The Pneumonia Etiology Research for Child Health Project (PERCH)

Study Protocol

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Introduction

1.1 Background

1.1.1 Pneumonia

Pneumonia remains the leading infectious cause of child deaths (Bryce, Boschi-Pinto et al. 2005). Current prevention and treatment strategies were developed primarily based on the pathogens identified in pneumonia etiology studies conducted in the 1980s (Programme for the Control of Acute Respiratory Infections and World Health Organization 1991; Rudan, Boschi-Pinto et al. 2008). Bacterial pathogens, especially *Haemophilus influenzae* and *Streptococcus pneumoniae,* were recognized to be the major etiologies of pneumonia mortality; consequently treatment and prevention strategies targeted these agents (Programme for the Control of Acute Respiratory Infections and World Health Organization 1991) (Shann 1986).

By 2015, three major changes will have taken place since those etiology studies were conducted that make a new round of etiology information essential. First, pneumococcal and Hib conjugate vaccine will be routinely used in 50% and 90% of all low-income countries, respectively. As a result, the primary underlying understanding about etiology which drives existing treatment algorithms may be invalid and consequently the treatments may be ineffective or inappropriate. Second, HIV infection is now widespread, and is driving both the frequency of pneumonia and the distribution of pathogens causing it (Calder and Qazi 2009). Third, substantial changes in nutrition, living conditions (e.g., urbanization), and access to health care will also modify the transmission of agents and the natural history of infection. Without this new pneumonia etiology information, our treatment algorithms will be ineffective or inappropriate and we may miss opportunities for prevention with new vaccines, biologics, or other strategies. Whereas in the past a large proportion of pneumonia cases were of unknown etiology even after an exhaustive diagnostic work-up, we now have highly sensitive molecular tools to advance the identification of pathogens.

A new foundation of data is required to ensure that childhood pneumonia treatment and prevention strategies are relevant and appropriate for the epidemiologic setting of the future. The Pneumonia Etiology Research for Child Health (PERCH) project is a large, multi-center case-control study to determine the etiology of pneumonia likely to occur in the future. The study is based on careful selection of representative sites and will be carried out using standardized clinical and laboratory methods and techniques, in combination with innovative diagnostic techniques and novel specimen collection methods. The data will be analyzed using advanced statistical methods and their interpretation will be considered carefully in advance of knowing the specific microbiologic results. Conducting these studies now, strategically designed to reflect what we expect the world to look like in 2015 and beyond, will provide important evidence to guide the next generation of pneumonia prevention and treatment approaches.

1.1.2 Known common pathogens

Establishing the proportion of childhood pneumonia episodes and the number of associated deaths that are attributable to a given bacterial pathogens is extremely difficult. Most studies, as will be seen in the review of the literature presented here, are based on case series of children with episodes of pneumonia, but with little comparative information among children who do not have pneumonia. Especially for pathogens which may cause mild disease along with

severe disease, or for pathogens which have the ability to cause colonization without disease, the interpretation of identifying the presence of the pathogen among a group of children with pneumonia is not evidence of causality. That being said, here we provide background information on the understanding of pathogens that are important causes of pneumonia in children.

Bacteria have been a major focus of detection among pneumonia cases because these episodes can be treated with antibiotics and because vaccine development has been possible. The highest estimates for the proportion of severe cases of pneumonia that are attributable to bacteria come from inpatient studies which include lung aspiration as a means of identifying the etiological agent. Lung aspirate studies, which have reported bacterial isolation rates between 28% and 84%, are widely regarded as the gold-standard for defining the etiology of pneumonia, although they can only practicably be undertaken in hospitalized patients who are clinically stable and have a well-defined peripheral consolidation (Scott and Hall 1999; Vuori-Holopainen and Peltola 2001; Vuori-Holopainen, Salo et al. 2002). Consequently, the spectrum of pathogens identified by lung aspirate studies may be biased toward those pathogens more likely to cause peripheral alveolar consolidation.

Taken as a whole the literature suggests that almost half of all cases of community acquired pneumonia characterized on chest radiograph by either lobar or broncho-pneumonic changes are due to bacteria (Scott and Hall 1999). The predominant organisms identified in these studies are *Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus*. Additional support for the conclusion that bacterial pathogens are responsible for a large number of severe episodes of pneumonia comes from the studies of *H influenzae* type b (Hib) vaccine and pneumococcal conjugate vaccine (PCV) which demonstrated a significant reduction in the incidence of radiologically confirmed pneumonia among vaccine recipients (Mulholland, Hilton et al. 1997; Cutts, Zaman et al. 2005; Madhi and Klugman 2008). Widespread use of Hib vaccine and PCV will prevent many, but certainly not all, cases of severe pneumonia. Among the pneumonia episodes that remain, treatment with antibiotics directed at these two primary pathogens is unlikely to be effective.

The introduction of PCV has led to a significant reduction in nasopharyngeal carriage of vaccine serotypes of pneumococci which, in turn, has produced a significant indirect vaccine effect among unvaccinated children and adults (Bogaert, De Groot et al. 2004; Reingold, Hadler et al. 2005). The ecology of the nasopharynx is dynamic, and it is possible that the changes in pneumococcal colonization as a result of PCV use could impact carriage of other bacteria along with the changes in serotype carriage of pneumococci that we know occur. Studies have suggested an inverse relationship between carriage of *S. pneumoniae* and *S. aureus,* which has led to speculation that the use of PCV covering a broader range of serotype than PCV7 may result in a shift, not only toward non-PCV7 type carriage, but also toward higher *S aureus* carriage rates (Bogaert, van Belkum et al. 2004; Regev-Yochay, Dagan et al. 2004). It is critical, therefore, to monitor for changes in the etiology of pneumonia that may occur with PCV use.

While the 'traditional' bacterial pathogens are clearly major contributors to the current pneumonia disease burden, clinical studies suggest that other pathogens may also be important in sub-groups of the inpatient population and their role would be underestimated using blood culture data alone. Respiratory viruses make a significant, though often highly seasonal, contribution to the pneumonia in-patient burden in children in developing countries. Other

important pediatric pathogens include *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella pneumophila*, *Bordetella pertussis, Pneumocystis jirovecii* (particularly in immunocompromised patients and including malnourished children) and *Mycobacterium tuberculosis*. The role and burden of other more rare or fastidious pathogens is unknown.

Respiratory syncytial virus (RSV) is the major cause of severe acute respiratory infection (ARI) in children, worldwide (Rudan, Boschi-Pinto et al. 2008; Nair, Nokes et al. 2010). In addition to RSV, other important viral pathogens include influenza viruses, adenovirus, and parainfluenza virus (Berkley, Munywoki et al. 2010). Rhinoviruses are important causes of upper respiratory infections, but their role in pneumonia is still being clarified. The newly identified human metapneumovirus (hMPV), human bocavirus, Mimi virus, WU virus, and KI virus can all causes respiratory infection and may be of clinical significance (Osterhaus 2008).

Newly developed molecular diagnostic methods now allow for the identification of potentially novel pathogens in children with pneumonia in whom traditional investigations were unrevealing (Briese, Palacios et al. 2005; Dominguez, Briese et al. 2008). Although only symptomatic treatment is available for many viral infections, the timely diagnosis of viral infection allows cohorting of affected children in the wards to prevent nosocomial transmission and also reduces unnecessary use of antibiotics (Byington, Castillo et al. 2002; Bhavnani, Phatinawin et al. 2007). Furthermore the clear detection of viral pathogens as significant causes of hospitalized respiratory disease using appropriate epidemiologic and laboratory methods, serves to focus attention on relevant vaccine development.

M. pneumoniae and *C. pneumoniae* are also noteworthy causes of community-acquired pneumonia which are readily treatable with macrolide antibiotics. Data are sparse on the burden of pediatric pneumonia in developing countries due to these so-called 'atypical' pathogens; however, they are estimated to cause ~20% of adult community-acquired pneumonia in Africa (Arnold, Summersgill et al. 2007). Among patients hospitalized with pneumonia in rural Thailand, *M. pneumoniae* and *C. pneumoniae* were found to cause 9% and 19% of pneumonia in persons aged 0-4 years and 5-14 years, respectively (Phares, Wangroongsarb et al. 2007). In Gabon, atypical pathogens were identified in 11% of children hospitalized with pneumonia, with pertussis accounting for over half of the cases (Lassmann, Poetschke et al. 2008). *C. trachomatis* has also been documented as a cause of pediatric pneumonia. A study of neonates hospitalized for pneumonia in Nairobi found *C. trachomatis* in 50% (Were, Govedi et al. 2002). *P. jirovecii (carinii),* has come to prominence as the cause of severe pneumonia in young African children, particularly among individuals who are immunocompromised (Graham, Mtitimila et al. 2000; McNally, Jeena et al. 2007). Recent data from Kenya and Uganda confirm that *Pneumocystis* pneumonia (PCP) is prevalent among both HIV-infected and HIV-uninfected children hospitalized with severe pneumonia, a finding established in Uganda several years ago (Bakeera-Kitaka, Musoke et al. 2004).

Mycobacterial infections are another important cause of pneumonia, however, the burden of disease has been difficult to establish in developing countries because of diagnostic challenges. There are an estimated 8 million new cases of TB worldwide each year, and 9 of the 10 countries with the highest incidence per capita are in Africa (Dye 2006). Clinical algorithms for diagnosing PTB in children are premised upon chronic clinical features,(Hesseling AC 2002) (Hesseling, Schaaf et al. 2002) despite emerging evidence that culture-confirmed PTB may present with acute symptoms in children. Jeena et al. reported that 43% of 138 culture-confirmed cases of

hospitalised PTB in children presented as acute pneumonia with less than 10 days of symptoms. (Jeena, Pillay et al. 2002) Additionally, other studies in Africa have identified MTB in 8-15% of children hospitalised with acute community-acquired pneumonia, irrespective of HIV infection status (Madhi, Petersen et al. 2000; Zar, Apolles et al. 2001; McNally, Jeena et al. 2007). The latter studies may under-estimate the role of MTB in the etiology of severe childhood pneumonia, as these studies generally used induced sputum or gastric washings, which only has a sensitivity of 23-30% for diagnosing PTB. The use of lung aspiration as a diagnostic procedure is expected to enhance our ability to detect pediatric cases of TB, as it has with adults (Scott, Hall et al. 2000). The role of non-tuberculous mycobacterial infections in the etiology of childhood pneumonia remains to be established. Although the burden of non-tuberculous mycobacterial disease is linked to the prevalence of HIV infection among children, diagnosis is essential for the proper management of affected patients. Furthermore, an unbiased approach to diagnostic sampling is the only way to discover new associations between pathogens and clinical pneumonia.

The purpose of finding unusual pathogens is to optimize therapy. At present the WHO pediatric guidelines for treating children with severe or very severe pneumonia do not include the use of macrolide antibiotics. Likewise, treatment for tuberculosis is available, but is often not used appropriately given the challenges in establishing a diagnosis of TB disease and the paucity of data on the role of MTB in the pathogenesis and etiology of severe childhood pneumonia. While the management of severe viral respiratory disease is essentially supportive, etiological diagnosis of viral infection permits patient cohorting to reduce nosocomial transmission. Additionally, progress is being made in the field of RSV vaccine and drug development and understanding the epidemiology of viral pneumonia will be critical in public health decision making(Empey 2010). For influenza, vaccines exist for both children and for pregnant women as does antiviral therapy. The relative importance of influenza among severe and very severe pneumonia cases is yet to be clearly understood. Finally, it is commonly understood, but poorly measured, that children with severe disease are often infected with more than one micro-organism and management or prevention of one of those may substantially affect the epidemiology and burden of the other micro-organism(s).

A wider question regarding the usefulness of the current WHO guidelines for treatment of moderate to severe pneumonia in the face of emerging antibiotic resistance remains. For many years penicillin has been the mainstay of treatment with chloramphenicol or gentamicin plus penicillin reserved for children with very severe disease. However resistance, particularly to penicillin, is now emerging as a potentially serious problem even amongst isolates of *S. pneumoniae* (Scott, Hall et al. 1998; Nyandiko, Greenberg et al. 2007). Recent data collected from HIV-infected children in South Africa found high rates of antibiotic resistance among colonizing organisms and pathogenic isolates, particularly among children on prophylactic cotrimoxazole (Cotton, Wasserman et al. 2008).

Finally, WHO have put considerable effort into the standardization the interpretation of chest radiographs in children to permit the comparison and generalizability of results from one region to another. Of particular interest is the extrapolation of vaccine efficacy trial results to other settings where the vaccine is not currently in use (Cherian, Mulholland et al. 2005). It will therefore be important to see how the etiology of WHO-defined radiographic confirmed pneumonia changes following the introduction of PCV and Hib vaccine across the developing world.

1.1.3 Past pneumonia etiology studies

Current strategies for prevention and treatment of childhood pneumonia in developing countries were developed primarily based on the pathogens identified in pneumonia etiology studies conducted in the early 1980s (Shann 1986; Wall, Corrah et al. 1986; Ikeogu 1988).

In 1983 the Board of Science and Technology for International Development (BOSTID) at the National Academy of Sciences, USA, defined ARI as one of six priority areas for research funding and convened an international ARI meeting. The participants identified three prerequisites for relevant research:

1. Studies should be undertaken in a wide variety of countries to give full geographical representation to the children of the developing world and they should be standardized to facilitate international comparisons.
2. The *etiology* of ARI should be investigated first because it would be essential for later research on prevention and case management.
3. The international research group should be coordinated by a centre that could provide technical assistance and quality control, and could foster active collaboration between investigators (Bale 1990).

Over the next 5 years, BOSTID undertook such a project, involving investigators from 12 sites who met on an annual basis to agree on clinical definitions, laboratory methods, study designs and analysis plans. The results of the program were published in 1990 in a supplement of the *Reviews of Infectious Diseases*. The anchor of the supplement is the paper "*The epidemiology of acute respiratory tract infection in young children: comparison of findings from several developing countries*" by Beatrice Selwyn on behalf of the BOSTID investigator group, reporting a standardized analysis of the epidemiology of ARI in young children from 10 sites.
The project combined results from 16 studies of upper and lower respiratory tract infections in both community- and hospital-based settings. It examined incidence, prevalence, duration, case-fatality and the effects of age, sex and season on the patterns of disease. It described bacterial and viral etiology and interrogated the clinical signs of respiratory tract infections to define these diseases more accurately. It evaluated risk factors for respiratory tract infections across several sites, including mother's age and education, weight-for-age percentiles, and crowding and smoking in the household. In these studies, *Haemophilus influenzae* and *Streptococcus pneumoniae* were the most frequently identified bacterial etiologies of pneumonia at all sites.

The BOSTID report revealed some of the many difficulties in creating an integrated global description of respiratory tract infections (Selwyn 1990). The inclusion of upper respiratory tract infection (URTI) affirmed its biological connection with LRTI but also undermined the public health impact of the studies, given the generally benign perception of URTI. Site selection gave preference to underprivileged populations but, because the sites had to be close to competent laboratories (which were rare in low-income countries), the representation of the developing world was uneven. For example, five out of the 12 sites were located in Central and South America. A standardized case-definition is essential for international comparisons but most of the BOSTID investigators amended the standardized definitions, thus producing, in some cases,

exceptional incidence results. The failure to obtain lung aspirate material reduced the sensitivity of the study to bacterial causes of pneumonia.

As a consequence of identifying bacterial pathogens as the primary cause of severe childhood pneumonia, global efforts to treat and prevent pneumonia mortality in children focused on using antibiotics effective against these bacteria and on developing vaccines to prevent these infections(Shann 1986). Subsequent intervention studies, including antibiotic treatment programs and vaccine trials, reaffirmed the findings of the etiology studies and confirmed the appropriate emphasis on these as dominant causes of pneumonia at that time (Sazawal and Black 2003; Cutts, Zaman et al. 2005; Madhi and Klugman 2008; Madhi, Levine et al. 2008).

1.1.4 Current pneumonia etiology studies

In recent years it has been recognized that pneumonia continues to be significant cause of childhood morbidity and mortality in developing countries but that it has attracted little attention at the level of scientific inquiry. Several global stakeholders, including the WHO and the Bill & Melinda Gates Foundation have given prominence to the research gap(Greenwood 2008; Scott JA 2008) and scientists across the globe have responded to this need.

In the first stage of PERCH we described the landscape of existing pneumonia research through an email survey reaching out to 5000 investigators. As of June 14, 2010 PERCH has received 74 responses. The results of the survey reveal that there are multiple childhood pneumonia etiology studies taking place throughout the world in both developed and developing countries. This reinforces the point that the PERCH study will provide a proportion, rather than the whole, of the picture of childhood pneumonia etiology in the developing world. However, the survey also highlights the challenges in interpreting various pneumonia etiology studies, particularly when comparing or combining the results. There are a multiplicity of case definitions, clinician involvement, facility types, specimens collected and laboratory tests across the studies.

The survey results also show that there is likely a greater depth of available data then previously recognized. For instance, 8 sites reported doing lung aspirates, 9 induced sputum and 3 conducting post-mortem examinations. The results of this survey will be combined with several other sources of information on current or recent work on pneumonia including (1) a literature review of recent pneumonia etiology studies using several search strategies including: (a) ("Pneumonia"[MESH] OR Pneumon* OR pulmon* OR lower respiratory tract infection OR bacteraemia OR sepsis OR septic*) AND (child* OR pedia* OR paedia* OR neonat* OR infant*) AND (etiol* OR aetiol*) with a filter of: Etiology/Broad , published within the past five years and describing activities taking place since 2000 ; (b) (pneumonia[majr] AND "etiology "[Subheading]) AND (Etiology/Narrow[filter]) , published within the past five years and describing activities taking place since 2000 ; (c) "Pneumonia/microbiology"[MAJR], published within the past five years and describing activities taking place since 2000; (2) studies identified in three recent etiology review articles (Rudan, Boschi-Pinto et al. 2008; Calder 2009) (Murdoch D, unpublished), and (3) studies identified by communication with researchers in the field. The information from the surveys and literature review will be crucial in piecing together the full picture of childhood pneumonia etiology in the world. Several investigators involved in, or planning, pneumonia etiology projects have contacted the PERCH project to request information on case definitions, epidemiological design and laboratory testing so they can harness the work

of PERCH and incorporate this into their own study designs. The PERCH methods have been made available on request but will also be published as a set of methodological papers.

1.1.5 Rationale for the PERCH pneumonia etiology study

By 2015, three major epidemiological changes will have taken place since the formative etiology studies of the 1980s and these changes make a new examination of pneumonia etiology essential (Scott 2008; Scott and English 2008). First, projections indicate that pneumococcal and Hib conjugate vaccination will be routinely used in 50-90% of all low-income countries, invalidating the underlying evidence base of etiological causes for existing treatment algorithms. Second, HIV-infection, which was absent from studies in the 1980s, is driving both the frequency of pneumonia and the distribution of pathogens causing it. Third, substantial changes in nutrition, urbanization, and access to health care will also modify transmission of agents and the natural history of infection. Without new pneumonia etiology information, our treatment algorithms will be ineffective and we will miss opportunities for prevention with new vaccines, biologics, or other strategies.

The strategy for achieving the goal of sufficient, appropriate information on pneumonia etiology to direct prevention and treatment efforts is complicated by various epidemiologic and microbiologic factors. First, pneumonia, an infection of the lung tissue, ranges in severity and outcome. Second, the pathogens causing pneumonia and the distributions of those pathogens may vary according to severity of the episode. The distribution of pathogens causing mild cases of pneumonia is not the same as that of pathogens causing fatal episodes as evidenced by Hib and PCV vaccine probe studies, as well as studies described above of the etiologic distribution of lung aspirate studies. This suggests either that some pneumonia pathogens are inherently more virulent or that different pathogens contribute to the pathogenesis of pneumonia at different stages of severity. Third, obtaining biologic samples for etiologic testing from the site of infection (the lung) is generally not possible. Fourth, the pathogens causing pneumonia are commonly also observed in the respiratory tracts of humans who do not have pneumonia, making the mere presence of the organism in a child with pneumonia difficult to interpret as causally related. Finally, cases of pneumonia are commonly the consequence of infection by more than one infectious agent.

One consequence of these features is that identification of a pathogen in a case of pneumonia does not necessarily imply that it is the cause of the illness, and conversely, failure to identify a pathogen does not necessarily imply it is unrelated (Murdoch, O'Brien et al. 2009). To establish an etiologic diagnosis it is necessary to consider multiple possible etiologies (exposures) as the cause of any one pneumonia episode. Case-control studies are the design of choice when studying an outcome and its relationship to multiple exposures or risk factors. As such, our approach to estimating the etiologies of hospitalized pneumonia is to conduct a case-control study using modern diagnostics, testing multiple specimens, and determining the proportionate contribution of each using appropriate statistical methods.

It is likely that the distribution of pathogens that cause pneumonia changes with the degree of clinical severity. Outpatient and population-based cohort studies help characterize the variation in etiologic distribution among more common, non-severe episodes of pneumonia, but these types of studies do not achieve the fundamental goal of this project. PERCH aims to guide new vaccine development and improve approaches to treat and control childhood pneumonia in developing countries, with the vision of reducing childhood mortality. Therefore, the strategic

approach of the project is to focus on severe cases, most readily identified among hospitalized pneumonia patients. This assumes that severe cases are those most likely to result in death. Hospitalization, as a filter or proxy for severity, is highly variable from setting to setting and cannot be used in itself as a predictable measure of severity. The designs that could be used to identify the etiology of severe pneumonia include longitudinal cohort studies, probe studies, and case-control studies. A longitudinal cohort study would be ill suited to the study of multiple etiologic causes given the relative scarcity of the outcomes in each etiology group. A vaccine or treatment probe study provides excellent causal inference but it can only isolate the etiologic fraction of one or a small group of pathogens at a time, it would be extremely expensive to conduct and would not provide sufficient information for the breadth of the project goal. We have therefore selected a case-control approach, paired with modeling work, to extend the information from the case-control study to regional and global settings.

We recognize there will be concern that the etiologic distribution of severe pneumonia cases identified in the hospital setting may not be entirely representative of the etiologic distribution of severe pneumonia cases or pneumonia deaths that occur outside of treatment facilities. However, a focus on hospitalized cases provides the most efficient design for case-capture, it allows in-depth sampling and testing of children that would not be possible in a community setting, and it provides a natural filter for severity. We acknowledge that the problem of pneumonia etiology is large and complex and that the findings of the PERCH project may be refined by further community-based studies using either observational cohorts or vaccine probe designs. However, as a first step, a case-control study of hospitalized cases will provide a wide net to capture the variety of pneumonia etiologies in an epidemiologically efficient design.

2 Study Objectives and Overview

2.1 Primary objectives

- Determine the association between pneumonia and infection with known and putative viral, bacterial, mycobacterial, and fungal pathogens.
- Estimate the fraction of pneumonia attributable to pathogens for which vaccines are currently under development (including *S. pneumoniae* common protein vaccines, respiratory syncytial virus (RSV), parainfluenza virus (PIV), influenza and *Staphylococcus aureus*) as well as other known, but poorly quantified causes of pneumonia in children including, but not limited to, non-typeable *H. influenzae*, non-typhoidal *Salmonellae*, human metapneumovirus, *M. tuberculosis*, *Pneumocystis jiroveci* ,and potentially fastidious bacteria.
- Assess putative risk factors for infection and/or disease due to novel or under-recognized pneumonia pathogens.

2.2 Secondary objectives

- Determine the association between disease severity and etiology.
- Develop a set of specimens for novel pathogen discovery among episodes with no known etiology (completely negative with comprehensive testing).
- Determine patterns of antimicrobial resistance among invasive isolates including, but not limited to, *M. tuberculosis*, *S. aureus*, *and S. pneumoniae*.
- Develop a set of isolates of key pathogens associated with pneumonia including, but not limited to, influenza, *S. pneumoniae*, non-typhoidal *Salmonellae*, and *S. aureus* for molecular epidemiologic analyses.

- Develop a robust clinical severity index based on analyses of PERCH putative criteria and outcomes.
- Provide a robust platform for ancillary studies of pneumonia epidemiology including, but not limited to, the utility of digital auscultation, chest radiograph, and viral quantification.

2.3 Overview

This will be a multi-country case-control study of severe pneumonia in children <5 years of age. The sites where the study will be conducted are projected to be representative of the areas where most of the severe pneumonia cases in children will occur in 2015 and where key interventions that are expected to be widespread from 2015 onwards are already in place.

Sites:

Seven PERCH sites have been selected:

- Johannesburg, South Africa
- Lusaka, Zambia
- Kilifi, Kenya
- Basse, the Gambia
- Bamako, Mali
- Sa Kaeo and Nakhon Phanom, Thailand
- Dhaka, Bangladesh

Estimated Timeline:

Case-control study timeline:

- June 30, 2010 – submission of final clinical protocol to BMGF
- July 30, 2010 – submission of sub-contract budgets to BMGF
- July-Sept 2010 (3 months) – review of final protocol, SOPs, budgets, etc. by BMGF
- Q4 2010 – Q2 2011 (3-6 months) – contract signing, site staff hiring, training, IRB approvals
- Jan 2011- April 2013 (2 years at each site) - enrollment of cases and controls
- April 2013 – May 2013 (2 months) – final specimen testing, data cleaning, close-out of controls and cases
- June 2013- July 2013 (2 months) – final analyses and report writing

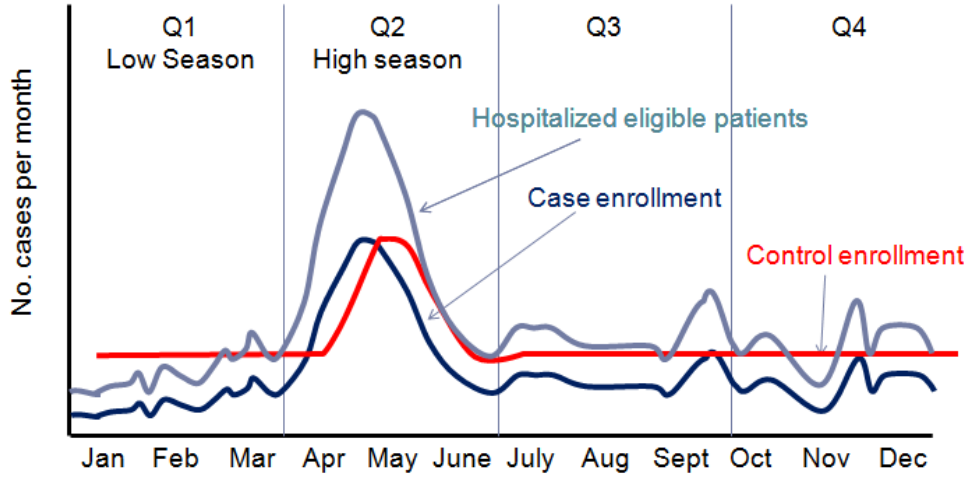
Enrollment of Cases and Controls

Cases and controls will be enrolled over a 2 year period at each site, approximately Jan 2011-April 2013. At larger sites where the total number of eligible patients exceeds the desired sample size, sampling of cases will be implemented. Enrollment will reflect the seasonal distribution of pneumonia patients. Controls will be enrolled on approximately a 1:1 basis to cases, with a minimum of 25 controls enrolled per month at each site. An HIV+ control group will also be enrolled at sites with high HIV prevalence.

Figure 1 illustrates how the monthly minimum enrolment of controls provides a stable set of controls in low incidence months, and how, in peak months, the number of controls above the minimum will be added and collected. The shape of the case and control enrolment curves (dark blue and red, respectively) illustrates that a small interval is anticipated between the number of cases enrolled and the collection of controls. This is a function of the expected lag in

adjustment based on observed cases and the communication of this new target figure to the field workers recruiting controls in the community.

Figure I. Illustration of case sampling proportional to the case detection rates



Cases:
Hospitalized patients 28 days-59 months of age with severe pneumonia.

Controls:
Children from the community without severe or very severe pneumonia who are frequency matched to cases on age and season. HIV+ controls at sites with high HIV prevalence (Zambia and South Africa) will be selected from patient support centers (PSCs) serving the hospital catchment area.

Specimens:
Multiple specimens will be obtained from each case and control:

- Acute blood
- Convalescent blood (cases only)
- Nasopharyngeal and oropharyngeal swabs
- Induced sputum or gastric aspirate if sputum is not obtained (cases only)
- Pleural fluid (cases only, when clinically indicated)
- Lung aspirates (cases only, in select sites)
- Urine
- Post mortem lung needle biopsy (fatal cases only, in select sites)

Follow-up:
A limited clinical evaluation of the cases will be conducted 24 and 48 hours after admission. All cases will also be followed up 30 days (window 21-90 days) post-admission to assess mortality status and to collect convalescent blood and a urine specimen at select sites. Collection of ancillary study data and verification of household environmental risk factors may also be

conducted at this time. Weight and height may be collected in some sites at the follow up visit to assess the impact of pneumonia on acute growth parameters.

Diagnostic tests:
We plan to use bacterial and mycobacterial culture and several highly sensitive non-culture based laboratory diagnostic tools to test the different specimens collected from each child (see 4.6.4 Laboratory Evaluation).

Data structure:
The observed data can be envisioned as a four-dimensional matrix of subjects (cases or controls), specimens, tests and pathogens.

Analysis:
PERCH will estimate (1) the prevalence of **infection** for each of approximately 30 specific pathogens among cases hospitalized with severe pneumonia and (2) the frequency of each of these pathogens as a putative **cause** of hospitalized, severe pneumonia. It will also estimate the proportion of cases with no pathogen identified. This "unknown" group will be a high priority for pathogen discovery efforts.

Sample size:
Over two years, the PERCH study aims to enroll between 5000-7000 patients with severe or very severe pneumonia and an approximately equal number of controls using a standardized methodology that will facilitate pooled analysis. As such, the study is expected to provide substantial power to detect new etiologies of pneumonia, to offer improved precision on existing estimates, and in case-control analyses, to determine associations with risk factors that may not have been possible previously.

