Maternally Derived Anti-Dengue Antibodies and Risk of DHF in Infants: a Case-Control Study

A Master's Thesis Presented

By

STEVEN HATCH, MD

Submitted to the Faculty of the
University of Massachusetts Medical School,
Graduate School of Biomedical Sciences, Worcester
In partial fulfillment of the requirements for the degree of

Master of Science in Clinical Investigation (MSCI)

August 1, 2010

Maternally Derived Anti-Dengue Antibodies and Risk of DHF in Infants: a Case-Control Study

A Master's Thesis Presented

By

Steven Hatch, MD

The signatures of the Master's Thesis Committee signifies completion and approval as to style and content of the Thesis

Daniel Libraty, MD, Chair of Committee

Peter Rice, MD, Member of Committee

Laura Gibson, MD, Member of Committee

Alan Rothman, MD, Member of Committee

The signature of the Dean of the Graduate School of Biomedical Sciences signifies that the student has met all master's degree graduation requirements of the school.

Anthony Carruthers, Ph.D.,
Dean of the Graduate School of Biomedical Sciences

Clinical Investigation Program

August 1, 2010

Abstract

This study proposes to directly test the hypothesis that antibody-dependent enhancement (ADE) is the critical factor in the development of dengue hemorrhagic fever (DHF) in infants. DHF occurs in two distinct clinical settings: a) in children and adults with secondary DENV infection, and b) in infants with primary DENV infection born to mothers with prior DENV infection. The ADE hypothesis proposes that pre-existing serotype-cross-reactive non-neutralizing anti-DENV antibodies bind the heterotypic DENV during secondary infection and enhance its uptake into immune cells, leading to increased viral load and DHF. This model suggests that DHF in DENV-infected infants is caused by the enhancing effect of waning maternal anti-DENV antibodies, thus causing a "physiologic secondary infection" during an infant's primary infection and thereby increasing the infant's risk for DHF.

The effect of maternal immunity on DHF in infants has been studied exclusively in Southeast Asia. However, the maternal DENV seroprevalence approaches 100% in this part of the world. As a consequence, the ADE model of infant DHF cannot truly be tested in Southeast Asia, because all infants possess anti-DENV antibody at birth. In the Western Hemisphere, by contrast, women may have experienced either a single DENV infection, more than one DENV infection, or no DENV infection at all. The ability to include DENV-seronegative mothers as controls allows for the ADE hypothesis to be directly tested in a clinical study. To our knowledge, no such study has been previously conducted.

This thesis presents a case-control study designed to evaluate the influence of positive maternal dengue seroprevalence on the risk of DHF in infants. As the MSCI program provides instruction in study design, this thesis does not present findings. The clinical trial described herein began in May 2010 and enrollment is expected to continue through May 2012 (see Table 4).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

Overview and Specific Aims

This study proposes to directly test the hypothesis that antibody-dependent enhancement (ADE) is the critical factor in the development of dengue hemorrhagic fever (DHF) in infants. DHF is the life-threatening syndrome associated with dengue virus (DENV) infection. DENV infection among humans has expanded rapidly over the past 50 years. The increasing incidence makes an accurate model of DHF pathogenesis imperative for vaccine development, clinical management and public health strategies.

DHF occurs in two distinct clinical settings: a) in children and adults with secondary DENV infection, and b) in infants with primary DENV infection born to mothers with prior DENV infection. One of the principal models for the pathogenesis of DHF in these two seemingly disparate groups is ADE. The ADE hypothesis proposes that pre-existing serotype-cross-reactive non-neutralizing anti-DENV antibodies bind the heterotypic DENV during secondary infection and enhance its uptake into immune cells, leading to increased viral load and DHF. This model suggests that DHF in DENV-infected infants is caused by the enhancing effect of waning maternal anti-DENV antibodies, thus causing a "physiologic secondary infection" during an infant's primary infection and thereby increasing the infant's risk for DHF.

The effect of maternal immunity on DHF in infants has been studied exclusively in Southeast Asia. However, the maternal DENV seroprevalence approaches 100% in this part of the world. As a consequence, the ADE model of infant DHF cannot truly be tested in Southeast Asia, because all infants possess anti-DENV antibody at birth. In the Western Hemisphere, by contrast, women may have experienced either a single DENV infection, more than one DENV infection, or no DENV infection at all. The ability to include DENV-seronegative mothers as controls allows for the ADE hypothesis to be directly tested in a clinical study. To our knowledge, no such study has been previously conducted.

In brief, the major objectives of my proposed research are:
#1: **Compare rates of dengue seroprevalence of mothers from two groups of infants: infants with DHF and those with symptomatic DENV infection but without DHF.** The null hypothesis of this study is that the proportion of dengue-seropositive mothers of infants with DHF infants is equal to the proportion of dengue-seropositive mothers of infants with symptomatic DF who do not meet criteria for DHF. To test this, we will compare the rates of prior DENV infection in mothers of these two groups of children. Serologic testing for both anti-dengue IgM and IgG of all serotypes will be performed on all participants using ELISA assays. Serum $PRNT_{50}$ assays—the plaque-reduction neutralization assay and the gold standard test in serologic analysis of flavivirus infections—will also be performed.

#2: **Test ADE in vitro using the serum from the mothers of both groups of DENV-infected infants (as a surrogate for ADE activity of pre-illness infant sera)**. Antibody-dependent enhancement assays are performed by using serial dilutions of serum, followed by incubation of DENV-serum mixtures to FcγIIa receptor-bearing K562 or BHK-21 cells at a fixed multiplicity of infection, with subsequent measurement of DENV levels by either qRT-PCR, plaque titration, or flow cytometry. We propose to measure the ADE activity of a maternal serum dilution that

estimates infant dengue antibody levels at the age of infection, comparing mothers of DHF infants versus mothers of "not-DHF infants."

#3: **Evaluate the relationship between ADE activity and estimated peak viremia levels.** The ADE model predicts that increased viral uptake into macrophages directly leads to higher viral loads; consequently, there should be a positive correlation between ADE activity and the peak viral load. We will perform PCR analysis on all infants with dengue, estimate the peak viremia level based on day of illness, and compare these data to ADE activity.

Research Plan

Background and Significance

DENV infection is the most common mosquito-borne viral infection in the world today, and has been an increasing public health menace over the past few decades. Currently there are an estimated 100-150 million annual infections, with an annual mortality of >20,000, the overwhelming majority of deaths being in infants and children (1). DENV is a member of the flavivirus family, with four distinct but closely related serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. Transmitted to humans primarily by *Aedes aegypti* mosquitoes, DENV is found mostly in tropical and subtropical areas worldwide. Classical dengue fever (DF) presents as an acute febrile illness characterized by headache, retro-orbital pain, and myalgias. A small group of patients go on to develop the manifestations of DHF, which is distinguished from DF principally by a vascular leakage phenomenon, leading to hemoconcentration, pleural or peritoneal effusions, and, in the most severe cases, dengue shock syndrome (DSS). While death is a relatively rare complication, the sheer volume of infections worldwide makes DENV the most lethal hemorrhagic fever virus by far—the total number of deaths worldwide from Ebola since 1976 is just over 1,000; DENV claims an equal number in less than a month.

The protective immune response against DENV remains poorly understood. There are no good animal models of human dengue disease (2); therefore, clinical and epidemiologic studies are necessary to answer questions relating to DENV pathophysiology. Further, although many successful viral vaccines were developed with a minimal understanding of basic viral and cellular processes, the development of an effective DENV vaccine requires a more comprehensive model of DENV pathophysiology (3). In particular, an understanding of which individuals are at risk for developing DHF is a priority in the coming decade.

Epidemic DHF and DSS emerged in the Philippines and Thailand only 50 years ago. Since then, the virus has become hyperendemic (i.e., endemic transmission of multiple serotypes) in Southeast Asia, where today it is among the top ten causes of pediatric hospitalization (4). By contrast, DENV infection in the Western Hemisphere remained relatively low until the 1970s, when DF incidence began to rise, followed by epidemics of DHF and DSS in the 1980's. Today, countries such as Brazil, Venezuela and Colombia are subject to epidemics in increasingly rapid succession. The seroprevalence in the West remains less than 100% based on ongoing studies, although hyperendemic circulation is becoming established in some areas (5).

Immunity and pathogenesis in DENV infection

One of the unique features of dengue is the role of heterologous infection in disease severity. Following infection with a given DENV serotype, an individual will develop long-lasting immunity to that serotype (homotypic immunity). However, heterotypic immunity to the remaining three serotypes lasts only a few months (6). One key observation in dengue pathogenesis is that heterotypic immunity is a risk factor for DHF: the overwhelming majority of DHF cases occur in people who are experiencing secondary infections with a different DENV serotype (7). The major exception to this group is in infants, where "primary DHF" is most commonly seen during the period from 6-12 months.

One widely accepted theory to account for the occurrence of DHF in these different groups is ADE (8) (9, 10)**.** The ADE model proposes that pre-existing heterotypic anti-DENV IgG antibodies (Abs) acquired during a previous infection can, under certain conditions, facilitate uptake of virus into macrophages (purportedly the main target cell for DENV) and possibly other immune cells via the binding of virus-antibody complexes to Fcγ receptors. The consequent increase in receptor-mediated endocytosis of non-neutralized virus particles causes something resembling a "Trojan Horse" phenomenon, leading to higher viral loads, which in turn triggers a host inflammatory cascade that leads to DHF.

The *sine qua non* of the ADE hypothesis is its prediction of a primary-infection DHF phenomenon in infants, where maternally derived anti-DENV IgG levels decay over time. Infant immune systems are "primed" in utero by passive transfer of DENV-specific Abs from DENV-immune mothers, thus creating a "physiologic secondary infection" during primary DENV infection, where waning maternal anti-DENV Abs enhance viral uptake, resulting in DHF. Figure 1 illustrates a schematic showing the risk of DHF based on the ADE hypothesis.

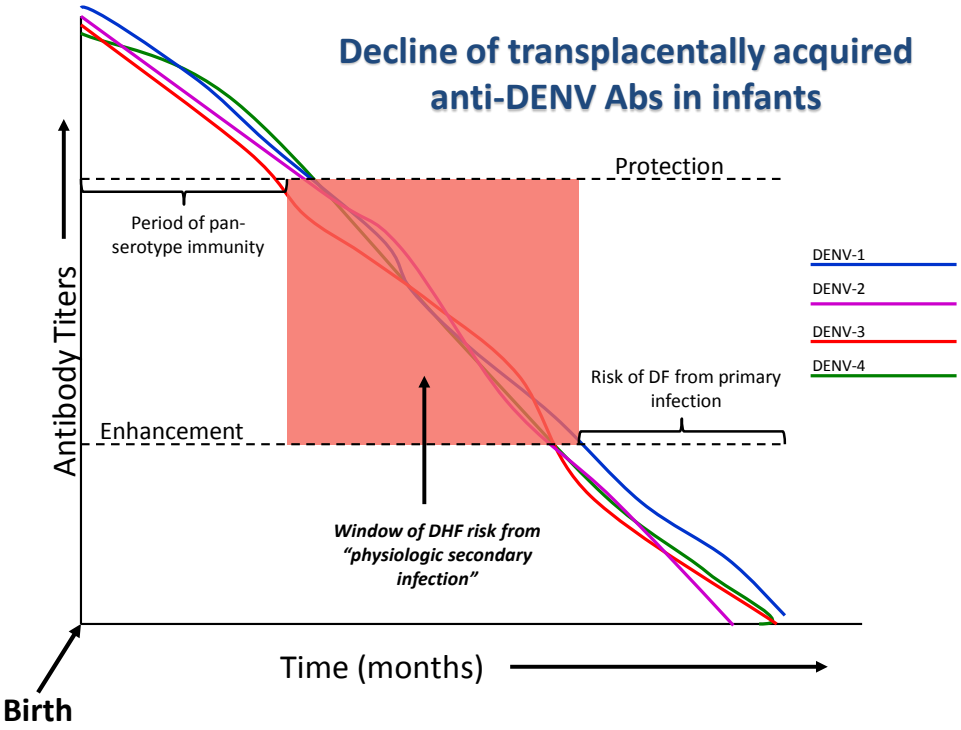

Figure 1. ADE hypothesis in an infant model. Waning maternal DENV Ab levels are initially able to neutralize all four DENV serotypes. However, as anti-DENV Ab levels decline (between approximately 4-6 months), the infant enters a "window phase" where DENV-specific Abs paradoxically facilitate intact virus uptake into immune cells, leading to abnormally high viral loads and increased risk for DHF. Following this "window" period, Ab levels are sufficiently low that they have no effect on subsequent infection, and infants exposed to DENV during this period are at risk of DF but have substantially reduced risk, or no risk, of DHF.

