Prospective Cohort Study of Severe Bronchiolitis and Risk of Recurrent Wheezing

Funded by:
National Institute of Allergy and Infectious Diseases (NIAID), Division of Allergy, Immunology and Transplantation (DAIT)

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Draft or
Version Number: 1.6

August 21, 2013
STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46)
- Completion of Human Subjects Protection Training
- NIH Clinical Terms of Award (1U01AI087881-01A1)
INVESTIGATOR SIGNATURE PAGE

Protocol:                                                   Version/Date:  
                                                        Version 1.6 / August 21, 2013

Site Principal Investigator:

Title: Prospective Cohort Study of Severe Bronchiolitis and Risk of Recurrent Wheezing

Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)

INSTRUCTIONS: The site Principal Investigator should print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent. After signature, please return the original of this form by surface mail to:

Carlos A. Camargo, Jr., MD, DrPH
Emergency Medicine Network (EMNet) Coordinating Center
Massachusetts General Hospital
326 Cambridge Street, Suite 410
Boston, MA 02114

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the NIAID and the IRB.

____________________________________  __________________________________  ____________
Site Principal Investigator (Print)                                                   Site Principal Investigator (Signature)  Date
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# LIST OF ABBREVIATIONS

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<th>Abbreviation</th>
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<tr>
<td>AAIB</td>
<td>Asthma, Allergy and Inflammation Branch</td>
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<td>API</td>
<td>Asthma Predictive Index</td>
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<td>ARI</td>
<td>Acute Respiratory Infection</td>
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<tr>
<td>ASIgE</td>
<td>Allergen Specific Immunoglobulin E</td>
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<tr>
<td>CBC</td>
<td>Complete blood count</td>
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<tr>
<td>DAIT</td>
<td>Division of Allergy, Immunology, and Transplantation</td>
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<tr>
<td>ED</td>
<td>Emergency Department</td>
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<td>EMNet</td>
<td>Emergency Medicine Network</td>
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<tr>
<td>HIPAA</td>
<td>Health Insurance Portability and Accountability Act</td>
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<tr>
<td>hMPV</td>
<td>Human metapneumovirus</td>
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<tr>
<td>IATA</td>
<td>International Air Transport Association</td>
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<tr>
<td>ICU</td>
<td>Intensive care unit</td>
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<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
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<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>LRTI</td>
<td>Lower respiratory tract infection</td>
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<td>mAPI</td>
<td>Modified Asthma Predictive Index</td>
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<td>N</td>
<td>Number</td>
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<tr>
<td>NIAID</td>
<td>National Institute of Allergy and Infectious Diseases</td>
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<td>NIH</td>
<td>National Institutes of Health</td>
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<tr>
<td>NPA</td>
<td>Nasopharyngeal aspirate</td>
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<tr>
<td>PHS</td>
<td>Partners HealthCare Systems</td>
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<tr>
<td>PI</td>
<td>Principal Investigator</td>
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<tr>
<td>PIV</td>
<td>Parainfluenza virus</td>
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<tr>
<td>RANTES</td>
<td>Regulated on activation normal T cell expressed and secreted</td>
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<tr>
<td>RBEL</td>
<td>RSV Bronchiolitis in Early Life</td>
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<tr>
<td>REDCap</td>
<td>Research Electronic Data Capture</td>
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<tr>
<td>RSV</td>
<td>Respiratory syncytial virus</td>
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<td>HRV</td>
<td>Human rhinovirus</td>
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<tr>
<td>WIND</td>
<td>Wheezing Index</td>
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<tr>
<td>25(OH)D</td>
<td>25-hydroxyvitamin D</td>
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PROTOCOL SUMMARY

Title: Prospective Cohort Study of Severe Bronchiolitis and Risk of Recurrent Wheezing

Population: 1,000 male and female children, age <1 year, who are admitted to the hospital with an attending physician-diagnosis of bronchiolitis.

Number of Sites: Approximately 20 hospitals

Study Duration: Study participant enrollment is for 6 months (November 1 to April 30) in up to 3 consecutive years. We will follow study participant until age 6 years. Total study duration is approximately 5-6 years depending on age at enrollment.

Duration of Individual Subject Participation:
1-2 hours in hospital, then approximately 30 minutes to 1 hour per year in follow-up activities.

Objectives:
Primary:
- Aim 1 - To examine the association between (a) the infectious etiology of a child's severe bronchiolitis, and (b) the severity of this illness, and the subsequent development of recurrent wheezing by age 3 years.

Secondary:
- Aim 2 - To examine the association between the level of serum 25-hydroxyvitamin D (25[OH]D) during severe bronchiolitis and the subsequent development of recurrent wheezing by age 3 years.

- Aim 3 - To combine these clinical and laboratory data to derive the wheezing index (WIND), a new clinical index that will identify children at higher risk of developing recurrent wheezing by age 3 years.

Exploratory:
- Exploratory Aim - To create a biorepository that will permit future testing of novel mechanistic hypotheses.
Future (funding to be obtained externally or internally):

- Future Aim 1 - To examine the persistence of the infectious etiology of a child’s severe bronchiolitis 3-weeks after the hospitalization and the subsequent risk of developing recurrent wheezing by age 3 years.

- Future Aim 2 – To examine the association between the infectious etiology of acute respiratory infections requiring health care utilization and the subsequent risk of developing recurrent wheezing by age 3 years.

- Future Aim 3 – To examine the association between the presence of virus when a child is healthy and the subsequent risk of developing recurrent wheezing by age 3 years.
Schematic of Study Design:

Pre-study preparation

In-hospital data collection

Planned ~50 study participants per site: - ~40 Medical Ward

Clinical data, blood serum to: EMNet Coordinating Center Massachusetts General Hospital

Nasopharyngeal aspirates and nasal swabs to: Baylor College of Medicine

Blood pellet to: University of Arizona

OVER 3 YEARS = 1,000 total

Post-hospital data collection

1. 1-week post discharge phone call
2. 3-week “clearance” nasal swab and call
3. Seasonal nasal swab x 2
4. ARI survey and nasal swab x 3 years
5. 6-month follow-up phone calls x 4 years
6. Primary care & hospital records x 4 years
1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Epidemiology of Bronchiolitis
Bronchiolitis is the leading cause of hospitalization for US infants (1, 2) and the associated hospitalization costs are >$500 million per year (3). In a nationally representative sample, bronchiolitis hospitalization rates increased 2.4-fold from 1980 to 1996 (1). In a Tennessee Medicaid database, from 1996 to 2003, there was a 41% increase in outpatient, emergency department (ED), and inpatient bronchiolitis episodes (4).

Microbiology of Bronchiolitis
Respiratory syncytial virus (RSV) is the most common pathogen associated with bronchiolitis (5, 6) and most children are infected with RSV by age 2 years (7, 8). Other viruses that have been linked to bronchiolitis include human rhinovirus (HRV) (9, 10), human metapneumovirus (hMPV) (11), influenza viruses types A & B (12, 13), parainfluenza viruses types 1, 2 and 3 (PIV-1, 2, & 3) (14), adenovirus (15, 16), coronaviruses 229E, OC43, NL 63, & HKU1 (17-22), human bocavirus, (23-26), and polyomaviruses WU (27) and KI (28). The clinical relevance of these two polyomaviruses is unclear (29).

Wheezing After a Bronchiolitis Hospitalization
Over the past 50 years (30), several research groups have found in small cohorts of children hospitalized with bronchiolitis (n=75 to 206) a higher rate of recurrent wheezing within 3 years of admission (23-60%) compared to healthy controls (1-12%) (31-33). Importantly, many of the children hospitalized with bronchiolitis develop childhood asthma (34) and may even have longer term respiratory morbidity (35, 36). In a Swedish cohort of 47 infants hospitalized with RSV bronchiolitis, the cumulative prevalence of asthma at age 7 years was 30% in the RSV group versus 3% in the control group (37). In the RSV Bronchiolitis in Early Life (RBEL) cohort (n=206), 47% of children hospitalized with RSV developed asthma by age 6 years (38). In the Tucson Children’s Respiratory Study prospective birth cohort, having a RSV lower respiratory tract infection (LRTI) before age 3 years was an independent risk factor for wheezing up to age 11 years, but not at age 13 (39). Unlike the Swedish study and RBEL, nearly all of the Tucson children with RSV LRTI had mild (outpatient) bronchiolitis.

Viral Etiology and Wheezing (Significance of Aim 1a)
Although RSV is the most common pathogen associated with bronchiolitis (5, 6) and has been effectively used to define cohorts of children with bronchiolitis (32, 33, 40), other pathogens may have a stronger association with recurrent wheezing (41-45). The most intriguing virus, in terms of recurrent wheezing and asthma, is HRV. Several recent single center studies have linked
HRV infection to: infant wheezing (46, 47), infants with recurrent respiratory symptoms and abnormal lung function (48), later development of recurrent wheezing of childhood (49), and asthma at age 6 years (50-52). Our prospective multicenter ED data show that children with HRV bronchiolitis resemble children with an asthma exacerbation (see section C1) (53). These data build on the other extensive data from our group about the role of rhinovirus in wheezing and asthma (52, 54-64). Confirming that HRV bronchiolitis is associated with recurrent wheezing and eventually asthma in a large multicenter cohort would support the addition of HRV to the standard viral panel now used by most hospitals, and re-examination of the short- and long-term clinical care of this large patient population (65-67).

Severity of Illness and Wheezing (Significance of Aim 1b)
In the Tucson Children’s Respiratory Study prospective birth cohort (68), over 40% of the children who had any wheezing in the first 3 years of life and other risk factors for asthma did not develop asthma. This pattern of illness was termed “transient early wheezing” (69). However, unlike the cohort in this proposal, the Tucson study was not a cohort of hospitalized children and the respiratory outcomes of these two populations (i.e., children with or without severe bronchiolitis) may be quite different. Indeed, based on a Tennessee Medicaid database there is a dose-response relationship between bronchiolitis severity (as defined by clinic visit, ED visit, and hospitalization) and the increased odds of both early childhood asthma and asthma-specific morbidity (70). Furthermore, data suggest that a child requiring intensive care unit (ICU) admission independently correlates with later wheezing and asthma (71). Although several demographic, environmental, and medical history factors have been associated with and may contribute to the severity of bronchiolitis (1, 4, 40, 72-92), there are limited prospective data examining the independent predictive value of an ICU admission or these other risk factors on the development of recurrent wheezing. If this study finds an association between severity of illness and recurrent wheezing, closer pulmonary follow-up care and future medication trials may be warranted for the children requiring intensive care for their bronchiolitis.

Vitamin D and Wheezing (Significance of Aim 2)
An emerging risk factor for both respiratory infection and childhood wheezing is vitamin D status, as measured by serum 25-hydroxyvitamin D (25[OH]D) level. Lifestyle changes over the past few decades have made vitamin D deficiency increasingly common (93-95). Dr Camargo and colleagues discovered in a prospective birth cohort in Massachusetts, that lower maternal intake of vitamin D during pregnancy is associated with increased risk of recurrent wheezing in the mothers’ young children (96). These findings were replicated in 5-year old Scottish children (97). Dr Camargo recently confirmed these novel findings in a separate birth cohort of 922 children from New Zealand (98); low 25(OH)D levels in umbilical cord blood were associated with increased risk of respiratory infections at 3 months, and childhood wheezing to age 5 years, but not incident asthma (99). There are data demonstrating an association between low levels of 25(OH)D and increased risk of asthma exacerbations (100, 101) and worse asthma control (102). The pathophysiology of these associations may relate to vitamin D’s role in the activity of the innate immune system (103-107), specifically cathelicidin (103, 108-113), and protection against respiratory infections. Indeed, Jartti, Camargo and colleagues recently found in children hospitalized with wheezing, that serum 25(OH)D levels had an inverse linear association with
HRV and RSV, but no association with bocavirus or enterovirus (114). This study will examine, for the first time, if low levels of 25(OH)D in the first year of life among children with severe bronchiolitis are associated with specific viral infections, recurrent wheezing, and/or eventual asthma. These data could quickly change clinical care, since vitamin D supplementation is a safe, accessible, and affordable intervention.

Predicting Asthma
Despite the generally strong associations between bronchiolitis and recurrent wheezing, no one has identified reliably the 20-60% with severe bronchiolitis who are at increased risk of developing recurrent wheezing (31-33) – or if this large group of children will ultimately develop asthma (115). Hampering this pursuit has been the terminology used to describe wheezing in preschool children (116) and the recent appreciation that asthma is a heterogeneous disease with multiple complex causes (117-119). The first widely-adopted predictive index for the development of childhood asthma (not recurrent wheezing) is entitled the Asthma Predictive Index (API) and based on The Tucson Children’s Respiratory Study cohort. The “stringent index” of the API includes clinically-based parameters and had a positive predictive value of 48% (120) for current asthma at age 6 years. If the stringent index was used to predict asthma symptoms once during the school years there was a positive predictive value of 77% (120). A modified API (mAPI) (121) was created by Guilbert et al. in order to enroll children at age 2-3 years for the Prevention of Early Asthma in Kids (PEAK) trial (122). In addition to the clinical values in the API, the mAPI adds allergic sensitization to aeroallergens and foods. These additions may be important, since a high-risk birth cohort from Australia (n=198) suggests it is the interaction between atopy and RSV or HRV infections early in life that promotes the development of asthma at age 5 years (123). However, no predictive value for recurrent wheezing or asthma has been calculated for the mAPI. Furthermore, neither the API nor the mAPI were developed for children with severe bronchiolitis, a group at very high-risk for recurrent wheezing and asthma. In addition, these two indices do not account for viral etiology, severity of LRTI, or 25(OH)D – factors likely to influence risk.

WIND (Significance of Aim 3)
Due to the high prevalence of bronchiolitis (1, 2) and potential for severe illness (40, 73, 89, 124), there have been several large scale treatment trials in the US and other countries (125-131). However, without data to identify clinically distinct sub-groups of children with bronchiolitis, lingering questions remain about the results of these otherwise large and scientifically rigorous studies (132-134). For example, two large multicenter bronchiolitis corticosteroid trials did not identify the infectious etiologies of the children (129, 131), and came to a different conclusion than a small corticosteroid study of children with HRV bronchiolitis (65, 67). One of the main thrusts of this proposal and WIND would be identifying those children with severe bronchiolitis at high-risk of developing recurrent wheezing and eventual asthma, which would not only help clinicians target follow-up care, but would also advance bronchiolitis research by better routing children into future treatment or prevention trials. Indeed, this proposal (e.g. WIND) would help overcome an important historical barrier to improving the care of children with this common condition by identifying children with bronchiolitis that may respond...
differently to medications and/or have different clinical outcomes. Furthermore, these data would be the first to examine all of the aforementioned risk factors – viral etiology, severity of illness, and 25(OH)D levels – in combination with other historical (parental/personal atopy), medical, and environmental (e.g., allergen exposure) factors, as well as those in the mAPI to predict recurrent wheezing by age 3 years. Our long-term goal would be to follow the proposed cohort until the children were age 6 years in order to assign a more formal diagnosis of childhood asthma.