2.4 Epidemiologic Rationale for Design

PERCH is designed as a case-control study to identify the etiology of severe pneumonia. Other designs that could be used include longitudinal cohort studies and probe studies. Because PERCH will focus on exploring many different potential etiologies of severe pneumonia, a longitudinal cohort study would be ill suited given the relative infrequency of the events of interest and the fact that cohort studies lend themselves to study of the effects of one or a small number of exposures on a wide range of outcomes. A vaccine or treatment probe study can only isolate the etiologic fraction of one or a small group of pathogens at a time, is extremely expensive to conduct and will not provide the information for the breadth of the project goal. We have therefore selected a case-control approach, paired with modeling work, to extend the information from the case-control study to regional and global settings.

The case control analysis will be used in two major ways, first to infer pneumonia causality from pathogens detected from the upper respiratory tract of pneumonia cases; second, to assess the strength of the association between pneumonia and known and suspected risk factors.

To maximize detection, PERCH will apply a variety of conventional and novel diagnostic approaches to a range of specimen types (including blood, urine, induced sputum and nasopharyngeal specimens). The case control analysis will be used to observe associations of pneumonia with organisms identified from the nasopharynx of cases. It is assumed that if

nasopharyngeal infection precedes pneumonia, then a greater odds of observing a pathogen in cases versus controls points to the causal role of that agent in the pneumonia pathogenesis.

PERCH also aims to model the global distribution of pathology to extend the information from the case-control study to regional and global settings. To do this effectively, data are needed to appropriately characterize the epidemiologic setting of the study sites. For example, knowledge of the distribution of risk factors for each site will facilitate site-to-site comparisons of the etiology distribution found by PERCH, will aid the interpretation PERCH's results relative to those from other studies and will inform the regional and global projection models. Knowing the prevalence of risk factors in the population allows calculation of population attributable risk percent. For this purpose, both individual-level and community-level data will be required. For example, background prevalence of known or potential risk factors will be described among controls. In addition, community-level variables, (e.g. pollution levels) will be gathered from existing sources, including national databases. Because these data will be sought from existing databases, we must ensure good comparability between PERCH data and that from existing data sources. Where possible, the design for collecting data and operationalizing variables in PERCH will attempt to match the methods and measures used in these global databases. Where needed, PERCH will develop adjustment factors to bridge data from study sites to existing sources.

3 Key study personnel, sites and participating institutions

3.1 Study Governance Structure

In establishing a governance structure for PERCH, the core team has done their best to assure that the structure accomplishes the following aims:

1. Assures a high-quality study by incorporating input from all key components of the PERCH structure, including both internal and external groups
2. Provides a clear set of roles and responsibilities for each component of the governance structure
3. Balances the priorities of all key stakeholders
4. Provides unambiguous processes for resolution of key issues
5. Balances the needs for consensus and efficiency, which may at times be in conflict with one another

The PERCH team has defined a governance structure that allows for collective ownership of the study across sites while maintaining functional management systems for what will be a complex operation. The chart and diagram below illustrate the roles and responsibilities of each group involved in study governance.

PERCH Body	**Responsibilities**
PERCH Expert Group (PEG)	• Primary responsibility is to provide sound, strategic advice during the conduct of the study • Represents a "pre-peer review" • Provides links with external research community • Provides a backward link with the Pneumonia Methods Working Group which guided the preliminary phase of PERCH

Executive Committee	• Charged with overall governance of the study, including: -Publications -Sharing of study results -Biorepository -Implementation issues -Monitoring of site performance -Establishing and maintaining standards
Working Groups	• Charged with operations and coordination • Responsible for establishing, maintaining and monitoring standardization across sites • Will collaborate as needed, with questions from sites directed to the working group leaders • Chair is responsible for organizing teleconferences and assuring that each meeting is productive and effective
Operational team	• Weekly monitoring of study progress • Clarification of operational issues that have been decided elsewhere • Identification of issues that require WG attention • Assuring implementation of WG decisions at operational level

The PERCH Core Team is led by Dr. Kate O'Brien, the overall Principal Investigator. Her roles include oversight of the entire project, providing strategic direction, management of personnel and budgets and assuring scientific conduct of high quality and integrity. She is supported in this leadership role by co-PIs (Anthony Scott and Maria Knoll). From a management perspective and study conduct perspective, the PERCH Core Team has a Secretariat consisting of Dr. Kate O'Brien and Dr. David Murdoch, who, along with the site PIs form the PERCH Executive Committee. Other PERCH core team members include pediatricians, epidemiologists, laboratory scientists, and statisticians based at JHSPH.

Each Working Group has a Team Leader from the PERCH Core Team whose responsibility is to direct that section of the project in keeping with the scientific objectives, in a consistent manner across Working Groups, whilst accounting for inherent variations in site capacity and practical setting. It is the responsibility for the Team Leader and the PERCH coordinators assigned to that Working Group to convene regular communication, and identify and propose approaches to the study design, conduct and monitoring. The Team Leaders report to the PERCH PI and co-PIs.

Core Team members participate in Working Groups according to their experience and knowledge. Dr. O'Brien participates in all Working Groups, assuring there is cross working group cohesion and integration. The Team Leaders report to the PERCH Core Team on a weekly basis thereby assuring that Working Group decisions, needs and approaches are kept on track from a strategic, budget and scientific perspective. The Core Team participation in the Working Groups is as follows but other Core Team Members will participate in an ad hoc fashion according to the needs of the Working Group.

An essential component of the PERCH project is monitoring and assurance of quality across all domains of the study. To this end we have established a Quality Management Strategy (QMS), overseen by the PERCH PI, Dr.Kate O'Brien. The QMS involves oversight of study quality in the following areas:

- Regulatory/GCP monitoring
- Clinical assessment standardization and monitoring
- Safety reporting and monitoring (see Section 5.7)

The QMS rests on the principle that each Working Group has a set of core activities whose quality metrics will be identified, measured and reported back regularly to the Core PERCH Team. It is the responsibility of the Team Lead of each Working Group to develop their comprehensive Quality Management approach in consultation with the Core PERCH Team.

PERCH will involve two other monitoring components as part of the QMS. The first is a Clinical Standardization and Monitoring component, led by Dr. Jane Crawley. Dr. Crawley is a pediatrician with more than 10 years of field experience; she will lead training across the sites to standardize the clinical assessment of study subjects at enrollment and follow up. Dr. Crawley will monitor the adherence to the standardized measures of clinical assessment throughout the trial through periodic field visits. The third monitoring component is the Safety Reporting and

Monitoring described in section 5.7. Each site will be responsible for recording and reporting serious adverse events through a local safety monitor. In addition to the immediate reporting (see section 5.7), the PERCH core team will describe any events in aggregate as part of the yearly reporting process to the JHSPH IRB.

Quality Management Sub-Teams
Data Quality, Regulatory and Monitoring: Maria Knoll (Team Leader), , Amanda Driscoll, Andrea DeLuca, Daniel Feikin, Kate O'Brien, Jane Crawley

Clinical/Epidemiology: Daniel Feikin (Team Leader), Andrea DeLuca, Maria Knoll, Kate O'Brien, Anthony Scott, Niranjan Bhat, Chizoba Wonodi, Jane Crawley

Lab: David Murdoch (Team Leader), Amanda Driscoll, Kate O'Brien, Ruth Karron, Niranjan Bhat, Anthony Scott

Data Analysis: Scott Zeger (Team Leader), Maria Knoll, Anthony Scott, Kate O'Brien, Daniel Feikin, Hope Johnson

3.2 Site descriptions

Table I. Site Characteristics

Site	Country	PI Name	Institution	HIV	Urban/ Rural	U5 Mortality Rate
Kilifi	Kenya	Anthony Scott	Kilifi-KEMRI Wellcome Trust	Medium	Rural	60
Dhaka, Matlab	Bangladesh	Abdullah Brooks	ICDDR, B	Low	Mixed	88
Basse	Gambia	Stephen Howie	MRC	Low	Rural	109
Johannesburg	South Africa	Shabir Madhi	University of Witwatersrand; RMPRU	Very high	Urban	63
Bamako	Mali	Karen Kotloff	University of Maryland	Low	Urban	196
Sa Kaeo, Nakhon Phanom	Thailand	Susan Maloney	CDC – Thailand	Low	Mixed	18
Lusaka	Zambia	Donald Thea	Boston University	High	Urban	135

Bangladesh Site
Bangladesh is the world's most densely populated nation-state with a per capita GDP of $1500, but is a country in transition. It has an official population of 140 million, but newer estimates place it a 160 million, with an urban/rural split of 22% and 78%, respectively. The site in Bangladesh covers urban and rural centers, each backed by well-defined catchment areas with ongoing population based demographic and morbidity surveillance. The site includes two

facilities: the Dhaka and Matlab hospitals of ICDDR,B (in the Dhaka and Chittagong Divisions of Bangladesh, respectively), the former urban and the latter rural. The Dhaka hospital is a 350-bed facility supported by the Kamalapur urban field site, which has both active demographic and morbidity surveillance among a population of 350,000, of whom 11.5% are children < 5y. Kamalapur has been involved in pneumonia studies since 1998, and has conducted pneumonia aetiology surveillance since 2004. The Matlab hospital is a 140-bed facility, and is supported by longstanding demographic and morbidity surveillance among a population of 114,000. Both hospitals have dedicated pneumonia wards.

The Gambia

The Gambia has a population of 1.7 million, and is located in the Sahel, a semi-arid savannah region of sub-Saharan Africa. Agriculture (groundnuts) and tourism are economic backbones of the The Gambia, which has a GNI per capita of $320, one-third of the average for sub-Saharan Africa (World Bank), and over half the population under the national poverty line. Ethnically there are at least a dozen tribes the largest of which are the Mandinka and the Wollof, and the population is 90% Muslim. The Gambian public healthcare delivery system comprises central referral hospitals, basic care facilities ('major' and 'minor' health centers), and village-based services. In 2005/06 69 per cent of care seeking for children under 5 with suspected pneumonia was referred to an appropriate provider, principally public health services (MICS 2005-06 data). The MRC Gambia unit has a long history of local and multi-centre research of infectious diseases. The childhood pneumonia research program is well-established, notably including large scale conjugate Hib and pneumococcal vaccine trials, and a range of other clinical pneumonia studies**.** MRC Gambia is currently participating in a Gates-funded multicentre pneumonia etiology study and a GAVI funded pneumococcal surveillance project coinciding with the expected introduction of pneumococcal conjugate vaccine in 2009. The MRC's Basse site, which will host the PERCH project, benefits from a well-established demographic surveillance system.

Mali Site

Mali is a land-locked country in sub-Saharan West Africa that has functioned as a stable constitutional democracy with multi-party elections since 1992. Most Malians inhabit rural areas, existing as subsistence farmers and herders; 10% of the ~12 million population live in the capital city of Bamako. The official national language is French, but this is largely the language of educated individuals (70% of adults are illiterate). In practice, >40 tribal languages are spoken. The Niger River traverses most of the country, which is desert or semi-arid Sahel in the north, and tropical Sudanese savanna in the south (where Bamako resides). Mali was described by the United Nations in 2008 as the world's 12th least developed country. The GNI per capita is $500. Infant and < 5 mortality have declined by only ~21% since 1990, suggesting that the millennium development goals for reduction in child mortality are unlikely to be met. The proposed site for conducting a case control study of severe pneumonia in Malian children 0-59 months of age is Hospital Gabriel Toure (HGT), where nearly all pediatric hospital admissions in the Bamako metropolitan area take place. The proposed study area is the city of Bamako, HGT's catchment area. Each year HGT treats ~25,000 children as outpatients and admits ~5,000 others (most <5 years old). Most children admitted to HGT are very ill. A survey in 2000 revealed that 71% of all admissions to HGT among children <16 years old were for infectious diseases and 21% of all admitted children died. Since 2001, we have been performing laboratory-confirmed disease surveillance for pediatric infectious diseases among children admitted to HGT or treated as outpatients.

Kenya Site

The site in Kilifi is the KEMRI-Wellcome Trust Research Programme – a research initiative that began in 1989 as a collaboration with The Kenya Medical Research Institute, The Wellcome Trust of Great Britain and Oxford University. A science research building, including state-of-the-art laboratories, is located immediately adjacent to Kilifi District Hospital and the clinical scientists at the Programme run the pediatric clinical service. A population of 250,000 people living around the hospital are followed in a demographic surveillance study with four-monthly household visits. The activities of the clinical service, the laboratories and the DSS are linked at each point of contact by real-time data entry into a comprehensive relational database. Kilifi District Hospital admits approximately 4,500 children per year; initially malaria was the dominant cause of morbidity but with effective malaria treatment and prevention pneumonia and neonatal illnesses are now the commonest causes of admission. The Programme employs a total staff of 700 with approximately 100 scientists and 20 pediatricians at consultant or trainee level.

Thailand Site

In Thailand, the study will be implemented by the International Emerging Infections Program (IEIP), a formal collaboration between the Thailand Ministry of Public Health (MoPH) and the U.S. Centers for Disease Control and Prevention (CDC), and also called the TUC or Thailand MoPH – U.S. CDC Collaboration. The study will be conducted in two rural provinces. Sa Kaeo province is in eastern Thailand on the Cambodian border and has a population of 537,000 (32,000 age <5 years). Nakhon Phanom borders Laos in northeastern Thailand and has 742,000 residents (48,000 age <5). The 2007 per capita annual GDP was $997 in Sa Kaeo province and $1,006 in Nakhon Phanom province(Information 2007). Active, population-based pneumonia surveillance is ongoing in all 20 hospitals in both provinces. Pneumonia epidemiology in the two provinces is similar except for the high incidence of melioidosis in Nakhon Phanom, typical of northeastern Thailand. The PERCH study will be conducted in the two largest hospitals in each province.

South Africa Site

The study-site is in Soweto, an urban low-income community with a diversity of ethnic backgrounds. Although the majority of households have access to running water, 25% of families live in informal settlements and use fossil fuels for heating and cooking. The community is severely affected by HIV, with one-third of mothers and at least 5% of children being HIV-infected. The under-5 mortality rate in South Africa, including the study site, increased from 60 to 69 per 1 000 live births between 1990 and 2005 (Countdown Coverage Writing Group; Countdown to 2015 Core Group 2008). Fifty-seven percent of all deaths occur in HIV infected children, of which at least one-third are due to pneumonia even in the era of anti-retroviral therapy (Violari A 2008).

There are 23 primary health care (PHC) clinics in the Soweto region and a single public hospital; i.e. Chris Hani Baragwanath Hospital (CHBH) which is the sole referral hospital for all these PHCs. All immunization in the community occurs at one of the 23 PHCs, and vaccines are provided for free with minimal numbers of children being immunized through the private sector, where the fees are charged. None of the PHCs admit children with severe pneumonia. All severe pneumonia cases are referred to CHBH, the largest hospital in the Southern Hemisphere with 450 pediatric beds with an average occupancy rate of 80%. The hospital primarily provides

secondary and tertiary level care to the population of Soweto, with an estimated 90% of all hospitalizations from the community occurring at CHBH. Children are either hospitalized in one of four general pediatric wards or in the short-stay ward if the duration of hospitalization is expected to last <72 hours. Children with features of severe pneumonia who do not require intravenous antibiotic therapy or supplemental oxygen therapy may also be admitted to the short-stay ward. All health care for children < 6 years, including diagnostic tests and treatment, provided at the hospital and PHCs are free. There is however a very low threshold for referring children to the hospital for evaluation and further management of pneumonia. Travel to the hospital is supported either through readily available public transport or by ambulance if clinically indicated.

Zambia Site

The Zambia PERCH study site will be located at University Teaching Hospital (UTH), a 1500-bed academic center and tertiary care facility with 425 pediatric in-patient beds located in the capital city, Lusaka and the home of Zambia's only medical school. In 2008 there were a total of 14,923 in-patient pediatric admissions, 3467 (23%) of which were ARI and 1035 (30%) occurred in children under 5 years old. The case fatality rate of severe pneumonia was 25.8%. UTH serves the greater Lusaka District, an area of 70 square kilometers and a population of 1.3 million. National per capita income (USD 395) is half that recorded at independence in 1964 and among the world's lowest. Consistent problems include: extreme poverty, overcrowding; poor access to water and sanitation (26% with flush toilets); food insecurity and high unemployment (9% formally employed). These conditions and a poor public health infrastructure contribute to one of the world's highest rates of pneumonia (59.5/1000 children) and pneumonia mortality. HIV (19% seroprevalence among women attending ante-natal clinics) and tuberculosis (506 cases per 100,000) are severe problems highly affecting the burden of pulmonary disease in Lusaka.

3.3 Data coordinating center

The EMMES Corporation (EMMES) will serve as the centralized data coordinating center (DCC) for the PERCH study. EMMES is a full-service Contract Research Organization (CRO) founded in 1977 providing support to scientists and medical researchers to evaluate medical products, drugs, and services for the improvement of medicine and the public health.

For PERCH, EMMES will maintain a centralized data entry system for clinical and lab data that all site staff and PERCH Core Team can access. EMMES will work with the sites and Core Team to deploy the AdvantageEDC^{SM} system for PERCH-specific functionality. EMMES will provide assistance with the preparation of study-related materials such as Standard Operating Procedures (SOPs), Manual of Procedures (MOP), electronic case report forms (CRFs) and/or source documents and tracking logs, as defined by the PERCH team. In collaboration with JHSPH biostatisticians, the DCC will be responsible for all routine statistical analyses related to the primary and secondary objectives of PERCH and will provide frequently updated reports on study progress and access to study data.

3.4 Reference Labs

Some of the PERCH study site laboratories will be established as regional reference laboratories. The main purposes of these laboratories are to coordinate regular quality assurance programs and to perform specialized testing according to their specific areas of experience/expertise.

With respect to the latter activity, a PERCH study site may already have experience with a specialized test and would therefore be well-placed to perform the testing for all study sites.

3.5 External Labs

Involvement of external laboratories will be restricted to areas of the study requiring laboratory expertise beyond that which is available at the PERCH study sites. External laboratory involvement may be required where independent review is needed, for provision of specialized technical support, for performance of highly specialized and/or esoteric tests, or when specifically requested by the sites themselves.

4 Study procedures

4.1 Case selection

The PERCH project is driven by the knowledge that pneumonia causes substantial child mortality worldwide and it aims to provide data that can reduce this mortality. The PERCH project will focus on severe cases for the following reasons:

- they provide the closest picture of the etiology of fatal cases.
- their association with lung inflammation is more specific
- they are admitted to hospital (and can be investigated thoroughly)

PERCH will evaluate children with WHO-defined severe and very severe pneumonia. Patients with WHO non-severe pneumonia, i.e. a raised respiratory rate but with no lower chest wall indrawing or signs of very severe pneumonia, will not be studied in PERCH. In order to draw inferences about etiology when comparing data across different sites, the case definition for study entry will be uniform across sites, precisely defined, and have rigorous implementation during the study period. This will enable us to determine if heterogeneity in findings from different sites represents true epidemiologic differences or simply differences in the mix of enrolled patients.

4.1.1 Inclusion criteria

Cases must meet the following inclusion criteria for eligibility to enter into the PERCH study:

- Admitted to hospital
- Meets the WHO clinical criteria for severe or very severe pneumonia on admission
- Aged 28 days*-59 months
- Accompanied by written informed parental/guardian consent
- Lives in defined study catchment area (may be defined as all or part of a geopolitically defined area or distance zone from the hospital)

*Note the day of birth is considered to be “day 1”. Thus a child born on September 1st will be 28 days old on September 28th.

WHO clinical criteria for severe and very severe pneumonia are defined as the presence of cough or difficulty breathing with the current illness and:

Table II. WHO Clinical Criteria for Severe and Very Severe Pneumonia	
Classification	**Cough or Difficulty breathing plus any of the following signs or symptoms:**
Severe Pneumonia	Lower chest wall indrawing

Very Severe Pneumonia*	Central cyanosis Unable to feed , or vomiting everything Convulsions, lethargy, or unconsciousness Head nodding

*Children age 28-59 days:

Definitions for certain signs for this age group should be modified:

1. Lethargy is defined as "an infant who does not wake up on stimulation or, on waking, subsequently moves only on stimulation or does not move at all"
2. Unable to drink/breastfeed is changed to—"difficulty feeding or not feeding well (in an infant who was previously feeding well)"

During the enrollment process, the danger sign for 'convulsions' will be collected by asking about the number and duration of seizures. In PERCH, children who have had multiple or prolonged (more than 15 minutes) seizures will be considered to have a danger sign for very severe pneumonia. In children who do not have multiple or prolonged seizures, this danger sign will not be counted (in order to exclude children with simple febrile convulsions, which is not a danger sign for severe pneumonia).

Note that this classification for severe and very severe pneumonia is intended to define the entry study sample. A more precise sub-classification of severity based on additional clinical features will be used for a stratified analysis of etiology.

Progression to case definition during hospitalization:

Cases that progress to severe pneumonia during hospitalization but do not meet the definition for severe or very severe pneumonia at the time of admission because the disease is in the early stages of illness will not be included. This is for two reasons: 1) recruiting patients at a fixed point, admission, is considerably simpler and less expensive than establishing continuous recruitment of inpatients, and 2) the project aims to define etiology to support prescribing policy for children "on admission" and cases that progress in severity after admission would not contribute to this process.

Chronic disease:

One of the limits to the specificity of the proposed case definition is that children with chronic lung or respiratory disease may be admitted to the study. The key variable, the duration of the history of symptoms, is unlikely to be reliable or precise. Therefore, we will not exclude patients from the study on the basis of a long history of symptoms, but will solicit this information and examine the effect of illness duration on the spectrum of pathogens in post hoc sub-category analyses.

4.1.2 Exclusion criteria

Cases that meet any of the following exclusion criteria will be ineligible to enroll in PERCH:

- Has "Hospital Associated Pneumonia", defined as hospitalized for any cause within the last 14 days

- One month (30 days) exclusion period following date of discharge from the hospital for an admission for which the child was enrolled in PERCH study. This will minimize the chances of enrollment for the same episode of pneumonia two times. (A child may become a 'case' two or more times but only if the episodes of illness are separated by at least 30 days – see section 4.4.4)

It should be noted that children who enroll in PERCH but have no results for any study specimen will be excluded from the primary etiology analysis.

Wheeze:

The intent of PERCH is to study children with severe or very severe pneumonia, rather than a study of children with reactive airway disease (RAD). It is well recognized that RAD and pneumonia can co-present in an individual child. However, children with reactive airway disease only (e.g. asthma) can present with clinical findings, such as lower chest wall indrawing, which are essential components of the pneumonia case definition, even though they don't have pneumonia. We do not want to include as PERCH cases children whose clinical signs, which result in their inclusion as severe or very severe pneumonia cases, resolve in a short period of time following acute therapy with bronchodilators. These children are unlikely to have pneumonia, instead their chest clinical signs are likely attributable to reactive airway disease, an important illness, but not the disease that PERCH is aiming to study. Therefore to differentiate patients whose severe or very severe pneumonia case-defining clinical signs are attributable to reactive airway disease only, we will use an algorithm of response to bronchodilation. The major clinical sign of concern with respect to differentiating RAD from pneumonia is lower chest indrawing. Wheezing patients with very severe pneumonia clinical signs are very unlikely to resolve these signs with bronchodilatory therapy. The remainder of the discussion pertains to children whose clinical signs and symptoms meets the case definition for severe pneumonia. All children who meet the case definition of very severe pneumonia will be recruited into the PERCH study regardless of their wheezing status or response to bronchodilator therapy.

Children who meet the case definition of severe pneumonia and whose case defining clinical signs (i.e. chest indrawing) remain after bronchodilation will be recognized as meeting the case definition in spite of bronchodilation and will be enrolled in the PERCH study whereas those children whose case-defining signs resolve with bronchodilation will be recognized to have primarily reactive airway disease causing their respiratory illness and will not be enrolled. Of note, the resolution or lack of resolution of wheezing in these children does not determine whether the child will be enrolled in the PERCH study; it is only the resolution or lack of resolution of the signs that contribute to the severe pneumonia case definition.

There are significant practical, clinical management and ethical issues to consider in operationalizing this decision tree. Ideally the bronchodilator challenge would take place immediately and an assessment of whether the child's illness meets the severe pneumonia case definition would follow that acute therapy. However, for PERCH we also want to assure that respiratory specimens are collected prior to antibiotic administration. If enrollment in PERCH must await the response to bronchodilation, and if antibiotic administration must await a decision regarding PERCH enrollment and specimen collection, it is possible this sequential approach could delay the receipt of antibiotics for some children at some sites. This is not acceptable; the PERCH study cannot create an impediment or delay in delivering essential clinical management for children with respiratory illness. Therefore PERCH will adopt a hybrid

approach which will assure that clinical care among wheezing children with signs of severe pneumonia prior to therapy is not delayed and simultaneously that a bias is not introduced into the PERCH study regarding collection of specimens among children with wheezing and pneumonia.

The hybrid approach for children who meet the severe pneumonia case definition but are also wheezing is as follows. These children will be enrolled into PERCH allowing for the immediate collection of diagnostic specimens prior to antibiotic therapy if it is eventually decided they require it. These children will be managed by the local clinician with bronchodilator therapy for their wheezing. For the purpose of PERCH we define this as "bronchodilator challenge" because it challenges whether their case-defining symptoms resolve. If their case-defining signs of severe pneumonia resolve with acute administration of bronchodilator therapy, they will remain in the PERCH study but will form a separate sub-analysis of etiology and the number of follow up procedures will be limited. See description below for more detail.

Core principles and definitions for the bronchodilator challenge and PERCH enrollment approach.

1. The purpose of bronchodilator therapy is to manage the child's acute wheezing illness, and is <u>not a study procedure</u>. The term 'bronchodilator challenge' refers only to the interpretation by the PERCH study of the clinical response to that medical care and whether the severe pneumonia defining symptoms resolve following the bronchodilator therapy.
2. Children with severe pneumonia (SP) *and* wheezing will have a bronchodilator challenge response contribute to the decision about full PERCH enrollment versus entry in to a sub-group analysis
3. Children with very severe pneumonia (VSP) and wheezing will be recruited to PERCH regardless of their response to acute bronchodilator therapy. The rationale is that children with very severe pneumonia are unlikely to resolve their pneumonia case-defining signs with bronchodilator challenge due to their greater likelihood of having true lung inflammation rather than only reversible reactive airway disease.
4. The term 'wheezing' refers to auscultatory wheeze, not just audible wheeze.
5. Response to acute bronchodilator therapy is defined as resolution of severe pneumonia defining signs/symptoms (i.e. resolution of lower chest wall indrawing), and not resolution of wheeze.
6. Enrollment into PERCH can take place before or after bronchodilator challenge assuming antibiotics have not been administered. If consideration for enrollment occurs after bronchodilator challenge and the child's pneumonia defining signs resolve, they will not be enrolled in PERCH. If enrollment consideration occurs before bronchodilator challenge, and their case defining signs resolve with bronchodilation the child will remain in the PERCH study but will be analyzed separately from other cases and will have no further clinical work up or follow up. This is relevant only for sites that cannot logistically or operationally assess the response to bronchodilation before PERCH enrollment.

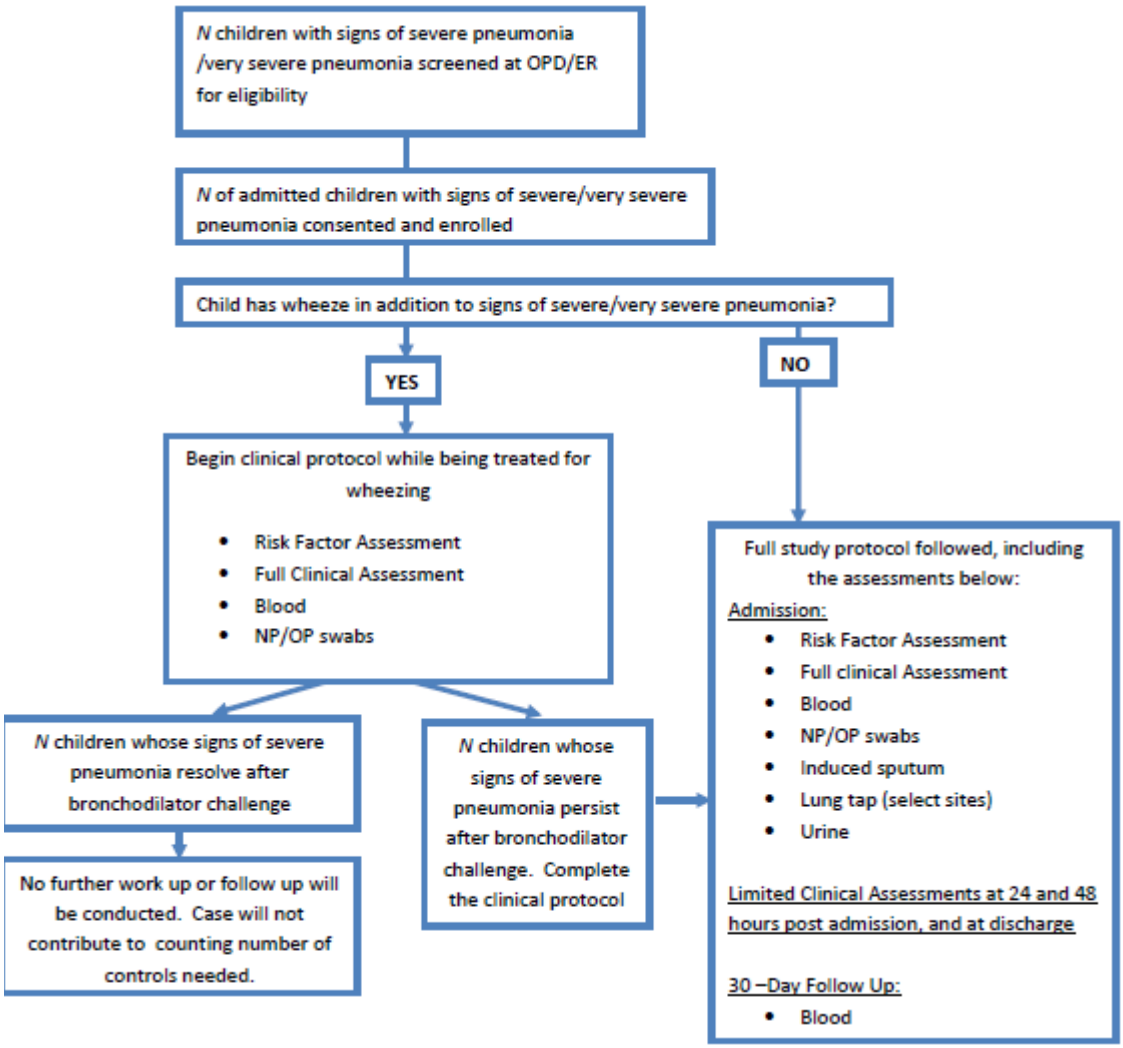
Study Subject Algorithm of PERCH Enrollment and Bronchodilator Challenge

The algorithm for including the response to bronchodilator therapy in relation to PERCH case status and enrollment is stratified by age. Younger children with signs of severe pneumonia and wheezing are less likely to have purely reactive airways disease and therefore are much less likely *a priori* to resolve their pneumonia defining signs/symptoms in response to bronchodilator therapy. We will therefore only use a single bronchodilator dose to assess response in young children, whereas among the older children we will use the response to three bronchodilator doses as described below.