Several epidemiologic studies have shown that the incidence of primary DHF in infants peaks approximately between 6 and 8 months of age (11). This time frame corresponds with predicted ADE activity, and in vitro studies have borne this out. Kliks et al were able to demonstrate a correlation between mothers neutralizing titers (to DENV-2) and infant age at the time of onset of DHF and Dengue Shock Syndrome (12), while Chau et al noted a strong temporal association between the enhancing activity of plasma and the age of the infant in a cohort of Vietnamese children (13). However, other studies have cast doubt on the ADE hypothesis: Laoprasopwattana et al were unable to demonstrate any correlation between the enhancing activity of DENV in K562 cells and the clinical severity or viral burden of Thai schoolchildren experiencing secondary DENV-2 or DENV-3 infection (14); Libraty et al evaluated a cohort of Filipino infants experiencing primary infection (largely DENV-3) and were unable to find significant differences in DENV-3 ADE between infants with DHF and those with less severe symptomatic disease (15). Reflecting these contradictory findings, one investigator recently noted that because measurements of ADE rely on in vitro models, its association with clinical outcomes remains controversial (16), stating that "to the best of our knowledge, DHF has not yet been described in an infant born to a dengue-naïve mother…further studies of infants with

DHF, particularly in regions with low or moderate dengue endemicity or where outbreaks occur among previously naïve populations, are needed to unequivocally confirm that maternally derived anti-DENV antibody is a critical risk factor for DHF in infants."

South America as a location to study the effect of maternal Ab on infant DHF

We have identified a promising site to study the influence of maternal dengue serostatus on the severity of infant DENV infection. Our collaborators are faculty members in the Department of Pediatrics, Universidad Surcolombiana (USCO) Hospital. We are currently working with them on a project evaluating a novel assay for the detection of dengue viremia. A city of approximately 300,000 people, Neiva is an ideal site for studying dengue in infants for two major reasons: it is a large enough city to have a sufficient number of cases of severe DENV infection, but it is small enough only to support one pediatric hospital allowing for a "funneling" of cases of severe dengue. USCO is not only the sole inpatient pediatric hospital for the city but for the surrounding region (Estado Huila). Thus, our collaborators and their department care for essentially all cases of pediatric DHF in Neiva.

One of the critical elements in a study to evaluate the effect of maternal antibody in primary DHF in infants is to have an adequate number of both seropositive and seronegative mothers, as well as a sufficient number of cases of infant DHF. That is, if the overall DENV seroprevalence in a study site is too low, then the likelihood of obtaining an adequate number of cases of infant DHF is likewise low; conversely, the higher the endemicity, the more difficult it is to find seronegative mothers. An ideal study would include not only seronegative mothers, but "monotypic" mothers (i.e. having been infected with only one serotype) and "multitypic" ones (those with a history of infections from at least two separate serotypes—current testing cannot distinguish between patients infected with two, three or all four serotypes).

In order to estimate the maternal DENV seroprevalence, we obtained sera from a cohort of 121 pregnant women who had been enrolled in a study in 2006 evaluating the protective effects of pre-natal pneumococcal vaccination on infants. The initial results of the anti-DENV IgG ELISA testing can be seen in Figure 2. Overall, the estimated seroprevalence based on these findings is approximately 32% (39 of 121). We assume this seroprevalence to be artificially low due to the fact that an estimated 20% of the women in this study came from the surrounding mountains, which support a climate too cool for the survival of *Aedes aegypti* and thus the transmission of dengue (personal communication). This would theoretically increase the overall seroprevalence among women living in the region at risk for dengue to roughly 40% as of 2006. The dengue seroprevalence likely has increased in the intervening years based on epidemiologic trends noted throughout northern South America over the past decade, though doubtfully to levels that would preclude the possibility of seronegative mothers. We have recently been shipped the samples and are performing $PRNT_{50}$ tests to evaluate the percentage of mothers with monotypic or multitypic infection.

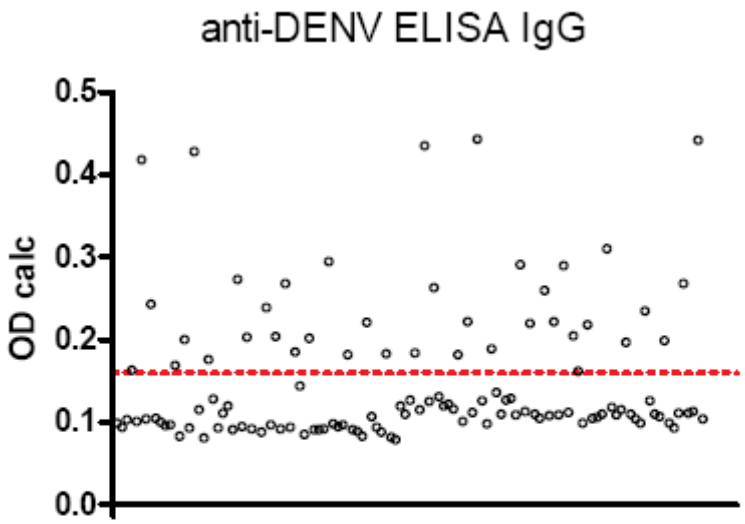

Figure 2: Maternal anti-DENV seroprevalence in a cohort of pregnant women in Neiva in 2006. Cutoff value to be considered positive was ODcalc=.160.

Table 1 shows the number of estimated number of cases of suspected DHF requiring hospitalization in Neiva in 2007 distributed by age. The number of cases in children <1yr of age in Neiva in 2007 was 94. A substantially higher number of DF cases were seen in infants who did not require hospitalization (personal communication), so the minimum sample size should be easily met (see "Study Design" below).

Table 1: 2007 cases of suspected DHF, by age group (n=639, laboratory-confirmed in 472)

Age (in years)	# Males	# Females
<1	***66***	***28***
1-4	75	90
5-14	99	102
15-44	80	55
45-64	10	12
>65	10	11

Study Design

We will conduct a case-control study comparing infants and mothers of the following groups: those with DHF and those with symptomatic DENV infection but without DHF. For appropriate categorization during the analysis phase, all infants will require positive laboratory evidence of acute DENV infection (serology or PCR). Infants with DHF will be defined according to the World Health Organization criteria, which include all of the following: thrombocytopenia, a bleeding tendency, and plasma leakage.

Infants brought by their mothers seeking medical care, and identified by clinical staff as having suspected symptomatic DENV infection, will be screened either in the inpatient or emergency

ward at USCO. Plasma leakage in DHF occurs in the period immediately following defervescence. Therefore, children in the symptomatic DENV cohort will be monitored clinically until after they have been documented to be free of any DHF manifestations 24 hours after defervescence in order to ensure appropriate categorization (see "Categorization of infants into DF versus DHF arms" under Laboratory and Study Procedures" below for further information).

At the initial visit clinical information on both the infant and the mother will be collected, and blood will be drawn from both the mother and infant. Mothers of infants at the outpatient clinic will be given information about warning signs of severe DENV disease and instructed to return to the clinic immediately if the infant develops such symptoms. Hospitalized infants with DHF will be cared for according to standard clinical procedures in place at USCO. To ensure that infants initially categorized in the non-DHF arm do not subsequently develop DHF, the study will involve two visits, the second focusing entirely on the infant's clinical progression, occurring between 48 and 72 hours after the initial visit. Furthermore, the second visit allows for a second blood draw in order to perform the study tests required for accurate diagnosis of acute DENV infection. At the second visit, infants in the outpatient clinic will be re-examined and have blood drawn. Infants at the second visit with ongoing fever will be re-evaluated in a third visit 24-72 hours later, but no further blood will be drawn except as required for standard clinical care. Infants with DHF will be re-evaluated at the second visit, and relevant clinical information (chest X-rays, hematocrit and platelet values, amount of IV fluid administered) will be recorded and additional blood will be drawn. Mothers will have their blood drawn at the first visit only. Figure 3 shows the timeline of visits and the main objectives of each visit.

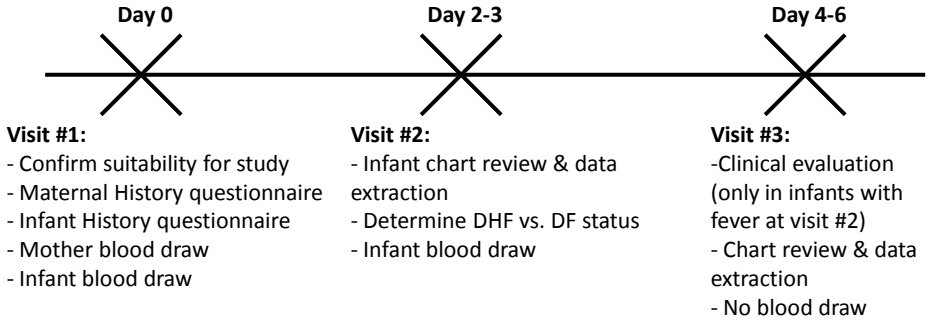

Figure 3. Study Visits

Patient Recruitment

Inclusion criteria include:

•Infants aged 12 months or less and their healthy mothers; these infants belong to two groups:
•Those hospitalized for suspected DHF*; and
•Those sick from suspected infection with dengue but who do not meet the criteria for DHF*;
•Active pregnancy in the mother does not affect inclusion or exclusion criteria.
*Identification of potential study subjects are initially based on clinician suspicion of dengue infection in the case of an acute febrile illness, and are accompanied by laboratory findings of leukopenia, thrombocytopenia, and/or elevated transaminases.

Exclusion criteria include:

•For mothers: any febrile illness since the time of delivery of the infant (to exclude an intervening DENV infection);
•Inability or unwillingness to provide informed consent
•HIV infection.

•For infants: any use of IVIG prior to study inclusion;
•HIV infection.

Clinical and demographic data will be collected on the mothers, including age, past or current medical history (including specifically a history of Yellow Fever vaccination, previous known dengue infection, previous hemorrhagic fever, diabetes, lupus or other autoimmune diseases, asthma, TB and HIV), and medications. Similar information will be collected on the infants; additionally, information on the length of illness, time from symptom onset to presentation, specific treatments or interventions used, serial CBCs and CXRs, serial blood pressure measurements, presence or absence of complications, serial weight and height measurements, and mortality will also be collected.

Laboratory and Study Procedures

Mothers will have 12 ml of blood collected at the first visit only; infants will have no more than the amount defined as minimal risk (see Appendix #3), as measured by infant weight, on two consecutive blood draws between 48 and 72 hours apart.

Serologic testing for both anti-dengue IgM and IgG of all serotypes will be performed on both mothers and infants using ELISA. The maternal IgM will be performed to evaluate for recent acute DENV infection, which could lead to misclassification of infant/mother pairs. To further delineate between DENV-naïve, monotypic and multitypic serostatus, serum plaque-reduction neutralization ($PRNT_{50}$) assays (17) will also be performed on maternal sera. Due to a nationwide vaccination campaign in 2006 in Colombia for the related flavivirus Yellow Fever, ELISA assays for IgG antibodies to Yellow Fever virus will also be utilized, consistent with standard dengue antibody research protocols.

In infants, serum NS1 assays, which have a higher sensitivity during acute infection than ELISA IgM, will also be used. Acute DENV infection in infants will be defined as either a positive anti-DENV IgM, a positive NS1 ELISA, or a positive DENV PCR test. Neutralizing antibody titers will

be interpreted as described in the literature (18). Along with the $PRNT_{50}$ assays, antibody-dependent enhancement assays will be performed following techniques described in the literature (14).

Table 2: Laboratory tests to be run on Mothers and Infants

Mothers	Infants
DENV IgM ELISA	DENV IgM ELISA
DENV IgG ELISA	DENV IgG ELISA
$PRNT_{50}$	DENV PCR
YF IgG ELISA	DENV NS1 ELISA

Categorization of infants into DF versus DHF arms. The WHO criteria for establishing DHF requires the presence of the following four components: **fever**; **hemorrhagic manifestations**, as defined either by a positive tourniquet test, the presence of skin bleeding (either petechiae or ecchymoses), or mucosal bleeding (either epistaxis, menorrhagia, or GI bleeding); **thrombocytopenia** (platelets <100,000 cells/ml); **and evidence of plasma leakage** in the form of pleural effusion, ascites, hypoproteinemia, or hemoconcentration to levels ≥20% baseline values). To ensure all infants included in the DHF arm meet the criteria, a separate Case Report Form will be used documenting that the infant has met the DHF criteria (see Appendix #1).