**CCL5 and Wheezing (Significance of Exploratory Aim)**

An exploratory aim of the study is to create a cohort biorepository that will permit testing of novel mechanistic hypotheses, some which may ultimately be added to future versions of WIND. In the present study, we will examine if children with higher nasopharyngeal aspirate levels of CCL5 (previously known as RANTES)(135) will have a higher risk of developing recurrent wheezing (136). Levels of CCL5 (previously known as RANTES [regulated on activation normal T cell expressed and secreted]) are increased in the nasal secretions of children with RSV (137) and polymorphisms of the CCL5 promoter gene increase the acute severity of RSV disease (138). Beyond the acute disease, CCL5 has been shown to predict recurrent wheezing after RSV bronchiolitis (139). Other data supporting the association between CCL5 and future wheezing include: 1) anti-CCL5 antibody in BALB/c mice decreased airway hyper-reactivity (140); 2) CCL5 released during RSV infection increased the inflammatory response to subsequent allergic airway challenges in mice (141); and 3) increased serum levels of CCL5 due to polymorphisms increased the risk of recurrent wheezing after RSV bronchiolitis (142). Of most relevance, is that among 206 infants in the RBEL cohort, which only includes children hospitalized with RSV bronchiolitis and first time wheezing, higher CCL5 was the best predictor of asthma at age 6 years (136). Confirming this association with RSV in a much larger, multicenter cohort and examining if CCL5 is associated with viruses beyond RSV, as well as recurrent wheezing, would advance scientific knowledge and open new avenues of research.

**Summary of Significance**

Bronchiolitis is the leading cause of hospitalization for US infants and many of these infants will develop recurrent wheezing. However, there have not been any comprehensive, large-scale, prospective studies of this high-risk group to examine the factors that are associated with recurrent wheezing. This study will address all of these knowledge gaps in this prospective, multicenter, cohort study of severe bronchiolitis. The combined results will help derive WIND, an improved, state-of-the-art predictive index for recurrent wheezing.

### 2.2 Scientific Rationale

Bronchiolitis is the most common cause of infant hospitalization in the US (1, 2). Small cohort studies of children hospitalized with bronchiolitis (n=51 to 206) have found that 20-60% of these children will develop recurrent wheezing of childhood (31-33); almost 50% will develop doctor-diagnosed asthma by age 6 years (43, 143). Despite this strong association, it remains unclear which children with severe bronchiolitis (as defined by the need for hospitalization) will develop
recurrent wheezing and eventually asthma. Indeed, no one has rigorously or effectively defined sub-groups of children with severe bronchiolitis who may respond differently to medications and/or have different clinical outcomes. As a result, lingering questions have followed other large studies designed to improve the treatment and understanding of children with severe bronchiolitis (132-134, 144).

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

This is an observational study without administration of any medication or interventions except collection of the nasopharyngeal aspirate (NPA), 5 mL of blood, and nasal swab collection. The NPA causes some temporary discomfort to the child and might cause a small amount of nasal bleeding. The minor procedure is not known to be associated with risk of substantive harm and this specimen is frequently obtained from children with bronchiolitis. The anterior nares swab causes very little discomfort and is not known to be associated with any substantive harm. The venipuncture is associated with some temporary discomfort and possibly bruising. This minor procedure is also not known to be associated with risk of substantive harm.

Most of the data we plan to collect are routinely ascertained in the course of current ED and inpatient management of children with bronchiolitis. Only the study investigators and study personnel will have access to the study data. All study data including those entered by study personnel and those that may be entered by participants via online surveys will be entered and stored in REDCap (Research Electronic Data Capture) a Health Insurance Portability and Accountability Act (HIPAA) compliant web-based application featuring audit trails for tracking data manipulation and export procedures. (See REDCap General Information, Appendix A1 and section 4 for more detailed information.) This application will be hosted by Partners HealthCare Systems (PHS). PHS has all necessary physical and operational securities in place to meet or exceed Federal and State security and privacy regulations. PHS restricts, monitors, and controls access to systems by authorized personnel who additionally have signed confidentiality and ethical pledges to safeguard data (a condition of employment with PHS) and have performed training in protecting Human Subject data (see PHS Research IT Facilities Security, Appendix A2 and PHS Research IT Facilities Infrastructure, Appendix A3.)

All study personnel involved in data collection will have human research protection training.

If laboratory testing identifies a vitamin D deficient child, the EMNet Coordinating Center will follow the safety plan used for K23 AI077801, “Vitamin D deficiency and the severity of bronchiolitis” (PI, Mansbach). This plan, includes parent/legal guardian and primary
provider letters that describe plans of action based on the 25(OH)D values and has been approved by the Children’s Hospital Boston Institutional Review Board (IRB.) EMNet Coordinating Center does not plan to report positive Immunoglobulin E (IgE) results to families. Although IgE results are critical for this research, they are of unclear clinical relevance and may cause undue family stress. Additionally, study design precludes timely communication of the infectious pathogen results to the families. However, we will send families the results of viral testing performed at the index hospitalization when it is available, which we expect to be the year following the enrollment year. During any of our follow-up, if the study participant reports any concerns about their child’s health, study personnel will advise the parent/legal guardian to consult with their primary care physician.

2.3.2 Known Potential Benefits

There are no direct benefits from participation in this study.

There is potential benefit to society. This study will address major knowledge gaps in the short and long term management of children with severe bronchiolitis.
3 OBJECTIVES

PRIMARY

Aim 1: To examine the association between (a) the infectious etiology of a child’s severe bronchiolitis, and (b) the severity of this illness, and the subsequent development of recurrent wheezing by age 3 years and asthma at age 6 years.

Among children age <1 year admitted to the hospital with severe bronchiolitis:

- Hypothesis 1a: Children infected with rhinovirus (either alone or in combination with another virus) will be at the highest risk of developing recurrent wheezing of childhood when compared to children infected by other common bronchiolitis pathogens.
- Hypothesis 1b: Children admitted to the intensive care unit will have a higher risk of developing recurrent wheezing of childhood when compared to children admitted to the regular ward.

SECONDARY

Aim 2: To examine the association between the level of serum 25-hydroxyvitamin D (25(OH)D) during severe bronchiolitis and the subsequent development of recurrent wheezing by age 3 years.

- Hypothesis 2: Serum 25(OH)D levels will have an inverse linear association with the development of recurrent wheezing of childhood (i.e. low levels of 25(OH)D will increase risk of recurrent wheezing).

Aim 3: To combine these clinical and laboratory data to derive WIND, a new clinical index that will identify children at higher risk of developing recurrent wheezing by age 3 years.

- Hypothesis 3: The infectious etiology, severity of illness, and the serum 25(OH)D level will all be independent predictors of recurrent wheezing of childhood. These factors in combination with the factors from the Asthma Predictive Index (i.e., atopic dermatitis, family history of asthma, allergic rhinitis, wheezing unrelated to colds, and eosinophilia) (120) will yield WIND. We anticipate that this new clinical index will have a positive predictive value ≥85% to identify children age <1 year with severe bronchiolitis who will develop recurrent wheezing.

Outcome
The outcome measure for all three Aims is recurrent wheezing of childhood by age 3 years (as defined by the date of birth, not the date of enrollment). Recurrent wheezing of childhood is
defined in the 2007 NIH asthma guidelines (145) as having at least 2 corticosteroid-requiring exacerbations in 6 months or having at least 4 wheezing episodes in one year that last at least one day and affect sleep. Among children with this clinical history and a positive mAPI (see section A7), the NIH guidelines recommend that clinicians begin inhaled corticosteroids for children age 0-4 years to reduce impairment and risk of exacerbations, but not to alter the underlying severity or progression of disease (122, 146).

**Exploratory Aim:** Create a cohort biorepository that will permit testing of novel mechanistic hypotheses, some which may ultimately be added to future versions of WIND. In the present study, we will examine if children with higher nasopharyngeal aspirate levels of CCL5 (previously known as RANTES)(135) will have a higher risk of developing recurrent wheezing (136).

**FUTURE**
The following future objectives are currently unfunded, but if new funding is not obtained, internal funds will be used to complete the study per protocol.

**Aim 1:** To examine the association between (a) the persistence of the infectious etiology of a child’s severe bronchiolitis, (b) the infectious etiology of acute respiratory infections requiring health care utilization until age 3 years, and (c) the presence of virus the summer following the index hospitalization when the child is healthy and during one other randomly assigned season when the child is healthy, and the subsequent development of recurrent wheezing by age 3 years and asthma at age 6 years.

- **Hypothesis 1a:** Children with persistence of the same virus 3 weeks after the index hospitalization will be at higher risk of developing recurrent wheezing of childhood when compared to children who are able to clear the virus.

- **Hypothesis 1b:** Children with rhinovirus ARIs (requiring health care utilization and with documented wheezing) will be at higher risk of developing recurrent wheezing of childhood when compared to children infected by other common ARI pathogens.

- **Hypothesis 1c:** Children with the presence of rhinovirus when healthy will be at higher risk of developing recurrent wheezing of childhood when compared to children without detectable rhinovirus when healthy.
4 STUDY DESIGN

We will conduct a prospective multicenter cohort study of children age <1 year with severe bronchiolitis (as defined by the need for hospitalization). Site investigators will enroll 1,100 children between November 1 and April 30 in up to 3 consecutive years (average 367 per year). We expect that sites will need to over-enroll by 10% to meet the overall enrollment goal of 1,000 children (average 333 per year.) to account for study participants who fail to meet run-in criteria (see section 6.1, Post-discharge 7-28 days: Short Term Follow-up / Run-in.) During the hospitalization, we will collect clinical data, a NPA, blood, and a nasal swab. The post-hospitalization follow-up involves phone calls (with the possible option of a REDCap online survey for some assessments), further collection of nasal swabs, and acquisition of medical records. Researchers at each site will call the families 1-week post discharge. Subsequently, EMNet staff will conduct all phone calls – starting at approximately 3 weeks post-admission, and then from the age of 6 months every 6 months for approximately 5-6 years depending on age at enrollment. (Note: 1U01AI087881-01A1 will fund phone calls for first 4 years of study, then we will seek new funding for calls to age 6 years, as permitted per original consent form. If new funding is not obtained, internal funds will be used to complete the study per protocol.) A nasal swab will be collected by the parent/legal guardians 3 weeks after the date of index admission or by study personnel if the study participant is still hospitalized (clearance swab). Parent/legal guardians will also collect a nasal swab during the first summer after enrollment sometime in June, July, or August and 1 other time during the second year after enrollment (seasonal swab). Additionally, until the child is age 3 years, the parent/legal guardian will collect a nasal swab each time the child has an outpatient, ED, or hospital visit related to an acute respiratory infection (ARI) called the ARI swab. Medical records will be acquired by site researchers and EMNet staff from birth until age 6 years. (Note: U01 1U01AI087881-01A1 will fund collection of medical records for first 4 years of study, then we will seek new funding for records to age 6 years, as permitted per original consent form. If new funding is not obtained, internal funds will be used to complete the study per protocol.)

A child may be hospitalized more than once over the course of the study but sites will enroll that child only once into the study. In other words, each child is eligible for enrollment only once across the three enrollment years. Parent/legal guardians will consent eligible children to the main study (see Sample Child Consent Form, Appendix B1) and an optional genetics component of the study (see Sample Tissue Bank Consent Form, Appendix B2.) The parent/legal guardian of an eligible child who consents to participate will complete the intake interview (composed of the Intake Form, Contact Form, and Maternal Pregnancy and Nutrition Form (see “Data Forms” below). Site study personnel will complete the child’s specimen collection (NPA, blood, nasal swab) in the hospital. The follow-up phase consists of a 1-week call, 3-week clearance swab and call, seasonal swab, ARI swabs, 6-month calls or possible online surveys, and medical records collection/review. The complete schedule of events is shown in Appendix C.
Data Forms

The data collection forms are created to be simple “structured interviews” that can be read to study participants. Data will be submitted to the EMNet Coordinating Center at Massachusetts General Hospital using REDCap, a HIPAA-compliant web-based application. Data will either be entered into REDCap by study personnel at the sites or EMNet Coordinating Center or directly into a REDCap online survey by the participants. The participant-completed online surveys have all the same security and protection as the REDCap data entry used by study personnel.

REDCap is a free, secure, HIPAA-compliant web-based application hosted by the Partners HealthCare Research Computing, Enterprise Research Infrastructure & Services (ERIS) group. Vanderbilt University, with collaboration from a consortium of academic and non-profit institutional partners, developed this software toolset and workflow methodology for electronic collection and management of research and clinical study data. The data collection tools will rely on a study-specific data dictionary defined by members of the research team with planning assistance from Harvard Catalyst | The Harvard Clinical and Translational Science Center EDC Support Staff.

REDCap provides an interface to enter data with real time validation (automated data type and range checks). The system offers easy data manipulation with audit trails, reports for monitoring and querying participant records, and an automated export mechanism to common statistical packages. The REDCap application hosted by Partners Healthcare ensures data storage that meets or exceeds all Federal and State security and privacy regulations (see PHS Research IT Facilities Security, Appendix A2.)

Data entered by study personnel will be of high-quality as a result of pre-study training, which includes this detailed protocol, a training teleconference with Power Point presentation, and the completion and review of sample forms before active enrollment begins. In addition, there will be a detailed manual to accompany data collection forms. The manual will provide written instruction for questions on the data forms, general recruiting information, decision rules for conflicting results, and how to interpret answers with qualifiers. The manual for data collection is a supplement to this protocol and will be continually revised as needed to address site questions that may arise throughout the course of the study. These materials and methods build on successful EMNet studies (53, 148-151).

Site and study participant data will come from multiple sources (Appendix D):

1. Site PI questionnaire (Site Form)
2. Study participant inclusion screening (Screening Form)
3. Hospital interview and chart review (Intake Form)
4. Family contact information (Contact Form)
5. Mother prenatal diet information (Maternal Pregnancy and Nutrition Form)
6. Inpatient chart review (Inpatient Form)
7. Readmission Intake Form
8. Readmission Inpatient Form
9. Telephone interview one week after discharge (1-week Follow-Up Form)
10. Telephone interview three weeks after admission (3-week Follow-Up Form)
11. Telephone interview/online survey every 6 months from birth (6-month Follow-Up Form)
12. Assessment of severity of acute respiratory infection (ARI swab Form)
13. Assessment of potential worsening of ARI (ARI swab Follow-Up Form)
14. Assessment of wellness at three weeks after admission (Clearance Swab Form)
15. Assessment of wellness before seasonal swab collection (Seasonal Swab Form)
16. Review of medical records from birth, including HIPAA-approved medical release (Chart Review Form)
17. Parental allergy data collected during child medical record review (Parent Chart Review Form)
18. Protocol Deviation Form
19. Adverse Event Case Report Form

Most of the questions on the forms have been used in previous EMNet studies and an inpatient bronchiolitis study (U01 AI-67693, “Prospective Multicenter Study of Bronchiolitis Admissions: Etiology & Disposition”). The two “readmission” forms listed above are short versions of the original admissions forms and are only administered in the event that the study participant is readmitted to the enrolling hospital after enrollment.

**Data Management and Quality Assurance**

The EMNet Coordinating Center will conduct two separate training sessions for all study personnel before the start of enrollment. A REDCap-specific training session will focus on all issues related to entering data in to the REDCap system accurately and completely. A second, general training session will focus on executing the protocol and will include training resources related to sample collection. Additionally, there will be online training resources made available to all study personnel for reference after the training is completed.

All study personnel will be trained to collect NPA and nasal swabs using a video and practice sessions. The site PI (Co-Investigator) will be responsible for assuring that local staff are competent in all study procedures including specimen collection. All site PI’s will provide written certification that the local staff are competent in all procedures to the EMNet Coordinating Center before starting enrollment. Sites may not begin recruitment until this written certification has been provided to the EMNet Coordinating Center.