1. Sites are <u>not required</u> to assess the response to bronchodilator therapy to enroll children with wheezing and severe pneumonia into PERCH.
2. For sites that can administer bronchodilator therapy <u>before</u> PERCH enrollment, we will exclude children whose case defining symptoms resolve, but there is no requirement for sites to assess the response to bronchodilators for PERCH enrollment.
3. For those sites that cannot administer bronchodilator therapy prior to PERCH enrollment, we will record the response to bronchodilator therapy (at least one dose for children < 2 years of age* and at least three doses for children 2-<5 years of age) after enrollment into PERCH and proceed as follows:
    a. Severe pneumonia case defining signs persist after one dose (children < 2 years) or three doses (children 2-<5 years) of bronchodilator treatment: Continue with the full study protocol
    b. <u>As a sub-group, s</u>evere pneumonia case defining signs resolve after one dose (children < 2 years) or three doses (children 2-<5 years) of bronchodilator treatment: Continue with study protocol modifying the specimen collection and control enrollment as follows.
        i. Collect:
            1. acute blood for culture, HIV, CBC
            2. NP/OP swabs for PCR**
        ii. Do not collect:
            1. Induced sputum
            2. Lung tap
            3. Urine
            4. Convalescent sera
        iii. Do not count case toward control enrollment numbers for that month

*If > 1 bronchodilator treatment is given at a site as routine practice that should continue.
**Refers only to patients admitted to hospital

Figure II: PERCH Case Enrollment



4.1.3 Selection/Sampling methods

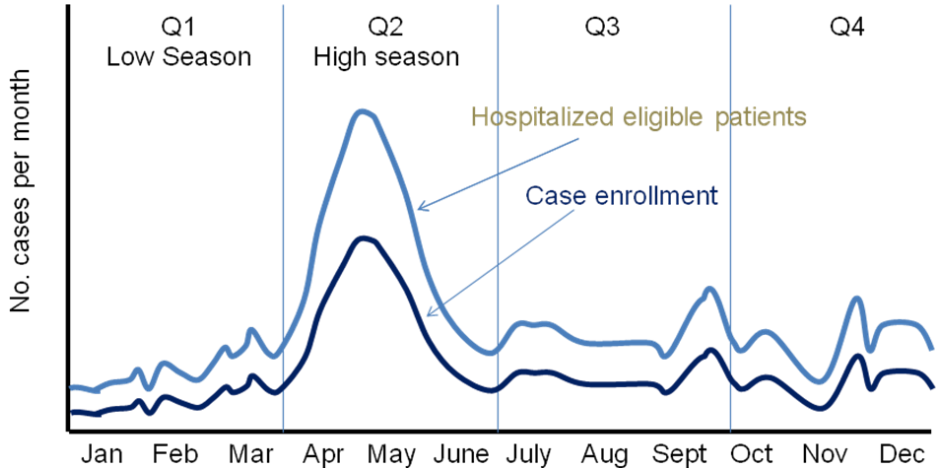
Because the overall study budget restricts the number of cases enrolled, it is necessary to limit enrollment at the largest sites. Because the focus of PERCH is on pathogens responsible for pneumonia mortality we will aim to optimize recruitment of children with very severe pneumonia despite the fact that in most sites they represent a minority of eligible patients. Accordingly we will aim to recruit equal numbers of severe and very severe cases, and therefore the sampling ratio will be higher for very severe cases compared to severe cases (at most sites all very severe cases will be enrolled).

Systematic sampling (e.g. every other case, or enrolling 2 days on/2 days off, or in 8 hour shifts that rotate on different days in which shifts enrollment occurs) will be used to determine which eligible cases will be invited to enroll in PERCH. Systematic sampling will help ensure representativeness since severity and clinical history vary during the day, week and year (more severe cases present at night and on weekends and severity varies seasonally). Because predictability of enrollment eligibility may influence which patients are invited to participate in the study, there is a possibility that patients who present to hospital during enrollment periods may differ from those who present during other times. To minimize selection bias we will

carefully monitor recruitment at each site through screening and tracking of all those eligible, those approached to enroll, and those who consent

Sites will enroll cases throughout the year to obtain seasonal distribution of cases and throughout the week and day, including weekends and evening/nights, to obtain a representative distribution of cases with respect to severity of disease. The enrollment rates will be proportional to the case detection rates, meaning a greater number of cases will be enrolled during peak times and during the high season (see Figure III). A strong study team (rather than regular hospital staff) will manage the detection and selection of cases to help minimize enrollment bias.

Figure III. Illustration of case sampling proportional to the case detection rates



4.2 Case evaluation

Table III presents an overview of the case evaluation schedule of possible activities, which may vary by site. Details for each time point are described in sub-section below.

Table III. Overview of Case Study Evaluations					
	Follow-up time point				
Activity	**Hospital Admission**	**24 and 48hrs Post admission**	**Hospital Discharge**	**Post Mortem (if applicable)**	**Post-Discharge Follow up****
Log of suspected cases	X				
Eligibility assessment	X				
Selection (if necessary)	X				
Enrollment/Consenting	X				
Specimen Collection:					

• Induced sputum	X				
• Blood	X				X
• Pleural fluid	X				
• Lung aspirate	X				
• Nasal and throat swabs	X				
• Urine	X				X***
• Post mortem biopsy				X	
Demographic, clinical history and risk factor assessment	X				
Full Clinical Assessment	X				
Clinical Procedures	X				
Limited Clinical Assessment *		X	X		
Vital Status assessment					X

*Limited Clinical Assessment: respiratory rate, oxygen saturation, amount of oxygen required, and lower chest wall indrawing.
** Weight and height may be collected in some sites at the 30-day visit to assess the impact of pneumonia on acute growth parameters.
***Urine will be collected for additional biomarker testing at select sites

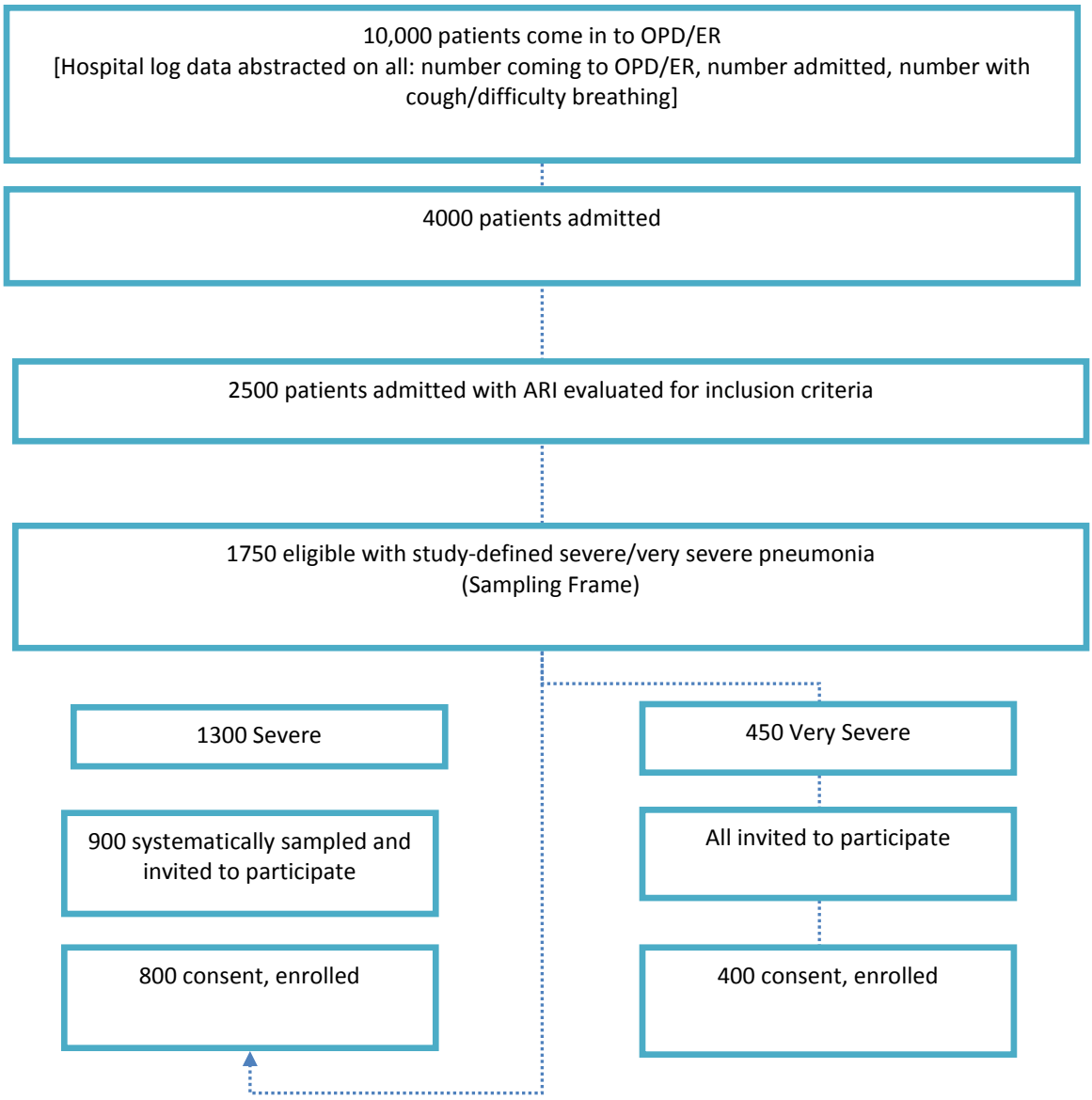
4.2.1 Surveillance and screening to identify all eligible cases

All suspected cases coming to the study hospitals will be assessed for eligibility using a pre-screening assessment tool and information pertaining to disease status will be collected and recorded on a screening log, as determined by the site-specific sampling frame. This will also help to ensure that cases of pneumonia are not missed. We will ask the JHSPH and other IRBs for a waiver of consent to collect this screening information.

Sampling frame:
The sampling frame is the set of all eligible cases from which to select those to be enrolled. Cases will be evaluated for their possible inclusion in the sampling frame as early as possible, at the time of admission, prior to the collection of specimens for routine clinical investigation and prior to administration of antibiotics. As part of this process, a case log may be created that captures key information (identity, eligibility criteria including results of clinical evaluations, risk factors) for all suspected cases. This can be used (1) to characterize the complete set of hospitalized cases at each site and (2) to monitor the eligibility assessment system and evaluate the success of the sampling frame (see Figure 4). This case log will allow us to obtain unbiased estimates of parameters of interest (e.g. outcomes, risk factors, etc.) for events (as opposed to individuals) since some children who have previously been recruited will be ineligible to be included in the sampling frame. The eligibility status for each case will then be determined– those deemed eligible will make up the sampling frame. For valid analyses, every eligible child

must have a known probability of selection and thus every eligible child must be captured in the sampling frame.

Figure IV. Patient flow chart (illustration)



The sampling frame is required at all sites, regardless if selection is used or not, since it identifies those eligible to be invited to participate. It is derived by evaluating data collected in the case log, which will also be created at all sites to assess representativeness and potential bias of those enrolled, and to calculate incidence where feasible.

One issue is what to do when selected individuals do not enroll, either by refusal or other reasons. Since replacement with patients not identified to be selected will lead to selection bias, a larger sample size than needed must be approached to cover non-enrollment. Basic demographic information on eligible children not enrolled, including the reason for not enrolling, will be collected and used to compare enrolled and non-enrolled children.

There may be cases of severe pneumonia in the community that will not be captured on the case log (e.g. where a child should be admitted to the hospital but is not.) If children present with severe/very severe pneumonia (and are diagnosed as such) there are no circumstances when they would be turned away. But parents may not permit admission either because they cannot afford the care or must return home (and cannot leave the child behind). These patients might represent a segment of the population at the greatest risk of disease and by missing them we are biasing our estimates for the risk factors and possibly the etiologic distribution. As noted, we will try to capture basic demographic information on such children to compare with enrolled children. However, it is likely that this group will be small at most sites, and regardless, the case log will be able to capture cases that should be admitted so we can count them. Our ability to represent the "community" experience of pneumonia is probably limited much more by cases failing to present rather than by failing to be admitted (e.g. at the Kilifi site, approximately 1/3 of children who die do not seek care during their final illness).

Another challenge will be to capture data on the children who die soon after presentation. There might not be time to properly assess and sample them before they die, which would create a potential bias. Case log data should be collected on them as well, and an attempt made to enroll them even after death if it is locally acceptable to collect post mortem specimens (please refer to the post mortem section in 4.2.5).

4.2.2 Admission assessment

4.2.2.1 Clinical

PERCH site investigators will collect the clinical variables that are required to define the entry of a case into the study prior to enrolment. Once a child is recruited to the study they will collect clinical specimens to define the etiology of pneumonia. They will also collect clinical and laboratory data that are not specifically related to etiology for the following three reasons:

1. *Controlling for case mix:* The case mix across different PERCH sites is likely to vary considerably by severity and by the prevalence of co-existing illnesses. Because both of these factors can influence the distribution of pathogens, this information is important for interpreting study findings. Geographic variations in etiology may be more attributable to the local mix of presenting cases than to geographic variation in pathogen distributions. The coarse differentiation of cases into two WHO classes, 'severe' and 'very severe' pneumonia, does not provide sufficient resolution to control adequately for variation in severity across sites. Co-existing illnesses also alter the pattern of etiology and the prevalence of coexisting illnesses varies geographically. Known examples include HIV and sickle cell disease, which are defined by laboratory tests, but other important conditions, such as malnutrition, are defined by clinical variables.

2. *Prediction model of etiology:* Through a comprehensive classification of etiology in a large number of cases, PERCH aims to explore whether different clinical or laboratory variables, or combinations of these, are associated with specific etiologies or, more probably, groups of etiologies and to what extent clinical variables can be used in a prediction model of etiology. This will be explored in a multi-variable model using the first half of the clinical data from all sites to define

the model. The second half of the data from each site will be used separately to provide a series of temporal/geographic validation tests of the model. To differentiate severity into finer strata, to classify existing illnesses and to associate the clinical presentation with different etiologies PERCH will collect data on additional clinical signs, beyond those that were collected at the time of enrollment eligibility, and these will be incorporated into the analysis at the end.

3. *Risk factors*:
PERCH intends to collect data on risk factors for three different pneumonia-related outcomes: 1) Severe hospitalized pneumonia; i.e. what makes a child more likely than his peers to get admitted with WHO severe or very severe pneumonia, referring to the primary PERCH case definition; these data will be collected from both cases and controls. 2) Severity of pneumonia; i.e., among cases, what makes a child more likely to present with very severe pneumonia compared to severe pneumonia. Another way to look at this is to say 'what are the markers of severity among cases?'. 3) Pneumonia of a specific etiology; i.e. among cases, what makes a child more likely to have a specific type of pneumonia e.g. risks for RSV pneumonia versus pneumococcal pneumonia. Risk factors for severity and for etiology will entail data collected from only the cases at admission and beyond.

Table IV. List of symptoms, signs and laboratory tests that may be evaluated at admission

	Variable	**Notes**
	General	
	Temperature (tympanic/axillary)	record as a continuous variable
	Vomiting everything	present / absent
	Convulsions	present / absent (not type)
	Lethargy	present / absent
	Unconsciousness	AVPU scale (Alert, Voice, Pain, Unresponsive)
	Respiratory system	
	Cough	3+ episodes during 15 minute time intervals (not associated with eating or drinking)
	Difficulty breathing	Present / absent
	Nasal discharge	present / absent
	Croup	present / absent
	Unable to breastfeed (infants) or drink due to breathlessness	Standard observed attempt at breastfeeding or to take a drink
	Respiratory rate	continuous variable
	Lower chest wall indrawing	Present / absent
	Stridor	distinguish between "calm" and agitated "stridor" (croup)
	Grunting	present / absent (not here restricted to young infants)
	Nasal flaring	present / absent
	Crackles/crepitations	present / absent and extent of disease (bilat) (recording

Table IV. List of symptoms, signs and laboratory tests that may be evaluated at admission		
	Variable	**Notes**
		sounds using digital stethoscope in some sites)
	Wheeze	present / absent (recording sounds using digital stethoscope in some sites)
	wheeze after bronchodilator	present / absent
	Decreased breath sounds	present / absent
	Central cyanosis	present / absent
	Deep breathing	present / absent
	Head nodding	present / absent
	Dullness on chest percussion	present / absent
	URTI Signs (sneezing, rhinorrhea, pulling on ears)	present / absent
	Clubbing	present / absent (collected at some sites)
Dehydration		
	Sunken eyes	present / absent
	Capillary refill time	Seconds (delayed = more than 3 seconds)
	Skin turgor/elasticity	Time in seconds to return to normal graded: immediate, slow, very slow (>2sec)
Malnutrition		
	Height/length	continuous scale
	Admission weight	continuous scale
	Pedal edema	present / absent
Shock		
	Heart rate	continuous scale
	Oxygen saturation	continuous scale (oximetry), adjusted for altitude
	Miscellaneous	
	Jaundice	present / absent
	Bulging Fontanelle (< 18 months of age)	present / absent
	Rash	Type: petechial, purpural, measles
Laboratory		
	Complete Blood Count, including the following:	
	Hemoglobin	continuous scale
	Peripheral white cell count	continuous scale
	Differential white cell count	Percentage
	Platelet count	continuous scale
	Malaria	rapid test or smears

Table IV. List of symptoms, signs and laboratory tests that may be evaluated at admission		
	Variable	**Notes**
	HIV serology	PCR confirmation in children under 18m; parent may decline HIV testing
	Sickle Cell Testing	in some African sites only, method may vary by site (PCR, HPLC or gel electrophoresis)
	Thalassemia	In Thailand only

Two PERCH sites, South Africa and Zambia, will also initiate voluntary HIV testing for the mothers of PERCH cases. Currently at both sites HIV testing is done routinely as part of antenatal care. These results may be recorded as exposure to HIV on a child's health card ('Road to Health Card' in South Africa and 'Under 5 Care' in Zambia), but are not captured as part of a child's record in PERCH. We propose either asking the mother for documentation of her recent HIV test result or offering her testing if she has not been tested recently. All testing will be done using trained nurses or HIV testing counselors. We will ask for consent to link these results with the child's PERCH data. We believe that knowing the mother's HIV status will be critical in evaluating a child's HIV exposure status at the time of enrollment in to PERCH. It has been shown that, even in the absence of HIV infection in an infant, HIV exposure during the pregnancy and peripartum period can lead to increased risk of severe or very severe pneumonia; we would like to evaluate this risk further.

The counseling and testing process will follow local ethics requirements, and the timing of the consent for a mother's HIV test will be done according to the clinic flow at each site.

4.2.2.2 Specimens Collected and Corresponding **Laboratory Analyses**

Multiple specimens will be obtained from each case and control. Specimen collection methods for these procedures are described in section 4.5.

Table V. Body Fluids collected from Cases	
Body Fluid	Laboratory Analyses
Acute blood 0-1kg child: 3mL 1-2kg child: 4.5 mL ≥3kg child: 5mL***	Blood culture (pneumococcal antigen on alarm+ subculture- specimens) Complete blood count with differential Singleplex PCR – pneumococcus Singleplex PCR – HIV (cases ≤18mo with positive antibody test (may be rapid test or ELISA)) HIV serology – Note: parents have the option to decline HIV testing. HIV positive subjects will be referred for further clinical care (e.g. at an HIV patient support center). C-reactive protein Malaria (microscopy or antigen testing, in endemic areas) Sickle Cell Testing (in endemic areas) Thalassemia (Thailand only)

Table V. Body Fluids collected from Cases	
Body Fluid	Laboratory Analyses
	Antibiotic activity testing Storage for other serology, other biomarker testing; and future host genetic testing
Convalescent blood ideally 4 mL, min 2 mL	Storage for convalescent serology CD4 testing for HIV+ cases in South Africa and Zambia only (plasma)
NP rayon swab	Bacterial culture for pneumococcus (and serotyping if applicable)
Throat rayon and NP flocked swabs	PCR for respiratory pathogens Archived for potential viral culture, cytokines/chemokines, singleplex PCR
Induced Sputum*	Microscopy and bacterial culture M. tb microscopy, culture PCR for respiratory pathogens
Lung Aspirate** (at select sites)	Microscopy and bacterial culture M.tb microscopy, culture PCR for respiratory pathogens
Pleural Fluid	Microscopy and bacterial culture Protein and glucose testing M.tb microscopy, culture PCR for respiratory pathogens Antigen detection (pneumococcus and possibly legionella)
Lung Tissue (from post mortem needle biopsy, at select sites)	Histology Gram Stain and bacterial culture PCR for respiratory pathogens
Gastric Aspirate	M. tb culture
Urine [5 mL]	Antibiotic activity testing (on a subset) Storage for future testing (antigens; biomarkers) Total Urinary Arsenic testing (on a subset) Urinary Creatinine (on a subset)

*at least one specimen. Delay collection of induced sputum from those with bronchospasm and severe hypoxia until clinical conditions permit

**in settings with a history of use and where possible; not recommended in areas with HIV+ infants

***volume may vary by site (i.e. in sites where more blood is collected as standard of care – up to 7.5 mL without weight –based adjustments)

4.2.2.3 Demographics and risk factors

Risk factors for severe pneumonia will be collected from each case and control. These data will be used to control for confounding in the case control analysis, to describe the epidemiologic setting of the sites and to inform the global projection models. Furthermore, other putative risk factors for severe pneumonia will be assessed by examining their occurrence among cases

compared with controls. The case control analysis for causal inference assumes that controls represent the population from which cases are derived. We will obtain demographic data, information on socio-economic status and access to health care, to assess this assumption. Case and control health care records may be reviewed to obtain some of this data (e.g. immunization history, ARV treatment history, current/previous enrollment in another study, and CD4 counts if HIV-positive).

Table VI. Risk Factors*			
Category	Risk factor /Variable	Category	Risk factor/Variable
Demographic	Age	**Co-morbidities/ intervention**	Diarrhea
	Sex		Sickle Cell Disease
	Mortality status of Mother/Father		Prematurity
	Maternal age		Anemia
	Father's education level		Antibiotic use
	Maternal literacy		Steroid treatment
	Race/Ethnicity		Birth weight
Nutrition	Breastfeeding		
	Vitamin A supplementation		
		Family environment	Rural or urban residence
	Zinc supplementation		Number of siblings
			Type of home (compound/single family)
			Sex of siblings
			Age of siblings
	Vitamin D status (lab test on stored samples)		Number of children living under one roof
	Malnutrition: will be assessed by z score from weight and height;		Birth order
			Number of people in the family
	Pedal edema		Temporary guests in the household
			Out-of-home care
Vaccination history	BCG		
			Ventilation in the main living area
	DPT3		
	Measles		Smoker in the house
	HiB		Maternal smoking

Table VI. Risk Factors*			
Category	Risk factor /Variable	Category	Risk factor/Variable
	PCV		Urinary cotinine levels in child (lab test)
	Influenza		Child being carried on mother's back while cooking
	Rotavirus		Type of cooking fuel
Co-morbidities/ intervention	Cold/ hypothermia (temp. threshold to be defined)		Type of stove
	Malaria Parasitemia (lab test)	**Socio-economic status**	Maternal Education
			Head of Household occupation
	Bednet ownership and use		Household Income
	HIV infection status		Source of water in the house
	AIDS		Types of wall
	ARV treatment		Household possession
			Electricity
			Type of toilet
			Communication devices
	Cotrimoxazole therapy/ prophylaxis	**Access to care**	Possession of transportation means
	Mother's HIV status during pregnancy		Means of reaching health facility
	Known TB		Distance to study facility
	DOTS/ TB treatment of adult contact		Cost of reaching study facility
	Past medical history: e.g. asthma, heart disease, etc		Time to reach study facility
			Place of birth (hospital, home)
	Previous Pneumonia		Mode of delivery (vaginal, c-section)
Environmental Exposure	Arsenic Exposure i.e., total urinary arsenic		

Table VI. Risk Factors*			
Category	Risk factor /Variable	Category	Risk factor/Variable
	concentration		

*Note: Some sites may collect only a subset of these risk factors

4.2.2.4 Chest Radiography

All cases will have a PA or AP chest x-ray. At some sites, lateral chest radiographs will also be obtained depending on local standards of care and feasibility. All radiographs will be digitized for the purpose of standardized readings on-site or off-site depending on the site capacity and for quality assurance /quality control readings off-site.

4.2.2.5 Digital Auscultation

All cases will have a clinical examination to include respiratory lung sounds. In some sites these sounds may be digitally recorded for standardized categorization. PERCH provides a unique opportunity to build a substantial library of breath sound recordings across multiple populations in a standardized manner. Using this large library, it may be possible to develop and validate a framework for describing and classifying breath sounds that would be consistent and reliable across observers, similar to the algorithm for interpreting chest radiographs described in the previous section. Such a framework could be applied in both clinical and research contexts. In addition, these classifications can be linked to clinical, radiologic, and microbiologic findings as part of the etiologic prediction model, or used to develop computer-based algorithms for the automated classification of breath sounds. The recording will be done using a digital stethoscope which looks and functions as a regular stethoscope but allows for recording of the sounds digitally. The audio-files of the sounds will then be reviewed and categorized by standardized auscultation or by computer analysis. The audio-files will be labeled only with study number and no names or initials. The audio files may be 'read' by on-site or off-site readers depending on site capacity and for quality assurance/ quality control.

4.2.3 Follow up

Standardized data on a limited set of clinical signs and symptoms (respiratory rate, oxygen saturation and amount of oxygen requirement) will be collected at admission, at 24 and 48 hrs after admission, and at discharge to allow inferences to be made about the association between disease progression and specific etiologies and to accurately assess severity. Treatment given during the hospitalization period will also be recorded.

4.2.3.1 24-hour/48-hour limited clinical assessment

A clinical assessment will take place at 24 and 48 hours following admission to measure certain important signs and assess response to therapy:

- O2 saturation
- Respiratory rate
- Volume of O2 required/min
- Lower chest wall indrawing

- Pneumonia danger signs
- Changes in treatment regimen(s)

The "day of admission" will be determined by calendar date, i.e., if enrolled after midnight the 24-hour assessment should be performed the following night or the second morning.
No study specimens will be collected.

4.2.3.2 Discharge assessment

The following clinical signs will be assessed at discharge:

- O2 saturation
- Respiratory rate

Discharge diagnosis and clinical status will also be evaluated (i.e., whether fully recovered, left against medical advice, died, etc.).

4.2.3.3 Post-discharge follow-up

All patients discharged alive will be followed up 30 days (window 21-90 days) post-admission to assess vital status and to collect a convalescent serum at all sites and a urine specimen at select sites. Ancillary study data or risk factor information will also be collected at this time. CD4 testing will be done on plasma collected from HIV-infected cases in South Africa and Zambia.

4.2.4 Post Mortem Assessment

The identification of organisms (bacterial, viral and fungal) causing pneumonia in children from a variety of different geographical and ethnic backgrounds will necessarily lead to a spectrum of potential etiologies. The introduction of vaccines against two of the commonest causes of severe pneumonia, *Streptococcus pneumoniae* and *Haemophilus influenzae b*, will also alter the prevalence of causative organisms in severe and fatal pneumonia. The PERCH study has been established to determine these different etiologies. Assessment of clinical samples such as upper respiratory tract washings, sputum or blood cultures will be tested with routine microbiology and molecular diagnostic methods as part of the PERCH study.

However, standard clinical and laboratory diagnostics often fail to identify the etiology of pneumonia (1). Furthermore, the low specificity of some tests – such as sputum or nasopharyngeal culture render them hard to interpret. Fatal outcome in severe pneumonia reflects infection and disease in the lower respiratory tract and lung parenchyma. This means that diagnosis of the cause of death (on a background of multiple potential commensal organisms within the upper tract) should be more accurate if a 'predominant' organism can be identified in the diseased lung, by localizing the virus/bacteria/fungi to the site of the lung pathology.

This argues for direct examination of lung tissue. The PERCH study will obtain ante-mortem lung aspirates on patients in four sites (i.e. the Gambia, Bangladesh, Mali and South Africa) but only a minority of cases have peripheral consolidation on chest x-ray amenable to lung aspirate. An alternative approach to examine lung material directly is to perform post-mortem percutaneous lung biopsy in fatal pneumonia cases. Full autopsy is difficult given the cultural and social constraints on post mortem examination in many of the study countries. Immediate post

mortem percutaneous lung biopsy offers a potentially simpler and less invasive approach to obtain lung tissue than full autopsy.

The advantages of directly obtaining tissue samples from post mortem lung include; (i) microbiological testing to compare post-mortem material with premortem samples on the same patient to validate premortem diagnostics (ii) to establish a diagnosis where these are lacking. (iii) to provide a diagnosis in children who arrive at hospital in extremis and die shortly after arrival, before research investigations can be initiated. In these cases a postmortem approach may provide vital etiological information about this group that is of greatest interest for the prevention of pneumonia mortality.