Data will be recorded on paper case report forms (CRFs; see Appendix 1), which will be kept at USCO. Copies will be transferred to UMass electronically, on a periodic basis for data analysis. The data will be retained on a password protected network drive at UMass.

No specific interventions will occur as part of the study. All medical therapy, including the use of oral or intravenous fluid supplementation, will be administered at the discretion of the attending physician and will follow standard medical care.

Study Objectives

Objective #1: Compare rates of dengue seroprevalence of mothers from two groups of infants: infants with DHF and those with symptomatic DENV infection but without DHF.
To test the main hypothesis, we will determine whether the DENV seroprevalence is significantly higher in mothers of infants with DHF than in mothers of infants without DHF. Based upon estimated seroprevalences of 100% among mothers of infants with DHF and 80% among mothers of infants without DHF, with α of .05 and β of .2, we calculate that the minimal sample size requirement would be 36 in each arm.

The 80% estimate of maternal seroprevalence in the non-DHF arm is highly conservative, with a considerably higher DENV seroprevalence than the 32% seroprevalence observed in the cohort from the maternal pneumococcal vaccination study (Fig. 2). We have used the 80% figure to ensure that the sample size is not an underestimate, and to account for potential discrepancies between current maternal DENV seroprevalence and that found in the cohort from pneumococcal immunization study, including but not limited to the participation of likely seronegative mothers living in surrounding mountainous regions (who are not expected to be

enrolled in our current study at the same rate, if at all), as well as the increase in seroprevalence in the years since the pneumococcal vaccination study. Since the ADE hypothesis requires the presence of declining maternal antibodies as a prerequisite for infant DHF, the seroprevalence in the DHF arm is, by definition, 100 percent.

We propose to recruit in a 1:2 case-control fashion, with a minimum of 50 mother/infant pairs in the DHF group and 100 mother/infant pairs in the symptomatic DENV group, allowing for withdrawals and loss to follow-up. Statistical analysis for the main study objective will be performed using Fisher's exact test to detect differences in proportions.

Objective #2: Test ADE in vitro using the serum from the mothers of both groups of DENV-infected infants (as a surrogate for ADE activity of pre-illness infant sera). Since the ADE hypothesis predicts that infants who develop DHF should have a higher degree of ADE activity than infants who develop symptomatic DF but not DHF, we plan to evaluate the enhancing activity of sera as part of this study. The recent study by Libraty et al (15), which was prospective in nature, allowed for the collection of pre-illness plasma samples from infants and therefore direct testing of enhancement activity from infant samples; this will not be possible in this case-control study. Consequently, maternal sera, diluted in order to estimate infant dengue antibody levels at the age of infection, will be used as a surrogate and tested for enhancing activity by ADE assays as described in the literature (14, 15, 19, 20). We will use the Student's *t*-test for comparisons of mean values between normally distributed continuous variables (assuming normality).

Objective #3: Evaluate the relationship between ADE activity and estimated peak viremia levels. The ADE model also predicts that infants with DHF have higher viral loads than those with DF but not DHF, and it has been demonstrated that higher viral loads are associated with DHF (21, 22). We plan to compare ADE activity and the level of viremia in both groups of infants. While we cannot know with certainty, based on the non-prospective design of this study, the peak viremia level during an infant's illness, we will use viremia levels within 3 days after illness onset as an estimate for peak viremia, consistent with previous studies. If we are unable to obtain infant sera within this time course, we plan to compare viremia levels between infants in both arms categorized by days after onset of illness. Pearson correlation test will be used to measure correlation between ADE activity and estimated peak viral load (assuming normality). Stata™ software will be used for all statistical analysis.

Future Directions & Speculative Areas

One of the potentially unique aspects of this study is the possibility that there will be a serologically heterogenous cohort of mothers, not merely in terms of the binary division of seronegative versus seropositive, but also in that we may be able to identify mothers with monotypic infection. Thus, we can study the influence of maternal antibodies to a particular serotype on subsequent infant infection, e.g. a mother with a $PRNT_{50}$ indicating a history of DENV-2 infection paired with an infant experiencing DENV-3 infection. Based on the overall sample size, the expected low frequency of monotypic immune mothers, the tendency for one or two DENV serotypes to predominate in any given year, and the fact that the number of possible sequential orders of infection is 12, we do not expect to enroll an adequate sample size for all

potential comparisons. Nevertheless, such serologic groups have never before been described to the best of our knowledge.

Additionally, we intend to evaluate the influence of serotype on disease severity. The circulating strains in Neiva are not currently known, but given the estimated time for patient recruitment (approximately 12-20 months), we would expect to have mostly or entirely one circulating serotype. While other studies have compared infants infected with predominantly one serotype (12, 15), this may be the first study to do so with a serologically heterogenous group, allowing for meaningful sub-group analysis.

Patient Protections

All procedures involving patient recruitment (including the use of Spanish-language consent forms), as well as use of Protected Health Information is being done with approval of the Institutional Review Boards of UMass and USCO. The UMass IRB application (docket #13644, approved 2/2/10), can be found in Appendix #2. As part of ongoing studies, all investigators and relevant personnel at UMass and USCO have taken the CITI Responsible Conduct of Research Course.

Proposed Timeline

Figure 4 illustrates the proposed timeline. Patient recruitment began in May 2010 and is expected to last approximately 15 months. Formal data analysis will begin in early 2011 and is expected to take approximately one year's time. Manuscript(s) will be prepared following the completion of all data analysis.

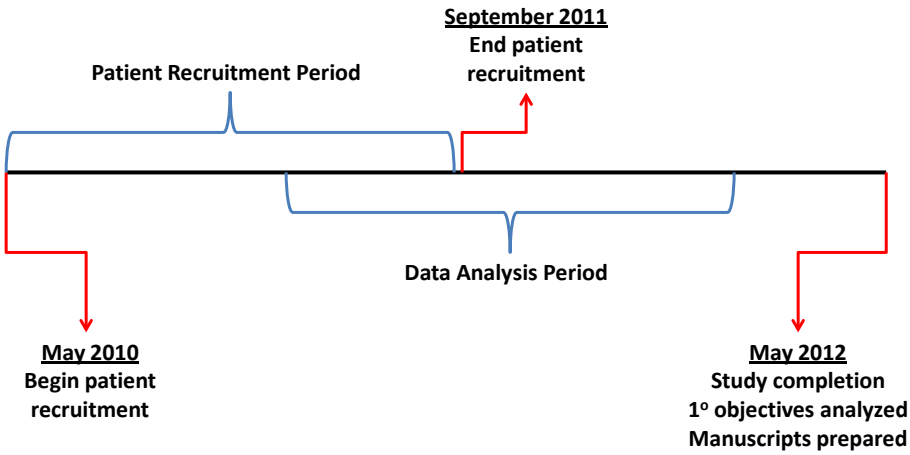

Figure 4. Study Timeline

References

1. Gubler, D. J. 2002. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol* 10:100-103.
2. Yauch, L. E., and S. Shresta. 2008. Mouse models of dengue virus infection and disease. *Antiviral research* 80:87-93.
3. Thomas, S. J., J. Hombach, and A. Barrett. 2009. Scientific consultation on cell mediated immunity (CMI) in dengue and dengue vaccine development. *Vaccine* 27:355-368.
4. Kyle, J. L., and E. Harris. 2008. Global spread and persistence of dengue. *Annu Rev Microbiol* 62:71-92.
5. Braga, C., C. F. Luna, C. M. Martelli, W. V. Souza, M. T. Cordeiro, N. Alexander, M. D. Albuquerque, J. C. Junior, and E. T. Marques. 2009. Seroprevalence and risk factors for dengue infection in socioeconomically distinct areas of Recife, Brazil. *Acta Trop*.
6. Sabin, A. B. 1952. Research on dengue during World War II. *The American journal of tropical medicine and hygiene* 1:30-50.
7. Sangkawibha, N., S. Rojanasuphot, S. Ahandrik, S. Viriyapongse, S. Jatanasen, V. Salitul, B. Phanthumachinda, and S. B. Halstead. 1984. Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. *Am J Epidemiol* 120:653-669.
8. Halstead, S. B., J. E. Scanlon, P. Umpaivit, and S. Udomsakdi. 1969. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. IV. Epidemiologic studies in the Bangkok metropolitan area. *The American journal of tropical medicine and hygiene* 18:997-1021.
9. Halstead, S. B., J. S. Chow, and N. J. Marchette. 1973. Immunological enhancement of dengue virus replication. *Nat New Biol* 243:24-26.
10. Jain, A., and U. C. Chaturvedi. Dengue in infants: an overview. *FEMS immunology and medical microbiology*.
11. Nguyen, T. H., H. Y. Lei, T. L. Nguyen, Y. S. Lin, K. J. Huang, B. L. Le, C. F. Lin, T. M. Yeh, Q. H. Do, T. Q. Vu, L. C. Chen, J. H. Huang, T. M. Lam, C. C. Liu, and S. B. Halstead. 2004. Dengue hemorrhagic fever in infants: a study of clinical and cytokine profiles. *The Journal of infectious diseases* 189:221-232.
12. Kliks, S. C., A. Nisalak, W. E. Brandt, L. Wahl, and D. S. Burke. 1989. Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. *The American journal of tropical medicine and hygiene* 40:444-451.
13. Chau, T. N., N. T. Quyen, T. T. Thuy, N. M. Tuan, D. M. Hoang, N. T. Dung, B. Lien le, N. T. Quy, N. T. Hieu, L. T. Hieu, T. T. Hien, N. T. Hung, J. Farrar, and C. P. Simmons. 2008. Dengue in Vietnamese infants--results of infection-enhancement assays correlate with age-related disease epidemiology, and cellular immune responses correlate with disease severity. *The Journal of infectious diseases* 198:516-524.
14. Laoprasopwattana, K., D. H. Libraty, T. P. Endy, A. Nisalak, S. Chunsuttiwat, D. W. Vaughn, G. Reed, F. A. Ennis, A. L. Rothman, and S. Green. 2005. Dengue Virus (DV) enhancing antibody activity in preillness plasma does not predict subsequent disease severity or viremia in secondary DV infection. *The Journal of infectious diseases* 192:510-519.
15. Libraty, D. H., L. P. Acosta, V. Tallo, E. Segubre-Mercado, A. Bautista, J. A. Potts, R. G. Jarman, I. K. Yoon, R. V. Gibbons, J. D. Brion, and R. Z. Capeding. 2009. A prospective nested case-control study of Dengue in infants: rethinking and refining the antibody-dependent enhancement dengue hemorrhagic fever model. *PLoS Med* 6:e1000171.
16. Simmons, C. P., T. N. Chau, T. T. Thuy, N. M. Tuan, D. M. Hoang, N. T. Thien, B. Lien le, N. T. Quy, N. T. Hieu, T. T. Hien, C. McElnea, P. Young, S. Whitehead, N. T. Hung,

and J. Farrar. 2007. Maternal antibody and viral factors in the pathogenesis of dengue virus in infants. *The Journal of infectious diseases* 196:416-424.

17. Roehrig, J. T., J. Hombach, and A. D. Barrett. 2008. Guidelines for Plaque-Reduction Neutralization Testing of Human Antibodies to Dengue Viruses. *Viral immunology* 21:123-132.
18. Endy, T. P., A. Nisalak, S. Chunsuttitwat, D. W. Vaughn, S. Green, F. A. Ennis, A. L. Rothman, and D. H. Libraty. 2004. Relationship of preexisting dengue virus (DV) neutralizing antibody levels to viremia and severity of disease in a prospective cohort study of DV infection in Thailand. *The Journal of infectious diseases* 189:990-1000.
19. Moi, M. L., C. K. Lim, A. Kotaki, T. Takasaki, and I. Kurane. Development of an antibody-dependent enhancement assay for dengue virus using stable BHK-21 cell lines expressing Fc gammaRIIA. *Journal of virological methods* 163:205-209.
20. Sadon, N., A. Delers, R. G. Jarman, C. Klungthong, A. Nisalak, R. V. Gibbons, and V. Vassilev. 2008. A new quantitative RT-PCR method for sensitive detection of dengue virus in serum samples. *Journal of virological methods* 153:1-6.
21. Libraty, D. H., T. P. Endy, H. S. Houng, S. Green, S. Kalayanarooj, S. Suntayakorn, W. Chansiriwongs, D. W. Vaughn, A. Nisalak, F. A. Ennis, and A. L. Rothman. 2002. Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. *The Journal of infectious diseases* 185:1213-1221.
22. Libraty, D. H., P. R. Young, D. Pickering, T. P. Endy, S. Kalayanarooj, S. Green, D. W. Vaughn, A. Nisalak, F. A. Ennis, and A. L. Rothman. 2002. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *The Journal of infectious diseases* 186:1165-1168.