Each site will send all ED related records (e.g., visit notes, laboratory results, discharge instructions) and the inpatient discharge summary for the index hospitalization to the EMNet Coordinating Center for the first 3 study participants enrolled at their site in the first enrollment year. The EMNet Coordinating Center will use these records to confirm that key information is being abstracted and recorded correctly into REDCap.
During enrollment, the EMNet Coordinating Center will continually monitor data entered by sites in “real-time” to identify any potential protocol deviations and assess data integrity. Additionally, the EMNet Coordinating Center will select 5 key questions from the Intake Form and randomly select 10% of study participants from each site and re-administer these 5 questions at the 3-week interview. If differences are discovered during this process, the EMNet Coordinating Center will set up a conference call with the site to review the procedures and examine the root causes of the discrepancies.

In order to ensure homogenous quality of the collected NPA specimens across the approximately 20 sites, the EMNet Coordinating Center will also compare the total volumes of the NPAs collected by all sites. If one site is sending significantly different volumes we will contact the site and review the procedures to correct any problems and ensure they are collecting the NPA correctly.
5 STUDY POPULATION

5.1 Selection of the Study Population

Each morning from November 1 until April 30 in year 1 (2011-2012), and year 2 (2012-2013), a member of the site research team will screen all children admitted with bronchiolitis to the medical ward, any “intermediate care” type of unit, and the ICU, in the past 24 hours. Using a variety of mechanisms (e.g. patient logs, communication with medical teams, computerized registry, on-site research assistants), site investigators will be aware of all children age <1 year admitted to the hospital with bronchiolitis and will record whether they were either approached, missed (no attempt to approach), or known to be ineligible from pre-screen of record in the screening form (Appendix D2.) If the patient was missed, study personnel will indicate if this was caused by lack of availability of personnel, being unaware the patient was in the hospital, or another reason. If the patient was known to be ineligible study personnel will record the reason the patient was ineligible. In year 3 (2013-2014), selected sites will screen ICU patients across the entire 6-month period but have the option to screen ward patients only during December, January, and February, which are typically the peak months for bronchiolitis. We will enroll children, male and female, of all races.

Once the general inclusion criteria (age <1 year, admitted to hospital, physician diagnosis of bronchiolitis, parent/legal guardian’s ability to give informed consent) have been confirmed, study personnel will approach the parent/legal guardian, relate a brief overview of the study and ask if he or she is interested in hearing more about the study. If interested, study personnel will administer the main section of the screening form (Appendix D2, Q3-15) to finally confirm eligibility and if the potential study participant is determined to be eligible will display a short, introductory video that contains information about the study (see Appendix G13.) If the parent/legal guardian indicates a willingness to participate, the site investigator or study personnel will obtain parent/legal guardian consent. Then, the study personnel will begin the Intake Form (see Appendix D3.)

Each site will enroll 30-50 study participants per year from November 1 – April 30 that meet all run-in requirements (able to be contacted for 1-week and 3-week calls.) Over the 3-year study, each site should enroll approximately 100 children. Included in the study participants enrolled each year by each site will be approximately 20% who were also admitted to the ICU. Because we estimate that 10% of the study participants who are initially enrolled will not meet run-in requirements (see section 6.1, Post-discharge 7-28 days: Short Term Follow-up / Run-in), over-enrollment of 33-55 study participants per year will be required to achieve the target sample.

Monthly variation in the overall number of children admitted to the hospital with bronchiolitis as well as children admitted to the ICU is expected, with the highest number of admissions occurring in December, January and February. Each site will determine how many study participants will be recruited each month during the enrollment period to ensure they meet the annual goal of 30-50
enrolled study participants with at least 20% from the ICU from November 1 – April 30. Sites that fall short of the annual goal will make up the difference in the subsequent year. To ensure that we reach recruiting goals, the EMNet Coordinating Center will run monthly reports to monitor enrollment by site. Based on the number of study participants enrolled monthly at each site, we may ask certain sites to increase their recruitment, to balance a site with an unexpectedly low volume or to meet either the overall enrollment goal or ICU enrollment goal for the study.

The site study team will consult the list of study participant names and medical record numbers of all enrolled study participants maintained in REDCap before enrolling any new study participant to ensure that the same person is not enrolled twice.

With the exception of specimen collection (the NPA, blood, and nasal swab), study participants will be evaluated and treated as usual and without regard to this observational study. Parent/legal guardians will be approached about participating after the medical team has finished their assessments and stabilized the study participant. If necessary, the recruiting will take place the morning after admission, but no later than 24 hours after admission to ward or ICU. Although necessary for patient care, we do not think this delay will compromise data collection, since the ED course and inpatient treatment before enrollment will be collected via chart review.

Consent will be obtained from the parent/legal guardian of all participants. The EMNet Coordinating Center will provide IRB approved samples of consent forms to each participating institution.

### 5.2 Inclusion/Exclusion Criteria

**Inclusion Criteria**
- Age <1 year
- Admitted to hospital with attending physician diagnosis of bronchiolitis, as defined by the American Academy of Pediatrics: acute respiratory illness with some combination of rhinitis, cough, tachypnea, wheezing, crackles, and retractions (152)
- Parent/legal guardian must have and provide us with a permanent address (i.e., not homeless), phone number, email address, alternate contact information, and primary care provider information and expect that this information will not change in the next 12 months*
- Ability of the parent/legal guardian to give informed consent ≤24 hours after admission to hospital or ICU
- Parent/legal guardian speaks English or Spanish

**Exclusion Criteria**
- Enrolled into the current study during an earlier bronchiolitis admission
- Parent/legal guardian who does not agree to the collection of the NPA or blood specimen or who does not agree the possible future use of either specimen
- Child transferred to participating hospital >48 hours after the original time of admission
- Time since child transferred to a participating site hospital >24 hours
- Current treatment is 2nd oral steroid course in 6 months, or 4th episode of wheezing in past year (i.e., already meets the primary endpoint)
- Known heart-lung disease, immunodeficiency, immunosuppression, or gestational age <32 weeks
- Insurmountable language barrier

*Legal guardians who are college students, whose current residence may not be their permanent residence or may not be at their current address for the next 12 months, may be enrolled assuming they meet the other inclusion criteria since this population is likely to have stable phone and email address. Military personnel not expecting to be deployed in the next 12 months may be enrolled assuming they meet the other inclusion criteria. Families who plan to move locally in the next 12 month (as defined by maintaining the same primary care provider) may also be enrolled into the study if all other eligibility requirements are met.
6 STUDY PROCEDURES/EVALUATIONS

6.1 Study Procedures

Site Data
Before beginning the study, the site PI should complete the Site Form (Appendix D1). This form provides site contact information and information about concurrent bronchiolitis clinical guidelines and research.

Post-admission 0-24 hours: Clinical Data
After approaching potential participants and confirming interest, study personnel will complete the Screening Form (Appendix D2) to confirm eligibility. Once eligibility has been confirmed study personnel will consent the study participant to the main study (see Sample Consent Form, Appendix B1) and optionally to the genetics portion of the study (see Sample Tissue Bank Consent Form, Appendix B2.) In addition, study personnel will obtain a signed medical record release form. Once consented, study personnel will assign the study participant with a unique study identification number or study ID, which is the principal method to identify study participants and their biological specimens. Each study ID is comprised of a 3-digit site number and a 3-digit individual study participant number. Only study personnel at the participating sites and at the EMNet Coordinating Center will have access to the personal information linked with this ID. Only study personnel at the EMNet Coordinating Center and the other two laboratories receiving biological specimens (the Baylor College of Medicine and the University of Arizona) will have the codes linking the site numbers and the participating sites. (The participating labs will not have access to the personal information linked with the study ID.) The REDCap software ensures that duplicate IDs cannot be created and that all data entered in to the application are associated with a unique ID.

After completing the Screening Form and obtaining consent of the child’s parent/legal guardian and assigning the study ID, the parent/legal guardian will undergo a 20-minute enrollment interview that consists of completing the Intake Form (see Intake Form, Appendix D3), and the Contact Form (see Contact Form, Appendix D4.) First, the interview portion of the Intake Form will be completed. Data collected on this form will provide basic demographic descriptors, medical history, pre-hospital medications, and details of the current illness. The interview will conclude with the completion of the Contact Form. All personal contact information including name, an address where the participant plans to live for the next 12 months, a phone number that is always in service, at least one email address the participant plans to use for the next 12 months, contact information for the child’s primary care provider and contact information for at least one alternate contact are all required to participate in the study. (Note: The specific, often private contact information for the alternate contact(s) [e.g., cell phone, email] may be provided at the 1-week call.) Additional information such as best times to call and whether the participant
prefers online surveys will also be collected. In order for the study participant to receive remuneration they will need to provide a social security number. However, they may still participate if they choose not to supply a social security number if they agree to forego these payments.

At the completion of the enrollment interview the study personnel will provide a nutritional survey (see Maternal Pregnancy and Nutrition Form, Appendix D5a) to the biological mother if she is present and instruct her to complete the form at some point during the hospitalization. The form will take approximately 5 minutes to complete and assesses maternal diet, vitamin, and supplement use during pregnancy. Study personnel will be available for questions while the mother completes the survey. After the survey is complete, study personnel will collect it and enter the data into REDCap.

If the biological mother is not present, but the consenting legal guardian who is present (for example, the father) indicates that he or she can deliver the survey to the mother, then study personnel will supply a copy of the survey to be delivered to the mother, which will contain instructions to return the completed survey in an included, stamped envelope, to the EMNet Coordinating Center or the mother will have the possibility of completing the form online. In the event that the nutrition survey is not received by EMNet within 2 weeks, EMNet staff will conduct reminder phone calls and if necessary, send replacement surveys to the mother. If the legal guardian indicates that the biological mother will not be available to complete this survey (e.g., death, unknown location), but is confident that he or she has the knowledge to answer all questions pertaining to the mother’s diet during pregnancy, the legal guardian will be allowed to complete a version of the survey where the same questions are addressed to an individual other than the mother (see Maternal Pregnancy and Nutrition Form for Non-Mother, Appendix D5b). An early question on the survey will indicate the status of the person answering the survey questions. If the legal guardian indicates that the biological mother will not be available to complete this survey (e.g., death, unknown location), and is NOT confident that he or she could answer all questions pertaining to the mother’s diet during pregnancy, study personnel will indicate in the survey that this form will not be able to be completed and no further efforts will be made to collect the data.

After the enrollment interview, the completion of the Contact Form, and presentation of the Maternal Pregnancy and Nutrition Form, study personnel will complete a brief chart review for the pre-admission visit (usually an ED visit) in order to complete the final section of the Intake Form. This portion of the form provides data about the pre-admission evaluation and treatment.

At the end of hospitalization, another chart review will be completed (see Inpatient Form, Appendix D6) for the entire inpatient stay of the child. This form will capture maximum and minimum values of important clinical measures as well as relevant treatments and lab results.

Also, the enrolling study personnel will send a notification to the PCP listed by the study participant that their patient has enrolled in the study (Appendix G11.) This letter will inform the
PCP about the study, provide contact information for the study, and inform him or her that we will be requesting medical records annually.

Participants will be given a brochure about the study that contains information contact information and reminders about study information that was covered during enrollment (see Study Introduction Brochure, Appendix G12.)

Post-admission 0-24 hours: Specimen Collection
All study personnel will be trained to collect the biological specimens using materials such as video instruction, PowerPoint presentations and practice sessions. Written certification that local staff are competent in all study procedures including specimen collection will be provided to the EMNet Coordinating Center by the local site PI before starting recruitment. The EMNet Coordinating Center will send sites all materials necessary for the specimen collections performed by study personnel (note that blood collection will be performed by phlebotomy teams at local sites.) To help the sites, the materials required for biological specimen collections will be pre-packaged into individual bags or kits.

Within 24 hours after admission, a NPA specimen will be collected using a standardized procedure for every study participant enrolled in the study. This will not affect local clinical practice or care. The total time required to perform all of the tasks involved in the NPA collection is expected to be about 15 minutes. NPA specimens are more sensitive for detection of respiratory viruses than are specimens on swabs (153). Indeed, Dr. Pedro Piedra, who will be performing the microbiologic testing of these specimens at Baylor College of Medicine, has detected a RNA virus in nearly 93% of the specimens collected in a recent inpatient bronchiolitis study (154). Once collected, the NPA specimen will then be added to transport medium with a virus stabilizer (15% glycerol in Iscove’s media) creating a 1:1 dilution. Each aspirated specimen will contain a maximum of 8 mL (range 6 to 8 mL) after dilution. After collection and labeling with the specimen collection date/time and a unique study ID, the specimens will be frozen at –70°C or –80°C. After collection, the biological specimen will be transported, on ice, to a 4°C refrigerator within 1 hour of collection (the biological specimen should be in the refrigerator no longer than 24 hours before transfer to –80°C freezer). In January and May, specimens from each site will be placed on dry ice for transport to Dr. Pedro Piedra at the Respiratory Virus Diagnostic Laboratory at Baylor College of Medicine, Houston, Texas where microbiologic studies will be conducted. Once in the Baylor laboratory, the specimens will be stored at –80°C. Upon the first thaw, the specimens will be divided into equal 1.0 mL aliquots and each aliquot will be labeled with a bar code. Please refer to Section 6.2 and the Manual of Procedures for Specimen Collection, Labeling, Storage, and Shipment for NPA (Appendix E1) for further details.

Also, ideally within 24 hours after admission, a blood specimen will be collected. However, it is permissible to collect the blood specimen at any time during the index hospitalization. The total amount of blood to be collected from a child will be a minimum of 5 mL and a maximum of 15 mL. If a complete blood count (CBC) and differential were performed before enrollment, the total minimum volume to participate in the study will be 4.5 mL. If less than the 5 mL of blood is
drawn or obtained from other sources such as previous venipunctures or discarded blood in red top tubes, study personnel will ask the parent/legal guardian if the blood draw can be redone to obtain the necessary volume of blood at that moment or at a later time during the admission. If after the blood draw is redone and all available blood from previous venipunctures and discarded blood is collected, the volume fails to meet the 5 mL minimum, but the study participant meets all other participation criteria and expresses a desire to continue in the study, sites should contact Project Coordinator Ashley Sullivan to discuss a possible waiver. Although availability of local phlebotomy teams can impact estimates, the average time of collection will be 5 minutes. For all study participants, we will draw extra blood from already scheduled venipunctures when possible, which will be a frequent occurrence in the ICU setting.

If a CBC and differential has not already been performed approximately 0.5 mL of blood should be drawn in a purple top tube and sent to the site’s lab so this test can be performed. Any additional blood specimens will be collected in red top tubes, which the EMNet Coordinating Center will provide to each site. The labeling procedures for the blood will be the same as those described above for the NPA. After collection and labeling with the specimen collection date/time and a unique study ID, the specimen(s) will be brought to the site’s lab where it will be centrifuged. The serum will be siphoned off with a pipette and placed in a plain cap tube, which the EMNet Coordinating Center will provide to each site. The remaining specimen (the pellet) will then be transferred to a separate plain cap tube, which the EMNet Coordinating Center will provide to each site. The biological specimen should be stored in the –80°C freezer no longer than 24 hours after collection. For those participants who agree to the genetic portion of the study, the pellet specimen will be shipped to the University of Arizona for the purpose of establishing a DNA biorepository to examine the possible genetic causes of severe bronchiolitis, recurrent wheezing, asthma and related concepts in accordance with the tissue bank consent form (see Sample Tissue Bank Consent Form, Appendix B2.) This testing will be performed under the future use clause of the main consent form (see Sample Consent Form, Appendix B1.) Consenting to the genetics portion of the study is not required to participate in the main study. If the participant chooses not to participate in the genetic portion of the study, the pellet will not be mailed to the University of Arizona, but will be shipped to the Massachusetts General Hospital for possible future use consistent with the main consent.