Lung biopsy samples can be examined by microbiological cultures and molecular diagnostics to detect unrecognized infections or additional infective agents. For example, a recent CDC study on Influenza A H1N1 deaths in the US, showed that testing of post-mortem lung tissue using PCR and immunochemistry assays identified many bacterial lung infections missed by standard clinical methods (2). They may also be examined using histology, where characteristic parenchymal changes may indicate the primary organism (such as granulomatous inflammation in TB, viral inclusion bodies or specific bacteria recognized on Gram staining).

There are a number of potential problems with an autopsy approach to diagnosis in fatal pneumonia. The first is sampling error, whereby the section of lung with the pathology is not sampled; this can be minimized by directing the sampling to the affected lobes. Second, in the perimortem and immediate post mortem period there is widespread cellular degradation and breakdown in physiological barriers (such as in the large bowel), associated with leakage of a mixed flora of intestinal bacteria into the bloodstream and contamination of tissues. The value of post mortem microbiological testing in autopsy tissues has been questioned in the light of these potential problems (3). Studies have revealed, in general, that a proportion of post mortem microbiological diagnoses will be uninterpretable due to contamination, and that success may require choosing the appropriate test to identify a suspected pathogen (such as with fungal pathogens or TB) which may not be a first line microbiological test.

However, despite these potential problems, successful autopsy-based studies of pneumonia etiology, utilizing a purely histopathological approach without supporting microbiology, have had value in revealing unsuspected pathogens (4,5) In our view these data argue that relying wholly on premortem samples and those distant to the diseased lung will result in a significant under- or mis-diagnosis of the causative organism. The extra information which could be derived from postmortem lung biopsy samples in fatal pneumonia cases may significantly add to the data available to the study and will provide information of much greater value in assigning causality in pneumonia than that obtained from peripheral specimens such as nasopharyngeal cultures.

4.2.4.1 ***Validation of antemortem etiologic diagnosis of fatal pneumonia***

Data from postmortem evaluation can also help to validate new and existing diagnostic methods implemented in PERCH. One traditional role that post-mortem examinations have played in clinical care is as a quality assurance mechanism to ensure the accuracy of diagnosis made during the care of patients in life. The PERCH project aims to use Bayesian models to estimate

the contribution of major pathogens to pneumonia etiology and evidence from a lung sample that has high specificity (e.g. lung biopsy material) in a minority of patients who die during treatment may be used to validate and extrapolate evidence derived from lower specificity samples (e.g. nasopharyngeal sampling) among the remaining cases who survive.

4.2.4.2 *Improved diagnosis of lung conditions contributing to fatal outcome*

Several of the possible primary pathogens in this group may produce 'final common pathway appearances' in the lung such as bacterial pneumonias. However evaluation of post-mortem lung tissue will also enhance TB diagnosis, and provide quality assurance for the methods used for antemortem diagnosis. The diagnosis of pulmonary tuberculosis is important, because it may contribute to a fatal outcome, but also challenging, especially in HIV infected children (6,7). Typically, tuberculosis is diagnosed antemortem on the basis of clinical and radiological findings, but these features are often shared with other infections such as PCP and cytomegalovirus pneumonitis, reducing their specificity in diagnosis. Chintu's study demonstrated a high prevalence of pulmonary tuberculosis in both HIV-positive and HIV-negative patients in Zambian patients (4). However in another study of 93 consecutive deaths of HIV-infected children in South Africa, Rennert *et al*. confirmed the diagnosis of tuberculosis from lung tissue in only 4% of the patients (8). Some 18% of the patients, who had been on empiric treatment for tuberculosis before death, had no postmortem evidence of active disease in the lung. This disparity may have been due to effective treatment or could indicate clinical misdiagnoses because other pathogens, such as cytomegalovirus and *P.jiroveci*, were found in all suspected tuberculosis cases. Histological evaluation of lung tissue will improve diagnosis, provide quality assurance of antemortem diagnosis and establish the prevelence of tuberculosis in different populations. This would significantly influence guidelines for national vaccination, prevention and control programs.

Post mortem examination may also help identify pathological processes which contribute to a fatal outcome such as diffuse alveolar damage, and facilitate the diagnosis of pneumonia-like conditions which simulate acute pyogenic pneumonia clinically, like such as Lymphoid Interstitial Pneumonia (LIP), malaria, non-infectious pneumonia (e.g. paraffin ingestion), or non-pneumonic etiology (e.g. anemic heart failure).

4.2.4.3 *Identification of Novel Pathogens*

Identification of novel pathogens is one of the objectives of PERCH. With post-mortem evaluations PERCH can describe the histopathology associated with any new pathogenic agents and determine the likelihood of their role in the etiology and pathogenesis of pneumonia. The potential resource of tissue and DNA available for some cases should also improve the chances of characterizing novel pathogens

4.2.4.4 **Study Aims**

1. Detect infections or co-infections missed during antemortem evaluations
2. Validate antemortem diagnosis
3. Assess the correlation of histopathological patterns of disease with etiology, particularly if novel pathogens are involved.
4. Improve diagnosis of pulmonary tuberculosis and pneumonia-like conditions

5. Interpret pathology results in conjunction with clinical data, as the lack of such an analysis has been a limitation of previous studies.
6. Form a biorepository for future investigations in this field.

The PERCH project is investigating the etiology of childhood pneumonia in seven different countries. PERCH plans to study the clinical course and determine the microbiological causes of pneumonia in children from 1 month to 5 years old. As part of the PERCH study, we will study tissues from the lung in patients who die of pneumonia in six of these study sites (excluding the Gambia), dependent on the outcome of local IRB approval. A full open autopsy is often difficult to perform for a number of reasons, so we plan to use postmortem percutaneous needle biopsies of the lung.

All PERCH cases will be monitored throughout their hospital stay. The parents of any fatal pneumonia case participating in the study will be approached for participation before or after death depending on the site customs, preference, and community acceptability.

4.2.4.5 **Post Mortem Inclusion criteria**

Cases must meet the following inclusion criteria for eligibility to enter into the PERCH lung biopsy study:

- Enrolled in the PERCH study
- Patient dies in hospital
- Not more than 12 hours have elapsed since death
- Parental/guardian consent given for lung biopsy

4.2.4.6 **Post Mortem Exclusion criteria**

Children that meet any of the following exclusion criteria will be ineligible to enroll in the postmortem needle biopsy study:

- Parents decline consent
- Lung biopsy procedure cannot be performed

4.2.5 *Post mortem Study subject evaluation methods*

4.2.5.1 **Specimen collection**

Prior to the biopsy and specimen collection procedures, any available case x-ray should be reviewed with the operator to determine likely site of disease and therefore the appropriate sampling protocol for either lobar or diffuse disease. Obtaining a representative sample of the lung is important. Pneumonia may be localized to one area of the lung or changes spread across patchy or wide areas of the lung.

By using a percutaneous procedure, some anatomical representativeness can be obtained by taking multiple cores from both sides.

- There will be one biopsy entry site on each side – one on the left and one on the right. All lobes of the lung can be sampled through one entry site. One entry per side will minimize physical evidence of the procedure on the body (leaving a punctate scar less than 0.5cm in diameter).
- The biopsy site is in the mid-axillary line in the 5th intercostal space

- The biopsy needle will be used to take multiple biopsies from both sides. Passing the needle in different directions will ensure sampling of different lobes.
- An automatic, reusable needle biopsy system will be used, with an 18G detachable biopsy system allowing sampling of 22mm length cores [Omni-RAM (Agoram) system for soft tissue biopsies].

The text below details the sampling strategy:

- Two Biopsy Sites, Bilaterally in all cases (1 left, 1 right) in mid axillary line
- A total of 16 biopsies will be taken. E.g. the biopsy needle will be reinserted through a single puncture site on each side 8 times to collect tissue samples from different lung segments.
- Providing 5 biopsy cores for Histology, 6 cores for Microbiology, 2 for Molecular pathology, 2 for Molecular microbiology, and 1 core for frozen tissue

Protocols for direction of biopsy sampling will be based on any antemortem evidence for localization of disease, for instance from clinical examination of chest X-ray results. The different biopsies will be preserved in separate fixatives according to their intended use:

A: Histology: 10% neutral buffered formalin for standard histology from formalin fixed, paraffin embedded blocks

B: Microbiology: No preservative, rapid transport to microbiology laboratory for

i) Gram stain, microscopy and standard agar plate culture
ii) mRNA extraction for Multiplex PCR
iii) TB culture

C: Molecular Microbiology: TE buffer for subsequent 16s rRNA typing

D: Molecular Pathology: RNaLater buffer for subsequent mRNA extraction and PCR

E: Frozen Tissue: For subsequent immunohistochemistry and PCR

As well as allowing routine histology, and comparative microbiology with any antemortem sampleson the same patient, preservation in RNAlater and Frozen tissue have both been validated for DNA/mRNA and microRNA extraction from autopsy tissues and production of microarrays in lung tumours and interstitial lung disease. (9-11).

4.3 Control selection

Controls selected from the community will be enrolled approximately on a 1:1 basis to cases, with a minimum of 25 controls enrolled per month. Parents/guardians of control subjects will be compensated according to the routine practice at each site. Local practices and IRB guidelines will govern the amount and type of compensation; the amount offered will not be so high as to induce someone to participate in the study. Food and transportation reimbursement are examples of the types of compensation that could be provided by the sites. Specific details will be included in site specific appendices. Basic demographic information on eligible children

not enrolled, including the reason for not enrolling, will be collected and used to compare enrolled and non-enrolled children.

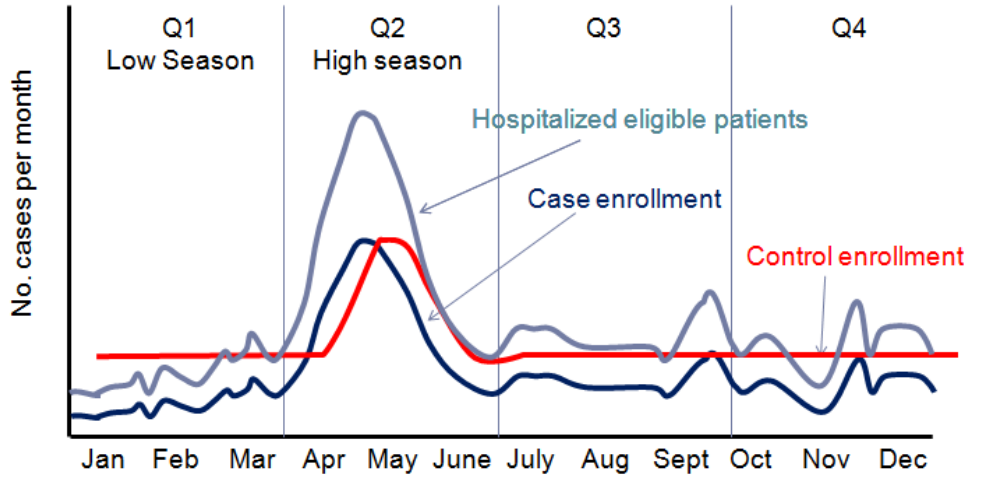
Table VII presents an overview of the control evaluation schedule of activities. Details for each time point are described in sub-section 4.4 below.

Table VII. Overview of Control Study Evaluations		
	Follow-up time point	
Activity	**Day screened**	**Day enrolled***
Log of assessed children	X	
Eligibility assessment	X	
Enrollment/Consenting		X
Specimen Collection:		
• Blood		X
• Nasal and throat swabs		X
• Urine		X
Demographic, clinical history and risk factor assessment		X
Limited clinical assessment (i.e. respiratory signs, respiratory rate, neurological signs, rash, temperature)		X

*May be the same day of screening for children enrolled at home.

Controls will be selected from the community from which the cases came (but not matched by geographic location). Approximately 1 control per case will be enrolled. PERCH sites will recruit a minimum of 25 control patients per month. In months where the number of cases exceeds 25, sites will enroll controls in an additional 1:1: ratio to achieve the same number as cases enrolled that month.

Figure V. Illustration of control selection rates relative to the case enrollment rates



In the two sites with high HIV prevalence (Zambia and South Africa), a separate control enrollment strategy will be deployed in addition to the above strategy to capture a second group of HIV-infected controls for the etiologic analysis (see section 4.4.6).

4.3.1 Strategy of community control selection

There will be two main stages to the control selection strategy – defining a catchment area and selecting eligible control children from within that catchment area. At the first stage in control selection, the catchment population from which cases are drawn will be clearly defined at each site by examining the geographic distribution of existing cases in the hospital(s). The catchment population includes all residents of the geographic catchment areas served by the study hospitals. The catchment area for the PERCH study could be the entire catchment area of the hospital, or a subset of the catchment area where the majority of cases come from. This latter strategy will be employed for logistical reasons so as not to have to enroll controls over a too expansive area, and for statistical reasons so that controls from very distant areas that rarely use the study hospital would not be over-represented in the controls. In some sites (i.e. Kilifi, Gambia and Bangladesh) a demographic surveillance system (DSS) area will be defined as the catchment area for PERCH. In the other 4 sites, existing case admission logbooks and local knowledge of the area will be used to define an appropriate catchment area for PERCH where the majority of severe pneumonia cases reside.

In the next stage of control selection, one of two strategies will be employed. In some sites, all children in the catchment area will already be enumerated. This will apply to areas with DSS sites (i.e. Kilifi, Gambia and Bangladesh) and with comprehensive birth registries (i.e. South Africa and Thailand). In these sites, a simple random sample of children, frequency matched by age, will be selected from the entire catchment area. There will not be matching by geographic location of the cases.

In the sites without individual household enumeration of the catchment area (i.e. Zambia, Mali), the catchment area will be divided into segments, such as villages or census enumeration areas where the approximate population size of these segments is known. Segments will be selected using simple random sampling with a probability proportional to estimated size as needed for the number of controls to be enrolled each month. To select a household in the selected

segment, either an enumerated list of households will be used and simple random sampling employed or the WHO EPI immunization cluster sample survey method will be used (www.who.int/vaccines-documents/document WHO/IVB/04.23). In brief, this is done by choosing a random direction from the center of the segment and walking from that center to the perimeter, enumerating all households on the line of the walk. Then, a starting household from the enumerated list is chosen randomly and visited to verify if an eligible control is available. If not, the next household in a predefined direction (e.g. left or right) is visited, and so on, until an eligible control is enrolled.

4.3.2 Inclusion criteria for community controls

Enrollment of community controls will not be restricted with respect to signs of non-severe pneumonia or presence of upper respiratory tract infection (URTI) symptoms. Analyses will be conducted both including and excluding ill controls.

Controls must meet the following inclusion criteria for eligibility to enter into the PERCH study:

- Aged 28 days-59 months
- Lives in defined study catchment area stratum (may be defined as a village or distance zone from the hospital)
- Accompanied by written informed parental/guardian consent

4.3.3 Exclusion criteria for community controls

Controls must not have any of the following exclusion criteria:

- Discharged from hospital within the last 14 days
- Severe/very severe pneumonia
- <30 days from discharge date of previous enrollment as a case
- Admitted to a hospital within the past 30 days because of an acute illness (in South Africa and Zambia only. CD4 counts will be done on HIV-infected community controls in these sites and an acute illness within the last 30 days may affect these results)

4.3.4 Rationale for HIV infected controls

Because of the very increased risk of pneumonia among HIV-infected children, it is expected that the cases will be disproportionately HIV-infected compared to the community. In the sites with high HIV prevalence (i.e. Zambia and South Africa), this might result in up to half of cases being HIV-infected. The community prevalence of HIV infection in children < 5 years, even in these high prevalence areas, will likely be <5%. Therefore, this will lead to an over-representation of HIV-infected cases compared with controls. This is the principle upon which to estimate the magnitude of the association between HIV and pneumonia. However, it is expected that the etiologic spectrum of respiratory pathogens will differ in HIV-infected children as will the pathogens colonizing the nasopharynx. An unconfounded analysis of the causal association between nasopharyngeal infection and pneumonia needs to compare HIV-infected cases with HIV-infected controls. To achieve sufficient numbers of HIV-infected controls to enable this comparison, a separate sampling strategy has been designed to enroll HIV-infected controls in these two sites.

HIV-infected controls will be enrolled from HIV patient support centers (PSC) serving the hospital catchment population. PSCs include ARV/ART clinics. We considered enrolling

hospitalized HIV-infected control subjects but rejected this option because of a 2-3 day lag-time in making the HIV diagnosis, by which time the naso/oropharyngeal flora will likely have become altered with nosocomially –acquired pathogens. Secondly, infants who are HIV-infected and admitted with non-pneumonia diagnoses have been difficult to find in South Africa due to improved prevention-of –mother –to-child-transmission and lower prevalence of HIV among newborns.

In Zambia and South Africa, HIV-infected controls will be enrolled in a 1:1 ratio with HIV-infected PERCH cases using the same age frequency matching approach described in section 4.3.7.

4.3.5 Inclusion criteria for HIV infected controls

HIV infected controls must meet the following inclusion criteria for eligibility to enter into the PERCH study:

- Documented to be HIV-infected
- Aged 28 days-59 months
- Screened at a PSC, either as follow-up or as a new patient, located in the catchment area from which the cases are being enrolled.
- Lives in defined study catchment area stratum (may be defined as a village or distance zone from the hospital)
- Accompanied by written informed parental/guardian consent

4.3.6 Exclusion criteria for HIV infected controls

Controls must not have any of the following exclusion criteria:

- Discharged from hospital within the last 14 days
- Severe/ very severe pneumonia
- Admitted to a hospital within the past 30 days because of an acute illness
- <30 days from discharge date of being previously enrolled as a case, or within the previous 3 months as a control
- Requires hospitalization on the day of admission

4.3.7 Matching and selection/sampling methods

Controls will be frequency matched on age and season to the cases to ensure that neither factor can confound the primary analysis, i.e. – the association between case-status and infection of the nasopharynx with respiratory pathogens.

Age strata for frequency matching to be used are the following: 28 days-5 months, 6-11 months, 12-23 months, 24-59 months. Seasonality matching will be done by month – attempts will be made to enroll the same number of cases and controls by month, with a minimum number of 25 controls per month. All analyses will incorporate stratification of age and season.

4.3.8 Case-control transitions

The following rules will be applied for case-control transitions:

a. Case-to-case. A case can be enrolled as a case again if admitted >30 days after date of discharge from the hospital for an admission for which the child was enrolled in PERCH study and >14 days after the discharge from the last hospital admission (any cause). The 30 day limit is to ensure that the same pneumonia episode is not

enrolled twice and the 14 day limit is to avoid enrollment of nosocomial pneumonias.

b. Case-to-control. To be consistent in the treatment of cases and controls, a previous case can be enrolled as a control if the control enrollment date is >30 days after the last PERCH date of discharge from the hospital for an admission for which the child was enrolled in PERCH study.
c. Control-to-control. A control can be enrolled a second time as a control if selected through the random selection process. There is no time period of exclusion between control selections.
d. Control-to-case. A control can be enrolled as a case at any time after he/she was enrolled as a control. However, if he/she is enrolled as a case within 48 hours of being enrolled as a control, he/she would be excluded as a control from the recent control enrollment. The reason for this is that any URTI that the child had at the time of control enrollment might have been the early stage of the severe or very severe pneumonia.

Study sites will track study participants with a unique identifier to link separate episodes when they become eligible as cases or controls.

4.4 Control evaluation

To correctly classify the pneumonia status of enrolled controls, respiratory symptoms will be assessed at the time of screening and enrollment. Parents of controls will asked about the child's history of cough and difficulty breathing and will be questioned about the symptoms of upper respiratory tract infection (i.e. cough, runny nose, sore throat). Those with a history of cough or difficulty breathing will be examined by the fieldworker to determine the temperature, respiratory rate and presence of lower chest wall indrawing. If a child has lower chest wall indrawing, he/she will not be enrolled as a control and will be referred for clinical care. If the child has elevated respiratory rate for age (≥60 for under 2m, ≥50 for 2-11m, ≥40 for children under 5y - WHO definition), he/she will be enrolled as a control (and have control samples taken) and will then be referred by the field worker for evaluation at the hospital where cases are being enrolled. Referred controls will be identified at the study hospital and if they are considered by the clinical staff to have severe or very severe pneumonia, they will be given the opportunity to enroll as a case instead of as a control. Control children with URTI only will be instructed to present themselves to the PERCH hospital if their illness progresses. If upon evaluation of the child at the PERCH hospital, they are deemed to have severe or very severe pneumonia within 48 hours of their enrolment as a control, they will be invited to enroll as a case instead of as a control. If they are diagnosed with severe or very severe pneumonia more than 48 hours after their enrolment as a control, they will be eligible to be enrolled as a case but will also be maintained in the database as a control (they will be enrolled as both a case and a control). When > 48 hours have elapsed between control enrolment and pneumonia diagnosis , the preceding "control" URTI will be considered as a separate episode from the "case" pneumonia since as URTIs often precede pneumonia.

At the analysis stage, analyses will be conducted both including and excluding controls with evidence of URTI or non-severe pneumonia. Controls with URTI (i.e. cough, runny nose, sore throat, etc) and non-severe pneumonia (i.e. cough/difficulty breathing and elevated respiratory

rate) will be used to assess whether the prevalence of pathogens exists along a spectrum between asymptomatic, URTI and LRTI patients.

4.4.1 Lab tests/specimens collected

At the time of recruitment, controls will have three sets of specimens collected (below). Methods for collection are described in Section 4.5

- Upper respiratory swabs– one each of the following: a flocked swab from the nasopharynx, a rayon swab from the nasopharynx, and an oropharyngeal swab
- Blood – 4 mL ideal, 2 mL minimum
    - Whole blood when possible either through venipuncture or fingerstick
- Urine – 5 mL (when possible)

Table VIII. Control Specimens	
Body Fluid	**Laboratory Analyses**
Venous blood **0-1kg: 3mL** **≥1kg: 4mL**	Pneumococcus PCR HIV serology – Note: parents have the option to decline HIV testing. HIV positive participants will be referred for further clinical care (e.g. at an HIV patient support center). Singleplex PCR – HIV (subjects ≤18mo with positive antibody test) CD4 testing for HIV+ controls (in South Africa and Zambia only) Malaria (microscopy or antigen testing, in endemic areas) Sickle Cell Testing (Gambia, Mali, Zambia and Kenya) Thalassemia (Thailand only) Hemogoblin (Thailand and the Gambia only) Other Serology Storage for host genetic studies Biomarker testing
NP rayon swab	Bacterial culture for pneumococcus (and serotyping if applicable)
Throat rayon swab/Flocked NP Swab	PCR for respiratory pathogens
Urine (5 mL)	Antibiotic activity testing (subset); storage for future testing (antigens; biomarkers) Total Urinary Arsenic testing (on a subset) Urinary Creatinine (on a subset)

4.4.2 Demographic and risk factors

Demographic and risk factor information will be the same as what is collected for cases. Refer to section 4.2.2.3.

4.4.3 Control follow-up

For tests that have clinical significance, the test results will be provided to the parents of control children. This will include results for HIV, CD4 counts, malaria, sickle cell, hemoglobin and thalessemia tests at applicable sites. Certain sites may also choose to provide the PCR results from the NP swab as required by the local IRB or local community feedback procedures. See table X (laboratory evaluations of controls) for more detail. Results that will be provided to controls will be specified in the site specific appendices.

If results are not immediately available (e.g. HIV PCR tests) at the time of control enrollment, the control child will be followed-up at the home or at the PSC to be given results and referred to appropriate ongoing care (e.g. HIV patient support center). The site of follow-up will be determined by each site according to local clinical practices.

4.5 Study subject evaluation methods

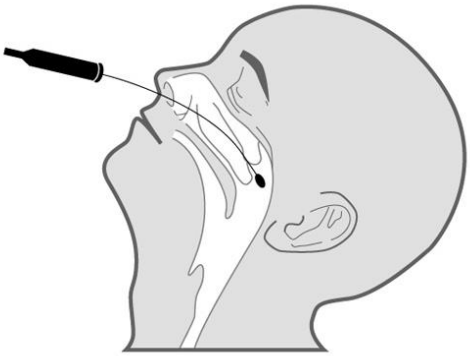
4.5.1 Specimen collection

4.5.1.1 Blood

Blood will be drawn from venipuncture in the amounts outlined above. Standard procedures for proper venipuncture will be employed, with particular attention to proper technique to minimize risk of contamination of blood cultures.

4.5.1.2 Nasopharyngeal swab

Two nasopharyngeal (NP) specimens will be collected from all cases and controls. One NP flocked swab will be collected and placed into viral transport medium, and a second flocked NP swab will be placed in skim milk tryptone-glucose-glycerin (STGG) medium.



4.5.1.3 Orpharyngeal swab

In addition to the NP swabs, all cases and controls will have an OP rayon swab collected and placed into the same vial of VTM as the NP flocked swab. The OP swab is taken by touching the swab to the posterior oropharynx and rubbing for 1-3 seconds.

4.5.1.4 Lung aspirates

Lung aspirates are very useful samples for determining pneumonia etiology, which can yield important information that guides patient therapy, but only a relatively small number of clinical

study sites have experience with this technique. The general technique used for pediatric lung aspiration is to insert a needle over the top of a rib into the area of consolidation or maximum physical findings, apply suction to the plunger of the syringe, maintaining constant suction while withdrawing the needle. Lung aspirates will be collected for clinical use and research; specimens will be tested in real time for clinical use purposes. Not all clinical sites will conduct lung aspirates; only those sites with sufficient site experience or physician experience will do this study procedure. Furthermore, only those children with large, peripheral consolidations that are amenable to lung aspirate will have the procedure conducted. Lung aspiration will not be performed on children with contraindications including: presence of pneumatocoeles on CXR, post measles pneumonia, patient is clinically unstable as determined by a clinician, CPR performed within the last 24 hours.

4.5.1.5 Induced sputum

Induced sputum will be collected from all cases except those with contraindications, which include the following: severe hypoxia (<92% on supplemental oxygen), inability to protect airways, severe bronchospasm at admission, seizure within the past 24 hours, or deemed inappropriate by a study clinician for another reason (e.g. mid-face trauma, inhalational injury, pulmonary edema, congestive heart failure, congenital heart disease, etc). If the above symptoms resolve during hospital course, induced sputum collection may be reconsidered at that point.

The following guidelines will be utilized for the collection of induced sputum.

- Sites will use Metered Dose Inhalers (MDIs) for bronchodilation
- Sites will employ light chest percussion in children < 2 years to improve mobilization of sputum. In children $\geq$ 2 years, their cough reflex should be sufficient to mobilize sputum after induction with nebulization of hypertonic saline
- The specimen should optimally be collected within 24 hours. After 24 hours, induced sputum can still be collected (to test for tuberculosis and *Pneumocystis jiroveci*), but bacteriology should not be performed unless antibiotic therapy fails.
- The quantity of the specimen should be at least 1 mL.
- The quality of the specimen will be assessed using the "Bartlett's Score". Specimens with suboptimal Bartlett's Score will still be processed by the lab for all specified testing, but the Bartlett Score and consequent microbiology results will be considered in the analysis. Suboptimal Bartlett Scores will also be reported to the clinical team doing the procedure as a performance indicator.
- Some sites will perform a second induced sputum to improve the diagnostic yield for tuberculosis, as has been shown in other studies (Zar et al. 2005). This will be stipulated in site specific appendices.

4.5.1.6 Urine

Urine samples will be collected from cases and controls for two purposes: (i) to examine for the presence or absence of antibiotic activity, a marker of recent administration of antibiotics which might influence the sensitivity of culture-based microbiological techniques and (ii) to be stored for future examination of antigens that determine etiology and relevant urinary biomarkers (e.g. biomarkers of smoke/air pollution exposure and urinary arsenic). Urine will be taken at the

earliest opportunity from children admitted as cases, ideally before the first dose of antibiotic therapy. However, antibiotic infusions will not be delayed to obtain a urine sample uncontaminated with hospital-administered antibiotics. If the urine specimen is not obtained by this time, antibiotic administration should begin. The specimen can still be collected and analyzed for detection of antigens.

For infants and young children, urine will be collected into a perineal adhesive bag and transferred to a sterile universal container. Older children may pass urine directly into a sterile universal container. If antibiotics have been given by the nursing or medical staff prior to capturing the specimen then the specimen data collection form will be documented appropriately and the sample container will also be marked. Samples will be taken to the laboratory and stored immediately at 4-8 ^{o}C until processed.

4.5.1.7 Gastric Aspirates

Gastric aspirate will be collected from all children for whom an induced sputum cannot be collected at the time of enrollment (with timing of collection based on clinical judgment). The general technique used for gastric aspirates is to insert a nasogastric tube through the nose into the stomach, and gently aspirate the tube with a syringe until the child produces enough liquid for collection. This is uncomfortable for the child insofar as a small tube is passed through the nose into the posterior pharynx and then in to the stomach. This is a routine, daily procedure in all hospitals and is used for a variety of purposes including feeding children, decompressing the stomach of gas when in respiratory distress and collection of gastric contents. There is very little risk associated with the procedure except some discomfort at the time of tube placement and the low risk of inadvertently passing the tube into the trachea and not the esophagus. This is readily recognized clinically, the tube is withdrawn and placement attempted again.

4.5.1.8 Pleural Fluid

Pleural fluid will be collected from a minority of cases as indicated by attending clinicians. These specimens will be used for bacterial culture, TB studies, and testing by PCR. The methodology for obtaining pleural fluid will follow local clinical practice guidelines, including standard safety precautions.

4.5.1.9 Post mortem Lung biopsy

Post-mortem lung biopsy will be obtained for microbiological, histological and immunocytochemical evaluations in order to establish the etiology in fatal cases of pneumonia, validate ante-mortem etiologic diagnosis of fatal pneumonias and asses the correlation of histo-pathological patterns of disease with etiology, particularly if novel pathogens are involved. Full open autopsy is not being considered, as it is unlikely to be acceptable to parents in the majority of sites. Instead PERCH proposes to collect limited amounts of lung material by percutaneous tru-cut needle biopsy from several sites in the lung using a standardized protocol. These samples would be collected within a 4-hour time frame after the child's death, with the consent of relatives. Timing of the collection of the post mortem lung biopsy is important as delays in obtaining the specimens will increase the risk of contamination and add "noise" to the results. Please refer to section 4.2.4 for more details.