(APPENDIX #1: CASE REPORT FORM #1)

Estudio de las Mamas y Infantes con dengue en Neiva

Información de los infantes

Criterios para DHF

(Versión: 26/03/2010)

SECCIÓN A: INFORMACIÓN GENERAL		
A1	Número de identificación (coloque la etiqueta)	__ __ __ - __ __ __
A2	Fecha de la admisión (dd/mm/aaaa)	__ __ / __ __ / __ __ __ __
A3	Fecha del inclusión en el estudio	__ __ / __ __ / __ __ __ __
A4	Iniciales de la persona que completa la forma	__ __ __ __
SECCIÓN B: CRITERIOS DE DIAGNOSIS DE DENGUE HEMORRAGICO (CRITERIA FOR DIAGNOSIS OF DHF [DATA ABSTRACTED FROM HOSPITAL CHART])		
B1	Sospecha de infección de dengue? (Suspicion for dengue infection?)	No ○ Sí ○
B2	Trombocitopenia (<100,000)? (Thrombocytopenia.)	No ○ Sí ○
B3	Hemorragia (Hemorrhage? If so, where?)	Ausente ○ Presente ○ Sitio: _____
B4	Presión arterial (ahora) (Current BP.)	Sistólica- __ __ __ mm Hg Diastólica- __ __ __ mm Hg
B5	Presión arterial (mínimum) (Lowest BP.)	Sistólica- __ __ __ mm Hg Diastólica- __ __ __ mm Hg
B6	Prueba de torniquete hecho (Was a tourniquet test done?)	No ○ Si ○
B7	Brazo de la prueba (Which arm was the test done, L or R?)	Derecho ○ Izquierdo ○
B8	Resultado cualitativo según el médico (Qualitative result according to MD)	Negativo ○ Positivo ○
B9	Cantidad de las petequias en el patrón (Si la cantidad está mas que 20, marque '21'.) (Amount of petechiae in area; if >20, mark	__ __

Número de identificación: __ __ __ - __ __ __

	'21'.)	
B10	Hematócrito (mínimum) (Minimum hematocrit.)	__ __ __ __
B11	Hematócrito (máximum) (Maximum hematocrit.)	__ __ __ __
B12	Presencia del derrame pleural (Presence of pleural effusion.)	Ausente ◯ Presente ◯
B13	Presencia de ascitis (Presence of ascites.)	Ausente ◯ Presente ◯

(APPENDIX #1: CASE REPORT FORM #2)

Estudio de las Mamas y Infantes con dengue en Neiva

Información de los infantes

(Versión:03/08/2010)

SECCIÓN A: INFORMACIÓN GENERAL		
A1	Número de identificación (coloque la etiqueta)	__ __ __ - __ __ __
A2	Fecha de la visita (dd/mm/aaaa)	__ __ / __ __ / __ __ __ __
A3	Fecha de la admisión (dd/mm/aaaa)	__ __ / __ __ / __ __ __ __
A4	Iniciales de la persona que completa la forma	__ __ __ __
A5	Localización	Hospital ◯ Clínica ◯

SECCIÓN B: CRITERIOS DE INCLUSIÓN (INCLUSION CRITERIA)		
B1	Edad <12 meses (Age <12 months)	No ◯ Sí ◯
B2	¿Temperatura ≥ 38.5°C o fiebre subjetiva en las últimas 5 días? (Temp >38.5 or subjective fever in the past 5 days?)	No ◯ Sí ◯
B3	Fecha inicio de fiebre (dd/mm/aaaa) (Date of fever onset.)	__ __ / __ __ / __ __ __ __
B4	¿Uno o más de los síntomas característicos (dolor retro-ocular o cefalea, mialgias, y erupción maculopapular)? (One or more of the following characteristic symptoms: retro-orbital pain or headache, myalgias, and maculopapular rash?)	No ◯ Sí ◯
B5	¿Uno o más de los signos de alarma: disminución brusca de la temperatura (hipotermia), dolor abdominal intenso, sangrado en encías/nariz/piel o otros sitios?	No ◯ Sí ◯

Número de identificación: __ __ __ - __ __ __

	(One or more of the following 'alarm signs': hypothermia, intense abdominal pain, bleeding in gums, nose, skin, or other sites?)	

SECCIÓN C. CRITERIOS DE EXCLUSIÓN (EXCLUSION CRITERIA)

C1	Edad >12 meses (Age >12 months)	No ◯ Sí ◯
C2	Uso de inmunoglobulina G en el neonato (Use of immunoglobulin G as a neonate?)	No ◯ Sí ◯

SECCIÓN D: DATOS DEMOGRÁFICOS (DEMOGRAPHIC DATA)

D1	Edad (meses y semanas) (Age, months & weeks.)	__ __meses __ __semanas
D2	Fecha de nacimiento (dd/mm/aaaa) (Date of birth.)	__ __ / __ __ / __ __ __ __
D3	Sexo (Sex, M/F.)	Masculino ◯ Feminino ◯

SECCIÓN E: FACTORES DE RIESGO (RISK FACTORS)

E1	Presencia de floreros (Presence of planters/flowerpots.)	No ◯ Sí ◯
E2	Presencia de matas sembradas en agua (Presence of bushes growing in wáter basins.)	No ◯ Sí ◯
E3	Presencia de recipientes al aire libre (Presence of open-air containers.)	No ◯ Sí ◯
E4	Criaderos en la casa o en los vecinos (Breeding areas in the house or vicinity.)	No ◯ Sí ◯

Número de identificación: ___ ___ ___ - ___ ___ ___

E5	Presencia de mosquitos en el área (Presence of mosquitos in the area?)	No ◯	Sí ◯
E6	Uso de tambores, pipas, o pipotes en la casa (Use of various types of wáter-holding containers in the house?)	No ◯	Sí ◯
E7	Suministro de agua en forma continua (Running wáter?)	No ◯	Sí ◯
E8	Almacena el agua en la casa (Water storage in the house?)	No ◯	Sí ◯
E9	Disponibilidad de recolección de basura (Availability of garbage collection?)	No ◯	Sí ◯
E10	Hay neumáticos o cauchos en la casa (Are there tires or other rubber tubes in the house?)	No ◯	Sí ◯
E11	Uso de tela metálica en ventanas (Are there screens in the windows?)	No ◯	Sí ◯
E12	Uso de insecticidas en la casa (Are insecticides used in the house?)	No ◯	Sí ◯
E13	Uso de repelente (Are insect repellents used?)	No ◯	Sí ◯
E14	Cerca a su casa hay llanterías, carros abandonados, pozos, construcciones (Are there wells, abandoned cars, or construction areas near the house?)	No ◯	Sí ◯
SECCIÓN F: SINTOMAS DE LA ENFERMEDAD (SYMPTOMS OF ILLNESS)			
F1	Malestar general (Malaise)	Ausente ◯	Presente ◯

Número de identificación: __ __ __ - __ __ __

F2	Astenia (Asthenia)	Ausente ○	Presente ○
F3	Fatiga (Fatigue)	Ausente ○	Presente ○
F4	Cansancio (Sleepiness)	Ausente ○	Presente ○
F5	Irritabilidad (Irritability)	Ausente ○	Presente ○
F6	Anorexia (Weight loss)	Ausente ○	Presente ○
F7	Nauseas (Nausea)	Ausente ○	Presente ○
F8	Vómitos ocasionales (Occasional vomiting)	Ausente ○	Presente ○
F9	Vómitos persistentes (Persistent vomiting)	Ausente ○	Presente ○
F10	Diarrea (Diarrhea)	Ausente ○	Presente ○
F11	Tos (Cough)	Ausente ○	Presente ○
F12	Rinorrea (Runny nose)	Ausente ○	Presente ○
F13	Disnea (Shortness of breath)	Ausente ○	Presente ○
F14	Diaforesis (Sweats)	Ausente ○	Presente ○
F15	Escalofríos (Chills)	Ausente ○	Presente ○
F16	Erupción (Rash)	Ausente ○	Presente ○
F17	Hematomas (Bruises)	Ausente ○	Presente ○
F18	Epistaxis (Nosebleed)	Ausente ○	Presente ○
F19	Hemoptisis (Hemoptysis)	Ausente ○	Presente ○
F20	Hematemesis	Ausente ○	Presente ○
F21	Melena	Ausente ○	Presente ○
F22	Hematuria	Ausente ○	Presente ○

SECCIÓN G: HISTORIA MÉDICA (MEDICAL HISTORY)

G2	Amamantando (Nursing?)	Ausente ○	Presente ○
G3	Hemofilia (Hemophilia?)	Ausente ○	Presente ○
G4	Otro tipo de hemorragia (Other hemorrhagic condition?)	Ausente ○	Presente ○
G6	SIDA (AIDS?)	Ausente ○	Presente ○
G8	Asma (Asthma?)	Ausente ○	Presente ○
G9	Diabetes mellitus	Ausente ○	Presente ○

Número de identificación: __ __ __ - __ __ __

SECCIÓN H: USO DE MEDICAMENTOS DURANTE LA ENFERMEDAD		
H1	Acetaminofen	No ◯ Sí ◯
H2	Aspirina	No ◯ Sí ◯
H3	Anti-inflamatorio no esteroide (NSAIDS)	No ◯ Sí ◯
H4	Corticoesteroides (Corticosteroids)	No ◯ Sí ◯
H8	Otros (Other; if so, specify.)	No ◯ Sí ◯ Especificar: _____
SECCIÓN I: EXPOSICIÓN A FLAVIVIRUSD		
I3	Otros casos de dengue en la familia en los últimos 14 días (Other cases of dengue in the family in the past 14 days?)	No ◯ Sí ◯
SECCIÓN J: HISTORIA DE VIAJES (TRAVEL HISTORY)		
J1	Ha viajado en los últimos 14 días (Has the infant traveled in the past 14 days? If so, where?)	No ◯ Sí ◯ Dónde: _____
SECCIÓN K: EXÁMEN FÍSICO (PHYSICAL EXAM)		
K1	Temperatura oral (Temp, oral)	__ __ , __ °C
K2	Talla (Height)	__ , __ __ M
K3	Peso (Weight)	__ __ __ , __ kg
K4	Frecuencia cardiaca (Heart rate, BPM)	__ __ __ latidos/minuto
K5	Presión arterial (Blood pressure)	Sistólica- __ __ __ mm Hg Diastólica- __ __ __ mm Hg
K6	Frecuencia respiratoria (Respiratory rate)	_____ respiraciones/minuto
K7	Letárgia (Lethargy)	Ausente ◯ Presente ◯
K8	Ictericia (Icterus)	Ausente ◯ Presente ◯
K9	Erupción (Rash)	Ausente ◯ Presente ◯
K10	Petequias (Petechiae)	Ausente ◯ Presente ◯
K11	Hematomas/equimosis (Hematomas or	Ausente ◯ Presente ◯

Número de identificación: __ __ __ - __ __ __

	ecchymoses)	
K12	Hemorragia (Frank hemorrhage; if so, where)	Ausente ○ Presente ○ Sitio: _____
K13	Derrame pleural (Pleural effusion)	Ausente ○ Presente ○
K14	Dolor abdominal a la palpación (Abdominal pain on palpation)	Ausente ○ Presente ○
K15	Ascitis (Ascites)	Ausente ○ Presente ○
K16	Hepatomegalia (Hepatomegaly)	Ausente ○ Presente ○
K17	Esplenomegalia (Splenomegaly)	Ausente ○ Presente ○
K18	Edema	Ausente ○ Presente ○
SECCIÓN L: PRUEBA DE TORNIQUETE (TOURNIQUET TEST)		
L1	Prueba de torniquete hecho (Has a tourniquet test been done?)	No ○ Si ○
L2	Brazo de la prueba (On which arm was the test done?)	Derecho ○ Izquierdo ○
L3	Resultado cualitativo según el médico (Qualitative results according to MD?)	Negativo ○ Positivo ○
L4	Cantidad de las petequias en el patrón (Si la cantidad está mas que 20, marque '21'.) (Amount of petechiae in area; if >20, mark '21'.)	__ __
SECCIÓN M: TOMA DE MUESTRA (BLOOD SAMPLE DRAW)		
M1	Hora de la toma (Time of draw)	__ __ __ __ h
M2	Identificación de la muestra (coloque el etiqueta) (Place ID number tag here.)	__ __ __ - __ __ __ __
SECCIÓN N: DISPOSICIÓN DEL PACIENTE (PATIENT DISPOSITION)		
N1	Disposición del paciente (Disposition of the infant: outpatient, hospitalized, referred, place where pt was referred)	Ambulatorio ○ Hospitalizado ○ Remitido ○ Lugar a donde fue remitido: _____