In addition to the 0.5 mL of blood required for the CBC and the additional blood obtained in red top tubes for the study, study personnel should collect any other discarded blood so that it can be shipped to the Massachusetts General Hospital for possible future use consistent with the main consent. This blood should be processed, stored and shipped with the same procedures outlined above and study personnel should clearly identify the source of this additional specimen on the specimen label and in the shipping list when sending at the end of the year. As an example, if there is additional blood drawn into the purple top tube not required in the CBC test the remaining specimen should be centrifuged and the plasma should be siphoned off with a pipette and placed in one of the plain cap tubes supplied by the EMNet Coordinating Center.
Center. This additional specimen should be clearly marked and sent with the serum specimens to the Massachusetts General Hospital with the study serum samples.

In May, the serum specimens from each site will be placed on dry ice for transport to Dr. Carlos Camargo at Massachusetts General Hospital and the pellet specimens from each site will be placed on dry ice for transport to Dr. Fernando Martinez at the Arizona Respiratory Center at the University of Arizona or at Massachusetts General Hospital. Once at Massachusetts General Hospital, the serum specimens will receive a bar code label and be stored at –80°C. Upon the first thaw, the specimens will be divided into equal 1 mL aliquots. Once at the University of Arizona or Massachusetts General Hospital, the pellet specimens will be stored at –80°C for future testing. Please refer to Section 6.2 and the Manual of Procedures for Specimen Collection, Labeling, Storage, and Shipment – Blood (Appendix E2) for further details.

Also within 24 hours after admission, an anterior nares swab specimen will be collected (index swab.) The total required time to perform all of the tasks associated with the simple nasal swab is approximately 1 minute. Although microbiologic testing of the NPA specimen is likely to be more sensitive than testing of the nasal swab, collecting the index swab during the hospitalization will allow: 1) comparison to the gold standard NPA results; 2) comparison to the other identical nasal swab collections that will be performed during follow-up by the parent/legal guardian (see Short Term Follow-up and Long Term Follow-up later in this section for more information about the parent/legal guardian-collected nasal swabs); and 3) site personnel to demonstrate how to collect the swab as well as the ease of the collection. This nasal swab collection will be taken no sooner than 2 hours after the NPA collection. It is permissible to collect the nasal swab prior to the NPA in instances where study personnel are concerned the participant will be discharge before this collection can be performed. After collection and labeling with the date and a unique study ID, study personnel will mail the swab to Dr. Carlos Camargo at Massachusetts General Hospital in the self-addressed stamped box provided with the nasal swab collection kit. This procedure emulates the participant nasal swab shipping to ensure maximum comparability of the specimens. Please see the end of this section, Storage of Nasal Swabs at Massachusetts General Hospital, refer to Section 6.2 and the Manual of Procedures for Specimen Collection, Storage, and Shipment – Nasal swabs (Appendix E3) for further details.

Study personnel will record the date and time of collection for all specimens collected during the index hospitalization in the Specimen/Enrollment Checklist (Appendix D21) and will also record the approximate volume collected for the NPA and blood specimens.

During the hospitalization, but after collecting the first swab, study personnel will supply the participant with 1 clearance swab kit and 1 ARI swab kit for self-collection during follow-up and provide complete instruction to the participants about the purpose and use of these kits (see ARI Swab Kit Contents, Appendix F1, and Clearance Swab Kit Contents, Appendix F4.) Study personnel will mark all specimen tubes and materials in these kits with the study participant’s unique study ID at the time of enrollment. Study personnel will also write the date the clearance
swab should be taken (3 weeks after admission) on the outside of the clearance swab kit. These kits will contain all the materials necessary for specimen collection, and training resources with instructions on when to use the kit (see ARI Swab Kit Letter, Appendix F2; ARI Swab Kit Instructions, Appendix F3; Clearance Swab Kits Letter, Appendix F5; Clearance Swab Kit Instructions, Appendix F6) and how to collect nasal swab specimens (see Nasal Swab Collection Instructions, Appendix F10.) Included in these materials will be a toll-free help line to reach the EMNet Coordinating Center at Massachusetts General Hospital to ask question about any aspect of the study or for additional instructions for the collection. In addition, a physical mailing address and email address will provide several ways to communicate with the EMNet Coordinating Center. Please refer to Section 6.2 and the Manual of Procedures for Specimen Collection, Labeling, Storage, and Shipment – Nasal Swabs (Appendix E3) for further details.

Post-discharge 7-28 days: Short Term Follow-up / Run-in
There are two elements to the short-term follow-up: a 1-week phone interview and a 3-week clearance nasal swab collection that also has an associated call, interview and form. These two items will define the run-in period and successful contact with the study participant for both of these events is required for continued participation in the main study.

The first element, the 1-week interview, is conducted by study personnel at the enrolling sites one week after their child’s discharge from the hospital and lasts approximately 10-minutes (1-week Follow-Up Form, Appendix D9). These follow-up interviews should be completed between eight (8) and 14 days after the hospital discharge. Ideally, 1-week follow-up interviews will be conducted on day 8 post-discharge when all participants would be asked about the same one-week period after discharge. Parent/legal guardians will be called at least 5 times over ≥3 separate days. If the parent/legal guardian is unable to be contacted in the first 3 attempts the enrolling site will additionally call the alternate contact(s) provided at the time of enrollment, if available, in order to update the study participant’s contact information. Data from the 1-week follow-up will help assess acute relapse, recent symptoms, and the disease’s impact on the family. Several questions (e.g. recent symptoms) are similar to those asked during the hospital interview and will provide additional endpoints for longitudinal analysis of specific symptoms and also if there are repeat hospitalizations. Before the follow-up interview, the caller will review the study participant’s Intake Form to reorient himself or herself to the study participant. The caller will confirm the dates of the hospitalization and read all of the questions in the 1-week Follow-Up Form. The caller will also confirm that all contact information on file, including alternate contacts, is accurate and complete.

Parent/legal guardians who refuse the 1-week follow-up interview (despite prior consent), who are deemed unreachable after 5 attempts over ≥3 days, or who do not undergo the 1-week follow-up interview for other reasons within the 14 day post-discharge window will not have successfully completed the established run-in period requirements for the main study. These study participants will not be part of the “chronic cohort” that constitutes the main study, but will be considered part of an “acute cohort” (see end of this section, Run-in Failure.)
The second element of the short-term follow-up is the 3-week clearance swab and the associated follow-up call, interview and form. The main purpose of the 3-week clearance swab is to assess whether the child has cleared the virus associated with the index hospitalization (see section 3, “Objectives”, Future Aims, Hypothesis 1a.) During the 3-week follow-up call the EMNet Coordinating Center will remind the parent/guardian to collect the 3-week clearance swab, will provide any necessary assistance with the collection, and administer a brief interview. The purpose of the 3-week follow-up interview is to assess acute relapse. The parent will also complete a brief form when they return their swab collection. The ability to successfully contact the parent/legal guardian for the follow-up call fulfills the run-in requirement.

All parents/legal guardians of study participants who are discharged within 21 days of the original admission date will be called by the EMNet Coordinating Center at least 10 times over ≥5 separate days beginning on day 21 post-admission. These parent/legal guardians will be asked to collect an anterior nares swab from the child between 21 and 35 days after the hospital admission using the clearance swab kit they were given at time of enrollment (see ARI swab and Clearance Swab kit Contents, Appendix F4) and complete a brief form regarding their child’s condition to include with the swab (Clearance Swab Form, Appendix D14.) If a clearance swab for the participant has not been received by day 29 post admission the EMNet Coordinating Center will send a reminder email and begin reminder phone calls on day 30. Occasionally, the EMNet Coordinating Center may ask the enrolling site to assist with attempts to contact hard to reach participants. Late collections (>35 days) will not be included in the study database. Parent/legal guardians will be able to refer to the training resources in the kit (see Nasal Swab Collection Instructions, Appendix F10) in order to perform the collection. Please refer to Section 6.2 and the Manual of Procedures for Specimen Collection, Storage, and Shipment – Nasal swabs (Appendix E3) for further details. During this call, the EMNet Coordinating Center will also administer a brief interview (see 3-Week Follow-Up Form, Appendix D10.) If the parent/legal guardian is not able to be contacted in the first 3 attempts the EMNet Coordinating Center will additionally call any alternate contacts provided at the time of enrollment in order to update the study participant’s contact information.

For those children who are not discharged by 21 days after their original admission date, study personnel will collect the 3-week clearance swab in hospital on day 21 post-admission using the same collection technique. In this situation, the 3-week follow-up call will be moved from 3 weeks after admission to 3 weeks after discharge. The parent/legal guardian’s of these children will be called by the EMNet Coordinating Center at least 10 times over ≥5 separate days beginning on day 21 post-discharge in order to confirm the ability of the EMNet Coordinating Center to contact the parent/legal guardian and fulfill the final run-in requirement. For those participants whose age 6 month or age 12 month assessments (described later in this section) would be scheduled at any point during the 3-week call window the EMNet Coordinating Center will complete these assessments simultaneously in order to reduce burden on the participant.

Once the swab has been collected, the parent/legal guardian (or study personnel at the enrolling hospital in the case of those study participants whose initial admission was ≥21 days)
will write the collection date/time on the specimen vial label, ensure the swab container is labeled with the correct study ID and mail the swab back to the EMNet Coordinating Center at Massachusetts General Hospital in the box provided. The EMNet Coordinating Center will record the receipt of the specimen and notify the participant of the successful receipt of the specimen via email, letter, or phone. A bar code label will be affixed to the specimens and be stored at –80°C at Massachusetts General Hospital. The clearance specimens will be placed on dry ice for transport to Dr. Pedro Piedra at the Respiratory Virus Diagnostic Laboratory at Baylor College of Medicine, Houston, Texas where microbiologic studies will be conducted. Once in the laboratory, the specimens will be stored at –80°C. Please refer to Section 6.2 and the Manual of Procedures for Specimen Collection, Storage, and Shipment – Nasal Swabs (Appendix E3) for further details.

Parent/legal guardians who are unreachable for the 3-week follow-up call after 10 attempts over ≥5 days will not have successfully completed the established run-in period requirements for the main study. These study participants will not be part of the “chronic cohort” that constitutes the main study, but will be considered part of an “acute cohort” (see end of this section, Run-in Failure.)

Parent/legal guardians who are able to be reached, but indicate that they no longer wish to participate in any aspect of the study will be withdrawn, but will have met the run-in requirement of being able to be contacted for the clearance swab collection phone call. So, their data will be used in main study analysis and they will be counted towards the site’s enrollment goal. The clearance swab itself is not required for participation in the study. If the parent/legal guardian does not indicate that they wish to withdraw from the study, but EMNet does not receive a clearance swab, they will continue to be an enrolled study participant and will continue to be contacted for long-term follow-up.

**Run-in Failure**

Those study participants who do not meet the run-in requirements of the study (1-week follow-up interview and being able to be contacted for the 3-week follow-up call) will not be part of the “chronic cohort” that constitutes the main study, but will be considered part of an “acute cohort.” Not being part of the chronic cohort means no further efforts will be made to follow-up with these individuals in this main study, they will not be included in any analysis related to the main study, and will not count toward the site goal of 30-50 enrolled participants per year. (We expect that approximately 10% of study participants will not meet the run-in requirements. Accordingly, sites may need to enroll more than 30-50 study participants in order to meet their enrollment goal of 30-50 children who successfully complete the run-in.)

However, consenting families who do not successfully complete the run-in will be considered part of an “acute cohort,” which means specimens previously collected will be stored for possible use in future studies associated with severe bronchiolitis, recurrent wheezing, asthma and related concepts under the clause in the consent form that allows for future use. That clause reads, “At the end of this study, we would like to keep any remaining samples for
possible future use. As we learn more about this common disease, we may wish to do further
testing associated with severe bronchiolitis, recurrent wheezing, asthma and related concepts
using any left over samples."

Post-discharge 3 weeks to age 3 years: Long Term Follow-up - Phase 1
There are four elements to the first phase of the long-term follow-up: 6-month follow-up form, an
annual medical records review, ARI nasal swab collections with an accompanying survey, and
healthy seasonal nasal swab collections. In addition to these follow-up elements, we will report
microbiologic testing results to the participants when those results become available, which we
expect to be sometime in the year following the enrollment year of the participant.

The first element, the 6-month follow-up form, is administered in a telephone interview by study
personnel at the EMNet Coordinating Center or completed online by the participants in 6 month
intervals from age 6 months until age 6 years. Please note that the first 6 month assessment will
always be conducted by phone with online surveys possibly being available at 12 months
forward. If the child is older than 6 months at admission, questions from the 6 month follow up
will be integrated into the Intake Form. All parent/legal guardians will undergo a 20-minute
telephone interview when the child is 6-months old (or at the 3-week call), one year old and then
every 6 months following this date (see 6-month Follow-Up Form, Appendix D11). After the 6-
month and 1-year follow-up forms have been completed as described above, follow-up
interviews/forms should be completed between age 6 and 7 months and in 6 month intervals
until age 6 years. If child is admitted after age 6 months then the 6 month intervals will
commence at age 1 year. Ideally, all follow-up interviews will be conducted exactly in 6 month
intervals. If the 6 month follow-up form is not completed within 28 days of the first day the
scheduled interview could have been completed, the participant will have missed the window for
that 6 month follow-up period.

For all parent/legal guardians one week before the planned the age 6-month interview a
reminder postcard or email will be sent by the EMNet Coordinating Center (see Reminder
Postcard for 6-month call, Appendix G1 and Birthday Postcard, Appendix G2). The
parents/legal guardian will then be called at least 10 times over ≥ 5 separate days. If the
parent/legal guardian is not able to be contacted in the first 3 attempts the EMNet Coordinating
Center will also use the provided mailing and email addresses and attempt to contact the
alternate contact(s) provided at the time of enrollment in order to update the study participant’s
contact information. If these efforts do not result in successful contact with the participant, the
EMNet Coordinating Center will perform searches of publically available information in an effort
to re-establish contact. Occasionally, the EMNet Coordinating Center may ask the enrolling site
to assist with attempts to contact hard to reach participants.

If an online survey for the assessment is created, beginning with the 12 month assessment or
after the first 6 month assessment has been completed those parent/legal guardians who
indicate a preference to complete online surveys will have a reminder email sent to the primary
email address on record containing a link the REDCap online survey for the 6-month follow-up
forms. If the online form is not completed 1 week after the email is sent a second reminder email will be sent. If the online form is still not completed one week after the second reminder email the EMNet Coordinating Center will attempt to administer the 6-month follow-up form by telephone interview using the same protocol described in the previous paragraph.

Data from the 6-month follow-up interview will help assess relapse events, recent symptoms, and the disease’s impact on the family. Several questions (e.g. recent symptoms) are similar to those asked during the hospital interview and will provide additional endpoints for longitudinal analysis of specific symptoms and also if there are repeat hospitalizations. In cases where the form is administered by telephone interview, the caller will review the study participant’s Intake Form and any prior follow-up forms to reorient himself or herself to the study participant before the follow-up interview. The caller will confirm the dates of the hospitalization and read all of the questions in the 6-month Follow-Up Form (Appendix D11). The caller will also confirm that the contact information on file is still accurate.