4.5.2 Clinical assessments

For each case enrolled in the study the following clinical measures will be collected according to the procedures described here and further described in the Standard Operating Procedures. A

subset of these (respiratory rate, temperature, cough/difficulty breathing, chest indrawing) will also be collected in all controls. In controls with signs of pneumonia respiratory symptoms and danger signs will also be collected.

Blood Pressure (cases): Arm or leg blood pressure will be collected using an automated or manual blood pressure measurement method. The systolic and diastolic BP will be recorded and mean BP recorded when collected by an automated method. Blood pressure will be collected whenever possible while the child is calm.

Respiratory rate (cases and controls): The child's respiratory rate will be recorded by counting the number of breaths in a full 60 second period. The respiratory rate will be observed and recorded, when the child is calm (i.e. not crying).

Temperature (cases and controls): The body temperature will be measured and recorded at the time of enrollment. Temperatures will be taken by axillary or tympanic thermometers. The temperature (in celsius) and the body site of measurement will be recorded.

Oxygen saturation (cases): Oxygen saturation will be measured using a pulse oximeter of a peripheral digit or an earlobe. The measure will be done on room air at the time of assessment whenever possible. For children who are already on oxygen at the time of assessment the oxygen will not be removed to measure the oxygen saturation. Oximetry measures will be recorded only when an adequate pulse is recorded on the oximeter which will verify that the probe is adequately placed and functioning. The oximeter reading will be recorded along with the amount of oxygen being received by the child at the time of the reading.

Height (cases and controls): Child heights will be measured using a flat surface and appropriate local methods for measurement.

Weight (cases and controls): Child weight (in kilograms) will be documented by weighing on an appropriately calibrated scale. The weight will be assessed immediately according to the age of the child. If the weight is outside the 5^{th}-95^{th} percentile for age, a second weight will be measured and the correct weight recorded.

Consciousness (cases): The level of child consciousness will be assessed according to categorical scale and recorded. The AVPU scale will be used and standardized across sites (Alert and Awake, responds to Voice, responds to Pain, Unresponsive or Unconscious).

Skin turgor (cases): Skin turgor will be assessed by pinching of the skin on the abdomen and recorded as normal, slow (1-2 sec) or very slow (>2 sec).

Pedal edema (cases): Presence or absence of pedal edema, as assessed by visual inspection and palpation will be recorded.

Capillary refill time (cases): The duration of time for capillary refill will be recorded in seconds. Delayed refill will be considered as >3 seconds.

Cough (cases and controls): The presence of observed cough at the time of assessment will be recorded as present or absent, and the duration of cough will be recorded.

Difficulty breathing (cases and controls): The observed presence of difficulty breathing will be recorded at the time of the assessment.

Grunting (cases): The presence of expiratory grunting will be documented as present or absent.

Stridor (cases): The presence of inspiratory stridor will be documented as present or absent.

Nasal flaring (cases): The presence of inspiratory or expiratory nasal flaring will be documented as present or absent. Whenever possible the observation will be made while the child is calm.

Wheezing (cases): Chest auscultation with a regular or digital stethoscope will be conducted according to the Standard Operating Procedure. The presence or absence of wheezing will be documented in the left and right lungs separately. Audible and auscultatory wheeze will be documented. Digital audio-files of the breath sounds will be recorded when digital stethoscopes are used. Digital audio-files will be stored centrally at the data coordinating center in Maryland, USA and will not contain names or other personal identifiers.

Crackles (cases): The presence or absence of crackles will be documented in the left and right lungs separately using a regular or digital stethoscope. . Digital audio-files of the breath sounds will be recorded when digital stethoscopes are used. Digital audio-files will be stored centrally at the data coordinating center in Maryland, USA and will not contain names or other personal identifiers.

Lower chest indrawing (cases and controls): The presence of the inward movement of the lower rib cage anteriorly on inspiration will be documented as present or absent. Standardization of this sign will be achieved by use of WHO training videos and on-site instruction by a senior clinician.

4.5.3 Chest X-Ray standardization and digitization

For the field evaluation of bacterial pneumonia vaccines, WHO developed a standardized method for interpreting the results of chest x-rays in children with suspected pneumonia. Currently this is the best available algorithm for systematically categorizing pediatric chest radiographs for pneumonia outcomes. The algorithm has been established with a set of validation and training films to provide a method for standardized reading of the films.

The WHO algorithm provides three categories of findings (primary consolidation, other consolidation and normal) that help to discriminate likely bacterial pneumonia from other outcomes. While PERCH would ideally prefer a system with the ability to differentiate further the x-ray findings, there is no other systematic, standardized chest radiograph reading framework that has sufficiently high inter- and intra-observer consistency. The WHO CXR reading algorithm will therefore be used in the PERCH study for the analyses of associations between chest radiography with disease severity and etiology.

Recognizing that this reading algorithm was designed for the purpose of standardizing the categorization of chest radiograph interpretation for bacterial vaccine trials (specifically pneumococcal conjugate vaccine trials, and Hib vaccine effectiveness trials) the PERCH project

will collect additional radiographic data to inform future reading algorithms. Therefore the PERCH study may use the existing reading framework for outcome purposes but the digitized films will be available for further more differentiated readings which can then be explored to determine those that are most reliably associated with etiologic categories and severity measures. The standardized readings as well as QA/QC readings may be done by trained expert readers based in countries other than those where the data was collected. Names and identifying information will be removed from the digitized film images.

Chest radiographs will be obtained at all sites on all enrolled cases of pneumonia. Chest radiographs will not be obtained on community control subjects. The chest radiographs will include a PA or AP on all cases and a lateral film on cases at selected sites at the time of initial assessment. The collection of lateral films will be determined by local standards of care and procedures. If further films are collected in the course of the child's illness, for the purpose of clinical management those films will also be included in the PERCH data collection. All chest radiographs will be captured as digital images either as digital radiographs, where available at sites, or by digitizing the plain film images using a flatbed scanner capable of producing high quality radiographic digital images. Digital cameras will not be used for digitizing the films because of variability in the procedures for collecting images. Digital chest x-ray images will be stored centrally at the data coordinating center in Maryland, USA in addition to the central coordinating center at Johns Hopkins, and will not contain names or other personal identifiers.

Readings of the digitized images will be done by PERCH CXR reading panel, made up of pediatricians and radiologists, who have been trained in the WHO CXR reading framework and have demonstrated proficiency in that reading framework. There will be fourteen readers total, along with four experts in the field who will arbitrate discrepant meetings. The arbitrators will be responsible for training all reading panel participants in the WHO framework. Film quality will be categorized as adequate, inadequate but readable for primary outcomes, or not readable. For those films that are adequate or inadequate but readable for primary outcomes two readers will independently read the film. Outcome categorization will be compared between readers. For those films where the reading is discrepant, the films will be referred to an adjudication panel whose reading will be final. In addition 10% of the "normal" films and 10% of the primary outcome films will be sent to the adjudication panel for assessment of reading calibration by the site. The QA readings described above will be used to provide feedback to the site but will not over-ride the reading of the site pediatrician and radiologist.

4.5.4 Laboratory evaluations

The planned laboratory tests for cases and controls are presented in the following tables:

Table IX. Laboratory Evaluations of Cases

Specimen	Subjects	Collection Container	Assay	Timing	Archive Sample	Centralized/ Decentralized Testing	Result reported to clinician (C); parent (P)
Acute Blood	All	Blood culture bottle	Blood culture*†	Real time	No	Decentralized	Yes –C/P
			Binax on blood culture alarm (+) culture (-) specimens	Real time			Site dependent
		EDTA Tube (Plasma)	CBC with differential*†	Real time	Yes	Decentralized	Yes – C/P
			Pneumococcus PCR	Batch		Decentralized	Site dependent
			HIV antibody test (and follow-up PCR for positive results in children < 18 months)* † (Some sites may use dried blood spots for HIV PCR testing, according to local practice)	Real time		Decentralized	Yes- C/P
			Sickle Cell Testing (selected sites) †	Batch		May be centralized	Yes-C/P if positive
			Thalassemia (Thailand only) †	Real time		Decentralized	Yes – C/P

Table IX. Laboratory Evaluations of Cases							
Specimen	**Subjects**	**Collection Container**	**Assay**	**Timing**	**Archive Sample**	**Centralized/ Decentralized Testing**	**Result reported to clinician (C); parent (P)**
			Malaria antigen testing or microscopy (selected sites) †	Real time		Decentralized	Yes – C/P
		Plain Tube (Serum)	Storage for future serologic testing	Batch	Yes	Centralized	No
			C reactive protein, other biomakers	Batch		May be centralized	No
			Antibiotic activity	Batch		Decentralized	No
			Host Genetic Studies	Store		Centralized	No
Convalescent Serum collected at 30 day follow up visit	All	Plain Tube (Serum)	Storage for future serologic testing	Batch	Yes	Centralized	No
Plasma collected at 30 day follow up visit	Selected	EDTA tube (Plasma)	CD4 Testing for HIV+ cases in South Africa and Zambia only**	Real time	Yes	Decentralized	Yes – C/P
Urine	All	Sterile container	Antibiotic activity (subset)	Batch	Yes	Decentralized	No
			Storage for future antigen testing, biomarkers	Batch		Centralized	No

Table IX. Laboratory Evaluations of Cases							
Specimen	**Subjects**	**Collection Container**	**Assay**	**Timing**	**Archive Sample**	**Centralized/ Decentralized Testing**	**Result reported to clinician (C); parent (P)**
			Total urinary arsenic testing using a Perkin-Elmer AAnalyst 600 graphite furnace system and urinary creatinine testing	Batch		Decentralized	No
NP flocked swab	All	Viral Transport Medium	PCR for respiratory pathogens†	Real time	Yes	Decentralized	Yes – C/P in selected sites
			Archived for potential viral culture, singleplex PCR	Batch		Centralized	No
NP rayon swab	All	STGG	Bacterial culture for pneumococcus and antibiotic susceptibility testing	Real time	Yes	Decentralized	No
			Serotyping/serogrouping for pneumococcal isolates			May be centralized	No
Throat Swab	All	Viral Transport Medium	PCR for respiratory pathogens† (esp mycoplasma)	Batch Test	Yes	Decentralized	Yes – C/P in selected sites
Induced Sputum	All, except when contraindicated	Sterile Container	Microscopy, bacterial culture and susceptibility testing†	Real time	Yes	Decentralized	Yes – C/P
			M. tuberculosis microscopy, culture†	Real time			Yes – C/P if positive

Table IX. Laboratory Evaluations of Cases							
Specimen	**Subjects**	**Collection Container**	**Assay**	**Timing**	**Archive Sample**	**Centralized/ Decentralized Testing**	**Result reported to clinician (C); parent (P)**
			PCR for respiratory pathogens	Real time			Site Dependent
Lung Aspirate (selected sites)	Select Cases	Sterile Container	Microscopy, bacterial culture and susceptibility testing†	Real time	Yes	Decentralized	Yes – C/P
			M. tuberculosis microscopy, culture†	Real time			Yes – C/P
			PCR for respiratory pathogens	Real time			Yes - C
Gastric Aspirate	Select Cases	Sterile container	*M. tuberculosis* culture*†	Real time	Yes	Decentralized	Yes – C/P, if positive
Pleural Fluid	Select Cases	Sterile container	Microscopy , bacterial culture and susceptibility testing*†	Real time	Yes	Decentralized	Yes – C/P
			Protein, glucose*†	Real time			Yes - C
			M. tuberculosis microscopy, culture*†	Real time			Yes – C/P if positive
			Antigen detection (pneumococcus and legionella)	Real time			Some sites may report according to local IRB Site appendices will specify
			PCR for respiratory pathogens	Real time			Yes - C

Table IX. Laboratory Evaluations of Cases							
Specimen	**Subjects**	**Collection Container**	**Assay**	**Timing**	**Archive Sample**	**Centralized/ Decentralized Testing**	**Result reported to clinician (C); parent (P)**
Lung Tissue (selected sites)	Post mortem cases	Formalin	Histology	Store	Yes	Centralized	Some sites may report according to local IRB or community requirements.
		Sterile Container	Gram Stain, bacterial culture and Susceptibility testing	Real time		Decentralized	Some sites may report according to local IRB or community requirements.
			Multiplex PCR	Store			Some sites may report according to local IRB or community requirements..

*Indicates tests done as part of clinical standard of care. All sites except the Mali site collect blood for culture and complete blood count on hospitalized pneumonia cases. HIV and malaria testing is done as standard of care in sites where they epidemiologically relevant. Gastric aspirates and pleural taps are standard of care when clinically indicated. Lung aspirates will be collected for clinical care and research; specimens will be tested in real time for clinical use purposes. All other tests will be done for research.

**CD4 results are for analysis purposes only; will not be used for clinical care.

†Indicates tests that may directly impact the child's care, or have some other benefit

Note: Some sites may utilize regional reference facilities for TB and HIV testing rather than doing this testing at the study site labs themselves. Details will be included in the site specific appendices. HIV antibody tests may done on either serum or plasma based on local protocols.

Table X. Laboratory Evaluations of Controls							
Specimen	**Subjects**	**Collection Container**	**Assay**	**Timing**	**Archive Sample**	**Centralized/ Decentralized Testing**	**Report results to parents**
Acute Blood	All	EDTA Tube (plasma)	Pneumococcus PCR	Batch	Yes	Decentralized	No
			HIV rapid antibody test(and follow-up PCR for positive in children < 18 months) † in selected sites only	Real time		Decentralized	Yes, in sites that do this test
			CD4 Testing (HIV+ controls, South Africa and Zambia only)*	Real time		Decentralized	Yes
			Sickle Cell Testing(selected sites) †	Batch		May be centralized	Yes, in sites that do this test
			Malaria antigen testing or microscopy (selected sites) †	Real time		Decentralized	Yes, in sites that do this test
			Thalassemia (Thailand only) †	Real time		Decentralized	Yes, in Thailand
			Hemoglobin (selected sites only)	Real time		Decentralized	Yes, in sites that do this test
		Plain Tube	Biomarkers	Batch	Yes	Some may be centralized	No

Table X. Laboratory Evaluations of Controls							
Specimen	**Subjects**	**Collection Container**	**Assay**	**Timing**	**Archive Sample**	**Centralized/ Decentralized Testing**	**Report results to parents**
		(Serum)	Storage for future serologic testing	Batch		Centralized	N/A
			Host Genetic Studies	Store		Centralized	No
			Antibiotic activity	Batch		Decentralized	No
NP flocked swab	All	Viral Transport Medium	PCR for respiratory pathogens	Real time	Yes	Decentralized	Yes, in selected sites
NP rayon swab	All	STGG	Bacterial culture for pneumococcus	Real time	Yes	Decentralized	No
Throat Swab	All	Viral Transport Medium	PCR for respiratory pathogens	Batch test	Yes	Decentralized	Yes, in selected sites
Urine	All	Sterile Collection Container	Antibiotic activity (if resources permit)	Batch test	Yes	Decentralized	No
			Store for future antigen testing	Batch test	Yes	Centralized	No
			Total urinary arsenic analysis testing using a Perkin-Elmer AAnalyst 600 graphite furnace system and urinary creatinine testing	Batch	Yes	Decentralized	No

*CD4 results are for analysis purposes only; not for clinical care. If available, these results may be obtained from the PSC from which the control is enrolled.

†Indicates a test that may benefit the participant. Note: Some sites may utilize regional reference facilities for TB and HIV testing rather than doing this testing at the study site labs themselves. Details will be included in the site specific appendices. HIV antibody tests may done on either serum or plasma based on local protocols.

4.5.5 Qualifications of local laboratories to report results:

Clinical results will be reported as indicated in tables IX and X above. Any reported results will be from tests performed in clinical laboratories that have been certified according to local standards or other certifying agencies. Details of site specific certifications will be included in the site specific appendices. For some of the tests or countries there is no specific certifying authority. The principle we are following is that results will only be returned if they are from a lab with stringent quality assurance/quality control and where the lab assay is a validated assay

4.6 Loss to follow up and missing data

Missing data:

The most important loss of data for cases may occur at admission due to difficulty in collection of many of the specimens. For example, induced sputum specimens will be not be available on those children in whom the procedure is contraindicated, which would tend to be the sickest children. In cases where the volume or quality of sputum is inadequate, these losses can be minimized by repeating the procedure. However, the timing will not be comparable to the specimens obtained from other cases which may affect comparability of the results.
In studies taking a wide variety of different samples from patients with a severe clinical syndrome that has a high early mortality it is usual to observe relatively high levels of missing data – because not all samples can be collected in all patients. However, except where missing data are highly correlated with other missing data this problem will be handled amply in the analysis using statistical procedures for imputing and incorporating missing data.

Loss to follow-up:

Because most of the data for the primary analysis will be collected on the day of study enrollment (at hospital admission), loss to follow-up will not greatly impact the ability of the PERCH study to evaluate the primary and secondary objectives. Follow-up for disease progression and documentation of mortality status will be used to refine the classification of the cases and to determine which etiologies are associated with the most severe pneumonias. Losses to follow-up of cases may occur at several time points: at 24 and 48 hours post admission where disease severity will be reassessed, at discharge, and 30 days post-admission where sera will be collected again and mortality status will be ascertained. Reasons for a child being lost to follow-up will be recorded (e.g. death, left hospital against medical advice). In the analysis phase, children who were lost to follow-up will be compared with the other children in the study to assess whether they were representative of the overall study population.

5 Risk assessment

The safety of the study participants is a central, over-riding principle of the study. This section describes the safety considerations and the ways in which the study procedures and study conduct minimize risk and maximize safety.

Because this is an observational study and does not involve an intervention, the main safety considerations in PERCH pertain to the collection of body fluids from cases and from controls. For cases, many of the body fluid collection procedures are already conducted as part of local, routine clinical care of patients hospitalized with severe or very severe pneumonia. Table XI below describes which ***body fluids*** are collected from ***cases*** as part of routine care and which are collected only for research purposes. Table VIII in section 4.6.4 indicates which ***lab tests*** on these body fluids are for research and which are part of standard care. Among ***controls***, all body fluid collection is for research only.

For specimens that are part of clinical standard of care, procedures will conform or exceed the safety and proficiency methods already in use at the sites. Efforts will be made to standardize the method of collection and equipment used for collection across sites which will both enhance safety for the subjects and will lead to more robust study results. The PERCH Clinical Standardization physician will provide training to sites when procedures differ from routine standard of care. Only those personnel who have been confirmed/certified to have proficiency

in the study procedure will collect specimens. That confirmation will be done by the site PI or his/her designee.

	Table XI. Body fluid specimen collection among cases		
Body Fluid Specimen	**Clinical Standard of Care or Research**	**Tests being done on this specimen that will benefit the participant (with respect to acute clinical care)**	**Activities to minimize risk**
Blood (all sites)	Standard of care. All sites consider blood cultures on hospitalized pneumonia cases as a component of routine care, not research.	• *Blood culture* • *Complete blood count* • *HIV test* • *Sickle Cell testing (selected sites)* • *Malaria test (selected sites)*	• *Limit volume to locally approved volume* • *Collected by trained, proficient, personnel* • *Sterile, single use equipment used* • *Follow SOP*
Urine (all sites)	Research	• *None*	• *Sterile, single use equipment used* • *Follow SOP*
Gastric aspirates (all sites)	Standard of care when clinically indicated	• *TB culture*	• *Collected by personnel who have been trained according to hospital guidelines* • *Single use equipment used*
Pleural taps (all sites)	Standard of care when clinically indicated in children with pleural fluid	• *Microscopy and bacterial culture* • *Protein and glucose* • *TB microscopy and culture*	• *Collected by personnel who have been trained according to hospital guidelines* • *Monitoring and observation of child post-procedure for bleeding, respiratory or cardiac decompensation* • *Sterile, single use equipment used* • *Follow SOP*

Nasopharyngeal and oropharyngeal swabs (all sites)	Research	• *In Thailand, positive influenza results from PCR testing may impact care as Oseltamivir treatment is available*	• *Collected by personnel certified to have been trained and proficient* • *Collected by personnel certified to have been trained and proficient* • *Follow SOP*
Induced sputum (all sites)	Research	• *Microscopy and bacterial culture* • *TB microscopy and culture*	• *Collected by personnel certified to have been trained and proficient* • *Monitor for clinical deterioration for 4 hours following procedure* • *Follow SOP*
Lung aspirates (only selected sites will undertake this procedure. The Gambia is the only site currently conducting lung aspirates)	The Gambia uses lung aspirates in routine care. This provides for much more accurate detection of the pathogens present in the lung tissue which otherwise may be unidentified or unappreciated as the cause of the pneumonia. This is particularly true of M. tb. Other sites will view this as a research procedure but specimens will be tested in real time for clinical use purposes.	• *Microscopy and bacterial culture* • *TB microscopy and culture* • *PCR for respiratory pathogens*	• *Collected by personnel certified to have been trained and proficient* • *Single use sterile equipment used* • *Monitor for clinical deterioration for 4 hours following procedure; monitor for death or pneumothorax for the remainder of the hospitalization* • *Follow SOP*

5.1 Lung aspirates

Lung aspirates have been and continue to be conducted in clinical management and for research purposes in a limited number of settings around the world, in both developed and developing countries. Lung aspirates can be an important diagnostic tool for children with serious pulmonary disease as it provides a direct sample of the infected lung and often results in a

microbiologic diagnosis that otherwise goes undetected. For example, lung aspiration has been used at the Medical Research Council (MRC) Research Unit in The Gambia for over 20 years with only infrequent minor complications in <3% of patients and a diagnostic yield of >50% (personal communication, Dr. Howie and Prof. Corrah).

The primary risks associated with lung aspiration are pneumothorax and hemoptysis. A review of children undergoing lung needle aspiration reported pneumothorax occurred in 27 per 1000 procedures, with chest tube drainage required in 5/1000 (0.5% of those undergoing lung aspirate) (Vuori-Holopainen and Peltola 2001). Serious non-fatal pulmonary haemorrhage is reported to occur at a rate of 1/1500 patients and transient pleural pain in 1/50 (Scott and Hall 1999). In over 6000 procedures in adults and children death was temporally, though not necessarily causally, associated with lung aspiration in 6 patients (1/1000), but the majority of these deaths occurred in historical literature, >30 years old, and could be averted by modern procedural and monitoring techniques(Scott and Hall 1999). These procedures will be done in clinical settings where there is 24 hour physician coverage of a seniority and experience where complications can be treated (e.g. chest tube placement).

Lung aspiration was also used in a recent study of pneumonia etiology in Malawi. In this setting, the lung aspirate provided a diagnosis in 47% of patients, as compared to an 11% yield from routine investigations. Among the 90 children who underwent lung aspirate, there was one complication requiring chest tube placement and that child did well (personal communication, Dr. Steve Graham). An unpublished review of all published literature and reported series of pediatric lung aspirates from the past 25 years revealed no deaths related to the procedure; pneumothorax requiring chest tube placement was reported in 2 (0.3%) of 741 procedures performed during that time period. In this same period it is expected that numerous children would have died from undiagnosed pulmonary infections which the lung aspirate allowed to be diagnosed and appropriately treated.

To minimize risks and maximize the benefit to subjects enrolled in the PERCH study the following steps will be implemented.

- Currently only the Gambia site conducts this procedure as they have done for over 25 years. The practices and procedures will continue to be followed at that site.
- For any other site that will implement the procedure they must have the capacity to management potential complications of lung aspirate at short notice for the 24 hour period following the procedure.
- The Gambian SOP and a training video will be available to all sites, and clinicians will learn the procedure by visiting the Gambian site for a sufficient period to be trained by local experts or by having Gambian physicians travel to the local site for training..
- The standard operating procedure for lung aspirates from the Gambia will be the basis for SOPs at other sites. In general the procedure involves inserting a needle (18 to 23 gauge) over the top of a rib (to avoid infracostal vessels and nerves) into the area of consolidation or maximum physical findings, applying suction to the plunger of the syringe, and maintaining constant suction while withdrawing the needle. The actual lung aspirate takes less than 5 seconds to perform. Chest radiography (1-view or 2- view) and/or physical signs typically determine the site of needle insertion.

- Lung aspiration will only be performed in patients with large peripheral consolidation on chest-xray , distant from great vessels, such that the risk of failing to get a specimen and the risk of inducing haemorrhage are both minimal. Cases with conditions that pre-dispose to complications (i.e. coagulopathy, suspected PCP, chest hyper-expansion, chest cysts or bullae) will be excluded from having this procedure performed. Cases with contraindications including the following will be excluded from lung aspirate specimen collection: presence of pneumatocoeles on CXR, post-measles pneumonia, patient is clinically unstable as determined by a clinician, CPR performed within the last 24 hours.
- Patients will be followed up closely for the 4 hours after the procedure and will be investigated with a chest radiograph if they show signs of clinical deterioration based on the clinical judgment of the physician.
- Performance of chest radiograph will be used to localize the consolidation. Following the procedure CXR will be repeated if clinically indicated. This is rarely needed but will be available at all sites.
- Specimens from lung aspirates will be tested in real time for the microbiologic cause of the pneumonia episode using all assays available at the site. This policy will maximize the opportunity for the subject to derive immediate clinical benefit from the procedure. The principal benefit to children undergoing the procedure is the early (and potentially life-saving) identification of pathogens that are not adequately treated with first line antimicrobial therapy. In developing country settings, using WHO treatment guidelines, these pathogens include *S. aureus*, most pathogenic gram-negative bacilli and tuberculosis. Without accurate diagnosis many of these children will be inadequately treated and a significant proportion will go on to chronic disease (in the case of TB) or death.

5.2 Induced sputum

Induced sputum is preceded by bronchodilator and then hypertonic saline inhalation to loosen up lower respiratory tract secretions, and induced by chest percussion and cough. The induced sputum that is coughed into the posterior pharynx is aspirated using suction. This procedure confers a mild increase over minimal risk because of the use of inhaled hypertonic saline that may, in theory, induce some airway hyper-reactivity. The child may also require an increase in their oxygen requirement for minutes to hours if the mobilized secretions cause some temporary reduction in the ventilation of lung sub-segments, or if the procedure induces some transient bronchospasm as described here. For the PERCH study, the agreed upon contraindications for the induced sputum procedure are oxygen saturation of less than 92% on oxygen, inability to protect airways, severe bronchospasm at admission, seizure within the past 24 hours, or deemed inappropriate by a study clinician for another reason (e.g. mid-face trauma, inhalational injury, pulmonary edema, congestive heart failure, congenital heart disease etc.) . If these conditions resolve during hospital course, an induced sputum can be considered at that point.

The sputum induction procedure itself can benefit the child because the inhaled bronchodilator may be therapeutic for a child with severe or very severe pneumonia as can the chest physiotherapy to assist in clearing secretions. The bacterial and M. tuberculosis results from the induced sputum can also provide benefit to the child to target or modify therapy if clinically indicated in relation to the sputum results.

While induced sputum is rarely *collected* in the course of routine clinical care from children for diagnostic purposes, the *procedure* to obtain this specimen ("chest physical therapy with acceleration of the expiratory flow") is done routinely to relieve difficulty breathing in hospitalized children in France and New Caledonia, and has also been shown to be of minimal risk in the absence of specific contra-indications such as neurologic disease compromising the airway, broken ribs, osteogenesis imperfect, significant thrombocytopenia (personal communication: Pr. P Brune, Hopital Antoine Béclère, Clamart, France).

Furthermore, we have identified at least 6 groups who routinely collect induced sputum from infants and children for research and/or clinical management purposes: SMadhi/Johannesburg, South Africa; H Zar/Cape Town, South Africa; A Scott/Kenya; S Mermond/New Caledonia; S Howie/the Gambia; E Lahti/Finland and S Bailleux /France. The most common sputum induction procedure involves: 1) administration of salbutamol, 2) delivery of hypertonic saline via nebulization to loosen secretions and induced bronchiole hyper-reactivity , 3) chest percussion, vibration and active breathing performed by a trained technician, 4) sputum expectoration through cough and 5) collection of the specimen via nasopharyngeal suction (for the youngest children) or expectoration (for those who can cooperate with instructions) .

The safety and feasibility of this sputum induction procedure has been verified in children and adults with asthma or pneumonia and adults with chronic obstructive pulmonary disease who have mildly to severely compromised airflow. The findings from the published pediatric assessments among asthmatics, a group most likely to have bronchospasm during the procedure, include children 6-16 years of age with either mild-moderate asthma or difficult to treat asthma, depending on the publication (Covar, Spahn et al. 2004; Lex, Payne et al. 2005). These studies were conducted when the children were well and the induced sputum was used to assess for degree of pulmonary eosinophilia. In total there were 155 patients who underwent an induced sputum procedure, with 16 of these known asthmatics developing some bronchospasm, of which all responded to inhaled bronchodilators. So, even in the group most likely to have bronchospasm during the IS procedure the observed frequency is about 10% and none of these were significant episodes.

Bailleux et al. from France evaluated a slightly different sputum induction technique to determine the tolerability and benefits of the procedure on children <2 years of age with bronchiolitis. The study included 250 children <2 months of age and 250 children 2-23 months of age. Half of the children in each age group received the induced sputum procedure and half received the standard clinical care for bronchiolitis. A total of seven hospitals in the Paris region participated between September 2004 and January 2008. The evaluated induced sputum procedure is performed by repeatedly massaging the abdominal area of the child to promote movement of sputum from the lower respiratory tract into the mouth where it is collected. The safety of this procedure was recently evaluated on children with pneumonia using the outcome measures: 1) drop in oxygen saturation, 2) malaise or unconsciousness, 3) worsening of the condition, 4) vomiting and 5) hypotonia. The results of this evaluation showed that the technique was not clinically risky and no worsening of the original condition was observed(Bailleux S 2008).

Induced sputum pilot studies have been ongoing in New Caledonia and Kilifi, Kenya for PERCH preparation. These systematically collected data have revealed no safety concerns. Between April and June 2010, 91 Kenyan children less than 5 years of age with WHO severe or very

severe pneumonia have had collection of induced sputum using the above described methods. There were no children in whom the procedures had to be stopped. No children needed new or increased oxygen therapy and no children needed nebulization as a result of the procedure. Furthermore, at the Kilifi site thousands of induced sputum specimens have been collected in the past 5 years for the purpose of tuberculosis and other pneumonia etiology studies. Systematic data are not collected on the need for increased oxygen administration or bronchospasm but anecdotally the clinical officer and nurse in charge of the procedure report that no such episodes have occurred.