Número de identificación: __ __ __ - __ __ __

DATOS DEL REGISTRO EN LA BASE DE DATOS (DATA FOR REGISTRY IN DATABASE)

(Solo para completar por personal admistrativo del estudio)

(To be completed only by study personnel)

Fecha del registro (dd/mm/aaaa): __ __ / __ __ / __ __ __ __ (Date of registration)

Iniciales de la persona que ingresa los datos: __ __ __ __ (Initials of person entering data)

Fecha de la verificación de los datos ingresados (dd/mm/aaaa): __ __ / __ __ / __ __ __ __

Iniciales de la persona que verifíca los datos: __ __ __ __

(Date of verification of data entry & initials)

(APPENDIX #1: CASE REPORT FORM #3)

Estudio de las Mamás y Infantes con dengue en Neiva

Información de las Mamás

(Versión: 26/03/2010)

SECCIÓN A: INFORMACIÓN GENERAL (GENERAL INFORMATION)		
A1	Número de identificación (coloque la etiqueta) (ID tag #)	__ __ __ - __ __ __
A2	Fecha de la visita (date of visit) (dd/mm/aaaa)	__ __ / __ __ / __ __ __ __
A3	Iniciales de la persona que completa la forma	__ __ __ __(initials of person completing form)
SECCIÓN B: CRITERIOS DE INCLUSIÓN (INCLUSION CRITERIA)		
B1	¿Firma de la hoja de consentimiento?	No ◯ Sí ◯
	(Did the person provide signed consent?)	
SECCIÓN C. CRITERIOS DE EXCLUSIÓN (EXCLUSION CRITERIA)		
C1	¿Temperatura ≥ 38.5°C o fiebre subjetiva desde el nacimiento del bebé?	No ◯ Sí ◯
	(Temp >38.5 or subjective fever since the birth of the baby.)	
SECCIÓN D: DATOS DEMOGRÁFICOS (DEMOGRAPHIC DATA)		
D1	Edad (años) Age, years	__ __
D2	Fecha de nacimiento del bebé (dd/mm/aaaa) (Date of birth of infant)	__ __ / __ __ / __ __ __ __
D3	Zona de residencia (código) (Residential Zone)	__ __ __
SECCIÓN G: HISTORIA MÉDICA (MEDICAL HISTORY)		
G1	Antecedentes de dengue (History of dengue infection; if yes, what year)	No ◯ Sí ◯ ¿Cuándo? (año)_____
G2	¿Enfermedad febril durante en embarazo? (Did you have a febrile illness during your	No ◯ Sí ◯

Número de identificación: __ __ __ - __ __ __

	pregnancy?)	
G3	¿Vacunación previa fiebre amarilla? (Have you been vaccinated against Yellow Fever? If yes, what year?)	No ◯ Sí ◯ ¿Cuándo? (año)_____
G4	SIDA (Does the mother have AIDS?)	Ausente ◯ Presente ◯
G5	Otros casos de dengue en la familia en los últimos 14 días (Are there other cases of dengue in the family in the past 14 days?)	No ◯ Sí ◯
SECCIÓN J: HISTORIA DE VIAJES (TRAVEL HISTORY)		
J1	Ha viajado en los últimos 14 días (Have you traveled in the past 14 days? If yes, where?)	No ◯ Sí ◯ Dónde: _____
SECCIÓN M: TOMA DE MUESTRA (BLOOD SAMPLE DRAWING)		
M1	Hora de la toma (Time of draw)	__ __ __ __ h
M2	Identificación de la muestra (coloque el etiqueta) (Place ID tag number here.)	__ __ __ - __ __ __ __
SECCIÓN N: DISPOSICIÓN DEL PACIENTE (PATIENT DISPOSITION)		
N1	Disposición del bebé (Disposition of the infant: outpatient, hospitalized, referred, place where pt was referred)	Ambulatorio ◯ Hospitalizado ◯ Remitido ◯ Lugar a donde fue remitido: _____

DATOS DEL REGISTRO EN LA BASE DE DATOS (DATA FOR REGISTRY IN DATABASE)

(Solo para completar por personal admistrativo del estudio)

(To be completed only by study personnel)

Número de identificación: __ __ __ - __ __ __

Fecha del registro (dd/mm/aaaa): __ __ / __ __ / __ __ __ __ (Date of registration)

Iniciales de la persona que ingresa los datos: __ __ __ __ (Initials of person entering data)

Fecha de la verificación de los datos ingresados (dd/mm/aaaa): __ __ / __ __ / __ __ __ __

Iniciales de la persona que verifíca los datos: __ __ __ __

(Date of verification of data entry & initials)

(APPENDIX #2: IRB APPLICATION)

APPLICATION FOR APPROVAL OF UMMS HUMAN STUDIES
UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL

NOTICE TO INVESTIGATOR

THE PRINCIPAL INVESTIGATOR IS RESPONSIBLE FOR THE CONTENT OF THIS APPLICATION AND *MUST* PROOF READ THE FINAL VERSION OF THE APPLICATION FORM BEFORE IT IS SUBMITTED. Errors in the application reflect poorly on the PI's oversight of the research. **FAILURE TO ADEQUATELY REVIEW THE APPLICATION WILL PUT THE STUDY AT HIGH RISK OF BEING TABLED UNTIL THE NEXT MEETING.**

Before the IRB meeting deadline, you must submit **ONE COPY** of the completed IRB application, including all 7 of the sections. All signatures and attachments **MUST** be in place. **The application and consent form must be numbered.** This packet will be pre-reviewed in the Human Subjects Office and returned to you. Copies should not be made until the pre-review is completed.

This administrative review is done to prevent receiving applications that are poorly prepared and unacceptable to the Committee. You are urged to prepare this application and consent form carefully. The two Human Subjects Committees review 10-20 protocols a month. The Committees are composed of individuals who donate a considerable amount of their time to this effort, and your careful attention to accurate, complete information and grammar are fully anticipated. The Human Subjects staff and the IRB reviewers will return the submission to you if this is not the case, resulting in an unnecessary delay in study review and your anticipated study initiation

INSTRUCTIONS

RADIATION OR DNA/CELL LINES

If the subjects receive any radiation, please contact the Radiation Safety Committee (RSC) at 508-856-3208 to discuss the possibility of RSC review. If RSC review is needed, IRB approval will not be given until RSC approval has been obtained.

If the subjects receive any rDNA vaccines, retroviruses, adenovirus derivative vectors, or autologous modified tissue, please contact the Institutional Biosafety Committee (IBC) at 508-856-5416 to discuss the possibility of IBC review and registration. If laboratory personnel will be involved with rDNA vaccines, retroviruses, adenovirus derivative vectors, or autologous modified tissue this must also be registered with the IBC. If review is needed, IRB approval will not be given until IBC approval has been obtained.

SIGNATURES

1. The PI signs Section III and Section VII the Informational Drug Data Form (IDDF).
2. The Chair and the Chief of the PI's Department/Division sign Section IV.
3. Additional signatures may also be required in section IV, please review.
3. All Research Personnel, including the PI, sign Section VI.

PROCEDURES FOR SUBMITTING A COMPLETED APPLICATION

If you are unsure about the type of review required by your study, or are inexperienced in completing IRB applications, it is strongly recommended that you provide a copy of a reasonably complete draft version. A substantive preliminary review by the Human Subjects Office will be performed. This preliminary review gives you the opportunity to address issues before the meeting and will save you time in the long run. Obviously, this review must be done well in advance of the IRB meeting deadline.

EXPEDITED REVIEW

If the study qualifies for Expedited Review (determined by the Human Subjects Office after review) the original and three copies of the final version of the application will be required. Two Committee members will review the protocol. This process usually takes approximately three weeks.

FULL COMMITTEE REVIEW

If the full Committee must review the study, the original and twenty copies will be needed (one for each member of the Committee). **Please note that the original copy of the full application and consent form must be sent to the Human Subjects Office for initial administrative review before the Principal Investigator makes twenty copies for the Committee.**

Meetings are scheduled for the first and third Tuesday of each month at 4:00 P.M. (except for the months of July and August when the Committee meets once each month). Meeting dates and deadline dates are available on our web page and are subject to change.

Each protocol is reviewed by two committee members prior to the meeting, and the investigator may be contacted to respond to concerns. You will be notified of the date and location of the meeting. Most Principal Investigators do not have to attend the meeting, but you are asked to be on call via your pager or telephone between the meeting hours of 4-6 p.m.

AMENDMENTS

The Human Subjects Office or the IRB must review any amendment or change in a protocol or consent form. No changes may be instituted until the investigator has received written approval of the revision from the Committee.

YEARLY REVIEW AND REAPPROVAL

Approved studies must receive re-approval at least once a year and more often if required by the Committee. A notice will be sent to you before the re-approval is due; approximately 2 months prior to the expiration date. Re-approval must occur within 30 days of the expiration date and appropriate planning must take place to meet this required deadline.

Please contact the Human Subjects Office at 856-4261 if you need additional information.

CONTENTS OF THIS APPLICATION

SECTION I

Before this application is submitted to the Research Subjects Office, the following must be done. P ease indicate by stating “YES” OR “N/A” (not applicable) that you have reviewed the packet and have accomplished these tasks as they apply to your study.

IN THE APPLICATION SECTION

_____ Completed Protocol Summary Sheet Section II

_____ Completed & obtained signatures on the P.I.’s Assurance Section III and Obtained signed agreement forms from all cooperating faculty Section V and departments Section VII.

_____ Obtained approval from Radiation Safety Committee or submitted protocol to the RSC

_____ Provided the Investigational New Drug (IND #) on the Protocol Summary Sheet Section II

_____ If the study is grant funded and you need additional information contact the Research Funding Office at 508-856-2119 . If the study is industry supported, please contact the Office of Clinical Research at 508-856-5015

_____ Provided 1 copy of the Sponsor Protocol or the “body” of the research grant (e.g. sections a through e of the Research Plan of an NIH grant). HUMAN SUBJECTS USE MUST BE IDENTICAL IN GRANT/COMPANY PROTOCOL AND IRB APPLICATION.

_____ Provided 1 copy of the Investigator’s Drug Brochure

_____ Numbered the pages of the Protocol body.

_____ Obtained approval from the Institutional Biosafety Committee (IBC) or submitted the protocol to the IBC Committee for review.

IN THE CONSENT FORM

_____ Indicated that subjects will sign a written consent form.

_____ Provided a consent form in standard UMMS format

_____ Wrote the consent form in the second person and at a 7th grade level.

_____ Numbered the pages of the consent form appropriately. (e.g. Page 1 of 4, Page 2 of 4)

_____ Indicated that verbal consent will be obtained if written consent is not being obtained.

_____ Provided a fact sheet for the patient. (A fact sheet should be included for complex, lengthy, or high risk studies.)

SECTION II
PROTOCOL SUMMARY SHEET

Today's Date:	December 14, 2009		
P.I. Name:	**Steven Hatch**	Degree:	MD
(PI Must be UMMS Faculty Member)		Faculty Title:	Instructor
Department:	Center for Infectious Disease and Vaccine Research		
Division Name:		Duration of the Study:	18 months
Phone #	508-856-4657	Total # of subjects at UMMHC:	None
Beeper/Pager#:	9426	Total # of subjects at off-site locations :	300
Email Address:	steven.hatch@umassmed.edu		
Title of Study: (type right) (Protocol # and version)	**Maternally Derived Anti-Dengue Antibodies and the Risk of Dengue Hemorrhagic Fever in Infants: a Case-Control Study**		

				"X" below which sites will participate	
Contact Person Name	Steven Hatch	Phone #	6-4657	University:	X
Pager #	9426			Memorial :	
Identify Condition being studied:	Dengue Infection (DF/DHF)			Marlborough:	
				Shriver Center:	
Source of Funding:	Departmental funds			Others:	

DEVICE INFORMATION

Please provide IDE# if not approved by FDA

Device Name	Approved	Investigational	IDE#

DRUG INFORMATION

In the table below, list all drugs being used. If the drug is considered investigational by the FDA you must include the IND# assigned by the FDA. Please "X" approved or investigational.