The second element of the long-term follow-up is an annual review of the child’s medical records. At some point between May and November, for each enrolled subject at the site, up to age 6 years, enrolling sites will acquire medical records available to the site investigators through the local medical records system for review by the EMNet Coordinating Center staff. The annual packet will include ED data, inpatient discharge summaries, outpatient visit notes (including primary care provider, allergist/immunologist, pulmonologist, infectious disease specialist, ancillary services such as nutrition, etc), and laboratory results, including blood tests (e.g., CBC, specific IgE), microbiology (e.g., virology results), radiology (e.g., chest film reading) and spirometry (e.g., FEV1). If records from the primary care provider (PCP) or other healthcare provider of a child are not available to the site because the provider is outside the local medical records system, the EMNet Coordinating Center will assume the responsibility of requesting the medical records from the “outside” providers. The EMNet Coordinating Center will use these ED, inpatient, and outpatient data to complete the Chart Review Form (see Chart Review Form, Appendix D16.) Note that data related to parental allergies, as revealed in the child’s medical record, will be recorded on a separate form (see Parent Chart Review Form, Appendix D17.)

The third element of the long-term follow-up (Phase 1 only) is the ARI swab collection. All parents/legal guardians will collect an anterior nares swab from the child participant until age 3 years each time the child has a respiratory infection that results in health care utilization (e.g. any doctor/clinic or hospital visit). Parent/legal guardians will be instructed to collect the swab as early as the point in time when they decide the will seek medical attention (e.g., scheduled a doctor’s visit, but have not gone yet), but if this is not possible they will be encouraged to collect the swab within 24 hours after the healthcare utilization. The purpose of this collection is to assess which viruses are associated with respiratory infections requiring medical attention up to age 3 years and to assess the severity of those infections (see section 3, “Objectives”, Future Aims, Hypothesis 1b.) Parents/legal guardians will initially use one of the nasal swab kits they were given at time of admission (see ARI Swab Kit Contents, Appendix F1) and will be able to refer to the training resources in the kit (see Nasal Swab Collection Instructions, Appendix F10).
In addition to the collection of the nasal swab, parents/legal guardians will complete a brief survey about the current infection, which will be contained in the kit (see ARI Swab Form, Appendix D12). The purpose of this brief survey is to assess the severity of the infection. Once the swab has been collected and the survey completed parents/legal guardians will ensure the specimen container is marked with their study ID and mail the swab and survey to Dr. Carlos Camargo at Massachusetts General Hospital in the self-addressed stamped box provided with the nasal swab collection kit.

When the ARI swab is delivered to the EMNet Coordinating Center, EMNet staff will 1) record the receipt of the specimen; 2) immediately send a replacement ARI swab kit to the participant personalized with the study participant’s study ID#; 3) communicate to the family via email, letter or a phone call that EMNet received the swab and that a replacement kit has been sent; and 4) at 1 week after the health care utilization that triggered the collection, conduct a brief survey via email or phone to assess whether the ARI has worsened since the time the specimen was taken (see ARI Swab Follow-up Form, Appendix D13). The purpose of this follow-up survey is to examine the clinical course and severity of the ARI. Please see the end of this section, Storage of Nasal Swabs at Massachusetts General Hospital, and refer to Section 6.2 and the Manual of Procedures for Specimen Collection, Storage, and Shipment –Nasal Swabs (Appendix E3) for further details.

The fourth element of the long-term follow-up (Phase 1 only) is the healthy seasonal swab collection. All parent/legal guardians will collect an anterior nares swab from the child in the first summer after enrollment sometime in June, July, or August and 1 additional time in the second year of enrollment. For the second seasonal swab, study participants will be randomly assigned to a specific season so that one quarter of the participants will have another swab collected during the summer (June, July, August), one quarter during the fall (September, October, November), one quarter during the winter (December, January, February), and one quarter during the spring (March, April, May). Parent/legal guardians will be instructed to collect the swab during a period when they believe the child does not have a cold or ARI (see Seasonal Swab Kit Instructions, Appendix F9.) The purpose of this collection is to assess the presence of viruses during a time of respiratory health (see section 3, “Objectives”, Future Aims, Hypothesis 1c.) The EMNet Coordinating Center will send these kits to the participants in June, July or August (see Seasonal Swab Kit Contents, Appendix F7). These kits will contain all the materials necessary for specimen collection, and training resources with instructions on when to use the kit and how to collect nasal swab specimens (see Nasal Swab Collection Instructions, Appendix F10.) Even though parents will be instructed to collect the swab during a time when their child does not have symptoms of an ARI, the seasonal swab kit will also contain a brief form that assess whether the child is exhibiting symptoms of a respiratory infection (see Seasonal Swab Form, Appendix D15.) Once the swab has been collected parent/legal guardians will ensure the specimen container is marked with their study ID and mail the swab to Dr. Carlos Camargo at Massachusetts General Hospital in the self-addressed stamped box provided with the nasal swab collection kit.
The EMNet Coordinating Center will record the receipt of the seasonal swab, and then communicate with the family via email, letter or phone call that we received the specimen. Please see the end of this section, Storage of Nasal Swabs at Massachusetts General Hospital, and refer to Section 6.2 and the Manual of Procedures for Specimen Collection, Storage, and Shipment – Nasal Swabs (Appendix E3) for further details.

**Storage of Nasal Swabs at Massachusetts General Hospital**

Once at Massachusetts General Hospital, the index, clearance, ARI and seasonal swab specimens will receive a bar code label and be stored at –80°C. EMNet will ship swab specimens on dry ice for transport to Dr. Pedro Piedra at the Respiratory Virus Diagnostic Laboratory at Baylor College of Medicine, Houston, Texas where microbiologic studies will be conducted. Once in the laboratory, the specimens will be stored at –80°C. Please refer to Section 6.2 and the Manual of Procedures for Specimen Collection, Storage, and Shipment – Nasal swabs (Appendix E3) for further details.

In addition to the follow-up activities, once the PCR testing of the NPA at Baylor is complete, sites will notify the parent/legal guardian and primary care physicians about the test results via letter as long as the parent/legal guardian requested notification during the initial study interview (see PCP Virology Results Notification Letter and Parent Virology Results Notification Letter, Appendix G3 and Appendix G4.) This testing will be performed at the end of each enrollment year. Due to the time it takes to complete this testing and communicate the results, we would expect parent/legal guardian and primary care physicians to receive these results letters sometime in the first half of the year after the enrollment year. Once the Vitamin D testing of the blood specimen is completed at MGH, EMNet will notify the parent/legal guardian and primary care physicians about the test results via letter as long as the parent/legal guardian requested notification during the initial study interview (see PCP Vitamin D Results Notification Letter Adequate/At Risk for Inadequacy/At Risk for Deficiency and Parent Vitamin D Results Notification Letter Adequate/At Risk for Inadequacy/At Risk for Deficiency G5, G6, G7, G8, G9, G10).

**Post-discharge age 3 years to age 6 years: Long Term Follow-up - Phase 2**

There are two elements to the second phase of the long-term follow-up, which continue from the first phase; the 6-month interviews and the annual medical record review. After age 3 years all collection of biological specimens associated with long-term follow-up (ARI-swab and seasonal swab) will cease. From age 3 years to age six years the EMNet Coordinating Center will continue the 6 month follow-up calls using the same protocol detailed in the previous section. (Note: 1U01AI087881-01A1 will fund phone calls for first 4 years of study, then we will seek new funding for calls to age 6 years, as permitted per original consent form. If new funding is not obtained, internal funds will be used to complete the study per protocol.) Also, the annual medical records review and completion of the chart review form will continue until age 6 years as described in the previous section. (Note: 1U01AI087881-01A1 will fund chart review activities for first 4 years of study, then we will seek new funding for chart review activities to age 6 years, as permitted per original consent form. If new funding is not obtained, internal funds will be used to complete the study per protocol.)
Other follow-up activities: Readmission Forms
After a study participant is discharged from the hospital, study personnel at the enrolling site will monitor admissions at their hospital to determine if the participant is readmitted with a bronchiolitis-related condition. In the course of screening eligible patients for enrollment in the study, sites will need to check potential participants against the list of existing participants to ensure they have not previously been enrolled. So, this will already occur during the enrollment season and this will likely account for the vast majority of readmissions. Additionally, sites will monitor admissions at their hospital in non-enrollment months for all participants until the participant reaches age 1 year.

Before leaving the hospital at the time of enrollment, participants should be instructed to call the toll free number of the study (855-815-9463) if they are readmitted to the any hospital to increase the chances a readmission to the enrollment hospital will be quickly recognized by the site team. Additionally, sites may choose to utilize any type of alert system available at their hospital to notify them of these readmissions or institute other measures to make sure they are aware of readmissions.

In the event that sites become aware an enrolled participant has been readmitted to their hospital for a bronchiolitis-related condition they will administer the Readmission Intake Form and the Readmission Inpatient Form, which are shortened versions of the Intake and Inpatient Forms administered at the index admission. After administering the readmission forms, they will inquire if the participant has completed an ARI swab collection and survey for this hospitalization. If not, they will answer any questions the participant has about that process and offer any assistance they can in completing the specimen collection and survey.

6.2 Laboratory Evaluations

6.2.1 Laboratory Evaluations/Assays

Virology
Dr. Pedro (Tony) Piedra, is a pediatric infectious disease specialist who since 1998 has been director of the CLIA-certified Respiratory Virus Diagnostic Laboratory at Baylor College of Medicine. This laboratory provides viral diagnostic support for multi- and single center studies (155-165), including U01 AI-67693 (Camargo, PI). Dr Piedra is an experienced respiratory virologist and has conducted numerous clinical trials in this area (157, 158, 161, 162, 164, 166-176), has extensive research experience with RSV (157, 159-162, 171, 177-182), hMPV (173, 175, 183), influenza (163, 164, 172, 176), and adenovirus (166, 167, 169, 174, 184, 185). Dr Piedra currently has real-time reverse transcriptase polymerase chain reaction (rt
RT-PCR) in his laboratory for the viruses being tested in this proposal. In U01 AI-67693, he detected one or more viruses in 93% of the specimens (154).

**Cytokines**

Dr Piedra’s lab has experience measuring cytokines in NPA specimens from children with bronchiolitis (186) and conducting other novel tests utilizing nasopharyngeal specimens, including lactate dehydrogenase (LDH) (187). In preparation for this U01, Dr Piedra detected CCL5 in NPA specimens from 100 children with bronchiolitis; higher NPA CCL5 was associated with RSV (P=0.037; manuscript in preparation).

**Nasopharyngeal and Nasal swab specimens: Infectious pathogens and CCL5**

**Infectious Pathogens**

The laboratory component of this study involves performing rtRT-PCR assays using the same successful techniques we used in U01 AI-67693: RSV (A and B) (188), HRV (165, 189), enterovirus (165, 189), hMPV (190), influenza types A and B (191), PIV types 1 (192), 2 (193) and 3 (194), and coronavirus (HKU1, NL63, 229E, and OC43) (165, 195), and a PCR assay to detect adenovirus (165, 196), human bocavirus (197) and respiratory polyomavirus WU (198) and KI (28).

**CCL5**

We will use Quantikine (Human CCL5/RANTES Immunoassay, R&D Systems, Minneapolis, MN) quantitative solid phase sandwich ELISA assay to measure human CCL5 in NPA samples. The minimum detectable concentration will range from 1.7 to 6.6 pg/ml (see section C7b for pilot data).

**Blood specimens: Allergen-Specific IgE, Total IgE, 25(OH)D, Genetic testing**

The CBC and differential will be performed locally at each hospital. The 25(OH)D will be tested by Massachusetts General Hospital. The Allergen Specific Immunoglobulin E (AS IgE) and Total IgE testing will be performed by Mayo Medical Laboratories. The genetic testing will be performed by the Arizona Research Laboratory at the University of Arizona.

6.2.2 Specimen Collection, Preparation, Handling and Shipping

6.2.2.1 Instructions for Specimen Preparation, Handling, and Storage

**Specimen Collection/ Preparation**

NPA:
Within 24 hours after admission, a trained study personnel member will collect the NPA specimen. This specimen collection will not affect local practice or care. If a NPA specimen is collected during the course of regular care before the collection of the specimen for the study, personnel will wait 1-2 hours before collecting the study specimen. All study personnel will be trained to collect NPA using a video and practice sessions. Written certification that local staff are competent in all study procedures including NPA collection will be provided to the EMNet Coordinating Center by the local site PI before starting enrollment. The EMNet Coordinating Center will send all the necessary materials to each site for specimen collection.

For the collection, the child will be placed supine, 1 mL of normal saline will be instilled into one naris, and then an 8 French suction catheter will be used to remove the mucus. This procedure will be performed once on each nostril. After the specimen collection from both nares, 2 mL of normal saline will be suctioned through the catheter to clear the tubing and to insure that a standard volume of aspirate is obtained. This procedure being standardized across sites is required for accurate detection of viral load. Once collected the nasal aspirate specimen will then be added to transport medium with a virus stabilizer (15% glycerol in Iscove’s media without antibiotics or antifungal) creating a 1:1 dilution. Each aspirated specimen will contain a maximum of 8 mL (range 6 to 8 mL. This virus stabilizer contains penicillin, gentamicin, and amphotericin B.

After collection and labeling with the date and a unique study ID, the NPA specimens will be placed on ice in a bio-hazard bag and stored at 4°C within one hour of collection, and frozen at –70°C or –80°C within 24 hours of collection. Specimens collected for the study must be easily distinguishable from other laboratory specimens stored in the freezer. Batches of specimens will be placed on dry ice for transport to Dr. Piedra at the Department of Molecular Virology and Microbiology at Baylor College of Medicine, Houston, Texas (see shipment details below). Once in Dr. Piedra’s laboratory, the specimens will be stored at –80°C. When the specimen is thawed for the first time, lab personnel will divide the specimen into six 1.0-1.5 mL aliquots. This division will help prevent repeated thaw/freeze cycles.

**Blood:**
A trained study personnel member or a member of the local phlebotomy team will collect a blood specimen. The blood draw may occur at any time during the child’s hospitalization. The 0.5 mL required for the CBC and differential will be collected in a purple top tube. All other blood specimens will be collected in red top tubes, which the EMNet Coordinating Center will provide to each site. At the discretion of investigator and parent/legal guardian, EMLA cream (lidocaine 2.5% and prilocaine 2.5%) will be applied to the skin to prevent pain associated with needle insertion. A minimum of 5 mL of blood is needed to participate in the study if a CBC has not been performed and 4.5 mL of blood if a CBC has already been performed. In the event the required volume of blood is not acquired through the blood draw and other sources such as prior
venipunctures or discarded blood from red top tubes, sites should attempt to schedule another blood draw. If after all these attempts the 5 mL of blood is still not able to be collected, enrolling sites should contact Project Coordinator Ashley Sullivan directly about the possibility of a waiver.

Once the blood in the red top tube is collected, the specimen will be centrifuged (3000 rpm at room temperature). The serum will be placed in a plain cap tube, which the EMNet Coordinating Center will provide to each site, and then stored at –70°C or –80°C. For those participants who have agreed to participate in the genetics portion of the study, the cap will be placed back on the original tube and the remaining contents (the pellet) will also be frozen at –70°C or –80°C. Batches of serum specimens will be placed on dry ice for transport to Massachusetts General Hospital, Boston, MA (see shipment details below). Once in Massachusetts General Hospital’s laboratory, the specimens will be stored at –80°C. When the specimen is thawed for the first time, lab personnel will divide the specimen into 1.0 mL aliquots. This division will help prevent repeated thaw/freeze cycles. Batches of pellet specimens will be placed on dry ice for transport to the University of Arizona, Tucson, AZ or Massachusetts General Hospital (see shipment details below). Pellet specimens will be stored for future use.