Similarly in New Caledonia between January 2010 and June 2010 there have been 63 children studied with no need for any adjunctive therapy during or following the procedure. In this site induced sputum is routinely performed by a physiotherapist and was not introduced for research purposes. Their feedback on the procedure is:

- They perform the procedure using oxygen therapy only if the child had an oxygen need before the IS procedure; after the procedure, the oxygen requirement is often lower or the same. No child has had an increased oxygen requirement as a result of the procedure.
- They perform a nebulization with bronchodilators to facilitate secretion drainage before the procedure, if the child has evidence of bronchospasm. No patient has required bronchodilator therapy as a result of the procedure.

5.3 Nasopharyngeal / Oropharyngeal swabs

Swabbing the posterior nasopharynx is a procedure with minimal risk. The main risks are discomfort and, rarely, transient bleeding from the nose. Studies of NP colonization have been conducted for a long time at many of these sites, hence they have experienced trained personnel already. Refresher or new training will occur and all personnel who will collect such specimens must demonstrate appropriate technique prior to initiating specimen collection. Collection of NP and OP specimens (i.e three swabs) is commonly done in studies and does not pose additional increased risk but does improve the microbial detection rate.

5.4 Urine

Collection of urine is a less than minimal risk procedure. Among small children, a urine bag will be placed on the child. Among those who are toilet trained, a specimen will be collected by urinating into a sterile cup. Urine may or may not be part of the routine work up of a child with pneumonia for pneumococcal or Legionella antigen testing. In the PERCH study sites this is not normally a part of clinical care of pneumonia patients thus the specimen is considered a research body fluid. The specimen will also be used to assess whether the child has received antibiotics prior to the time of body fluid collection, and at the Matlab, Bangladesh site will be used to measure urinary arsenic concentrations and creatinine.

5.5 Other Diagnostic Procedures (Blood, gastric aspirates, pleural taps)

Other diagnostic procedures being done as part of PERCH are considered routine standard of care. These include pleural tap (thoracentesis), gastric aspirates when clinically indicated, and blood draws. The only one of these procedures with associated risk that would be considered beyond minimal is pleural tap (thoracentesis). This procedure is done to collect a pleural fluid sample for testing and identification of infectious etiology among patients with a pleural effusion or empyema thoracis. Common risks of a thoracentesis include bleeding and bruising at

the puncture site and pneumothorax (collapsed lung). If the patient has a medical condition, or is using a medication or supplement that causes excessive bleeding, he/she is at a higher risk of bleeding from the puncture site. Rare risks include hemothorax (blood in the chest cavity), pulmonary edema (accumulation of fluid in the lungs), and venous air embolism (air bubble in a vein). The liver or spleen may be damaged by the needle used during the procedure.

Gastric aspirate is recommended as a safe and effective investigation of tuberculosis in children in the guidelines of the American Thoracic Society, when a standard protocol is followed (Pomputius 1997). The procedure is uncomfortable and can result in minor hemoptysis or aspiration but the risk is very small (Society 2000).

Taking blood samples by finger prick or venipuncture carries a low risk for the patient. However there are potential rare risks. Needle stick injuries resulting from re-used needles can lead to the transmission of potentially life-threatening infections such us Hepatitis B and HIV. Therefore, it is of utmost importance to adhere to safety principles during the procedure and throughout the process of handling biological samples including proper disposal of clinical/laboratory waste. This includes ensuring that persons involved in blood taking and handling of blood or blood products are vaccinated against Hepatitis B and are wearing gloves whenever samples are obtained or handled.

To minimize the risk of infection of the puncture site and reduce contamination of blood cultures these procedures need to be done under appropriate aseptic conditions, and should only be done after the patient and the parent have been reassured and the patient is as comfortable possible.

Drawing of blood can result in bruising and discomfort, or in rare cases infection at the site of venipuncture. These risks are minimized by appropriate training of personnel, use of sterile equipment, and pressure at the venipuncture site following the procedure. The risk is judged to be no more than minimal because it is part of routine care.

5.6 Ethical Considerations/risk assessment

PERCH is an observational study, not an intervention study. As stated, most of the procedures done to collect specimens for the study are routinely done for children with respiratory infections and considered minimal risk. We will require active surveillance of clinical status following induced sputum and lung aspirates, because while potentially valuable for clinical management, these procedures are not standard of care in all clinical settings. Any SAE (as defined in section 5.7 below) that occurs following one of these two procedures will be recorded and reported. In addition, any other safety events that study physicians consider to be serious and related to study procedures and unexpected will be reported.

Lung aspirates will only be performed as part of PERCH in sites that have appropriate pediatric expertise and under circumstances where the procedure is deemed safe and diagnostic (i.e. peripheral consolidation on chest x-ray). Induced sputum on the other hand will be a routine procedure performed on most cases, except those with contraindication. Because children admitted with severe pneumonia will be very sick by definition, it is expected that some children will deteriorate due to their illness soon after diagnostic procedures are done. Children will be closely monitored before, during, and after the procedure for clinical deterioration, and these clinical measures will be tracked and reported to the JHSPH IRB. However, we will only record

severe events that meet the SAE definition described in section 5.7 below following an induced sputum procedure.

PERCH is not designed as a study of case management of pneumonia. Standard of care will be followed in each site according to locally-observed guidelines (e.g. Ministry of Health, WHO). All PERCH sites were selected due to their high-capacity to follow the rigorous PERCH protocol, and as such are all embedded within relatively high functioning institutions. All sites treat severe pneumonia with intravenous antibiotics, although the first-line antibiotics might differ by site (see site-specific addendums for local treatment guidelines). In addition, all sites will be able to administer oxygen therapy to children with pneumonia. Beyond these two standard treatments, there is likely to be variability in the care received by site based on the level of staff training and hospital infrastructure. For example, some sites (e.g. Thailand) will have ventilators, while others (e.g. Kilifi, the Gambia) will not. This variability in the level of care, along with the case mix and severity of children hospitalized, will likely lead to variability in outcome of the hospitalization. This will be captured as data by the PERCH study, and measurable through variables such as case-fatality ratio in the hospital and at the 30-day follow-up. This variability in outcome, including case-fatality ratios, is anticipated as PERCH sites were selected to represent the spectrum of health-care settings in the developing world. Deaths will not be reported as serious adverse events, unless they follow certain procedures (i.e. lung aspirate and induced sputum). While PERCH will not alter local standard of care for pneumonia, local investigators will be responsible for assuring that PERCH participants are receiving the local standard of care. All sites have long term relationships at the facilities where they are working and are embedded in meaningful ways in the healthcare facilities where the research is being carried out. If the study staff observe suboptimal care (i.e. relative to the locally prescribed treatment guidelines) this will be addressed at the local institutional level by the PI or his/her designee and corrective actions delineated.

The diagnostic tests offered through PERCH provide significant opportunity for benefit to the child through the provision of results that can improve the child's treatment. This includes many of the real time tests (see Table IX). Testing of blood, induced sputum, lung aspirate, and pleural fluid, and gastric aspirate can identify pathogens such as *M. tuberculosis* or gram-negative bacteria that may not be adequately treated by empiric therapies. Changes in therapy based on these results could be life-saving. Beyond these etiologic specific microbiologic assays, the assays for underlying conditions (e.g. anemia, malaria, HIV and sickle cell disease and Thalassemia) offer the child the opportunity for improved treatment and/or prevention. In sum the clinical benefits afforded in real time to the child are significant.

5.6.1 Post Mortem Risk Assessment Considerations

All safety considerations are for the staff who will be handling the lung biopsy specimens. The procedures outlined above must comply with local standards. It is important to note that <u>all tissue samples must be handled as if potentially infectious.</u>

Special care must also be taken for approaching parents for consent. Because all care-takers will be under significant emotional distress, staff will be trained to approach all potential participants in a respectful manner.

Post-mortem studies in research settings have typically recorded acceptance rates of up to 25% (4). Families may decline consent for a variety of reasons – religious or cultural objections, concerns about mutilation of the body, interference with burial arrangements, perception that procedure is of no benefit to the patient, objections expressed by patients before death and perception that patient is too young or too old (12, 13). Taking a biopsy approach confined to the lungs may allay some of these fears, for example, about scarring or delay in burial arrangements.

We aim to take the biopsies as soon as possible after death allowing for the interval of the consent process, but preferably within 4 hours of death (ideally 1-2 hours) in order to reduce interference with the normal arrangements for dealing with the body (transport to a mortuary or funeral rites etc). This would also ensure optimal tissue quality and the least chance of post mortem microbiological contamination.

5.7 Serious Adverse Events

5.7.1 Serious Adverse Event definition:

Among children who had a lung aspirate:

- Death during hospitalization
- Pneumothorax or significant haemoptysis (>5 mLs) at any time following the procedure, during the hospitalization
- Clinical deterioration within 4 hours following the lung aspirate procedure defined as:
    - Drop in oxygen saturation below 92%, resulting in increased supply of supplemental oxygen for 10 minutes or more
    - Deterioration in AVPU score
    - Worsening of the respiratory condition as evidenced by
        - new requirement for bronchodilator or increased frequency of bronchodilator treatment.

Among children who had an induced sputum:

- Death within 4 hours following the procedure
- Clinical deterioration within 4 hours following the induced sputum procedure defined as:
    - Drop in oxygen saturation below 92%, resulting in increased supply of supplemental oxygen for 10 minutes or more
    - Deterioration in AVPU score
    - Worsening of the respiratory condition as evidenced by:
        - new requirement for bronchodilator or increased frequency of bronchodilator treatment.

5.7.2 Serious Adverse Event Reporting

All serious adverse events as defined above will be recorded at each site and reported to the PERCH Safety Monitor, DCC, PERCH PI, and the IRB. The SAEs will be graded by severity and by relatedness to the procedure.

This is a study of serious pneumonia in children and we do not plan to report every death to the JHSPH IRB, unless the death meets the definition of an SAE (section 5.7.1). The PERCH PI will, however, report to the IRB any deaths or other safety events which are considered to be serious and *related to study procedures* and unexpected. Non-serious adverse events will not be reported.

When SAE defining clinical events occur all attempts will be made to provide stabilization and clinical management to protect the safety and well-being of the child. All SAEs will be followed to resolution. The flow of information is described here and in Figure 4. If a complication meets any of the above conditions, it will be documented by the PI or designee using a standardized SAE reporting form and reported to a site-specific Local Safety Monitor (LSM, medical officer/physician) within 48 hours of becoming aware of the event. At all sites, a Local Safety Monitor will be identified prior to study initiation. That contact information will be maintained in the PERCH Study Manual. The site-specific LSM will assess each report of an SAE and provide feedback to the sites.

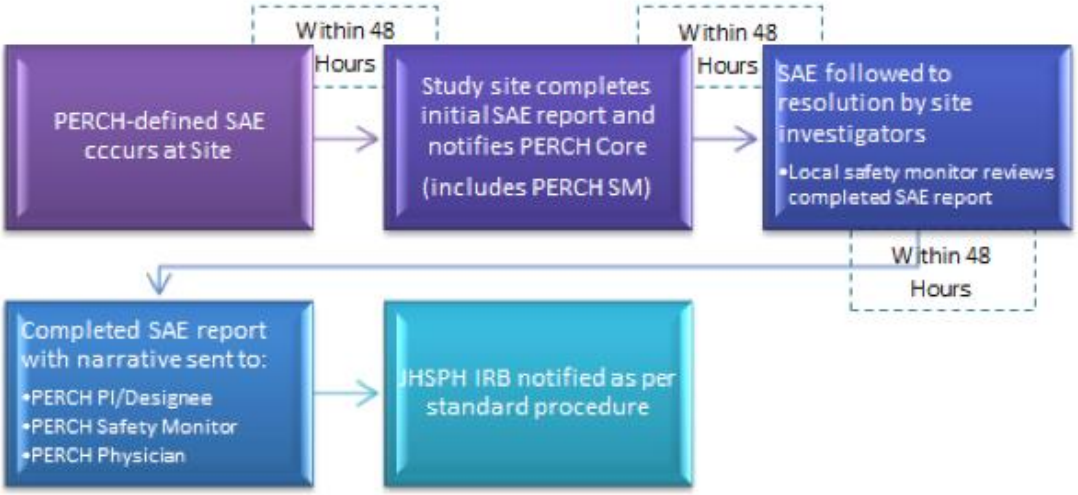
All completed SAE forms will be sent to the designated PERCH Safety Monitor, DCC and to the PERCH PI. The PERCH Safety Monitor (Dr. Julia Kim) will be a physician who is not directly involved with the PERCH study. The LSM will report all SAEs to the DCC for compilation. The DCC will receive updates of the SAE events until resolution of the SAE. The DCC will prepare reports of all SAEs by category, outcome, severity and relatedness to the procedure on a monthly schedule. This report will be shared with the designated PERCH Safety Monitor, for review, and will summarize the real-time reporting described in Figure 7.

The PERCH Safety Monitor can ask for more information if needed from the DCC or the sites. The PERCH Safety Monitor can also ask for more information or review of collected SAE data prior to the regular summary reports from the DCC if he/she observes worrisome trends in SAE reporting.

The DCC will prepare monthly reports of SAE data and report these to the PERCH Safety Monitor, PERCH PI, and all site PIs. The PERCH Safety Monitor will make an assessment on the basis of the cumulative monthly report about whether there is any indication that action needs to be taken at one or more sites and report this assessment to the PERCH PI and the Site PIs. The PERCH PI will have ultimate responsibility in determining if these procedures can be continued in the PERCH study based on the safety reports. PERCH will follow JHSPH reporting requirements for unanticipated event reporting.

SAEs will also be reported by the Site PIs to local IRBs and ERCs depending on their reporting requirements.

Figure VI. SAE Reporting Format



5.8 Study PI role in safety assessment and reporting

The PERCH study PI will hold overall responsibility for the safe conduct of the study. This will be achieved through several mechanisms. All study procedures will be implemented by the Site PIs according to the study Standard Operating Procedures. It is the responsibility of the Site PI to assure adherence to the SOPs, training of local personnel, and corrective action for deviations from the SOPs. Site PIs will report to the PERCH Study PI when significant deviations from the SOPs occur which might incur risk to study participants. The PERCH-independent external Safety Monitor will report directly to the PERCH Study PI as well as to the Site PI relevant to the safety event. The PERCH Study PI and or the Site PI has the authority to pause any or all PERCH study activities at a clinical field site if there are safety concerns. The PERCH Study PI will report to the Johns Hopkins IRB and to each Study Field Site PIs on all related and unanticipated SAEs received. Field Site PIs will report these to their own IRBs if required by the procedures and regulations established at the site.

5.9 DCC role in safety assessment and reporting

The DCC will be responsible for receiving initial SAE reports and updating the initial reports to create final reports. The DCC will be responsible for reporting the initial SAE to the PERCH Safety Monitor, the PERCH PI and the Site PIs. The DCC will also be responsible for compiling cumulative monthly SAE reports and disseminating them to the PERCH PI, PERCH Safety Monitor and the Site PIs.

5.10 Role of PERCH Core Team in safety assessment and reporting

The PERCH Core Team at JHSPH will serve as a 'study coordinating center' and support the responsibilities of the PERCH PI in safety assessment and reporting. The PERCH Core Team will receive all reports from the PERCH Safety Monitors on behalf of the PERCH PI, assure that these are assessed by the PERCH PI or his designee within 48 hours of receiving the report and will be responsible for providing reports to the Johns Hopkins IRB. In addition staff from the PERCH Core Team will report to the PERCH Study PI and to the relevant Field Site PI any safety concerns observed during site visits.

6 Statistical considerations

6.1 Overview

The overarching goal of PERCH is to describe the distribution of pathogens causing severe and very severe pneumonia in children around the developing world. This information will be useful for determining new treatment algorithms and for prioritizing development of new diagnostics, treatments, and vaccines.

To achieve its goal, PERCH is collecting multiple specimens from patients with pneumonia and controls and testing them thoroughly with highly sensitive detection methods. Equally important to this goal, PERCH is developing a statistical analysis and data interpretation approach that will use this information to accurately describe the pathogens associated with pneumonia. Specifically, the PERCH approach to statistical analysis will estimate (1) the prevalence of **infection** for each of approximately 30 specific pathogens among cases of hospitalized severe pneumonia and (2) the frequency of each of these pathogens as a putative **cause** of hospitalized, severe pneumonia. It will also accurately estimate the proportion of cases with no pathogen identified. This "unknown" group will be a high priority for pathogen discovery efforts.

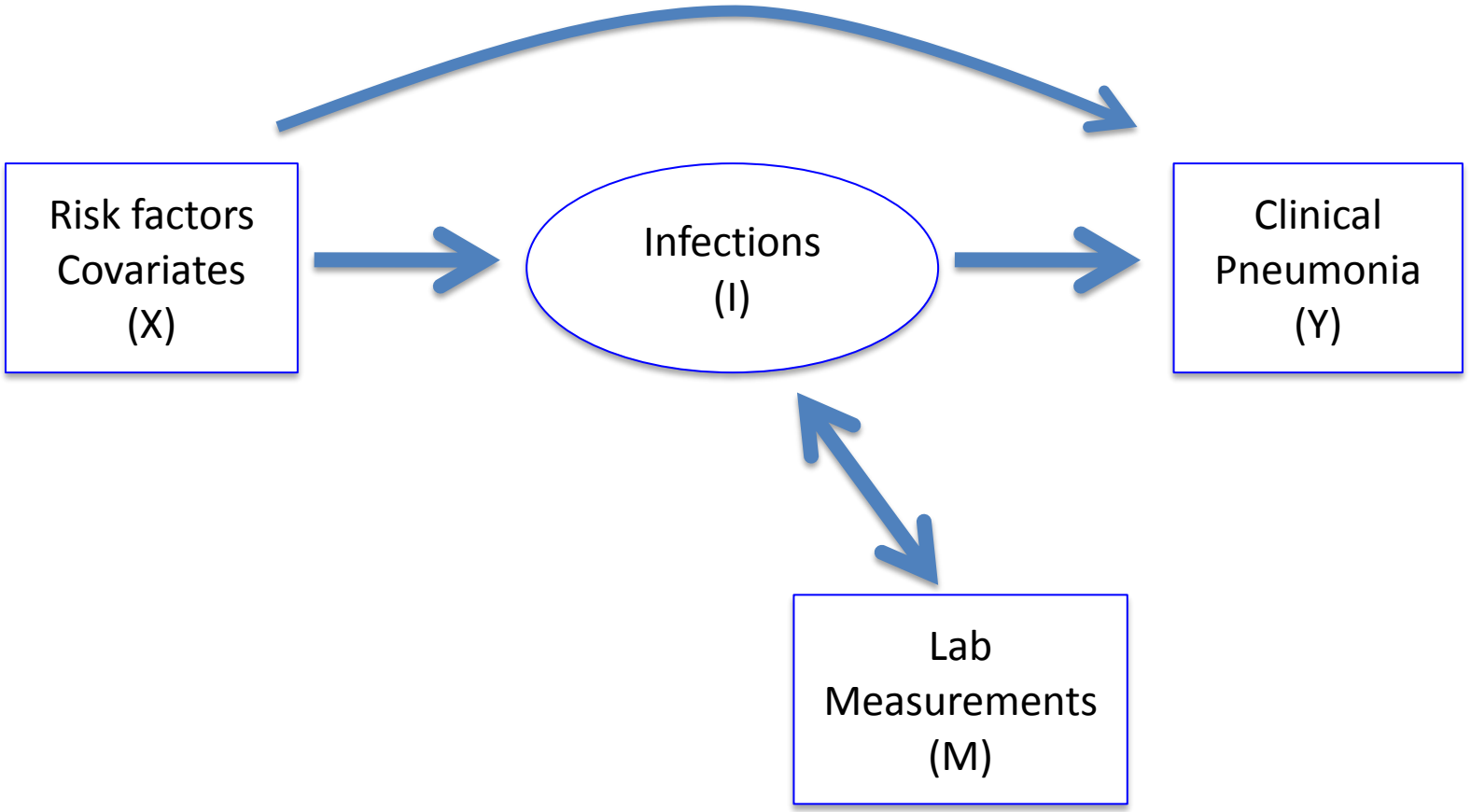
6.2 Analysis plan for each objective

6.2.1 Determine the association between pneumonia and infection with known putative viral, bacterial, mycobacterial and fungal pathogens

The basic structure of the analytic problem is pictured in Figure 5 below. There are a set of risk factors or other attributes (denoted "X") of children that influence the risk of being infected (denoted "I") with a given pathogen. We cannot know in truth whether children are truly infected, but we infer this based on a series of laboratory measurements ("M") which include among others, for example, multiplex PCR of nasal secretions, culture of blood for bacterial pathogens, and assessment of induced sputum for acid fast bacilli. Among the infections children have, only some of these pathogens are causally linked to their disease, namely hospitalized, severe pneumonia (denoted as "Y").

[Note: For the sake of simplicity we will use "30" pathogens as an example of how many different individual pathogens we will identify in PERCH and we use "40" as an example of how many lab measurements there are. These numbers are approximations of the number of pathogens that can be identified or the number of lab measurements being collected, and are merely place holders for the sake of clarity. Also, one of the "pathogens" is the "no pathogen identified" category that is relevant to future pathogen discovery efforts.]

Figure VII. Basic structure of the measurement of and relationship between infection and disease in cases


Risk factors
Covariates
(X)
Infections
(I)
Clinical
Pneumonia
(Y)
Lab
Measurements
(M)

The key features of this formulation of the epidemiologic problem include the following:

1. Infection status is a binary indicator for each of the 30 pathogens that can be measured, plus one additional broad category that includes "all others that are not identified by the lab testing". As there are 31 binary indicators of infection status for each child, there are 2^{31} (i.e., over 2 billion) possible infection states (i.e. infected or not infected for each of 31 "pathogens"). This enormous number of possible infection states, most of which have 0 people in them, creates statistical and modeling complexity which must be addressed. Below we discuss ways of reducing this dimension by smoothing (aggregating) across the "neighboring" cells to make estimation feasible.

2. Risk factors for infection with a given pathogen, including among other variables age, vaccination status, socioeconomic status, nutritional status, HIV infection, and antibiotic use are allowed to affect the risk of having each of the 30+1 infections. Some of these risk factors may also affect the general probability of developing 'hospitalized pneumonia' by a pathway other than the specific infection, for example, by reducing access to medical care.

3. Hospitalized, severe pneumonia is caused by one or more of the 30+1 pathogens causing infection and possibly by two-way interactions between them.

4. We do not directly observe the presence of each infection. Rather the presence of infection by each pathogen must be inferred from up to 40 laboratory test results ("M") (illustrated in Table XII).

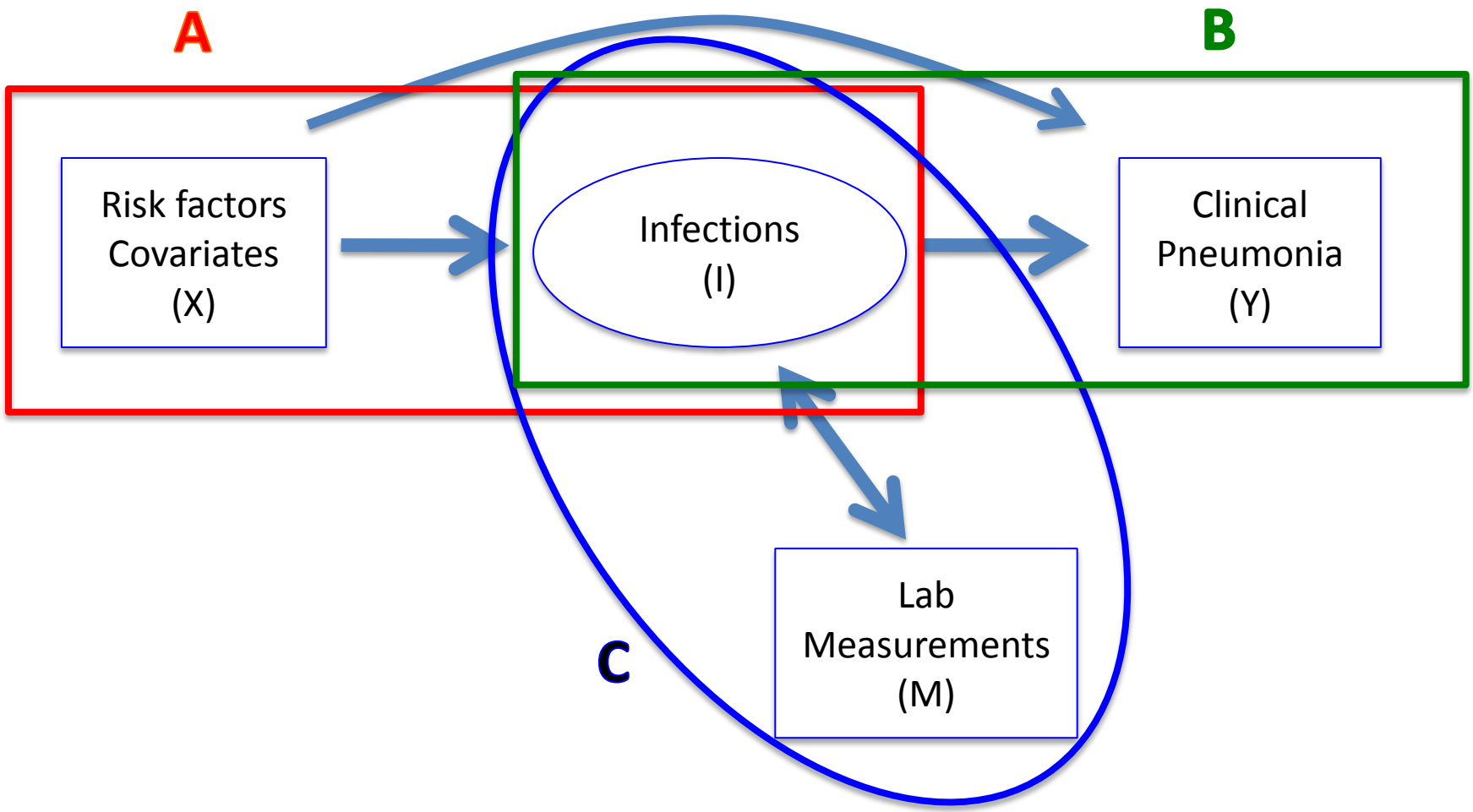
Table XII. Determination of infection status for 30 pathogens using results from 40 lab tests

	Path 1	Path 2…	Path 30
Lab test 1	infected	NA	NA
Lab test 2	NA	infected	
…Lab test 40	Infected	Infected	NA

5. The sensitivity and specificity of each laboratory test will be represented by a distribution on (0,1) (i.e., estimates of sensitivity and specificity can range from >0% up to and including 100%). Information from existing literature on the laboratory assays will be used to estimate their sensitivity and specificity. Uncertainty bounds around the sensitivity and specificity measures of the lab tests will be incorporated.

Data analysis ignoring measurement error. If the infection status for each of the 30 pathogens was known for each pneumonia case and for controls, the approach to analysis would have two parts, denoted A, in red, and B, in green, in Figure 6 below.

Figure VIII. Analysis approach


A
B
Risk factors
Covariates
(X)
Infections
(I)
Clinical
Pneumonia
(Y)
Lab
Measurements
(M)
C

In Part A, among the cases and among the controls we will regress the presence/absence of infection for each pathogen on risk factors. The co-occurrence of infections would be of particular interest. We will therefore estimate the pairwise odds ratio for each of the 30 choose 2 = 435 pairs of infections, focusing on a smaller subset (e.g. top 30) with substantial prevalence. *Using marginal models* (e.g. Diggle, Heagerty, Liang and Zeger, 2002), the odds ratios could be regressed on covariates to build an understanding of what factors (e.g. age, nutritional status, vaccination status) influence the co-occurrence of pairs of infections (Diggle PJ 2002).

The analysis of Part A would permit construction of the histogram (or pie chart) of the frequency of pathogens, separately for cases and for controls, for any given set of risk factor values.

In Part B, using the cases and the controls we will regress the presence/absence of pneumonia on the indicators of infection and on selected interactions. Each coefficient represents the contribution of that infection (or pair of infections) on the risk of pneumonia. Given the large number of predictors relative to the expected number of pneumonia cases, *we propose to use the LASSO approach* to select a smaller subset of main effects and interactions that best predict case status (i.e. hospitalized pneumonia) (R 1996; Park 2007).

Because the analysis of Part B will be done with case-control data, only the relative odds of pneumonia for each pathogen-specific infection can be estimated. Specifically, we will be estimating for each pathogen and for various co-pathogen pairs the likelihood of infection given that the child has pneumonia compared with the likelihood of infection given that the child does not have pneumonia. Odds ratios that are near or approaching one are interpreted therefore to mean that there was a near equal likelihood of infection among the cases and the controls and no specific evidence that the pathogen was causally associated with pneumonia status.

The lack of association does not mean that the pathogen does not cause pneumonia; it simply means that there is <u>no evidence</u> of a causal association using measurement "M". In other words we are not assessing the relative *risk* of pneumonia given infection, we are only assessing the relative *likelihood* (or odds) of infection given pneumonia status compared with the likelihood of infection given control status. These relative odds values will be applied to the infection prevalences in the first histogram to obtain the histogram of causal effects, given covariates. The LASSO procedure will tend to shrink most regression coefficients in Part B towards 0. This will give the corresponding infections 0 as their estimated causal effect. If this is unsatisfactory, we will use alternatives to the LASSO that shrink less.

Pneumonia must be caused by a pathogen (or other exposure), although not necessarily by the ones in our list of 30. In cross-sectional study designs, the absolute prevalence of "other" pathogens (which could include, for example, toxic environmental exposures or as yet undiscovered pathogens) would be estimated by the intercept in the logistic regression in Part B. However, with the proposed case-control study design, an auxiliary source of information about hospitalized pneumonia case prevalence will be required to estimate the absolute prevalence of pathogen specific pneumonia episodes (i.e., going from percent of enrolled study cases with a given pathogen to prevalence in all community cases of a given pathogen).