USE SPACE BELOW FOR COMMENTS OR ADDITIONAL DRUG INFORMATION

Drug Name:	Approved	Inves.	IND#

DECRIBE THE RESEARCH BY CHECKING ALL THE ITEMS "YES" OR "NO"

(NB: Pregnant Women and Teenage Mothers are eligible though not targeted for study, and so are included in parentheses.)**

Yes	No		Yes	No		Yes	No	
	x	On Site at UMMS/UMMMC	x		Adults		x	Questionnaires (please provide)
x		Multicenter Study	(x)		Pregnant Women		x	Filming/video/audio
x		Cooperating Institutions	x		Minors (under 18)		x	Marketed drugs
x		Research Currently Funded	(x)		Teenagers (12-17)		x	Diagnostic Radiation
	x	Financial interest involved		x	Prisoners		x	Therapeutic Radiation
	x	Funding applied for		x	Fetuses / Abortuses		x	MRI
	x	UMMMC inpatients		x	Randomization		x	Ultrasound
	x	UMMMC outpatients		x	Placebo		x	Radioisotopes
x		Normal volunteers		x	Investigational drugs/device		x	Radiation involved?
x		Other		x	Increased hospital costs		x	Would receive radiation regardless
x		Males		x	Mental Impairment		x	Radiation Safety Approval Needed?
x		Females		x	Data bank		x	Biosafety Review Needed?
	x	Phase I Study		x	Phase II Study		x	Phase III Study

SECTION III
PRINCIPAL INVESTIGATOR'S ASSURANCE

As Principal Investigator for this study, I acknowledge and accept my responsibility, as mandated by the UMMS Assurance of Compliance for Protecting the rights and welfare of the human subjects taking part in this research study.

Assuring that the risks to an individual are outweighed by the potential benefits to him/her or by the importance of the knowledge to be gained.

Complying with all the applicable requirements specified by the UMMS Institutional Review Board as a condition of IRB approval.

Completing the required education either by reading the Guidelines for the Protection of Human Subjects in Research and subsequently answering at least 25 out of 28 questions correctly on the true/false, multiple choice, Human Subjects Exam **or** by completing the required modules for the CITI Course in the Protection of Human Research Subjects.

Providing each research subject with a signed copy of the IRB-approved consent form at the time of consent.

Retaining the original signed forms in a reasonably secure and confidential area for at least three years after termination of the research project.

Obtaining approval from the UMMS IRB of any proposed changes in a previously approved study. The proposed changes will not be implemented before IRB review and approval, unless necessary to eliminate apparent immediate hazards to subjects.

Informing the IRB immediately if I become aware of any violations of HHS regulations (45CFR46), FDA regulations (21CFR50, 56) or IRB requirements for the protection of human subjects.

Submitting progress reports of approved research as often as, and in the manner prescribed by, the UMMS IRB (the frequency of these will be on the basis of risk to subjects, but will be at least annually).

Within 48 hours (in-house events) or five working days (sponsor-reported events) report any unanticipated serious adverse experiences, injuries, and other unanticipated problems that involve risks to subjects and others, either physical, psychological, or threats to privacy.

Reporting any research subject's death within five working days, regardless of cause.

Understanding that the failure to comply with all applicable HHS and FDA regulations, IRB requirements/policies, and the provisions of the protocol as approved by the IRB may result in suspension or termination of my research project.

The Principal Investigator's signature <u>must</u> be obtained before submitting.

Signature of Principal Investigator: _____ Date: _____

Type PI name and title: Steven Hatch, MD

SECTION IV

DEPARTMENTAL / DIVISIONAL APPROVAL

I have reviewed the attached research project for both ethical considerations and technical merit and recommend its approval.

I certify that there are adequate resources and facilities to carry out this research, including staff, funding, space, recordkeeping capability, and resources to address serious adverse events and possible research-related injuries.

Signature of PI's Department Chair: _____ Date:_____

Type Name and title of Chair: Francis Ennis, MD

Signature of PI's Division Chief: _____ Date:_____

Type Name and title of Division Chief: _____

Will this research involve faculty or recruit patients from another department besides the department listed above?

Yes No

If yes, please complete

MY SIGNATURE BELOW INDICATES THAT I AM AWARE OF AND AGREE TO INVOLVE MY DEPARTMENT IN THIS RESEARCH PROJECT

Department Name: _____

Department Chair Name: _____

Signature of Department Chair: _____ Date: _____

Please "X" boxes below that apply

Faculty from my department will be involved in this research study
Patients from my department will be involved in this research study

The section above may be duplicated if there is more than one additional department or faculty member from another department that is participating in this study.

SECTION V
DESCRIPTION OF RESEARCH PROJECT

1. PERSONNEL ENGAGED IN THE RESEARCH STUDY. List all personnel engaged in the study. This list must agree with that in Section VI (Delegation of roles/responsibilities).
Steven Hatch, Alan Rothman

2. GENERAL STATEMENT OF PROBLEM
Purpose: Include concise hypothesis to be tested by proposed research.

This study proposes to directly test the hypothesis that antibody-dependent enhancement (ADE) is the critical factor in the development of dengue hemorrhagic fever (DHF) in infants. DHF is the life-threatening syndrome associated with dengue virus (DENV) infection. The increasing incidence makes an accurate model of DHF pathogenesis imperative for vaccine development, clinical management and public health strategies. The primary study aim will be to test the hypothesis by comparing the rates of dengue seroprevalence of mothers from two groups of infants: infants with DHF and those with symptomatic DENV infection but without DHF. Secondary aims include evaluating the relationship between viral load, serotype, and disease severity in dengue infected infants; and testing ADE in vitro using the serum from the mothers of both groups of dengue infected infants (as a surrogate for ADE activity of pre-illness infant sera).

3. BACKGROUND AND SIGNIFICANCE:
a. Provide a summary of the facts which led to selection of the problem.

DENV infection is the most common mosquito-borne viral infection in the world today, and has been an increasing public health menace over the past few decades. Currently there are an estimated 100-150 million annual infections, with an annual mortality of >20,000, the overwhelming majority of deaths being in infants and children [1]. DENV is a member of the flavivirus family, with four distinct but closely related serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. Transmitted to humans primarily by Aedes aegypti mosquitoes, DENV is found mostly in tropical and subtropical areas worldwide. Classic dengue fever (DF) presents as an acute febrile illness characterized by headache, retro-orbital pain, and myalgias. A small group of patients go on to develop DHF, which is distinguished from DF principally by a vascular leakage phenomenon, leading to hemoconcentration, pleural or peritoneal effusions, and in the most severe cases, dengue shock syndrome (DSS). While death is a relatively rare complication, the sheer volume of infections worldwide makes DENV the most lethal hemorrhagic fever virus by far—the total number of deaths worldwide from Ebola since 1976 is just over 1,000; DENV claims an equal number in less than a month.

The protective immune response against DENV remains poorly understood. There are no good animal models of human dengue disease [2]; therefore, clinical and epidemiologic studies are necessary to answer questions relating to DENV pathophysiology. Further, the development of an effective vaccine requires a more comprehensive model of DENV pathophysiology [3]. In particular, an understanding of which individuals are at risk for developing DHF is a priority in the coming decade.

Epidemic DHF and DSS emerged in the Philippines and Thailand only 50 years ago. Since then, the virus has become hyperendemic in Southeast Asia. Today, it is among the top ten causes of pediatric hospitalization in Southeast Asia [4]. By contrast, DENV infection in the Western Hemisphere remained relatively low until the 1970s, when DF incidence began to rise, followed by epidemics of DHF and DSS in the 1980's. Today, countries such as Brazil, Venezuela and Colombia are subject to epidemics in increasingly rapid succession. The seroprevalence in the West remains less than 100% based on

ongoing studies, although hyperendemic circulation is becoming established in some areas [5].

One of the unique features of dengue is the role of heterologous infection in disease severity. Following infection with a given DENV serotype, an individual will develop long-lasting immunity to that serotype (homotypic immunity). However, heterotypic immunity to the remaining three serotypes lasts only a few months [6]. One key observation in dengue pathogenesis is that heterotypic immunity is a risk factor for DHF: the overwhelming majority of DHF cases occur in people who are experiencing secondary infections with a different DENV serotype [7]. The major exception to this group is in infants, where "primary DHF" is most commonly seen during the period from 6-12 months of age.

One widely accepted theory to account for the occurrence of DHF in these different groups is ADE [8]. This model—which has been demonstrated in vitro—proposes that pre-existing heterotypic anti-DENV IgG antibodies (Abs) acquired during a previous infection can, under certain conditions, facilitate uptake of virus into macrophages and other immune cells via the binding of virus-antibody complexes to Fcγ receptors. The consequent increase in receptor-mediated endocytosis leads to higher viral loads, which in turn triggers a host inflammatory cascade that leads to DHF.

The sine qua non of the ADE hypothesis is its prediction of DHF during primary DENV infection in infants, where maternally derived anti-DENV IgG levels decay over time. Infant immune systems are "primed" in utero by passive transfer of DENV-specific Abs from DENV-immune mothers, thus creating a "physiologic secondary infection" during primary DENV infection, where waning maternal anti-DENV Abs enhance viral uptake, resulting in DHF.

Several epidemiologic studies have shown that the incidence of primary DHF in infants peaks approximately between 6 and 8 months [9]. This time frame corresponds with predicted ADE activity, and in vitro studies have borne this out [10]. However, other studies have cast doubt on the ADE hypothesis [11] [12]. Reflecting these contradictory findings, one investigator recently noted that because measurements of ADE rely on in vitro models, its association with clinical outcomes remains controversial [13]. They note that "to the best of our knowledge, DHF has not yet been described in an infant born to a dengue-naïve mother…further studies of infants with DHF, particularly in regions with low or moderate dengue endemicity or where outbreaks occur among previously naïve populations, are needed to unequivocally confirm that maternally derived anti-DENV antibody is a critical risk factor for DHF in infants."

We have identified a promising site to study the influence of maternal dengue serostatus on the severity of infant DENV infection. Our collaborators are faculty members in the Department of Pediatrics, Universidad Surcolombiana (USCO) Hospital. We are currently working with them on a project evaluating a novel assay for the detection of dengue viremia. A city of approximately 300,000 people, Neiva is an ideal site for studying dengue in infants for two major reasons: it is a small enough city to allow for relatively easy follow-up for outpatient study subjects, while it is large enough to have a sufficient number of cases of DENV infection. Additionally, USCO is the sole inpatient pediatric hospital not only for the city but for the surrounding region; our collaborators and their department care for essentially all cases of DHF in Neiva.

b. Please describe the Investigator's previous work on the problem.

The PI (Hatch) has been working on dengue pathophysiology since the start his research fellowship in Infectious Disease in the fall of 2006. He is currently involved in the U01 grant evaluating the novel assay for detection of dengue viremia noted above. Dr. Rothman holds that grant and has been involved in the study of dengue pathogenesis for over two decades and currently holds several NIH-funded grants devoted to the study of dengue.

c What are the aspects that justify the use of human subjects, human data, or specimens as part of this research?

Given that dengue infection causes significant morbidity, as well as a negative economic impact, in not only Colombia but throughout much of Latin America as well as a small portion of the US, the need to establish an accurate model of DHF pathogenesis would be of great value to the field. It is not possible to study DHF in animal models because laboratory animals do not develop the salient pathologic features of DHF.

d. Attach references as appropriate.