If study personnel identify available discarded blood that has been collected in tubes other than a red top tube, this blood should be processed, stored and shipped with the same procedures outlined above and study personnel should clearly identify the source of this additional specimen on the specimen label and in the shipping list when sending with the serum samples to the Massachusetts General Hospital at the end of the year. These samples will not count towards the total required for the study.

Nasal Swabs:
The nasal swab specimens will be collected by two different parties. Study personnel will collect one nasal swab specimen at the time of enrollment (index swab) and parent/legal guardian will collect multiple other nasal swab specimens during follow-up (follow-up nasal swab specimens.) The method for collecting, preparing and shipping these specimens is identical.

Index nasal swab specimen
Within 24 hours after admission, a trained study personnel member will collect an anterior nares swab. The specimen collection will not affect local practice or care. Written certification that local staff are competent in all study procedures including nasal swab collection will be provided to the EMNet Coordinating Center by the local site PI before starting enrollment. We will send all the necessary materials to each site for specimen collection. For the collection, the child will be placed supine and will be secured by the parent/legal guardian or by study personnel. Study personnel will gently insert the swab into the child’s nostril, and rub the swab gently against the inner wall of the nostril, getting a good sample of mucus. The swab will then be
removed from the nostril, and using the same swab mucus from the other nostril will be collected. The swab will then be placed in the provided cryogenic vial, which will already contain the proper amount of transport medium and the cap will be securely tightened. This vial will be placed in the transport tube supplied with the EXAKT-PAK shipping system, which contains the required amount of absorbent material. This transport tube will then be wrapped in provided bubble wrap and inserted into a pre-addressed, pre-paid, outer box that has all necessary markings for shipment to Massachusetts General Hospital (see shipment details below.) Once in Massachusetts General Hospital’s laboratory, the specimens will be stored at –80°C.

During the index nasal swab collection at the time of enrollment study personnel will ensure the parent/legal guardian witnesses and understands the collection procedure so he or she can repeat the procedure for all follow-up nasal swab collections.

Follow-up nasal swab specimens
The parent/legal guardian will repeat anterior nares collection for all follow-up collections (clearance swab, ARI swabs, and seasonal swabs) using the same collection materials and methods described above.

The parent/legal guardian will have been given or sent a kit containing everything needed for the collection including nasal swab collection instructions (see ARI Kit Contents, Appendix F1.) After collecting the specimen following the method described above and securing the swab in the transport vial, the parent/legal guardian will record the collection date and time on the label, ensure the correct, unique study ID has been affixed, then seal the vial inside the provided plastic transport tube that already contains a sufficient amount of material to absorb any spilled liquid and ship the specimen to Dr. Carlos Camargo at Massachusetts General Hospital using the same preparation and shipping procedures described above.

Nasal swab specimens received by Massachusetts General Hospital during follow-up will be placed on dry ice for transport to Dr. Pedro Piedra at the Respiratory Virus Diagnostic Laboratory at Baylor College of Medicine, Houston, Texas where microbiologic studies will be conducted (see shipment details below.) Once in the laboratory, the specimens will be stored at –80°C.

Labeling
Study participants will be assigned a unique study ID at time of consent when data are entered into REDCap. The REDCap software ensures that duplicate IDs cannot be created and that all data entered in to the application are associated with a unique ID. Prior to specimen collection, study personnel will label all specimen
collection kits and containers with this unique study ID. These kits include the materials for NPA, blood, and nasal swab specimens collected at time of admission, as well as the clearance swab and 2 ARI swab specimen collection kits that are given to the parent/guardian for future collections. Labels with IDs will be pre-printed the EMNet Coordinating Center and sent to sites. Additionally, the date (mm/dd/yyyy) and time (hh:mm) of specimen collection must be written on the labels of the specimens collected. The ID number used in REDCap must match the label ID number used on the specimen. Study personnel will also ensure that the transport tubes and materials in all replacement kits that are sent from the EMNet Coordinating Center at Massachusetts General Hospital also are marked with the proper study ID prior to being sent to participants.

To help keep track of the specimens collected and received at Baylor and at the Massachusetts General Hospital, there will be shipment lists that will be sent with each shipment of specimens. On the shipment list will be the specimen ID number, collection date and collection time of each specimen (see Manuals of Procedures for Specimen Collection, Labeling, Storage, and Shipment for each specimen collection, Appendix E1-3).

All specimens stored at the Baylor Laboratory and the Massachusetts General Hospital will be labeled with a bar code upon arrival.

### 6.2.2.2 Specimen Shipment

**NPA**

Only lab personnel with International Air Transport Association (IATA) certification may package and ship NPA specimens. Site PIs should be present during this process to ensure that all NPA specimens are included in the mailing, to give the necessary supplies to the person preparing the package, and to include the completed shipment list in the mailing. Specimens will be shipped twice during each recruiting season (once in January and once in May). We will stagger the shipments so that 5 sites ship NPA specimens to the Baylor Laboratory during one of 2 calendar weeks in January and May. The EMNet Coordinating Center site will determine which sites ship during which week.

FedEx is the preferred carrier for NPA shipments. Place specimens and absorbent material into biohazard bags and place them in the sample box(es). Place the sample box(es) in the Styrofoam shipping box and surround the sample box(es) with 5 lbs (2.27 kgs) of dry ice. A completed NPA Shipment List must be placed on top of the Styrofoam lid. Place the Styrofoam shipping box into an outer cardboard box. Seal the cardboard box and affix the completed dry ice and Biological Substance, Category B labels. Mindful of holidays, specimens should be sent only on Mondays,
Tuesdays, or Wednesdays so that the specimens do not arrive on a Saturday, Sunday, or holiday.

NPA specimens should be shipped (overnight) to:
   Pedro A. Piedra, M.D.
   Attention: Kirtida Patel
   Baylor College of Medicine
   Department of Molecular Virology and Microbiology
   Mail stop code: BCM 280, Room 248E,
   One Baylor Plaza
   Houston, Texas 77030
   Lab Tel: 713-798-8339
   Office Tel: 713-798-5240

   On the day that the specimens are sent, the site PI should email both
   afsullivan@partners.org and ppiedra@bcm.tmc.edu the FedEx tracking number
   for the mailing.

See Appendix E1 for a manual of procedures for specimen collection, labeling, storage, and shipment for NPA

**Serum and Blood Pellet**

Only lab personnel with IATA certification may package and ship blood specimens. Site PIs should be present during this process to ensure that all blood specimens are included in the mailing, to give the necessary supplies to the person preparing the package, and to include the completed shipment list in the mailing. Specimens will be shipped once during each recruiting season in May.

FedEx is the preferred carrier for blood shipments. Place specimens and absorbent material into biohazard bags and place them in the sample box(es). Place the sample box(es) in the Styrofoam shipping box and surround the sample box(es) with 5 lbs (2.27 kgs) of dry ice. A completed blood Shipment List must be placed on top of the Styrofoam lid. Place the Styrofoam shipping box into an outer cardboard box. Seal the cardboard box and affix the completed dry ice and ‘Exempt Human Specimen’ labels. Mindful of holidays, specimens should be sent only on Mondays, Tuesdays, or Wednesdays so that the specimens do not arrive on a Saturday, Sunday, or holiday.

Only pellets from participants that did not provide consent for the genetic testing components of the study will be shipped to Massachusetts General Hospital. All pellets from participants that provided consent for genetic testing will be shipped to University of Arizona.
Serum and pellet specimens from participants who did not provide consent for genetic testing should be shipped (overnight) to:

Carlos A. Camargo, M.D.
Attention: Ashley Sullivan
Massachusetts General Hospital
326 Cambridge Street, Suite 410
Boston, MA 02114
Tel: 617-726-5276

On the day that the specimens are sent, the site PI should email both afsullivan@partners.org and ccamargo@partners.org the FedEx tracking number for the mailing.

Blood pellet specimens from participants who provided consent for genetic testing should be shipped (overnight) to:

Fernando Martinez, M.D.
Arizona Respiratory Center
University of Arizona
Keating Building
1657 East Helen Street
Tucson, Arizona 85721-0240
Tel: 520-626-7670

On the day that the specimens are sent, the site PI should email both afsullivan@partners.org and fernando@arc.arizona.edu the FedEx tracking number for the mailing.

See Appendix E2 for a manual of procedures for specimen collection, labeling, storage, and shipment for blood.

Nasal swabs
The nasal swab specimens will be collected by two different parties. Site study personnel will collect one nasal swab specimen at the time of enrollment (index nasal swab specimen) and parents/legal guardians will collect multiple other nasal swab specimens during follow-up (follow-up nasal swab specimens.) The method for collecting, preparing and shipping these specimens is identical.

Study personnel and parent/legal guardians who package the nasal swab shipments do not need to have IATA certification and the packages do not need to have biohazard labels since the shipments will meet all criteria to be classified as exempt human specimens; First, it has been determined that there is a minimal likelihood that pathogens are present and secondly the packaging meets all requirements in accordance with IATA Dangerous Goods Regulations 50th Edition, Effective 1
January - 31 December, 2009 and the Code of Federal Regulations, 49CFR Parts 100 to 185, revised as of October 1, 2007 (see Manuals of Procedures for Specimen Collection, Labeling, Storage, and Shipment – Nasal Swabs, Appendix E3.)

Shipments to Massachusetts General Hospital
Study personnel at enrolling sites and the parent/legal guardian will ship all nasal swab specimens collected during enrollment and follow-up (index swab, clearance swab, ARI swabs, and seasonal swabs) directly to the Massachusetts General Hospital where the specimens will be stored for 0-6 months. Massachusetts General Hospital will then ship all received specimens to the Baylor Laboratory using the same shipping procedure for transporting nasal swab specimens to the Baylor Laboratory used by the enrolling sites. The procedures for these two different shippers are described below.

Parent/legal guardian shipments to the Massachusetts General Hospital
After parent/legal guardians have prepared the nasal swab shipment using the provided pre-addressed, pre-paid, outer box (see section 6.2.2.1, Nasal swab – collection during follow-up) they will mail this box directly to the Massachusetts General Hospital via first class mail. All relevant regulations governing the mailing of biological specimens will be followed and the package will be properly labeled to meet all USPS guidelines and regulations.

Parent/legal guardian nasal swab specimens should be shipped to:

Carlos A. Camargo, M.D.
Attention: Ashley Sullivan
Massachusetts General Hospital
326 Cambridge Street, Suite 410
Boston, MA 02114
Tel: 617-726-5276

Shipment of specimens to Massachusetts General Hospital for participants not fully enrolled

If allowed as part of the site-specific consent form, sites with specimens from participants who consented to the study, but who were subsequently excluded (e.g., multiple wheeze episodes uncovered during the intake evaluation) or who did not fulfill all enrollment requirements (e.g., insufficient blood volume) should ship these specimens to Massachusetts General Hospital. These specimens may be complete for some subjects (i.e., NPA, serum, pellet, and nasal swab), and partial for others (e.g., less than required blood volume and no other samples collected).

These specimens will be run for biomarkers on inflammation, severe bronchiolitis, recurrent wheezing, asthma and related concepts (as outlined in the consent form) to
determine if there are differences between participants who were included and excluded in the study.

These specimens should be shipped (overnight) to:
Carlos A. Camargo, M.D.
Attention: Ashley Sullivan
Massachusetts General Hospital
326 Cambridge Street, Suite 410
Boston, MA 02114
Tel: 617-726-5276

On the day that the specimens are sent, the site PI should email both
afsullivan@partners.org and ccamargo@partners.org

For participants who withdraw from the study during their index hospitalization, sites should not ship these subjects’ specimens to Massachusetts General Hospital. The specimens of subjects who withdraw from the study during their index hospitalization should be destroyed. (Note: Depending on the site-specific consent form, specimens from participants who consented to the study but who withdraw after the index hospitalization are eligible for testing and sites should ship these subjects’ specimens to Massachusetts General Hospital.)

Massachusetts General Hospital shipments to the Baylor Laboratory
Only lab personnel with IATA certification may package and ship the nasal swab specimens to Baylor Laboratory because the shipment involves dry ice. The study coordinator should be present during this process to ensure that all nasal swab specimens are included in the mailing, to give the necessary supplies to the person preparing the package, and to include the completed shipment list in the mailing.

FedEx is the preferred carrier for frozen nasal swab specimen shipments to the Baylor Laboratory. Place specimens and absorbent material into biohazard bags and place them in the sample box(es). Place the sample box(es) in the Styrofoam shipping box and surround the sample box(es) with 5 lbs (2.27 kgs) of dry ice. A completed Nasal Swab Shipment List must be placed on top of the Styrofoam lid. Place the Styrofoam shipping box into an outer cardboard box. Seal the cardboard box and affix a completed dry ice label and ‘Exempt Human Specimen’ label. Mindful of holidays, specimens should be sent only on Mondays, Tuesdays, or Wednesdays so that the specimens do not arrive on a Saturday, Sunday, or holiday.

Frozen nasal swab specimens should be shipped (overnight) to:
Pedro A. Piedra, M.D.
Attention: Kirtida Patel
Baylor College of Medicine
Department of Molecular Virology and Microbiology
Mail stop code: BCM 280, Room 248E,
One Baylor Plaza
Houston, Texas 77030
Lab Tel: 713-798-8339
Office Tel: 713-798-5240

On the day that the specimens are sent, MGH study personnel will email ppiedra@bcm.tmc.edu the FedEx tracking number for the mailing.

See Appendix E3 for a manual of procedures for specimen collection, labeling, storage, and shipment for nasal swabs.
7 SAFETY CONSIDERATIONS

7.1 Adverse Events
This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and ICH E6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events version 4.0 (June, 14, 2010). These criteria have been reviewed by the study investigators and have been determined appropriate for this study population.

7.1.1 Definitions

7.1.1.1 Adverse Events
For this study, an adverse event (AE) is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that is experienced during participation in the study and is related to a study procedure. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with participation in the study related to any study procedure(s). Any medical condition that is present at the time that the study participant is screened will be considered as baseline and not recorded as an AE.

7.1.1.2 Adverse Events Associated with Study Procedures
The following clinical situations, when associated with study procedures are defined as adverse events and will be recorded on the AE CRF. These situations do not limit the study investigators from reporting any other events, associated or not with these procedures, from being recorded and reported as AEs.

Blood Draws
- Bruising at puncture site larger than 2 cm diameter
- Bleeding from puncture site lasting more than 5 minutes
- Swelling at puncture site larger than 2 cm

Nasopharyngeal Aspirate Collection and Nasal Swab
- Epistaxis within 24 hours from the procedure in which bleeding does not subside spontaneously within 5 minutes
7.1.1.3 Serious Adverse Events
A SAE is defined as “any adverse event that suggests a significant hazard, contraindication, side effect, or precaution.” Because this is an observation study, medical events that would otherwise be called a SAE will not be reported as a SAE unless related to a study procedure.

This includes but is not limited to any of the following events related to a study procedure:
1. Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up.
2. A life-threatening event. A life-threatening event is any adverse experience that, in the view of the investigator, places the study participant at immediate risk of death from the reaction as it occurred.
3. An inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability.
5. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

The event will be reported as a SAE if it meets any of the above definitions and is related to a study procedure.

7.1.1.4 Unexpected Adverse Event
An adverse event is considered “unexpected” when its nature, severity or frequency is not consistent with the information that is provided in the Protocol and is related to a study procedure. Additionally, assessment can be based on the medical judgment of the PI with concurrence of the NIAID Medical Officer and the study Medical Monitor if the event is Grade 2 or higher.

