	200 nM	Kodani et al[14]	2011
hRV	CY·**A** GCC **T**GC GTG GY	1000 nM	GAA ACA CGG ACA CCC AAA GTA	1000 nM	‡*	FAM-TCC TCC GGC CCC TGA ATG YGG C-BHQ1	100 nM	Harvey et al[16]	2016
EV	GGT GGC TGC GTT GGC	1000 nM	GAA ACA CGG ACA CCC AAA GTA	1000 nM		FAM-TCC TCC GGC CCC TGA ATG YGG C-BHQ1	100 nM	Harvey et al[16]	2016
ADV	GCC CCA GTG GTC TTA CAT GCA CAT C	500 nM	GCC ACG GTG GGG TTT CTA AAC TT	500 nM	*	FAM-TGC ACC AGA CCC GGG CTC AGG TAC TCC GA-BHQ1	100 nM	Heim et al[17] Kodani et al[14]	2003 2011
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 CGA CCA CGT TGT GGT TCA-BHQ1	50 nM	Dare et al[18]	2007
Coronavirus 2 (NL63)	GAC CAA AGC ACT GAA TAA CAT TTT CC	250 nM	ACC TAA TAA GCC TCT TTC TCA ACC C	250 nM	†	FAM-AAC ACG CT"T" CCA ACG AGG TTT CTT CAA CTG AG	50 nM	Dare et al[18]	2007
Coronavirus 3 (OC43)	CGA TGA GGC TAT TCC GAC TAG GT	500 nM	CCT TCC TGA GCC TTC AAT ATA GTA ACC	750 nM	*	FAM-TCC GCC TGG CAC GGT ACT CCC T-BHQ1	50 nM	Dare et al[18]	2007
Coronavirus 4 (HKU1)	CCT TGC GAA TGA ATG TGC T	100 nM	TTG CAT CAC CAC TGC TAG TAC CAC	750 nM	*	FAM-TGT GTG GCG GTT GCT ATT ATG TTA AGC CTG-BHQ1	50 nM	Dare et al[18]	2007
RNP3	CCA AGT GTG AGG GCT GAA AAG	600 nM	TGT TGT GGC TGA TGA ACT ATA AAA GG	600 nM	*	FAM-CC CCA GTC TCT GTC AGC ACT CCC TTC-BHQ1	200 nM	Weinberg et al[15]	2013
GAPDH	Life Technologies								
hMPV	CAA GTG TGA CAT TGC TGA YCT RAA	600 nM	ACT GCC GCA CAA CAT TTA GRA A	600 nM	*	FAM-TGG CYG TYA GCT TCA GTC AAT TCA ACA GA-BHQ1	100 nM	Kodani et al[14]	2011
hBoV	TGC AGA CAA CGC YTA GTT GTT T	500 nM	CTG TCC CGC CCA AGA TAC A	500 nM	*	FAM-CCA GGA TTG GGT GGA ACC TGC AAA-BHQ1	100 nM	Lu et al[19]	2006
hPeV	GTA ACA SWW GCC TCT GGG SCC AAA AG	400 nM	GGC CCC WGR TCA GAT CCA YAG T	400 nM	*	FAM-CCT RYG GGT ACC TYC WGG GCA TCC TTC-BHQ1	200 nM	Nix et al[20] Kodani et al[14]	2008 2011
H influenzae	ATG GCG GGA ACA TCA ATG A	300 nM	ACG CAT AGG AGG GAA ATG GTT	300 nM	§	FAM-CGG TAA TTG GGA TCC AT-MGB	100 nM	Meyler et a.[21]	2012
S pneumoniae	ACG CAA TCT AGC AGA TGA AGC A	500 nM	TCG TGC GTT TTA ATT CCA GCT	500 nM	*	FAM-TGC CGA AAA CGC TTG ATA CAG GGA G-BHQ1	100 nM	Carvalho et al[22] Kodani et al[14]	2007 2011
M pneumoniae	TTT GGT AGC TGG TTA CGG GAA T	500 nM	GGT CGG CAC GAA TTT CAT ATA AG	500 nM	*	FAM-TGT ACC AGA GCA CCC CAG AAG GGC T- BHQ1	100 nM	Winchell et al[23] Kodani et al[14]	2008 2011
C pneumoniae	GGG CTA TAA AGG CGT TGC TTT	500 nM	AGA CTT TGT TCC AGT AGC TGT TGC T	500 nM	*	FAM-CC TTG CCA ACA GAC GCT GGC G-BHQ1	100 nM	Mitchell et al[24] Kodani et al[14]	2009 2011
M hominis	TCA CTA AAC CGG GTA TTT TCT AAC AA	300 nM	TTG GCA TAT ATT GCG ATA GTG CTT	300 nM	*	FAM-CTA CCA ATA ATT TTA ATA TCT GTC GGT ATG-BHQ1	200 nM	Ferandon et al[25]	2011
Ureaplasma	CATACAGAAGGTGCTGGTGG	500 nM	CTTAGGATTTAAGTGGTGACATAC	500 nM	*	FAM-AGC TTC TAC AAA CCC AAC TAT TCC-BHQ1	400 nM	Xiao et al[26] Yi et al[27]	2010 2005
B pertussis (target I)	CAA GGC CGA ACG CTT CAT	300 nM	GAG TTC TGG TAG GTG TGA GCG TAA	300 nM	*	FAM-CAG TCG GCC TTG CGT GAG TGG G-BHQ1	300 nM	Tatti et al[28] Kodani et al.[14]	2008 2011
Bordetella pertussis (target II)	CGC CAG CTC GTA CTT C	700 nM	GAT ACG GCC GGC ATT	700 nM	*	FAM-AAT ACG TCG ACA CTT ATG GCG A-BHQ1	300 nM	Tatti et al[28] Kodani et al[14]	2008 2011

ADV, adenovirus; *B pertussis*, *Bordetella pertussis*; *C pneumoniae*, *Chlamydophila pneumonia*; *conc.* concentration; *EV*, enterovirus; *GAPDH*, Glyceraldehyde 3-phosphate dehydrogenase; *H influenzae*, *Haemophilus influenzae*; *hBoV*, human bocavirus; *hMPV*, human metapneumovirus; *hPeV*, human parechovirus; *M hominis*, *Mycoplasma hominis*; *M pneumoniae*, *Mycoplasma pneumoniae*; *PIV*, parainfluenza virus; *RNP3*, Human RNAse P; *S pneumoniae*, *Streptococcus pneumonia*.

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

*5′FAM 3′BHQ1.

†Internally labeled probes:5′FAM "T" = BHQ1-dT 3′ = phosphorylated.

‡**Y** = mix of C and T (pyrimidine) nucleosides, similar to "P" as listed in Harvey et al.[16] P is a universal base; (P) = dP-CE (pyrimidine derivative), designed to base pair with either A or G.

§5′FAM 3′MGB.

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,[2] transfer to outside hospital,[2] continuing hospitalization.[12]

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	%	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 o death,[2] transfer to outside hospital,[2] continuing hospitalization.[12]