1. *Gubler, D.J., Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol, 2002. **10**(2): p. 100-3.*
2. *Yauch, L.E. and S. Shresta, Mouse models of dengue virus infection and disease. Antiviral Res, 2008. **80**(2): p. 87-93.*
3. *Thomas, S.J., J. Hombach, and A. Barrett, Scientific consultation on cell mediated immunity (CMI) in dengue and dengue vaccine development. Vaccine, 2009. **27**(3): p. 355-68.*
4. *Kyle, J.L. and E. Harris, Global spread and persistence of dengue. Annu Rev Microbiol, 2008. **62**: p. 71-92.*
5. *Braga, C., et al., Seroprevalence and risk factors for dengue infection in socioeconomically distinct areas of Recife, Brazil. Acta Trop, 2009.*
6. *Sabin, A.B., Research on dengue during World War II. Am J Trop Med Hyg, 1952. **1**(1): p. 30-50.*
7. *Sangkawibha, N., et al., Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayong, Thailand. I. The 1980 outbreak. Am J Epidemiol, 1984. **120**(5): p. 653-69.*
8. *Halstead, S.B., J.S. Chow, and N.J. Marchette, Immunological enhancement of dengue virus replication. Nat New Biol, 1973. **243**(122): p. 24-6.*
9. *Nguyen, T.H., et al., Dengue hemorrhagic fever in infants: a study of clinical and cytokine profiles. J Infect Dis, 2004. **189**(2): p. 221-32.*
10. *Kliks, S.C., et al., Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. Am J Trop Med Hyg, 1989. **40**(4): p. 444-51.*
11. *Laoprasopwattana, K., et al., Dengue Virus (DV) enhancing antibody activity in preillness plasma does not predict subsequent disease severity or viremia in secondary DV infection. J Infect Dis, 2005. **192**(3): p. 510-9.*
12. *Libraty, D.H., et al., A prospective nested case-control study of Dengue in infants: rethinking and refining the antibody-dependent enhancement dengue hemorrhagic fever model. PLoS Med, 2009. **6**(10): p. e1000171.*
13. *Simmons, C.P., et al., Maternal antibody and viral factors in the pathogenesis of dengue virus in infants. J Infect Dis, 2007. **196**(3): p. 416-24.*

4. DETAILED DESCRIPTION OF RESEARCH PLAN (especially as it affects the subject)

a. Include a schematic representation of what the research will entail (e.g. a table with the number of visits and what will happen at each visit or flow diagram of subject's involvement over time).

We will conduct a case-control study comparing infants and mothers of the following groups: those with DHF and those with symptomatic DENV infection but without DHF. All infants will require positive laboratory evidence of DENV infection (serology or PCR). Infants with DHF will be defined according to the World Health Organization criteria, which include all of the following: thrombocytopenia, a bleeding tendency, and plasma leakage.

Infants with symptomatic DENV infection will be screened at an outpatient pediatric clinic affiliated with UISCO. Plasma leakage in DHF occurs in the period immediately following defervescence. Therefore,

children in the symptomatic DENV cohort will be considered eligible for analysis only after they have been documented to be free of any DHF manifestations 24 hours after defervescence, although this will not affect their initial enrollment status.

To document appropriate clinical follow-up, the study will involve two visits, the second between 48 and 72 hours after the initial visit. At the initial visit clinical information on both the infant and the mother will be collected, and blood will be drawn from both the mother and infant. Mothers of infants at the outpatient clinic will be given information about warning signs of severe DENV disease and instructed to return to the clinic immediately if the infant develops such symptoms. Hospitalized infants with DHF will be cared for according to standard clinical procedures in place at USCO. At the second visit, infants in the outpatient clinic will be re-examined and have blood drawn. Infants at the second visit with ongoing fever will be re-evaluated in a third visit 24-72 hours later, but no further blood will be drawn except as part of standard clinical practice. Infants with DHF will be re-evaluated at the second visit, and relevant clinical information (chest X-rays, hematocrit and platelet values, amount of IV fluid administered) will be recorded and additional blood will be drawn. Mothers will have their blood drawn at the first visit only.

Clinical and demographic data will be collected on the mothers, including age, past or current medical history (including specifically a history of Yellow Fever vaccination, previous known dengue infection, previous hemorrhagic fever, diabetes, lupus or other autoimmune diseases, asthma, TB and HIV), and medications. Similar information will be collected on the infants; additionally, information on the length of illness, time from symptom onset to presentation, specific treatments or interventions used, serial CBCs and CXRs, serial blood pressure measurements, presence or absence of complications, serial weight and height measurements, and mortality will also be collected.

b. Inclusion/Exclusion Criteria - As appropriate, explain what steps will be taken to insure that subjects meet the criteria (e.g. healthy, not pregnant, etc).

Inclusion criteria include:
•Infants aged 12 months or less and their healthy mothers; these infants belong to two groups:
•Those hospitalized for DHF (diagnosed according to World Health Organization Criteria); and
•Those sick from infection with dengue but who do not meet the criteria for DHF;
•Active pregnancy in the mother does not affect inclusion or exclusion criteria.

Exclusion criteria include:
•For mothers: any febrile illness since the time of delivery of the infant (to exclude an intervening DENV infection);
•Inability or unwillingness to provide informed consent.

c. Discuss the number of experimental and control subjects, and explain the statistical basis for the numbers.

To test the main hypothesis, we will determine whether the DENV seroprevalence is higher in mothers of infants with DHF than in mothers of infants without DHF. Based upon estimated seroprevalences of 100% among mothers of infants with DHF and 80% among mothers of infants without DHF, with α of .05 and β of .2, we calculate that the minimal sample size requirement would be 36 in each arm. We propose to recruit in a 1:2 case-control fashion, with a minimum of 50 mother/infant pairs in the DHF group and 100 mother/infant pairs in the symptomatic DENV group, allowing for withdrawals, loss to follow-up, and misdiagnosed patients who do not have dengue (this is far less likely in the DHF arm).

d. Does the study involve randomization?

Yes **No**

If yes, please describe process.

e. How long will each subject be enrolled in the study?

We anticipate following patients for two or three days from the time of study inclusion. Mothers participating in the study will only be required to give blood at the first visit. Outpatients who remain febrile at the second visit will be followed until their fever resolves, although no further blood samples will be needed after the second visit.

f. Provide a *brief* overview of what participation in the study will mean to each participant in terms of what he/she will experience. Describe in order, each procedure, how long each procedure will take and how often each procedure will be performed. Include doses & route of administration of any drugs and whether the procedure or drugs would **always**, **sometimes** or **never** be required as part of the subject's standard of care.

After written consent is provided, the infants will have their blood drawn at each of two visits; mothers will have their blood drawn only at the first visit. No medications will be administered as part of the study, and all medical therapy, including the use of oral or intravenous fluid supplementation, will be administered at the discretion of the attending physician and will follow standard medical care. Basic data from the physical examination of the patients, as part of usual clinical practice (such as temperature, blood pressure, pulse etc.), in addition to information on the subsequent hospital course of those patients who happen to be hospitalized, will also be recorded for the study.

g. Is any aspect of this research study being conducted in the Medical School or a non-UMMMC facility? If yes, please explain.

This study is being conducted at the Department of Pediatrics, Universidad Surcolombiana (USCO), Neiva, Colombia. This site represents a place where dengue infection is endemic, and where we have established relationships with collaborators interested in various projects on dengue research.

h. Will hospitalization be required as part of this research study?

Yes **No**

If yes, how long will subjects be hospitalized?

i. Will there be any material inducements or recruitment incentives given to research staff or research subjects as part of this research study? (e.g., direct payments, free hospitalization, care)

Yes No

If yes, explain how much, the pay schedule, or any partial payments that will be given.

As compensation for completing the study, participants will be given gifts of less than US $10 value. Transportation reimbursement, in the form of taxi vouchers, bus passes, or subway fare, will be made available to all participants who require it.

The committee is exceedingly sensitive to the threat of coercion that can stem from excessive compensation for participation in research. The IRB recommends hourly payments of $20/hr for every hour (or fraction thereof) the subject is involved in the study. This should include time in the hospital or clinic that is solely for the study, travel time, and time spent recovering from a procedure or an anesthetic agent used for a procedure. Time that the subject is unable to perform his/her routine activities of daily living due to study related issues should be included in this time. (Time required to perform multiple minor tasks should be lumped together; that is, filling out a questionnaire that takes 15 minutes on four different days constitutes one hour

of labor, not four hours.) If reimbursed, cost of transportation ($0.35/mile), parking and meals should be noted. A bonus of up to $50 may be given for completion of a long term study or for studies that involve low risk but uncomfortable procedures (such as endoscopy, multiple blood samples for pharmacokinetic studies, gynecological examinations, etc).

5. DISCLOSURE OF CONFLICT OF INTEREST

Investigators should disclose any financial arrangement they may have with a company whose product figures prominently in their research or financial arrangements they may have with company making a competing product. **The relationship should also be described in the informed consent documents**. In the case where the only relationship is that a company is sponsoring the research study, it is sufficient to prominently identify the sponsor on the front page of the consent form and to simply state "NONE" in the consent form under Conflict of Interest.

Is there a conflict of interest? Yes **No**

6. RELATIONSHIP TO STANDARD THERAPY.

Describe the standard therapy that patients would receive if not in the research study. Explain how this research intervention deviates from or replaces generally accepted standard therapy and justify the deviation.

For this study, the patients will be evaluated either in an outpatient clinic or on the hospital wards at USCO. Their initial evaluation and subsequent care (whether inpatient or outpatient) will not be influenced in any way by this study, other than additional blood will be drawn for the research described in this proposal (in accordance with amounts considered minimal risk as defined by 45 U.S. Code of Federal Regulations (CFR) 46.102). For those study participants who are hospitalized, information on subsequent hospital course (including laboratory values described above, chest x-rays, ultrasounds, and amount of IV fluid administered) will be abstracted by study personnel.

7. DESCRIBE THE POTENTIAL BENEFITS OF THIS PROJECT.

a. Include hoped-for benefit to society, to the group of subjects or to individual subjects.
b. Address the risk/benefit ratio of the study. If there are no direct subject benefits, this should be stated.

There is no direct benefit to the patients in participating in the study, however the risk/benefit ratio is likewise small, given the only risk is by serial phlebotomy. The potential benefit to society lies in the possibility that this study may influence the direction of DHF pathophysiologic studies, as well as help vaccine researchers consider optimal strategies to measure vaccine effectiveness.

8. DESCRIBE THE POTENTIAL RISKS TO SUBJECTS INCLUDE PSYCHOLOGICAL, ECONOMIC, LEGAL OR SOCIAL RISKS AS WELL AS PHYSICAL RISKS.

Include the following information:

a. Estimate likelihood of occurrence, severity, and duration. If generally accepted quantitative estimates are available based on previous data, these should be stated. Otherwise, qualitative estimates such as "rare", "occasionally", or "frequently" may be used. *The committee needs scientific information about drug/device side effects so as to best judge the pros and cons of the study.* ***Do not simply cut and paste the consent form "Risk" section into this part of the protocol.***

As noted above, phlebotomy is the only procedure of the study that entails risk to the patient, and as such

is in the category of minimal risk. Risks of phlebotomy include lightheadedness (frequent), anemia (rare), and venipuncture site infection (rare).

b. Explain what steps will be taken to protect against its occurrence, minimizing the harm, methods for early detection of harm, and what procedures will be followed to avoid serious injury (e.g. withdraw from study or dose reduction).

Phlebotomy will be performed by trained medical staff at the clinical sites (physicians, nurses, or medical technologists).

c Explain whether or not these risks are from a procedure performed with the **intent** and **reasonable prospect** of yielding **direct** health related benefit to the subject.

These risks are not from a procedure performed with the reasonable prospect of direct health-related benefit to the subject.

d. Do you, as the PI, have equipoise regarding the study? That is, are you comfortable with the risks in relationship to the knowledge gained? If the study involves randomization, do you believe in the equality of the treatment arms?

We do believe that we have equipoise for the purposes of this study. The risks to the patients are minor, and the knowledge gained may help contribute to an understanding of DHF pathogenesis.