7.1.2 Medical Monitoring
The NIAID/DAIT Medical Officer will review all SAEs to assess for possible changes to the overall risk of the study.

7.1.3 Collecting, Recording and Managing Adverse Events

7.1.3.1 Identifying Adverse Events
Any adverse event related to a study procedure that occurs from the moment the study participant has signed the consent form will be recorded and is reportable. Adverse events may be discovered through any of these methods:
1. Observing the participant.
2. Questioning the participant.
3. Receiving an unsolicited complaint from the participant.

7.1.3.2 Recording AEs
Throughout the study all identified adverse events (serious and non-serious) will be recorded on an appropriate source document and an adverse event case report form (Appendix D19) regardless of their severity.

A complete description of all adverse events will include event description, time of onset, investigator assessment of severity, relationship to study procedures(s), time of resolution/stabilization of the event, expectedness, determination of whether the AE qualifies as a SAE, and action taken. A change in the severity of the AE will also be documented. The PI will document assessment of severity and relationship on the CRF.

7.1.3.3 Recording SAEs
Serious adverse events will be recorded on the serious adverse event case report form (Appendix D20), which will include all of the information stated above as well as a narrative of the event signed and dated by one of the study investigators and the NIAID Medical Officer.

7.1.3.4 Managing Adverse Events
The site investigator will apply his or her clinical judgment as to whether an AE is of sufficient severity to require that the participant immediately be removed from further participation in the study. The site investigator will institute any necessary medical therapy to protect a participant from any immediate dangers.

An adverse event will be followed until any of the following takes place: a) it is resolved, b) participant is stable, c) a minimum of 30 days after participant is terminated from the study and the NIAID Medical Officer and the study investigators determine that follow-up is complete.

7.1.4 Grading and Attribution

7.1.4.1 Grading criteria
In addition to determining whether an adverse event fulfills criteria for a serious adverse event or not, the severity of adverse events experienced by study participants will be graded according to the criteria set forth in the National Cancer Institute’s Common Terminology Criteria for Adverse Events Version 4.0). This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.
All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (A semicolon indicates ‘or’ within the description of the grade.):

Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).
Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
Grade 4 = Life-threatening consequences; or urgent intervention indicated.
Grade 5 = Death related to AE.

7.1.4.2 Definition of Attribution
The attribution of an adverse event to a study procedure will be determined by the site investigator or designated physician co/sub-investigator. The site investigator or designee will record the determination of attribution on the appropriate adverse event or serious adverse event form. The attribution of an adverse event to a study procedure(s) will be determined using the descriptors in the following table. For the purpose of this study, the following procedures will be considered when determining attribution:
- Nasopharyngeal Aspirate Collection and Nasal Swab
- Blood collection

<table>
<thead>
<tr>
<th>Code</th>
<th>Descriptor</th>
<th>Definition (guidelines)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UNRELATED CATEGORY</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Unrelated</td>
<td>The adverse event is clearly not related to study. The event is completely related to an etiology other than the study procedures (the alternative etiology must be documented in the study participant’s medical record)</td>
</tr>
<tr>
<td>2</td>
<td>Unlikely</td>
<td>The adverse event is doubtfully related to study and likely to be related to factors other than study procedures.</td>
</tr>
<tr>
<td></td>
<td>RELATED CATEGORIES</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Possible</td>
<td>The adverse event may be related to study procedure. There is an association between the event and study procedure and there is a plausible mechanism for the event</td>
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<td></td>
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<tr>
<td>4</td>
<td>Probable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The adverse event is likely related to study procedure. There is (1) an association between the event and the study procedure, (2) a plausible mechanism for the event to be related to the study procedure, and (3) the event could not be reasonably explained by known characteristics of the study participant’s clinical status and/or an alternative etiology is not apparent.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Definite</td>
<td></td>
</tr>
<tr>
<td></td>
<td>The adverse event is clearly related to study procedure. There is (1) an association between the event and the study procedure, (2) a plausible mechanism for the event to be related to the study procedure, and (3) causes other than the study procedure have been ruled out.</td>
<td></td>
</tr>
</tbody>
</table>

(For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE website: http://ctep.cancer.gov/reporting/ctc.html) will be consulted.

### 7.1.5 Serious Adverse Events (SAE) Reporting Criteria and Procedures

The site Principal Investigator will be notified by the study staff as soon as a staff member becomes aware of the SAE. In the absence of the site Principal Investigator, a physician sub-investigator will be notified.

#### 7.1.5.1 Notifying the NIAID Medical Officer

The site Principal Investigator will complete an adverse event form in REDCap no later than 24 hours after he/she becomes aware of the SAE related to the study procedure. The NIAID Medical Officer will be notified by the overall Principal Investigator (Carlos Camargo) or overall Project Coordinator (Ashley Sullivan) no later than 24 hours after they become aware of the SAE related to the study procedure. Reporting to the NIAID Medical Officer will utilize an initial SAE case report form in draft format. Contact information for the NIAID Medical Officer is listed below:

Alkis Togias, M.D.  
DAIT/NIAID/NIH  
6610 Rockledge Dr.  
Bethesda, MD 20892  
Office: 301-451-3104  
Cell: 240-507-9697
Within another 24 hours, the NIAID Medical Officer and the Principal Investigator will discuss the impact of the SAE on the participant and on the study. Within another 24 hours, a finalized, initial SAE case report form will be generated by the site Principal Investigator and must be approved by the NIAID Medical Officer. The finalized, NIAID-approved case report form will be placed in the participant study chart. Both forms will be sent to the NIAID Medical Officer. As additional clinical information is obtained by the site Principal Investigator regarding the SAE, the SAE case report form will be revised and submitted to the NIAID Medical Officer.

7.1.5.2 Notifying the Institutional Review Board
The Principal Investigator will ensure the timely dissemination of SAE information, including expedited reports, to the IRB in accordance with IRB regulations and guidelines.

7.1.6 Non Serious Adverse Events Reporting
All non-serious AEs will be reported to the NIAID Medical Officer by the EMNet Coordinating Center in monthly progress reports.

In addition, the Principal Investigator will ensure the timely dissemination of AE information to the IRB in accordance with applicable regulations and guidelines.
8 PROTOCOL DEVIATIONS

The practices below are consistent with Good Clinical Practice (GCP ICH E6) Sections:

- Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- Quality Assurance and Quality Control, section 5.1.1
- Noncompliance sections 5.20.1, and 5.20.2.

8.1 Protocol Deviation Definitions

8.1.1 Protocol Deviation

Any change, divergence, or departure from the study design or procedures of a research protocol that affects the study participant's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data constitutes a major protocol deviation (protocol violation). Changes or alterations in the conduct of the study which do not have a major impact on the study participant's rights, safety or well-being, or the completeness, accuracy and reliability of the study data are considered non-major protocol deviations. The Principal Investigator is responsible for reporting protocol deviations to the IRB using a standard reporting form. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

8.1.2 Major Protocol Deviation

A protocol violation is a deviation from the IRB approved protocol that may affect the study participant's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data.

If the deviation meets any of the following criteria, it is considered a major protocol deviation (protocol violation).

Example list is not exhaustive.

1. The deviation has harmed or posed a significant or substantive risk of harm to the research participant.

2. The deviation compromises the scientific integrity of the data collected for the study.

Examples:

- A research participant was enrolled but does not meet the protocol's eligibility criteria.
- Changing the protocol without prior IRB approval.
3. The deviation is a willful or knowing breach of human subject protection regulations, policies, or procedures on the part of the investigator(s).
Examples:
- Failure to obtain informed consent prior to initiation of study-related procedures
- Use of outdated or incorrect consent forms
- Falsifying research or medical records.
- Performing tests or procedures beyond the individual’s professional scope or privilege status (credentialing)

4. The deviation involves a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.
Examples:
- Working under an expired professional license or certification
- Failure to follow federal and/or local regulations, and intramural research
- Repeated minor deviations.

5. The deviation is inconsistent with the NIH Human Research Protection Program’s research, medical, and ethical principles.
Examples:
- A breach of confidentiality.
- Inadequate or improper informed consent procedure.

8.1.3 Non-major Protocol Deviation

A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that has not been approved by the IRB and which DOES NOT have a major impact on the study participant's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

8.2 Reporting Protocol Deviations

Upon determination that a protocol deviation has occurred, the study staff will a) notify the Principal Investigator, b) will complete the Protocol Deviation form. If the protocol deviation is considered “major” according to protocol definitions, the NIAID Medical Monitor needs to be notified within 48 hrs by the overall Principal Investigator (Carlos Camargo) or overall Project Coordinator (Ashley Sullivan). NIAID may request discussion with the site Principal Investigator to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study and corrective actions. The site Principal Investigator will complete and sign the Protocol Deviation form and submit it to the EMNet Coordinating Center (afsullivan@partners.org) and to the site IRB, per IRB regulations. The EMNet Coordinating Center will notify the NIAID Medical Officer.
Non-major protocol deviations involving missing samples or visits outside of the time window will be reported by the EMNet Coordinating Center every two months to the NIAID in the form of log sheets.
9 STATISTICAL CONSIDERATIONS

9.1 Study Outcome Measures
The outcome measure for the three main aims of the study is recurrent wheezing of childhood by age 3 years (as defined by the date of birth, not the date of enrollment). Recurrent wheezing of childhood is defined in the 2007 NIH asthma guidelines (145) as having at least 2 corticosteroid-requiring exacerbations in 6 months or having at least 4 wheezing episodes in one year that last at least one day and affect sleep. Among children with this clinical history and a positive mAPI (see section A7), the NIH guidelines recommend that clinicians begin inhaled corticosteroids for children age 0-4 years to reduce impairment and risk of exacerbations, but not to alter the underlying severity or progression of disease (122, 146).

9.2 Sample Size Considerations
We plan to enroll an average of approximately 333 study participants per year for 3 years (total n=1000). Based on our collective experience, we anticipate that each of the selected site teams will enroll and have ≥3-year follow-up for an average of ≥43 of 50 children per enrollment year (ie, ≥85%). From November to April, the average number of admissions of children age <1 year with bronchiolitis per site is 400, allowing for <15% enrollment rate. The run-in requirement (see section 6c) will require over-enrollment of ~10% to achieve the target sample. In previous EMNet studies of children presenting to the ED, we have had 90% two-week follow-up (151, 199) and in Years 1 and 2 of our inpatient study (including all-comers and sites not in this study) we had 88% short-term follow-up. In other populations, we achieved a 6-month follow-up of 90% (children visiting ED with acute asthma (200)) and 12-month follow-up of >80% (adults visiting ED with acute asthma (150)). In three cohorts, with much more intensive follow-up requirements, we have achieved ~90% follow-up at 3 years (96) and 6 years (52, 99). In our pilot cohort study (see section C1) we achieved 100% follow-up at 6 months and 92% follow-up at 1 year. Using our screening form and based on this previous work, we estimate that we will have ≥85% follow-up rate for at least 3 years. We use a total sample size of 850 in all of the power calculations below.

Aim #1 – Hypothesis 1a is that HRV will increase risk of recurrent wheezing, as compared to the risk of children with other pathogens. Assuming 16% of 850 children are infected with HRV (n=136) and 60% with RSV (n=510) (53) and that between 20% and 40% of infants with RSV will have recurrent wheezing (32, 33), we will have 80% power to detect a 1.35- to 1.60-fold increase in the relative risk of having the study outcome between the two groups at the significance level of 0.05. Even if our follow-up is only 80%, we will still have 80% power to detect a 1.37- to 1.65-fold increase in the relative risk.
Hypothesis 1b is that children admitted to the ICU will be more likely to develop recurrent wheezing compared to children admitted to the regular ward. Similar to our ongoing inpatient bronchiolitis study (U01 AI-67693), we plan to purposefully over-enroll children admitted to the ICU and will have the patient population at the participating hospitals to meet our goals: approximately 20% of the cohort will come from the ICU and 80% from the ward. The study will have >80% power to detect a risk ratio of 1.55 if 20% of the ward patients develop recurrent wheezing, or to detect a risk ratio of 1.32 if 40% of the ward patients develop recurrent wheezing. Even restricting the outcome to patients who require intubation or CPAP (50% of total ICU patients), we will have 80% power to detect a 1.43- to 1.75-fold increase in the relative risk of having the study outcome between the two groups at the significance level of 0.05.

Aim #2 – Since few foods contain vitamin D precursors (201), exposure to ultraviolet (UV) B rays is the primary determinant of vitamin D status in humans. In northern latitudes between November and March there are insufficient UVB rays to produce vitamin D (93). In this study, we have a wide spread of latitudes from Boston (42 °N) to Houston (29 °N). We estimate that in sites north of Louisville, KY (38°N), 10% of the enrolled children will have 25(OH)D <25 nmol/L and 40% will have 25(OH)D ≥75 nmol/L. We estimate that in sites south of and including Louisville (38°N) that 3% of the enrolled children will have 25(OH)D <25 nmol/L and 60% will have 25(OH)D ≥75 nmol/L (94, 95). Overall, we estimate 55 study participants with 25(OH)D <25 nmol/L, 370 with 25(OH)D 25-74.9 nmol/L, and 425 with 25(OH)D ≥75 nmol/L. The study will have >80% power to detect a risk ratio of 1.95 among study participants with 25(OH)D <25 nmol/L compared to the 25(OH)D ≥75 nmol/L group if 20% of the 25(OH)D ≥75 nmol/L study participants develop recurrent wheezing, or to detect a risk ratio of 1.53 if 40% of the 25(OH)D ≥75 nmol/L study participants develop recurrent wheezing. Treating 25(OH)D as continuous, at a 0.05 significance level, we have 80% power to detect 1.2 to 1.3-fold increase in the odds of recurrent wheezing for a 1 SD (19 nmol/L) increase of 25(OH)D.

Aim #3
To address Aim #3, we plan to develop a prediction rule, which will need to balance complexity and accuracy. After creating the prediction rule based on the first year of our cohort (total n=850; half n=425) in which we expect to have 40% of children with recurrent wheezing, we will validate the prediction model based on data from the second year (n=425, cases=170). Assuming the sensitivity of the first year sample ranging from 80% to 95%, we will choose a threshold so that the specificity will be ≥90% in order to achieve a positive predictive value of ≥85% (the corresponding positive likelihood ratios ranges from 8 to 19). With a positive predictive value of 85% from the Year 2 data, the width of the two-sided 95%CI will be limited to 5.7% from the each side.

Exploratory Aim is that children with higher levels of CCL5 in their NPA will have a higher risk of developing recurrent wheezing of childhood compared to children with lower CCL5 levels. Treating NPA CCL5 as a continuous variable, with α set at 0.05 and a sample size of 850, we will have 80% power to detect 1.3-fold increase in the odds of recurrent wheezing for an NPA CCL5 increase of 1 SD (21.6 pg/mL).
9.3 Participant Enrollment and Follow-Up

From November 1 until April 30, investigators at the enrolling sites will enroll study participants until reaching the annual goal. The total number of study participants who successfully complete the run-in will be an average of 50 study participants per site per year, or 1,000 study participants over the 3-year enrollment period. Based on the number of study participants enrolled monthly at each site, we may ask certain sites to increase their recruitment, to balance a site with an unexpectedly low volume or to meet either the overall enrollment goal or ICU enrollment goal for the study.

We will use many techniques in order to enhance our follow-up:

1) During enrollment we will collect multiple phone numbers for the child’s parent/legal guardian on the Contact Form (Appendix D4) including at least one alternate contact. Providing at least one alternate contact is required to enroll in the study, since this contact will increase the chances of successful follow-up.