Dimension reduction: A challenge of this study is the large number of infections that can be observed. One way to address this problem is to assume that there are a small number of "classes" of children and that the infection profile can be approximated by a common prevalence histogram within each class. The classes and infection prevalences can then be

estimated using a *latent class model* (Goodman 1974). One extension that allows each child's profile to vary in a space defined by the latent class profiles is *the grade of membership model* (e.g. Erosheva, 2003)(J. M. Bernardo 2003). We will explore these and other dimension reduction methods as needed.

We have explored the use of the latent class model approach and recognize at the outset some limitations. Primarily the assumption of independence of observations (i.e. lab measurements) that form the data inputs is violated in the data of the PERCH study. For example, an NP specimen from a child will be tested for multiple pathogens using a multiplex PCR approach. Anything that impairs the sample (e.g. poor handling of the sample) will uniformly affect the lab results from this sample for a wide range of pathogens, thus they will all be negative. A second problem with LCA is that the classes cannot be defined *a priori*, but instead are defined by the model output itself. As a result we will not have pathogen by pathogen etiologic proportions, which is the desired primary goal for the PERCH analysis.

Measurement error: The main challenge is that infection status will not be observed exactly for any child. Rather a series of laboratory test results will provide noisy evidence about each infection. For each test, there will be some degree of prior knowledge about its sensitivity and specificity. *A measurement error model* will be included in the analysis as represented by Part C in Figure 6 above.

We will consider two approaches for incorporating measurement error. In the first we will assume that the lab test results are independent of one another. We will use the external knowledge about sensitivity and specificity to set prior distributions for each. The joint probability distribution of the unknown infection status of a child will be inferred from the test results given the child's values of X and Y. We will only infer the univariate and bivariate marginal distributions. It will be possible to infer the class probabilities in the latent class version.

This approach ignores the likely correlation among test results that use the same body fluid or extraction process. We will extend the independence model by allowing for fluid and extraction "errors" that cause all of their test results to be corrupted (usually toward zero).

Approach to analysis: Once the measurement error is acknowledged, analysis will be implemented *using a Bayesian hierarchical model and Markov chain Monte Carlo (MCMC) methods of inference*. We will repeatedly simulate from the conditional distributions of the infection statuses (I) and the model parameters given the observable (X, Y, M). The histograms that are the target of inference will be constructed, with uncertainty, by summarizing the distribution of the I's and of the I's from the analysis. In the first months of the project, the detailed specification of the hierarchical model will be developed and tested on simulated data.

6.2.2 Estimate the fraction of pneumonia attributable to pathogens for which vaccines are currently under development

(See section 6.2.1. above).

6.2.3 Analysis of risk factors for pneumonia for etiology specific causes of pneumonia

The proportion with the risk factor in cases and controls, and between etiologies among cases, will be compared using χ^2 and Fisher's exact test as appropriate. Continuous data will be

compared using *t* test or Kruskal-Wallis rank sum test. Multivariable logistic regression will be used to adjust for other risk factors and to assess interactions between risk factors and etiologies.

All patient information related to severity will be considered and logistic regression will be used to identify putative indicators of severity by associating them with fatal cases.

6.2.4 Develop a severity index and use to assess association between etiology and severity

Putative indicators of severity at admission will be identified by associating them with fatal cases. Logistic regression will be used to determine the association between the indicators of severity (e.g., hypoxemia, chest wall indrawing, cyanosis, etc.) and severe outcomes of pneumonia (immediate (in-hospital) death, death after leaving hospital, and duration of hospital stay among those who did not die). A severity index will be developed (such as a score from 1=less severe to 7=death). Latent class analysis will be explored for its potential to distinguish severity classes of cases. For developing the index, all patient information related to severity will be considered independent of etiology. The distribution of the severity index among the etiologies will then described. Pairwise comparisons of the severity index between etiologies will be tested using 2 x n χ^2 or Fisher's exact test.

6.2.5 Determine patterns of antimicrobial resistance.

The proportion of invasive isolates resistant to antibiotics will be estimated for important pneumonia pathogens including *M. tuberculosis*, *S. aureus*, *Salmonellae* spp. and others such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* Frequencies of susceptibility by pathogen and antimicrobial will be generated. Analyses will be stratified by pre-hospital exposure to antibiotics. The proportion of isolates that are resistant to multiple antimicrobials will also be calculated.

6.2.6 Determine the association between pneumonia etiologies and digital chest auscultation, with and without chest radiography

Logistic regression will be used to determine the association between the breath sounds (wheeze, crepitations, stridor, no sounds) and etiology, stratified by CXR findings (e.g., alveolar consolidation, otherwise abnormal, normal). The distribution of the breath sounds (and CXR findings) for each etiology will be described. Pairwise comparisons of breath sounds between etiologies will be tested using 2 x n χ^2 or Fisher's exact test.

6.3 Sample size and power calculations

Over two years, the PERCH study aims to enroll 5000-7000 patients with severe or very severe pneumonia and approximately an equal number of controls. The study will be conducted using a standardized methodology that will facilitate pooled analysis. This sample size will represent probably the largest multi-center pneumonia etiology study in children in recent times. This surpasses even the BOSTID studies in the 1980s, which included 12 sites and nearly 4000 hospitalized episodes of lower respiratory tract infection. As such, the study is expected to provide substantial power to detect new etiologies of pneumonia, to offer improved precision on existing estimates, and in case-control comparisons, to determine associations with risk factors that may not have been possible previously.

The large study size will allow for many stratified or subgroup analyses. Because differences in epidemiologic and environmental factors between sites or between risk groups may result in differences in the distribution of the pathogens, power calculations are provided for a range of sample sizes for likely site-specific and sub-group analyses, as well as overall.

Likely sub-group analyses include:

- HIV status
- antibiotic pre-treated
- disease severity
- symptomatic vs. asymptomatic controls
- age
- presence of an epidemic such as H1N1
- season
- year
- particular pathogens (for pathogen-specific risk factor analysis)
- malaria
- sickle cell disease
- fatal cases
- other risk groups (e.g., Vitamin D deficient, malnourished, low SES, etc.)
- specimens in limited quantify such as lung taps or post-mortem lung biopsies

The main drivers of the number of cases enrolled will be the requirement to provide a reasonably specified description of pneumonia etiology at seven different sites representing different socio-epidemiological settings. Each site varies in the number of cases eligible for enrollment. Table 13 summarizes the number of cases that will likely meet the PERCH case definition and would consent to be enrolled at each site. Estimates are conservative.

Number of available cases per site and overall

Table XIII summarizes the number of cases that will likely meet the PERCH case definition and consent to be enrolled at each site.

Table XIII. Estimated total number of eligible, consenting severe and very severe cases available for enrollment/analysis over 2 years.

	2-year enrollment potential		
Site	**Total cases**	**Severe***	**Very severe**
Bamako, Mali	700	525	175
Basse, The Gambia	1170	1016	154
Sa Kaeo/Nakhon Phanom, Thailand	500	400	100
Soweto/Johannesburg, South Africa	2000	1460	540
Dhaka, Bangladesh	3000	2250	750

Kilifi, Kenya	1800	1440	360
Lusaka, Zambia	3400	2000	1400
Total	12570	9091	3479

*For sites with unknown ratio of severe to very severe, 25% very severe was assumed.

6.3.1 Sample size

The target enrollment sample size was based on a series of power calculations and incorporates the need to capture seasonal variations in risk factors for pneumonia and in disease incidence, as well as the cost of testing each subject enrolled. The sample size calculations permit sufficient power to undertake site-specific analyses with a reasonable degree of precision, to conduct sub-analyses of pooled data while controlling for age, season, and site, and to support the identification of novel pathogens.

Cases: Small sites (Bamako, Thailand) will enroll all eligible cases. At the other large sites, sampling of cases will be implemented to restrict the target number of PERCH cases to 6300.

Controls: The target number of controls is based on a minimum background enrollment rate of 25 per month (i.e., 600 over 2 years per site) combined with a 1:1 case:control ratio when monthly case enrollment exceeds the minimum control background rate. For large sites, we anticipate that the overall case:control ratio will be approximately 1:1. For small sites, the overall ratio is anticipated to be slightly higher. For Lusaka and Soweto (i.e., sites with high HIV prevalence), an additional HIV+ control group will be added assuming a 1:1 ratio with the number of enrolled HIV+ cases.

Table XIV. Target case and control enrollment by site and total

Site	Cases	Controls	
		Community Controls	HIV+ controls
Bamako, Mali	700	900	
Basse, The Gambia	700	900	
Sa Kaeo/Nakhon Phanom, Thailand	500	600	
Soweto/Johannesburg, South Africa	1200	800	400
Dhaka, Bangladesh	1200	1200	
Kilifi, Kenya	1200	1200	
Lusaka, Zambia	800	850	350
Total	6300	7200	

While PERCH has set a target of 6300 cases to be enrolled, the number actually available may range from 5000-7000. This is due either to losses in data for analysis or higher than projected numbers of cases consenting at some sites. Losses may result from inability to collect and test all specimens, ineligibility due to severe pneumonia signs resolving upon administration of bronchodilators, changes in procedures over time that result in excluding some cases from analysis, and because some sites may not be prepared to start in Jan 2011 (due to minor site capacity development or IRB processes) and thus may not achieve a full 2 years enrollment before the study ends. To account for these potential losses, a low (70%) estimate is provided below.

Table XV. Target number of cases		
	Target	70% of target
Site	**Total cases**	**Total cases**
Bamako, Mali	700	490
Basse, The Gambia	700	490
Sa Kaeo/Nakhon Phanom, Thailand	500	350
Soweto/Johannesburg, South Africa	1200	840
Dhaka, Bangladesh	1200	840
Kilifi, Kenya	1200	840
Lusaka, Zambia	800	560
Total	6300	4410

HIV-infected cases and controls: Four of the 7 sites have measureable numbers of HIV-infected cases that could be included in an HIV-infected sub-group analysis, either pooled or as a site-specific analysis at the two largest sites. Since HIV status is associated with pathogen infection (i.e., higher carriage in HIV+ than HIV-), and with disease (e.g., higher rates of invasive pneumococcal disease and PCP in HIV+), stratifying analyses by HIV status is desired. Table XV describes the projected number of cases and controls enrolled with HIV. At the two sites with high HIV prevalence (Johannesburg and Lusaka), a second control group of HIV-infected children will be recruited from HIV patient support centers to ensure a sufficient number of HIV+ controls.

Table XVI. Estimated number of HIV+ cases and controls available in PERCH, by site					
	HIV+ cases	HIV+ controls			
Site		community controls	controls recruited from PSCs	Total	Assumptions re: HIV prevalence in cases and community controls
Kilifi	84	24	0	24	7% of cases are HIV+. Assume 2% of children in community are HIV+.
Soweto	400	40	400	440	5% of U5 are infected and 35% of hosp sev pn are HIV+
Bamako	63	15	0	15	9% of OP Spn bacteremia were HIV+ vs 1.7% controls
Lusaka	400	50	350	400	30% of children with access to care are HIV+ (assume 50% of sev pn and 20% of controls are HIV+)
Total	947	129	750	879	

Severe versus very severe cases: Because the proportion of very severe pneumonia cases is small compared to severe pneumonia cases, indiscriminant selection may result in insufficient enrollment of very severe pneumonia cases. Therefore, at most sites, all very severe cases will

be invited to participate. At the largest sites, an equal number of severe and very severe cases will be targeted by applying a sampling ratio that is higher for very severe cases.

Table XVII. Target number of severe and very severe pneumonia cases				
	Target		70% of target	
Site	**Severe**	**Very severe**	**Severe**	**Very severe**
Bamako, Mali	525	175	368	123
Basse, The Gambia	526	174	368	122
Sa Kaeo/Nakhon Phanom, Thailand	400	100	280	70
Soweto/Johannesburg, South Africa	660	540	462	378
Dhaka, Bangladesh	900	300	630	210
Kilifi, Kenya	840	360	588	252
Lusaka, Zambia	400	400	280	280
Total	4251	2049	2976	1434

Number of cases available by age group: Assuming the age distribution of cases is 50% 1-11m, 30% 12-23m, and 20% 24-59m, the estimated number of cases by age group per site and overall are provided in Table XVII.

Table XVII. Estimated number of severe and very severe pneumonia cases by age

(A) age 28 days-11 months

	Target			70% of target		
Site	**Total cases**	**Severe**	**Very severe**	**Total cases**	**Severe**	**Very severe**
Bamako, Mali	350	263	88	245	184	61
Basse, The Gambia	350	263	87	245	184	61
Sa Kaeo/Nakhon Phanom, Thailand	250	200	50	175	140	35
Soweto/Johannesburg, South Africa	600	330	270	420	231	189
Dhaka, Bangladesh	600	450	150	420	315	105
Kilifi, Kenya	600	420	180	420	294	126
Lusaka, Zambia	400	200	200	280	140	140
Total	3150	2126	1025	2205	1488	717

(B) age 12-23 months

	Target			70% of Target		
Site	**Total cases**	**Severe**	**Very severe**	**Total cases**	**Severe**	**Very severe**
Bamako, Mali	210	158	53	147	110	37
Basse, The Gambia	210	158	52	147	110	37
Sa Kaeo/Nakhon	150	120	30	105	84	21

Phanom, Thailand						
Soweto/Johannesburg, South Africa	360	198	162	252	139	113
Dhaka, Bangladesh	360	270	90	252	189	63
Kilifi, Kenya	360	252	108	252	176	76
Lusaka, Zambia	240	120	120	168	84	84
Total	1890	1275	615	1323	893	430

(C) age 24-59 months

	Target			70% of Target		
Site	**Total cases**	**Severe**	**Very severe**	**Total cases**	**Severe**	**Very severe**
Bamako, Mali	140	105	35	98	73	25
Basse, The Gambia	140	105	35	98	74	24
Sa Kaeo/Nakhon Phanom, Thailand	100	80	20	70	56	14
Soweto/Johannesburg, South Africa	240	132	108	168	92	76
Dhaka, Bangladesh	240	180	60	168	126	42
Kilifi, Kenya	240	168	72	168	118	50
Lusaka, Zambia	160	80	80	112	56	56
Total	1260	850	410	882	595	287

6.3.2 Post Mortem Sample Size

Overall PERCH aims to capture at least 6000 patients at 7 sites. The mortality rate will vary between sites (estimated to be between 1-5%), and this would translate to approximately 350 deaths. Operating at 5/7 sites and assuming a consent rate of no greater than 50%, this would mean a total potential of 5-50 fatal cases for biopsy for each site for 18 months with a maximum total sample size of ~ 150 cases. This may be nearer 75-100 cases if consent is lower (which may be the case), but a sample size of even 100 (lower end of expectations) would be realistic to answer the basic question of whether the microbiology results are interpretable, whether the histology is additive, and whether molecular diagnostics have any utility.

6.3.3 Power

Key analyses addressed in this section include determining:

i. the association of pneumonia with detection of pathogens in nasopharyngeal specimens
ii. the statistical precision of estimates for individual pathogen prevalence
iii. the risk factors associated with pneumonia
iv. the background prevalence of risk factors in the study population

Case-control analyses will be used to address (i) and (iii), and case-series analyses will be used to address (ii) and (iv).

For case-control analyses, an equal number of cases and controls is assumed. Analyses will be conducted both by site and pooled across sites. Site-specific and between-site analyses will illuminate which etiologies are common globally and which vary in importance depending on geography and local epidemiologic settings.

6.3.3.1 Determine association of pneumonia with detection of pathogens in nasopharyngeal specimens

Many positive test results in NP specimens are likely to represent carriage only and are not indicative of a causal role in the infection. PERCH plans to evaluate the utility of the lab results from NP specimens by comparing the prevalence of pathogens in the cases to that in the controls. This is the first step in a multiple step process for determining the preventable fraction of pneumonia attributable to specific pathogens. Odds of infection given case or control status will determine the strength of inference of 'causality' of NP sample results. If the pathogen is found more frequently in cases than would be expected (i.e., OR>1.0), this would indicate a causal link between pathogen and disease, assuming no bias or confounding. Odds ratios ≤1.0 will be considered non-informative and NP results for those pathogens will not be considered when attributing etiology to the cases. Power is calculated to detect clinically meaningful ORs≥2.0.

Analysis: the proportion positive for a given pathogen will be compared between cases and controls. Power is calculated for detection of differences in proportions equivalent to odds ratios ≥2.0, ruling out a lower bound of OR=1.0.

The hypothesis to be tested is the following:

$$\begin{cases} \text{Ho: } p_1 = p_0 \\ \text{Ha: } p_1 \neq p_0 \end{cases}$$

p_0: Proportion of nasopharyngeal aspirates in which the pathogen is detected in controls

p_1: Proportion of nasopharyngeal aspirates in which the pathogen is detected in cases

For all analyses a 2-sided alpha will be used. However, a 1-sided test would be appropriate for this analysis because ORs<1.0 are not of interest since it is unclear how to use a negative association for a particular pathogen when determining etiologic fraction of the cases. Therefore, a 2-sided alpha is conservative.

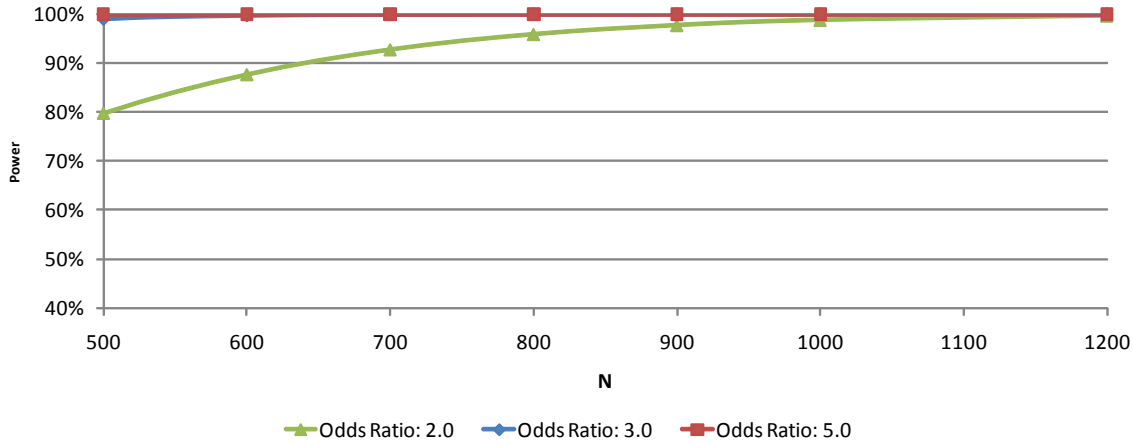
Dealing with multiple testing: Multiple tests will be performed on multiple specimens to detect up to 30 pathogens for each case. Per test and per specimen, the Bonferroni correction for 30 pathogens given a desired alpha of 0.05 is 0.00167. But analyses will be conducted at each site (analogous to repeating the study 7 times), which will increase the confidence (reduce the Type I error) of the results. As a balance between multiple testing and replication of analyses, the type I error is set to alpha=0.01.

Power: Results of power calculations are presented in the figure below to detect OR≥2.0, OR≥3.0, and OR≥5.0, for various estimates of prevalence of the pathogen in the cases. For pathogens found present in 15% of cases, all sites have at least 80% power to detect ORs ≥2.0. Overall, PERCH has 80% power to detect an OR of 2.0 for case prevalences as low as 1% in

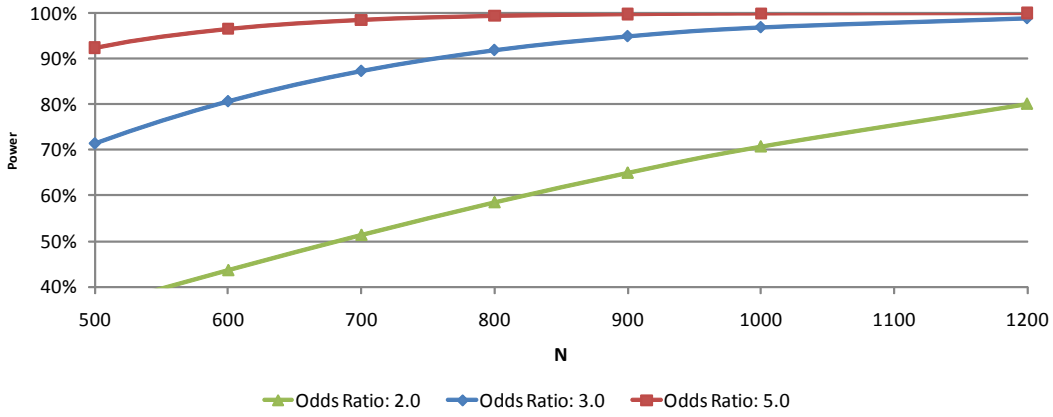
pooled analyses of all 6300 cases, and as low as 1.5% with 5000 cases. This is potentially important for pathogen discovery.

Figure IX. Power to detect odds ratios of 2.0, 3.0, and 5.0, for varying N (500 to 1200 cases), for 2-sided alpha=0.01, assuming an equal number of controls.

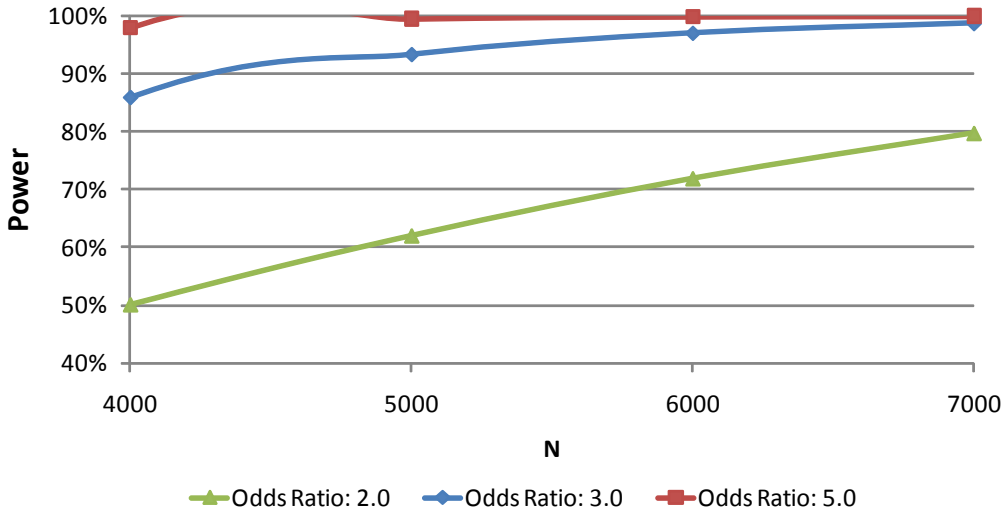
(A) 15% pathogen prevalence in the cases



(B) 6% pathogen prevalence in the cases



(C) 1% pathogen prevalence in the cases

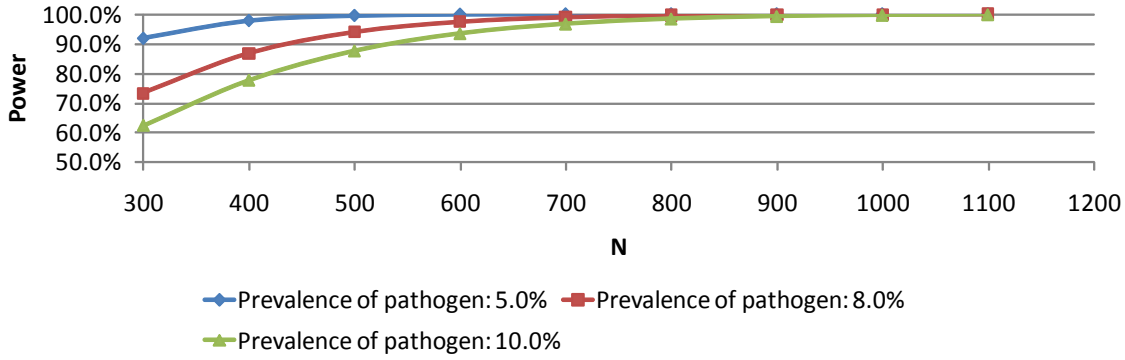


6.3.3.2 Determine statistical precision of estimates for individual pathogen prevalence

The large number of cases will allow for a very high degree of power for detecting novel pathogens and for precision around the estimates of all pathogens.

All PERCH sites are expected to have >85% power to detect pathogens with prevalences of 5-10% with precision ± 5%. Larger sites will provide high power for stratified analyses (i.e., for determining if etiology differs by severity of disease, or by HIV status). Pooled analyses will provide high power to detect rare and novel pathogens and will enable us to conduct and explore many small sub-group analyses while adjusting for age, site, and season/year.

Figure VIII. Power to detect pathogens with 5%, 8% and 10% prevalence, ruling out lower bounds of 0%, 3%, and 5%, respectively, for varying N (range 300 cases to 1200 cases), for 2-sided alpha=0.01.



6.3.3.3 Determine risk factors associated with pneumonia

Cases and controls will be used to evaluate the epidemiological risk factors for pneumonia and to estimate risk factors for significant etiological sub-groups of pneumonia (e.g. pneumococcal pneumonia, RSV, tuberculous pneumonia, etc.). For detecting ORs≥2.0, refer to the power calculations for 6.4.2.1 above.

Illustration: Vitamin D has recently been shown to be an important risk factor for morbidity in persons of all ages. To assess if it is also an important contributor to development of pneumonia in young children, the proportion deficient in Vitamin D will be compared between cases and controls. If prevalence of Vitamin D deficiency is 30% or more in the cases, we have over 95% power to detect an OR≥2.0 at each of the sites.

6.3.3.4 Determine background prevalence of risk factors in the study population

The community prevalence of risk factors will be used to characterize the study population, to interpret differences in the etiologic distribution of pneumonia cases between sites, and to extrapolate the study findings globally. Refer to the power calculations for 6.4.2.2 above.

7 Data collection, management and monitoring

7.1 Overview of data management system

Each site participating in the PERCH trial will be responsible for developing and maintaining a data management system for the purpose of data entry, cleaning and storage. The DCC will be the central data repository for all sites participating in the trial. The DCC's goal is to maintain the integrity of the data and to ensure that they are as accurate as possible. The DCC will perform a secondary study database clean-up working closely with the participating sites to ensure that all collected data has been received and that all quality control checks were performed.

The DCC will develop a standard data definition table (DDT) which will be used to consolidate all sites data into a consistent format for storage in the central database. The DCC will maintain a specimen tracking process and link them to clinical data. The DCC will work closely with data managers from the sites to assure proper quality control checks are performed at the site level based on the central data editing plan. Data integrity will be validated by producing standard reports and distributing to the sites on a routine basis. Data collected electronically or via CRFs will be transferred from the sites to the DCC using a secure FTP server or other means as deemed appropriate.

The DCC will provide, as needed, support for sites needing data management assistance including system setup, data collection, and data cleaning. Possible data systems to be used may include DataFax or InfoPath as a means of data collection. In addition the DCC may provide assistance in modifying or enhancing existing systems in use by the sites. The details of the data cleaning process will depend on the data system being used but will adhere to procedures defined in the standard data editing plan.

7.2 Data entry, error checking, cleaning

When possible, data editing will be performed in real time during data collection, while the subject is still present. In the event this is not possible, data entry and error checking will be completed within a timely manner to maximize the ability to resolve detected errors. Each site will be responsible for developing data entry error checking code, based on the standard data editing plan provided by the DCC. The sites will be responsible for following up, in a timely manner, on any queries produced by this code, to ensure the data are as complete and accurate as possible. On a predefined cycle, the sites will send updated data to the DCC. When the data are received, the DCC will run a second round of edit checks on the data.

7.3 Data sharing/access

PERCH will use an electronic data system developed by the EMMES corporation at six of seven sites (the Kenya site will maintain a site-specific data capture system). Each site will be responsible for uploading their data in an agreed upon format and will only have access to their data. Study leadership and DCC personnel will be given access to data from all sites. The DCC will then retrieve and merge all site data into one master database for centralized storage, cleaning and analysis.

Results from the multiplex respiratory PCR panel (identified by numeric study ID only) will be made available to the Azure PCR company and the Fast-track Diagnostics company for the purposes of validating software that will be used in our study to automate the interpretation of PCR results. The process will be overseen by Dr. David Murdoch, chair of the PERCH laboratory working group.

All sites will maintain 'ownership' of the data that is generated locally, and would not be restricted from using/reporting these data in accordance with the PERCH publications policy (see Appendix A).

7.4 QA/QC procedures

Prior to enrollment of the first subject at each site a complete test of the data management system will be performed by each site in coordination with the DCC. The DCC will provide standard test data which will be entered and processed at each site. The sites will verify that error checking is compliant with the data editing plan through use of a checklist provided by the DCC. When the data entry is completed, the sites will export the test data in a predefined format, and transfer them to the DCC where the data will be validated to make sure it matches the original test data.

During the first few months of the study the DCC will frequently review the data for quality control (QC) to identify trends and other peculiarities in the data. Once a threshold has been reached, a regular schedule will be established for these QC procedures. Data that have been accepted into the central database will be reviewed with a more advanced set of data quality checks. Any discrepancies (i.e. missing forms or failed univariate or multivariate checks) that are discovered during the verification process will be flagged with quality control notes for clinical site clarification.

The data management system developed by the DCC in consultation with the PERCH study group will produce the following data quality assurance reports:

- Queries
- Missing forms
- Center performance
- Monitoring site work flow
- Follow-up visit schedules

This combination of reports will allow the sites, the DCC and the study leadership to monitor the quality of the data. In addition, they will provide the tools necessary to identify if the site may be falling behind with the submission of their data and corrections.

The DCC data management staff will work closely with the staff at the sites to help them understand the problems that the data quality assurance reports detect and to assist them with resolution. The DCC data management staff, with guidance from the study leadership, will serve as a first-line resource to the participating sites on how to complete the study forms accurately.

7.5 Data Security

The DCC has a commitment to maintain data security and subject privacy. Standard DCC practices and policies for the conduct of clinical research studies will be implemented and reviewed periodically. The DCC Director is responsible for assuring all CSPCC data security policies are enforced within the DCC. Employees are responsible for following all data security policies when conducting study work. All study data collected will be handled, maintained and stored at the DCC according to DCC standard practices and policies. The central database will be stored on a server behind the firewall provided by the Department of Veterans Affairs, in compliance with their data security standards.

Each site participating in the PERCH trial will be responsible for maintaining data security as defined by local procedures and minimum requirements provided by the DCC. These minimum requirements include:

- Access to rooms housing computers or systems containing study data must be restricted to authorized personnel
- Network access to study data must be restricted to study personnel
- Computers that contain or have access to study data and have access to the Internet must be protected by firewalls as appropriate
- Study data stored on laptops or other portable devices must be encrypted
- Computers used by study personnel must maintain current anti-malware protection as appropriate

Data transferred between sites and the DCC will be performed over secure file transfer protocol (SFTP) or other secure encrypted channel as appropriate.

7.6 Monitoring

Good Clinical Practice (GCP) is “an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety and well-being of trial subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki and that the clinical trial data are credible” (ICH E6 Introduction). Although this PERCH study does not involve the administration of a treatment or product, the critical importance of this data in the later development of interventions to improve child health provide a rationale for adherence to the principles and guidelines of GCP. As such, monitoring of the study will be conducted within the applicable guidelines outlined in section 5.18 of ICH GCP.

The PERCH Core Team and local site teams will monitor all aspects of the study to ensure that the study is conducted, recorded and reported in compliance with the protocol, standard operating procedures, ICH GCP and with applicable local and government regulations.
The purpose of study monitoring is to verify that: (1) the safety and well-being of the subjects are protected during the study; (2) the reported trial data are accurate, complete and verifiable

from source documents; (3) the conduct of the study reflects the currently approved protocol/amendment(s); and (4) the conduct of the study is in compliance with GCP and the applicable local and national requirements (GCP 5.18). The objectives of a monitoring program will be to evaluate standardization and implementation of the protocol across the study sites, verify quality of data, ensure safety of the cases and controls, track performance indicators and provide real-time feedback to improve performance and study rigor.

Specific monitoring activities will be outlined in a monitoring plan and supporting Standard Operating Procedures (SOPs). The monitoring activities are divided into 4 areas: regulatory, data, laboratory and clinical standardization. Prior to the start of the study, a detailed monitoring plan will be provided to each investigator. Investigators will be informed of the frequency of monitoring visits and will be given reasonable notifications prior to each visit.

7.6.1 Regulatory monitoring

The objective of regulatory monitoring is to ensure that compliance to the protocol and relevant regulations and guidelines which include ICH GCP E6 Guidelines for Good Clinical Practice, Declaration of Helsinki and applicable local and national laws. This involves quality assurance (QA) and quality control (QC) program with written SOPs for protocol compliance and site performance. Quality Assurance monitoring will involve an overall systematic review of processes and activities to evaluate compliance with the protocol standards and GCP including eligibility and informed consent. Quality Control will involve techniques and activities for verification of the completeness, accuracy and consistency of the records.

Essential documents are a key component of regulatory monitoring. Essential documents are those documents that individually and collectively provide a record of the conduct of the study to evaluate the integrity and quality of the data produced (ICH GCP 8.0). Each study site will maintain a regulatory binder with applicable essential documents tailored to local and national requirements. These are records maintained by the investigator including: investigator agreements and CV, relevant certifications, the protocol/amendment(s), ethical and national approval documents, informed consent documents, source documents such as subject medical records (office, clinic or hospital), Case Report Forms (CRF), safety reports, and monitoring reports.

7.6.2 Data monitoring

The DCC will conduct site visits to ensure that data integrity is maintained as data progresses from collection to export to acceptance into the central database. Data management systems employed by the sites will be reviewed to ensure compliance with established operating procedures and the standard data editing plan. Other issues and potential problems may be identified and discussed as necessary. Subsequent site visits will be performed as needed, see section 7.4, QA/QC procedures, for more details.

The PERCH Core Team will ensure that each site establishes and maintains a quality management system for study conduct. An internal quality management system is essential to ensure, on an ongoing basis, that study charts are complete and accurate prior to data entry and external monitoring by the PERCH Core Team.

7.6.3 Laboratory Monitoring

The objective of laboratory monitoring is to ensure that specimens are collected, handled, transported, processed and stored in an accurate way to ensure integrity of the lab results, which are the linchpin of the PERCH primary analyses. David Murdoch will lead the Laboratory Standardization and oversee the monitoring activities. Local internal QA and QC will be set up at each site that includes a system of ongoing internal training and audit. An external quality monitoring system will be designed to confirm local lab results and to assess quality of lab test practices. Shipping will be done according to international regulations (IATA). Required permits will be secured per country regulations.

A master database will be maintained of PERCH specimens obtained from all sites noting their location.,

7.6.4 Clinical Standardization

To ensure comparability of results across sites it is essential that clinical signs used to define cases of pneumonia and to assess the severity of each case are interpreted in the same way at each site. Clinical standardization monitoring will ensure that clinical procedures are carried out in a systematic and accurate way while ensuring the safety and welfare of the subjects, and that clinical endpoints are identified, collected, documented and reported systematically and accurately.

Jane Crawley MD will lead the Clinical Standardization Monitoring program. She will work with the site PIs to: (1) set standards in the interpretation of clinical signs relevant to research on pediatric pneumonia, (2) design and implement a refresher course for clinical researchers in the standard interpretation of physical signs at the sites, (3) set up local quality assurance monitoring for clinical interpretation, and (4) design and implement an external quality monitoring system.

To achieve clinical standardization:

- A directory of the interpretation of respiratory clinical signs for key signs relevant to case definition and severity assessment in the PERCH project will be written that builds on WHO/IMCI reference materials.
- 2D images and video clips will be assembled and integrated with this written definition and communications specialists in the PERCH project will provide an internet reference site for field investigators.
- The directory will be shared with pediatricians in the expert group (PERCH Expert Group, or PEG) and with site investigators to optimize the interpretation of clinical signs and ensure wide acceptability of specific interpretations.
- All clinical-research staff at the 7 sites will be trained on site, including a clinical skills refresher course with a focus on respiratory clinical signs.
- Local internal quality assurance will be set up at each site that includes a system of ongoing internal training and audit.
- A clinical quality coordinator will be identified at each site who will be responsible for continuous internal monitoring of clinical sign interpretations and instrument standardization and timely relay of monitoring results from sites to the PERCH Core Team.

- An external quality monitoring system will be designed to audit and quantify any deviation from the established standards.

8 Human subjects

8.1 Ethical considerations

The study will be conducted in accordance with internationally recognized standards for ethical research and the International Conference on Harmonisation (ICH E6).

The protocol will be reviewed and approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) of the Johns Hopkins Bloomberg School of Public Health, and the study field sites. This will include community level approval procedures as used by each of the study field sites. Serious adverse events will be recorded at each site and reported to the PERCH Safety Monitor, DCC and to the PERCH PI, as described in section 5.7. SAEs will also be reported by the Site PIs to local IRBs and ERCs depending on their reporting requirements.

In addition to the above mentioned SAE events, we will report other events related to the conduct of the study as described here: breaches of confidentiality, enrollment violations, regulatory violations (e.g. changes made to protocol without IRB approval), as well as any protocol violation that puts participants at increase risk

Pre-screening will determine which parents are approached, using an assessment tool that documents clinical signs and symptoms of pneumonia. This should minimize approaching parents of severely ill children who do not have suspected pneumonia or approaching a child more than once. For control identification, a similar prescreening instrument will be used for to gather information on age eligible children in the communities. In both situations, the pre-screening information will be collected at the site as source data and we are asking the IRB for a waiver of consent for this prescreening information. If children meet basic eligibility, informed consent will be obtained as describe below.

The local site investigators will explain the nature of the study and will inform the parent/guardian that participation is voluntary, that their care will not be affected if they choose not to participate, and that they can withdraw the child at any time even after they consent to the study. Following an explanation of what study participation entails including the body fluid specimens that will be obtained, the clinical follow up visits and the clinical examinations, the parent or guardian will be offered an opportunity to have any and all of their questions answered before consenting to participation in the study.

Written informed and or oral consent will be obtained for each child prior to entry into the study. Written or oral format will be determined by the local ethics committee. For written informed consent, a copy of the consent form will be given to the parent/guardian of every participant in most sites and the signed original will be maintained with the child's records. If a parent indicates prior to signing consent that they do not intend to allow collection of specimens the child will be excluded from enrollment. Every attempt will be made to enroll all selected cases of severe pneumonia at each site. However, the children being recruited are very ill and their clinical care and management takes precedent over the enrollment in PERCH. If consent for PERCH could not be obtained at the time of admission for a child with suspected severe or very severe pneumonia, the parent will be approached as soon as possible and asked

for consent to enroll. By providing consent, the parent is agreeing that previously collected samples and data collected by the hospital for this admission will be included in the PERCH analysis. If a parent does not consent, that child will not be enrolled and those samples will not be included. In either case, however, after consent has been obtained, a parent may refuse collection of a particular specimen or clinical assessment. As long as the child has had a blood culture, an induced sputum, or an NP specimen obtained they will remain in the study for analysis purposes.

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records on individual subjects will be identified by a coded number and subject initials only. All study records will be kept in a secure location and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet or equivalent procedures. Clinical information will not be released without written permission of the patient, except as necessary for clinical care. Specifically this will include HIV results, sickle cell results, malaria, thalassemia, results of testing for tuberculosis and in the case of HIV infected subjects results of pneumocystis testing.

As part of written/oral informed consent, the PERCH study will obtain permission from parents/guardians of the study participants for retention of body fluid specimens and of microbiologic organisms, including bacterial isolates and their nucleic acid for future testing and study in relation to infectious diseases. PERCH will retain a link between study data and patient samples, but not necessarily between patient samples and personal identifiers. Because the specimens will be de-identified, after the specimens are stored they cannot be removed from the biorepository. "De-identified" specimens are specimens that are double-coded and labeled with a unique second number. The link between the clinical study subject number and the unique second number is maintained, but unknown to investigators and patients.

A key aspect of this study is the risk:benefit balance to the study subjects. As we have outlined in Section 5 on safety, the study is based on the notion that most of the body fluid specimens that are being collected from the children are either part of routine care already, or are of minimal risk. The exception to this is the collection of lung aspirates from children with lung pathology that is of an appropriate character amenable to needle aspiration. The benefit that can be conferred from that procedure is significant because the lab tests on the specimen will be done in real-time and will therefore be communicated back to the treating physician and will influence the care of the child. Furthermore, lung aspirates, when conducted by trained personnel in settings with the ability to manage the rare complications that occur, confer low risk and have the potential for very significant clinical benefit. The study also offers the subjects assured or enhanced clinical care insofar as the study provides additional supportive infrastructure of the clinical assessments, lab testing and patient management that is provided by the routine clinical hospital services. In summary the risk:benefit ratio for this study is strongly in favor of individual benefit to the child as well as essential societal and public health benefit.

The costs associated with routine clinical management of patients are expected to be covered by the same methods as preceded the initiation of PERCH, and PERCH funding is not intended to displace or substitute for existing, on-going resources for clinical management. PERCH may supplement existing resources as needed on a case-by-case basis.

8.2 Institutional Review Board (IRB) or Independent Ethics Committee (IEC)

JHSPH IRB:

The generic PERCH protocol and informed consent templates were developed by the Executive Committee, which consists of the individual site principal investigators as well as leadership from the PERCH Core Team. Once the JHSPH IRB has approved the generic protocol, and the informed consent documents, these may be adapted by the local sites as relevant for their study setting. The principle the sites must follow is that they cannot remove or add procedures to the generic protocol or change the content of the consent form without an amendment to the JHSPH IRB. Any local modification to the PERCH project that affects the risk/benefit ratio of the enrolled cases or controls will need to be approved by the JHSPH IRB as an amendment.

Local IRBs:

Each PERCH site will be responsible for submitting the locally-amended protocol, informed consents, recruitment materials, and case report forms (CRFs) to their local/institutional ethics committee. Local ethics board approval must be obtained before the start of the study, and the study must be kept current and in good standing with the local IRB until the end of the clinical enrollment, follow up and specimen testing or as required by the local IRB. Each site should maintain a regulatory binder that includes the following:

- Local IRB/EC Approval
- Local IRB/EC Membership
- JHSPH IRB Approval
- Current Protocol
- Current Consents
- Previous Versions of the Protocol and Consent, if any
- Master copies of the CRFs
- IRB Correspondence (e.g. annual renewals, approvals of amendments)
- Ethics Training Certificates/Staff GCP Qualifications
- CVs for all Key Personnel
- Monitoring Reports
- Log of Reportable Serious Adverse Events
- Delegation of responsibility documentation

8.3 Informed Consent

The principles of informed consent in the current edition of the Declaration of Helsinki should be implemented before any protocol-specified procedures or interventions are carried out. Informed consent will be obtained in accordance with the JHSPH IRB approved form with local modifications as described above.

Information should be given in both oral and written form, and the child's legal representatives must be given ample opportunity to inquire about details of the study and have any and all of their questions answered. The informed consent documents and recruitment materials must be made available as translations in to locally spoken languages. The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki, and ICH GCP section 4.8 and will also comply with local regulations.

Parents/guardians must be informed about the aims, expected benefits, and possible risks (including a statement that the particular treatment or procedure may involve risks to the child that are currently unforeseeable). They must also be informed of alternative procedures. Parents/guardians must receive an explanation as to whether any compensation and any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained. They must be informed whom to contact for answers to any questions relating to the research project.

The parents/guardians must be informed that participation is voluntary and that they are free to withdraw the child from the study for any reason at any time, without penalty or loss of benefits to which they are otherwise entitled.

The extent of the confidentiality of patient records must be defined, and parents/guardians must be informed that applicable data protection legislation will be complied with. Parents/guardians must be informed that the monitor(s), auditor(s), IRB/IEC members, and the regulatory authorities will be granted direct access to the patient's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the patient, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the patient's legally acceptable representative is authorizing such access.

Each PERCH site will be responsible for some level of community engagement at their site. This may include, and is not limited to, review of study procedures and documents by local leaders, participation of a community advisory group, and ongoing feedback to the community about the progress of the study.

Separate consent forms will be used for the post mortem study. The timing of consent will be locally determined, based on the site PIs recommendation about what is culturally acceptable and common local procedure.

The PERCH pneumonia methods working group discussed a number of issues regarding the planning of a post mortem needle biopsy study in October 2009. From previous experience of large autopsy trials in Viet Nam and Malawi (in malaria) and Zambia, obtaining consent for full or even partial open autopsy is difficult in these settings and unlikely to be acceptable to the majority of parents in the PERCH study sites. In the experience of the members of the PERCH advisory group (Pneumonia Methods Working Group) in developing countries an immediate post-mortem percutaneous biopsy specimen is likely to be considerably more acceptable than open autopsy.

Percutaneous needle biopsy is minimally invasive, not time consuming, so would not delay funeral arrangements. Obtaining parental consent at the time of death will be challenging, and require local clinicians or trained study nurses, familiar with the aims of the study, to explain the reasons for the request. Even given this we would anticipate up to 50% may decline.

Genome-wide association studies

Genome-wide association studies (GWAS) are an increasingly important tool of understanding host susceptibility to infectious diseases. PERCH provides a platform for GWAS that could

identify common genetic variants associated with susceptibility to specific pneumonia pathogens and/or associations with disease severity and outcome. The sample size, the collection of specimens from both cases and controls, and the careful collection of phenotypic data will provide the opportunity for future GWAS. Consequently, the focus of PERCH will be on the collection of specimens that provide sufficient quantity of host DNA, careful archiving of these specimens, and obtaining the appropriate consent and IRB approvals for these activities. DNA extraction and genotyping would be performed by future studies with separate funding.

To ensure that the storage of specimens is standardized across sites, the PERCH sites will use specimen tracking systems, which will be monitored by the DCC. In addition, metrics for tracking the quality of specimen storage will be part of the laboratory QA/QC plan.

9 Study completion

The investigators will notify the IRBs/IEC when the study has completed all contact with subjects, when the protocol specified lab testing has been complete and when the study itself is considered complete. Closure of the study at any individual IRB will be according to the rules/regulations of that IRB. At some IRBs the protocol must remain open while any manuscripts are still in preparation, while at other IRBs the protocol may be closed once all the lab testing and contact with subjects is complete.

The DCC will do a final clean-up of the study database working closely with the participating sites to ensure that all collected data have been received into the database and that all received data are as correct as can be made possible. Within three to four months after the last patient follow-up, the DCC will provide the study leadership with the final, locked study database.

10 Publications

Providing information and results from the study for use by the clinical and public health community to improve child survival and health is a primary objective of the study. To that end the study investigators aim to place in the public domain the entirety of the study procedures and results so the maximum benefit can be derived from the study. Notwithstanding this objective, principles of publication order and authorship will be adhered to. These are described in a Publication Agreement (Appendix A) agreed to by the PERCH and site investigators.

11 Changes in protocol and documentation of IRB approvals

The protocol may not be modified without written approval of the Executive Committee. All amendments to the protocol must be submitted to the JHSPH IRB and the local IRB that is affected by the change. All changes must be approved by the IRBs/IEC prior to their implementation. For changes that are relevant only to one site this will be clearly documented in the submissions to the JHSPH IRB. Documentation of IRB/IEC approval must be sent to the PERCH Core Team immediately upon receipt. The PERCH Core Team is responsible for keeping documentation of all IRB approvals at all sites but will not seek to have all sites maintain the approvals from all other sites.

12 Appendix A Publication Agreement

As a multi-site, multi-investigator project PERCH needs a transparent, fair process that is endorsed by all investigators for determining authorship and publication criteria. The study data

from PERCH will be a tremendous resource and provide great opportunity for pneumonia investigations. It is important to establish at the outset of enrollment principles that will govern authorship and publication rights. The PERCH team proposes the following principles:

1. The PERCH Executive Committee (EC) will be charged with developing and implementing publications policy governance. This group will be composed of three PERCH secretariats and all of the site principle investigators, and will be responsible for protecting the interests of all stakeholders in the writing and publication of study information.

2. The findings from PERCH primary objectives based on aggregated data from all seven study sites will be published before any individual sites publish their conclusions (this will be within a defined time limit).

3. Authorship expectations and responsibilities will be established and defined at the outset of the study for all investigators including the senior site investigators, the core PERCH team, and any collaborators.

4. PERCH should establish *a priori* lists of potential investigations that are anticipated from the outset so that the expectations for these publications can be established as soon as possible.

5. PERCH encourages the development of junior investigators, and is supportive of this aim. The responsibility for their development falls within the responsibilities of the site principal investigators (site, in this instance, includes the PERCH core team).

6. PERCH will establish time limits for "ownership" of a publication. After these periods are up, the initial allocations of responsibility or authorship can be revised if the obligations of "ownership" were not met.

Key Elements of PERCH Publication Policy/Procedures

1. Investigators develop ideas for publications and determines which classification of project it meets (multi-site primary, multi-side sub-study, or individual site project).
2. Investigators complete concept sheet according to governing principles, and outline paper objectives and authorship.
3. The PERCH Executive Committee (EC) reviews the concept sheet to:
    a. Consider the study type
    b. Considers the governing principles and their application
    c. Determines whether notification/approval is required
4. All site investigators are notified of the status of proposed publications 3 times per year.

II. Definition of an author

PERCH will define the contributions of authors according to the policy and criteria laid out by the International Committee of Medical Journal Editors (ICMJE). According to ICMJE, "an 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study, and biomedical authorship continues to have important academic, social, and financial implications." They go on to stress the three criteria for authorship: "Authorship credit should be based on 1) substantial contributions to conception and design, acquisition of data, or

analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3."[i] (Editors 2010)

The full ICMJE description of authorship guidelines are provided below.

Byline Authors

An "author" is generally considered to be someone who has made substantive intellectual contributions to a published study, and biomedical authorship continues to have important academic, social, and financial implications (1). In the past, readers were rarely provided with information about contributions to studies from persons listed as authors and in Acknowledgments (2). Some journals now request and publish information about the contributions of each person named as having participated in a submitted study, at least for original research. Editors are strongly encouraged to develop and implement a contributorship policy, as well as a policy on identifying who is responsible for the integrity of the work as a whole.

While contributorship and guarantorship policies obviously remove much of the ambiguity surrounding contributions, they leave unresolved the question of the quantity and quality of contribution that qualify for authorship. The ICJME has recommended the following criteria for authorship; these criteria are still appropriate for journals that distinguish authors from other contributors.

Authorship credit should be based on 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

When a large, multicenter group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript (3). These individuals should fully meet the criteria for authorship/contributorship defined above and editors will ask these individuals to complete journal-specific author and conflict-of-interest disclosure forms. When submitting a manuscript authored by a group, the corresponding author should clearly indicate the preferred citation and identify all individual authors as well as the group name. Journals generally list other members of the group in the Acknowledgments. The NLM indexes the group name and the names of individuals the group has identified as being directly responsible for the manuscript; it also lists the names of collaborators if they are listed in Acknowledgments.

Acquisition of funding, collection of data, or general supervision of the research group alone does not constitute authorship.

All persons designated as authors should qualify for authorship, and all those who qualify should be listed. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

Some journals now also request that one or more authors, referred to as "guarantors," be identified as the persons who take responsibility for the integrity of the work as a whole, from inception to published article, and publish that information.

Increasingly, authorship of multicenter trials is attributed to a group. All members of the group who are named as authors should fully meet the above criteria for authorship/contributorship.

The group should jointly make decisions about contributors/authors before submitting the manuscript for publication. The corresponding author/guarantor should be prepared to explain the presence and order of these individuals. It is not the role of editors to make authorship/contributorship decisions or to arbitrate conflicts related to authorship.

Contributors Listed in Acknowledgments

All contributors who do not meet the criteria for authorship should be listed in an acknowledgments section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Editors should ask corresponding authors to declare whether they had assistance with study design, data collection, data analysis, or manuscript preparation. If such assistance was available, the authors should disclose the identity of the individuals who provided this assistance and the entity that supported it in the published article. Financial and material support should also be acknowledged.

Groups of persons who have contributed materially to the paper but whose contributions do not justify authorship may be listed under such headings as "clinical investigators" or "participating investigators," and their function or contribution should be described—for example, "served as scientific advisors," "critically reviewed the study proposal," "collected data," or "provided and cared for study patients." Because readers may infer their endorsement of the data and conclusions, these persons must give written permission to be acknowledged.

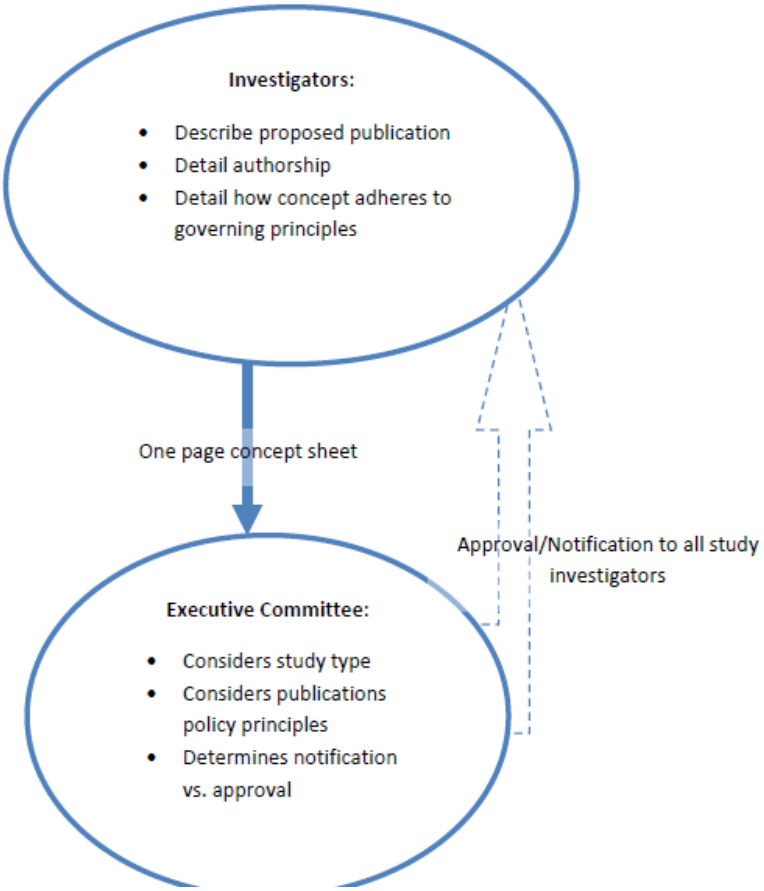
II. Authorship Roles and Planned Publications

The PERCH publications policy is meant to help guide all stakeholders through the publication process of investigations that utilize PERCH study data. This project will involve a number of interests, and definitions contained in this publications policy will need to balance the needs of the PERCH PIs and co-PIs, the local site investigators, and the opportunity for young investigators to obtain lead authorship on ancillary studies. This publications policy will utilize the following authorship groupings (Adapted from the "Multicenter AIDS Cohort Study Publication/Publicity Policy". September 17, 2008):

Title	Definition	Requires EC Approval/Notification
Joint Investigation	PERCH Investigators + Outside Groups	Approval
Core Investigation	PERCH Investigators only	Approval
Site-Specific Investigation	One PERCH site only	Notification
Multi-Site Investigations	More than two PERCH sites	Approval
PERCH Core Author's Group	Group of core authors	Approval

Based on expected study results, PERCH aims to have a series of several key papers to be published in 2013. When meeting with the site investigators, PERCH will outline authorship responsibilities for these key publications.

III. Diagram of PERCH Publications Process



13 Protocol Changes by Version

Version 2.0

- Adding the collection of a urine specimen during the 30-day follow up visit at select sites. This specimen would be stored for the purposes of future biomarker testing (page 14).
- Removing the list of working group members, as changes in staff could cause an un-due number of amendments to remove/add investigators (pages 16 – 17).
- Removing Karen Charron from the Quality Management Team. Ms. Charron was unable to continue her participation in PERCH. The sites will now share the responsibility of GCP and regulatory duties with the PERCH core team (page 18).
- Reducing the number of study sites in Bangladesh from 3 to 2. The third site was dropped due to operational difficulties (pages 19-20).
- Revising the description of our Data Coordinating Center. In May, the Perry Point VA withdrew from that role, and we have replaced them with the EMMES Corporation. This change has been noted both in the informed consent documents and in the protocol (page 22).
- Correcting the case definition (page 24).
- Correcting the process for collecting information on children who are screened for study participation (pages 30 – 31).
- Updating Tables IV, V, VI. The sites piloting study case report forms extensively and several changes were made during this process (pages 33- 36).
- Detailing the study parameters for the post mortem investigations (pages 39 – 44).
- Updating contraindications for the induced sputum procedure, per our correspondence with the JHSPH IRB (pages 51 and 69).
- Updating results reporting tables. The PERCH team is currently discussing with the JHSPH IRB and the tables now reflect some flexibility (pages 57 – 63).
- Adding in a description of clinical monitoring for children who have the induced sputum procedure, per our correspondence with the JHSPH IRB (page 71).
- Adding in post mortem risk assessment considerations (pages 73 – 74).
- Correcting the SAE reporting criteria for the induced sputum and lung aspirate procedures, based on correspondence with the JHSPH IRB (page 74).
- Updating the role of the Local Safety Monitors (LSMs). The site investigators felt that it was inappropriate for the LSMs to assign relatedness and severity for an SAE, and that this would be better assessed by the treating clinicians. Instead, they are asking the LSMs to review each SAE report and provide feedback as required (page 75).
- Adding a paragraph to describe the post mortem sample size (page 88).
- Updating data monitoring plan to reflect the departure of Ms. Karron (page 95).
- Describing rationale for timing of post mortem consent (page 100).

Version 3.0:

- Change the Principal Investigator from Orin Levine to Kate O'Brien (page 17).

- Clarifying the data that will be contributed by PERCH cases who do not contribute any study specimens (page 25)

- Adding a process for approaching the mothers of PERCH cases to confirm HIV status using voluntary counseling and testing procedures in the South Africa and Zambia sites (page 35).

- Clarifying process for control matching in the South Africa and Zambia sites. Previously we had required an additional step for controls to be matched to HIV positive PERCH cases based on antiretroviral status. This was found to be unworkable at the sites due to difficulty finding and recruiting children who were ART-naïve (page 47).

- Providing further detail on the panel of radiographers and pediatricians who will be responsible for interpreting the PERCH chest x-rays (page 56).

- Correcting lab tables to show the tests that are done on study specimens (page 57-62).

- Clarifying the definition of one of the SAE parameters, from 'New onset of unconsiousness or prostration' to 'Deterioration in AVPU score' as per the recommendation of the clinical standardization officer (page 73).

- Correcting the description of the data sharing process to reflect how sites are utlizing the electronic data capture system (page 91).

- Adding a description of a validation exercise using PERCH PCR results (page 91 - 92).

- Correcting information on the type of database that will be maintained for all PERCH specimens (page 94).

- Adding a list of changes to the end of the protocol, for tracking purposes

Version 4.0:

- Correcting the spelling of Dr. Kate O'Brien's name (page 17).
- Adding Mali as a site where lung aspirates will be performed (page 40).
- Correcting the concentration of saline used to perform IS (page 52).

Version 5.0:

- Added urinary arsenic and creatinine analysis to Table V. Body Fluids collected from Cases (Page 37)
- Added urinary arsenic and creatinine analysis to Table VI. Risk Factors (Page 39)

- Added urinary arsenic and creatinine analysis to Table VIII. Control Specimens (Page 51)
- Added urinary arsenic to Section 4.5.1.6. Urine. (page 53)
- Added total urinary arsenic and creatinine analysis to Table IX. Laboratory Evaluations of Cases (Page 60)
- Added total urinary arsenic and creatinine analysis to Table X. Laboratory Evaluations of Controls (Page 64)
- Added total urinary arsenic and creatinine analysis to Section 5.4 Urine (Page 72)

Version 6.0

- Updated contraindications for induced sputum specimen collection per discussions with JHSPH IRB (Page 53, 70).

- Updated contraindications for lung aspirate specimen collection per discussions with JHSPH IRB (Page 53, 70).

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