2) After discharge from the hospital the run-in period will begin. Children who cannot be contacted by the site 1-week after discharge or contacted by EMNet 3-weeks after admission for the clearance swab will be removed from the long-term or “chronic cohort”, but will still be part of the “acute cohort.” Since these former participants will no longer be considered enrolled study participants in the chronic cohort, no further efforts will be made to follow-up with these individuals through clinical data or biological specimen collection, they will not be included in any analysis related to the main study, and will not count toward the site goal of 30-50 enrolled participants per year. However, these fully consented study participants will be considered part of an “acute cohort” and the specimens previously collected will be stored for possible use in future studies related to vitamin D, infections, allergies, and respiratory illnesses. See section 6.1 – “Post-discharge 7-28 days: Short Term Follow-up” for further details.

3) During the follow-up period we will maintain contact with the families regularly using a variety of techniques: having a toll-free number for families to contact the EMNet Coordinating Center, reminder postcards/emails/calls, birthday cards, and reporting 25(OH)D and virology results. Furthermore, until the child is age 3 years the EMNet Coordinating Center will remind families about the ARI swabs and the seasonal swabs. Moreover, the families will be contacted each time a specimen is mailed to the EMNet Coordinating Center. Families will be contacted every 6 month for follow-up interviews until age 6 years.

4) Participants will be offered remuneration for each completed follow-up phone call in the amount of $20 for the initial 6-month call and $30 for each 6-month call after until the final call of the study for which they will receive $50 (maximum of $250.) (Note that current funding (1U01AI087881-01A1) covers a total of 4 years of follow-up calls – and not the 5-6 years
outlined here. We will seek funding for the later phone calls, which are part of the original consent form. If new funding is not obtained, internal funds will be used to complete the study per protocol.)

9.4 Analysis Plan

A variety of statistical techniques will be used to analyze the data. We will perform descriptive analyses, as well as more analytic work using contingency tables, analysis of variance, and correlation. Our primary analysis strategy will focus on the dichotomized outcome (recurrent wheezing yes/no by age 3) using multivariable logistic regression models. We will also use survival analysis as a secondary approach to examine time to recurrent wheezing taking into account censoring. In addition to the primary outcome (i.e. “recurrent wheezing” ≥2 steroid-requiring exacerbations in 6 months or having ≥4 wheezing episodes in one year that last ≥1 day), we will also explore the impact of using other definitions of recurrent wheezing (39, 43, 49, 67, 83, 202, 203) as the outcome measure. We will also examine the stability of the recurrent wheezing diagnosis beyond age 3 years (43). Although clinician prescribing behaviors are variable for children with wheezing (204, 205), we will consider them as further validation of the wheezing event. From most to least likely, the ranking will be as follows: 1) parent/legal guardian report with physician confirmation; 2) physician report; and 3) parent/legal guardian report.

8a1. Bivariate Analyses

Bivariate analyses will be used to initially test our main hypotheses in the first 2 Aims:

**Aim #1** – Recurrent wheezing will be compared across infectious agents (including no detectable pathogen and multiple pathogens) using chi-squared test and Fisher’s exact test, as appropriate (Aim 1a). The ICU versus ward comparison will be made using the same statistical tests (Aim 1b).

**Aim #2** – For all analyses, we will initially stratify the children into 3 groups based on the exposure variable, vitamin D. As discussed in section D3, we will use a 25(OH)D level of <25 nmol/L as the cutoff for deficiency. Based on Dr Camargo’s respiratory outcome data (99) we will initially define the middle group as 25-74.9 nmol/L and the highest group as ≥75 nmol/L, however, we will perform sensitivity analyses using different cutoffs based on the distribution of vitamin D in children with bronchiolitis. We will use chi-squared test and Fisher’s exact test, as appropriate for bivariate analyses and when treating 25(OH)D levels as a continuous variable, we will use univariate logistic regression.

**Exploratory Aim** – The CCL5-recurrent wheezing association will be examined using logistic regression.

8a2. Multivariable Analyses
For **Aim #3**, we will build multivariable logistic regression models to determine the independent association of each suspected risk factor with recurrent wheezing. Corresponding to the potential predictors listed in Figure 2, the conceptual model is:

\[
\text{logit (p)} = \text{demographics factors} + \text{prior history} + \text{parental factors} + \text{environmental factors} + \text{clinical factors} + \text{laboratory findings},
\]

where \( p \) is the probability of recurrent wheezing by age 3.

Predictors deemed to have clinical importance will be included as candidates regardless of their level of statistical significance. The model will be built on a “training set” of children enrolled in Year 1 and "tested" on the children enrolled in Year 2. Depending on the data, we may develop and validate the model on the entire dataset using the methods of bootstrapping and cross-validation (206, 207). However, the use of an independent testing set with the former approach may provide for a more convincing argument of model validation for clinicians and will be the basis for the initial analysis (208, 209). Two-way interactions and non-linear representations of continuous predictors will be explored. In addition, the alternative method of recursive partitioning will be considered for building a prediction rule and also for identifying important interactions or risk-thresholds to include in the model (210). Discrimination will be based on the area under receiver operating characteristic curve that is determined by the model’s predicted risk estimates (211, 212). Calibration will be assessed by the Hosmer-Lemeshow goodness-of-fit statistic, comparing predicted versus actual outcomes in the testing set (211, 212). Regression coefficients from the final model will be used to derive WIND.
10 SUBJECT CONFIDENTIALITY

Subject confidentiality is held strictly in trust by the participating investigators, their staff, and the sponsor and their agents. This confidentiality is extended to cover testing of biological specimens and all data collected. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of both the PI and NIAID/DAIT.

Study sites will permit access to all documents and records that may require inspection by the sponsor or its authorized representatives, including but not limited to, medical records (office, clinic or hospital) for the subjects in this study.

All study data will be entered and stored in REDCap (Research Electronic Data Capture) a HIPAA compliant web-based application featuring audit trails for tracking data manipulation and export procedures. (See section 4 for more on REDCap.) This application will be hosted by Partners HealthCare Systems (PHS). PHS has all necessary physical and operational securities in place to meet or exceed Federal and State security and privacy regulations. PHS restricts, monitors, and controls access to systems by authorized personnel who additionally have signed confidentiality and ethical pledges to safeguard data (a condition of employment with PHS) and have performed training in protecting Human Subject data (see PHS Research IT Facilities Security, Appendix A2.)

Every effort will be made to protect the privacy of the study participant. Unique identifiers will not be stored in the final database that will be used for analytic purposes. Information such as age, sex, and race will be collected in order to examine the prevalence of bronchiolitis among demographic subgroups. This information will help to establish efforts to improve bronchiolitis care in previously or newly identified high-risk subgroups.

At each site the biological specimens will be stored in a in a freezer is a secured room. Each specimen will be labeled with the study ID. Only study personnel will have access to the personal information linked with this ID. Only personnel at the EMNet Coordinating Center and the laboratories receiving specimens for testing (Baylor College of Medicine and University of Arizona) will have the codes linking the site numbers and the participating sites.

All biological specimens will be sent to either Baylor College of Medicine, Massachusetts General Hospital or the University of Arizona for processing. Specimens will be coded with study identifying numbers, but will not have any personal information that would allow lab staff to identify the individual who gave the specimen. Once Dr. Piedra, Dr. Camargo, and Dr. Martinez receive the specimens, they will be stored in a freezer in a secured room in the laboratory. Each specimen will have two labels: 1) a barcode label and 2) a study identification number (i.e., 3-digit site number + 3-digit individual study participant number).
Upon completion of the study, electronic data will be retained by the EMNet Coordinating Center at Massachusetts General Hospital. Electronic files will be password-protected. Once the site personnel have entered any data they may have collected on paper sheets into the electronic file, have answered all queries, successfully sent the information to the EMNet Coordinating Center, and the site monitor has signed off on the site, the site PI will be able to destroy the records as soon as institutional guidelines allow. However, the existence of any paper forms will be rare since all data will be entered directly in to the REDCap database.

The PI, co-investigators, and designated study personnel will use the data to prepare manuscripts. Site PIs will have the opportunity to write manuscripts based on secondary analyses. (For details, see the Publication Policy below.)

Publication Policy
Study data will be held at the EMNet Coordinating Center at Massachusetts General Hospital. Site investigators from participating sites may submit a proposal for a secondary analysis of the data by using an online form (http://www.emnet-usa.org/Coordinating_Center/SAPF.cfm). This online form will be posted on the EMNet website and, when proposals are submitted, this will generate an automatic email to Dr. Camargo notifying him of the submission. All secondary analysis proposals should state the hypothesis to be tested, the data required, the analytic methods to be used, and the individual responsible for writing the manuscript. Once the data for a specific secondary analysis have begun to be analyzed, the expected time to completion of a submittable manuscript will be 3 to 6 months. The study leadership will retain the data and conduct the analysis according to specifications agreed upon with the applicant. If necessary, funding for programming and statistical time may be requested from the applicant or the team leader. If no manuscript has been completed within the projected timeline, then the study leadership reserves the right to allow another investigator to approach the same question, if a competing application has been received.

10.1 Future Use of Stored Specimens
As stated in the consent forms, any residual biological specimens will be maintained for future use. Residual NPA and nasal swab specimens not utilized in testing for the study will remain at the Respiratory Virus Diagnostic Laboratory at Baylor College of Medicine, Houston, Texas. Residual serum specimens not utilized in testing for the study will remain at the Massachusetts General Hospital, Boston, Massachusetts. Blood pellet specimens will remain at the Arizona Respiratory Center at the University of Arizona, Tucson, Arizona and at Massachusetts General Hospital. Any additional blood specimens will remain at Massachusetts General Hospital. Participating sites’ IRBs will review future studies that involve the use of stored specimens that goes beyond the current informed consent.

Specimen labels will include the date (mm/dd/yyyy) and time (hh:nn) of specimen collection and an ID number (site number + study participant number). This label will not include study
participant identifying information. Only the EMNet Coordinating Center and the laboratories receiving specimens for testing (Baylor College of Medicine and University of Arizona) will have the links between the site number and the hospital. (The participating labs will not have access to the personal information linked with the study ID.) The study participant ID number and study participant’s laboratory data will remain securely stored. This coding scheme will protect subject confidentiality for any future studies with the stored specimens.
11 INFORMED CONSENT PROCESS

11.1 Informed Consent/Assent Process (in Case of a Minor or Others Unable to Consent for Themselves)

Informed consent is a process that is initiated prior to the child’s parent/legal guardian agreeing to participate in the study and continuing throughout the child’s study participation.

Parents/legal guardians will be approached about participating after the medical team has finished their assessments and stabilized the study participant. By delaying the recruiting process, we believe the parent/legal guardian will be better able to focus on the proposed study, including the consent. Members of the research team will recruit, consent, and interview the parent/legal guardian of the child utilizing a number of informational materials including an introductory video. Consent will be obtained from all parents/legal guardians. If the parent/legal guardian expresses any desire to discontinue, the assessment will be either postponed or terminated, based on his or her wishes.

Information about the risks and possible benefits of participation in this study will be provided to the parent/legal guardian. Consent forms describing in detail the study procedures and risks are given to the child’s parent/legal guardian and written documentation of informed consent is required prior to data collection. Consent forms will be IRB approved and the parent/guardian will be asked to read and review the document. Upon reviewing the document, study personnel will explain the study to the parent/guardian and answer any questions that may arise. The parent/legal guardian should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The parent/legal guardian may withdraw consent at any time throughout the course of the study. If the parent/legal guardian expresses any desire to discontinue, the assessment will be either postponed or terminated, based on his or her wishes. A copy of the informed consent document will be given to the parent/legal guardian for their records. The rights and welfare of the subjects will be protected by emphasizing that the quality of the child’s medical care will not be adversely affected if they decline to participate in this study.

See Appendix B for sample consent forms for subject participation.
12 LITERATURE REFERENCES


56. Busse WW, Lemanske RF, Jr., Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet 2010 Sep 4;376(9743):826-34.


86. Meissner HC. Selected populations at increased risk from respiratory syncytial virus infection. Pediatr Infect Dis J 2003 Feb;22(2 Suppl):S40-4; discussion S4-5.


114. Jartti T, Ruuskanen O, Mansbach JM, Vuorinen T, Camargo CA, Jr. Low serum 25-hydroxyvitamin D levels are associated with increased risk of viral infections in wheezing children. Journal of Allergy and Clinical Immunology 2010 Nov;126(5):1074-6, 6 e1-4.


118. Reed CE. The natural history of asthma. Journal of Allergy and Clinical Immunology 2006 Sep;118(3):543-8; quiz 9-50.


135. Levy JA. The unexpected pleiotropic activities of RANTES. Journal of Immunology 2009 Apr 1;182(7):3945-6.


SUPPLEMENTS/APPENDICES

A. REDCap Information
   1. REDCap General Information
   2. PHS Research IT Facilities Security
   3. PHS Research IT Facilities Infrastructure
B. Sample Consent Forms
   1. Sample Consent Form
   2. Sample Tissue Bank Consent Form
C. Schedule of Events
D. Data Forms
   1. Site Form
   2. Screening Form
   3. Intake Form
   4. Contact Form
   5. a. Maternal Pregnancy and Nutrition Form
      b. Maternal Pregnancy and Nutrition Form for Non-Mother
   6. Inpatient Form
   7. Readmission Intake Form
   8. Readmission Inpatient Form
   9. 1-Week Follow-Up Form
10. 3-Week Follow-Up Form (Administered only by the Emergency Medicine Network Coordinating Center (EMNet))
11. 6-month Follow-Up Form (EMNet only)
12. ARI swab Form

13. ARI swab Follow-Up Form (EMNet only)

14. Clearance Swab Form

15. Seasonal Swab Form (EMNet only)

16. Chart Review Form (EMNet only)

17. Parent Chart Review Form (EMNet only)

18. Protocol Deviation Form

19. Adverse Event Case Report Form

20. Serious Adverse Event Case Report Form

21. Specimen/Enrollment Checklist

E. Manuals of Procedures for Laboratory Specimen Collection, Labeling, Storage, and Shipment

1. NPA

2. Serum and Blood Pellet

3. Nasal Swabs

F. Nasal swab Collection kits

1. ARI Swab Kit Contents

2. ARI Swab Kit Letter

3. ARI Swab Kit Instructions

4. Clearance Swab Kit Contents

5. Clearance Swab Kit Letter

6. Clearance Swab Kit Instructions

7. Seasonal Swab Kit Contents (EMNet only)

8. Seasonal Swab Kit Letter (EMNet only)
9. Seasonal Swab Kit Instructions (EMNet only)
10. Nasal Swab Collection Instructions

G. Participant Materials
   1. Reminder Postcard for 6-month Call (EMNet only)
   2. Birthday Postcard (EMNet only)
   3. PCP Virology Results Notification Letter
   4. Parent Virology Results Notification Letter
   5. PCP Vitamin D Results Notification Letter - Adequate
   6. PCP Vitamin D Results Notification Letter - At Risk for Inadequacy
   7. PCP Vitamin D Results Notification Letter - At Risk for Deficiency
   8. Parent Vitamin D Results Notification Letter - Adequate
   9. Parent Vitamin D Results Notification Letter - At Risk for Inadequacy
  10. Parent Vitamin D Results Notification Letter - At Risk for Deficiency
  11. PCP Study Introduction Letter
  12. Study Introduction Brochure
  13. Study Introduction Video