9. CONFIDENTIALITY CONSIDERATIONS: EXPLAIN STEPS THAT WILL BE TAKEN TO INSURE THE CONFIDENTIALITY OF INFORMATION THAT IS OBTAINED IN THE COURSE OF THIS RESEARCH PROJECT. INCLUDE THE FOLLOWING:

a. How will identifiers be used? *Subjects will receive a study ID code identifying the sequence of enrollment. Blood samples collected at follow-up visits will be identified by a different alphanumeric specimen ID code. Data will be recorded on paper case report forms (CRFs) appropriate to the study visit. Blood sample collection data and diagnostic test results will also be recorded on paper CRFs.*

b. Where will data be stored? *The paper CRFs will be kept at USCO. Copies will be transferred to UMass, either by direct transfer of documents or electronically, on a periodic basis for data analysis. Analytic files will retain only the unique study ID code attached to each subject in the study. The data will be retained on a password protected network drive at CIDVR, or on documents that remain at USCO.*

c. Besides the UMMS IRB and their representatives, who will have access to the research data? *The principal investigator and collaborators.*

d. When will the data/specimens be destroyed? *The intended use of the archived samples is to further work on dengue immunopathogenesis, e.g. Antibody Dependent Enhancement assays, cytokine level measurements, plaque reduction neutralization titer (PRNT 50) assays, viral load assessments, etc. The well-characterized clinical samples planned to be collected in this study will represent an invaluable repository for such studies. The specific assays to be performed will be dependent on the initial data analyses from the clinical study. Therefore, we anticipate these research studies to extend for approximately three to five years based on our experience with similar clinical studies in Thailand performed at CIDVR.*

Thus, the data and specimens will be retained for at least 5 years. The paper CRFs will be destroyed after 5 years; electronic databases (without personal information) and specimens will be retained indefinitely.

e. In the future, might other use be made of specimens collected as part of the research? If yes, please describe. *A sample repository, when sample banking is possible, will be established, with plasma collection occurring for storage and future research investigation. These specimens will be invaluable for novel studies not currently anticipated, for example, involving novel assays of DENV-specific antibodies or other viral infections relevant to infants and mothers in Colombia. Throughout and after completion of the study, proposals for the use of the specimen repository will be solicited from participating investigators as well as the scientific community. Proposals will detail the hypotheses to be tested, test(s) to be performed, and number of samples required, with appropriate justifications. Proposals will initially be evaluated by the Principal Investigator, foreign collaborators, and the UMMS IRB.*

10. ECONOMIC CONSIDERATIONS:

a. In the course of this research project, might the subjects experience any additional expenses as a result of study participation? This includes both out-of-pocket costs and expenses that might not be covered by medical insurance.

Yes **No**

If yes, please explain and justify.

b. Please explain potential increase in standard hospital costs if any.

11. DESCRIBE THE CHARACTERISTICS OF THE SUBJECT POPULATION.

a. The subject population includes:

ADULTS ___x___ CHILDREN ___X___

b. Is the subject population restricted in respect to any of the following characteristics?

Please "x" those that apply	Yes	No
Age Range	x	
Health Status		x
Gender	(x)	
Racial/Ethnic composition		x

If you responded **YES** to any of the above, include a clear rationale for this restriction.
See above—the study is targeting mothers of infants suffering from either symptomatic dengue infection or from DHF, and the infants themselves. The phenomenon of DHF outlined in #3a can only be studied in this group. Infants can be of either sex, but because of maternal passage of antibodies to fetuses, mothers are the only eligible parents for this study.

12. WILL THE STUDY POPULATION SPECIFICALLY INCLUDE A POPULATION OF SUBJECTS CONSIDERED "VULNERABLE"? VULNERABLE POPULATIONS ARE CHILDREN, MENTALLY IMPAIRED, PREGNANT WOMEN, PRISONERS, OR FETUSES.

Yes No

If yes, please explain.
Per above, the study populations include both infants and potentially pregnant women (although given the targeted age of infants we do not anticipate many mothers to be pregnant). While these are both vulnerable populations, this study poses only minimal risk to participants and is of short duration.

13. WHAT IS THE SOURCE OF THE SUBJECT POPULATION?
The study population consists of infants with DHF or those suspected of having symptomatic dengue infection, and the mothers of both groups of infants, living in the catchment area of the USCO hospital.

14. EXPLAIN ANY STEPS TAKEN TO INSURE THAT THE SUBJECT POPULATION IS REPRESENTATIVE.
Not applicable.

15. HOW AND WHERE WILL SUBJECTS BE RECRUITED FOR THE STUDY? CONSULT THE IRB GUIDELINES FOR THE RESTRICTIONS ON RECRUITMENT OF EMPLOYEES, STUDENTS, AND INPATIENTS. ATTACH COPIES OF ALL RECRUITMENT MATERIALS TO BE USED AS PART OF THIS RESEARCH STUDY. THESE MATERIALS MUST BE APPROVED BY THE IRB BEFORE BEING USED. Recruitment guidance can be found on our website under HSC Forms.
We will recruit study participants among patients presenting to either with suspected DHF (at the USCO hospital wards) or suspected symptomatic dengue infection (at the outpatient clinic). The project staff (consisting of physicians and nurses) will identify patients who meet the inclusion criteria for the study. Such patients will then be approached by project staff. They will explain the study protocol and consent forms (in Spanish) in detail. Any questions regarding the study will be addressed, and written informed consent will be taken at the day of enrollment. Mothers will be excluded if they are unable or unwilling to provide consent. Participants may leave the study at any time without penalty.

16. WILL PROTECTED HEALTH INFORMATION (PHI) BE USED AS PART OF THIS RESEARCH STUDY? PLEASE VISIT OUR WEBSITE FOR MORE INFORMATION ABOUT PHI OR THE HEALTH INSURANCE PORTABILITY AND ACCOUNTABILITY ACT (HIPAA).

Yes No

If **yes**, please answer the following questions.

a. How and where will the PHI be accessed (i.e. meditech, database,medical records, another site)?

No PHI will be sent to UMMS; only coded information will be sent to UMMS, with the link to the code kept at USCO.

b. W

Yes—infants considered for inclusion into the DHF arm will be identified by physicians or nurses after a review of their medical record. We emphasize that this information will be obtained by staff actively engaged in the care of the infants and therefore the PHI is not being accessed outside of the normal course of events. The infants in the symptomatic dengue infection arm will not have their PHI accessed before study enrollment. Mothers participating in the study will not have any PHI accessed beyond that given at the time of study enrollment.

c. Please list the PHI to be used as part of this research study (i.e. name, DOB, medical record #).

DOB; age; past or current medical history (including specifically a history of Yellow Fever vaccination, TB, previous dengue infection, previous hemorrhagic fever, diabetes, lupus or other autoimmune diseases, asthma, and HIV); medications; and for infants in the DHF arm (or those infants in the symptomatic dengue infection arm that require hospitalization) data collected during the course of illness or hospitalization (including specifically treatments or interventions, serial CBCs and CXRs).

17. METHOD FOR OBTAINING INFORMED CONSENT

a. Are you requesting a waiver of the requirement for obtaining consent?

Yes **No**

If yes, please justify the request in the box below. Consent may be waived if research is minimal risk; the waiver does not adversely affect the subject **and the research could not practically be carried out without the waiver**. Your justification must address these issues. Specific regulations can be found at 45 CFR 46.117 (c) (1) (2) and 21 CFR 56.109 (c) (1)

Do not complete the following questions if you are requesting a waiver of informed consent.

18. WILL VERBAL CONSENT BE OBTAINED?

Yes **No**

If **yes**, will an unsigned "fact sheet" be given to subjects before verbal consent is obtained?

Yes No

If **yes**, please provide a copy of the "fact sheet".

19. WILL A SIGNED CONSENT FORM BE REQUIRED?

Yes No

20. AS A GROUP, ARE THESE SUBJECTS EXPECTED TO BE COMPETENT TO GIVE CONSENT FOR THEMSELVES?

Yes **No**

If **no**, please explain why and how consent will be obtained. *Mothers will consent for themselves and for their infants.*

21. EXPLAIN THE CIRCUMSTANCES UNDER WHICH CONSENT WILL BE OBTAINED. HOW WILL YOU

INSURE THAT POTENTIAL SUBJECTS HAVE ADEQUATE TIME TO CONSIDER THEIR OPTIONS, AND THAT POSSIBLE COERCION IS MINIMAL?

After proper identification of appropriate subjects for the study, potential participants will be approached by study staff (all of whom have taken NIH-mandated courses in ethics of human subjects research), explained the purpose of the study in non-technical language and provided with consent forms. All questions will be addressed in due course without respect to their number or length. Research staff will make clear at the initial encounter that no aspect of the participants' subsequent health care will be affected by their decision to participate in the study, and moreover that no penalty will ensue should they elect to withdraw from the study. Participants will be offered assistance with transportation costs associated with the study and told that they will be given a "gift" of small value as part of minimal compensation for their participation (as noted above); however there are no financial incentives that are sufficient to induce participation in those who would otherwise be reluctant to do so.

Consent and assent forms will be in Spanish. The English translations of these forms are in the appendix.

22. IF THE SUBJECT POPULATION INCLUDES MINORS, AND SIGNED CONSENT WILL BE OBTAINED, WILL AN ASSENT FORM BE USED AS PART OF THE CONSENTING PROCESS? CONSULT IRB GUIDELINES **FOR INFORMATION ABOUT CHILDREN IN RESEARCH STUDIES.**

Yes No Minors enrolled Verbal consent requested

NOTE: In general, it is expected that minors from age 8 to 15 will read and sign an assent form. Older adolescents (16 and 17) will usually read and sign the same consent form as the parents signed. The assent form template is available on our website.

23. IF YES, PLEASE EXPLAIN WHO WILL APPROACH THE MINORS AND HOW AND WHERE THE ASSENTING PROCUDURE WILL TAKE PLACE.

SECTION VI
CERTIFICATION OF APPROVAL

PI Name: _____
DELEGATION OF ROLES/RESPONSIBILITIES*: Checklist/Signature List

Please type Name and Credentials	Role*	Signature	Department/Campus	Delegation of responsibilities: Please use key** in box below to summarize your study activities and place an "x" in the appropriate column									
				A	B	C	D	E	F	G	H	I	J
Steven Hatch, MD	PI		CIDVR/University						X		X	X	X
Alan Rothman, MD	1		CIDVR/University						X		X	X	

***Roles: (choose appropriate # below)**

1. Sub or Co-Investigator **2. Study Nurse Coordinator** **3. Study Coordinator** **4. Other:**

****Delegation of Responsibility Codes: (choose all that apply)**

A. Consent Subjects
B. Take Medical History
C. Conduct Physical Exam
D. Phlebotomy
E. Monitor Vital Signs/Nursing Assessment
F. Maintain Regulatory Documents
G. CRF Completion and Query Resolution
H. SAE/AE Monitoring/Reporting
I. IRB Communications and Continuing Review
J. Other (explain): obtain and ensure accuracy of data; perform lab analysis, write abstracts and manuscripts

Although the Principal Investigator is ultimately responsible for every element of study activity, this form serves to clarify to whom the PI has delegated specific study activities and responsibilities.

APPENDIX #3: Maximum Allowable Total Blood Draw Volumes (Clinical + Research)

Body Wt (Kg)	Body Wt (lbs)	Total blood volume (mL)	Maximum allowable volume (mL) in one blood draw (= 2.5% of total blood volume)	Total volume (clinical + research) maximum volume (mL) drawn in a 30-day period	Minimum Hgb required at time of blood draw	Minimum Hgb required at time of blood draw if subject has respiratory/CV compromise
1	2.2	100	2.5	5	7.0	9.0 -10.0
2	4.4	200	5	10	7.0	9.0-10.0
3	6.3	240	6	12	7.0	9.0-10.0
4	8.8	320	8	16	7.0	9.0-10.0
5	11	400	10	20	7.0	9.0-10.0
6	13.2	480	12	24	7.0	9.0-10.0
7	15.4	560	14	28	7.0	9.0-10.0
8	17.6	640	16	32	7.0	9.0-10.0
9	19.8	720	18	36	7.0	9.0-10.0
10	22	800	20	40	7.0	9.0-10.0
11-15	24-33	880-1200	22-30	44-60	7.0	9.0-10.0
16-20	35-44	1280-1600	32-40	64-80	7.0	9.0-10.0
21-25	46-55	1680-2000	42-50	64-100	7.0	9.0-10.0
26-30	57-66	2080-2400	52-60	104-120	7.0	9.0-10.0
31-35	68-77	2480-2800	62-70	124-140	7.0	9.0-10.0
36-40	79-88	2880-3200	72-80	144-160	7.0	9.0-10.0
41-45	90-99	3280-3600	82-90	164-180	7.0	9.0-10.0
46-50	101-110	3680-4000	92-100	184-200	7.0	9.0-10.0
51-55	112-121	4080-4400	102-110	204-220	7.0	9.0-10.0
56-60	123-132	4480-4800	112-120	224-240	7.0	9.0-10.0
61-65	134-143	4880-5200	122-130	244-260	7.0	9.0-10.0
68-70	145-154	5280-5600	132-140	264-280	7.0	9.0-10.0
71-75	156-185	5680-6000	142-150	284-300	7.0	9.0-10.0
76-80	167-176	6080-6400	152-160	304-360	7.0	9.0-10.0
81-85	178-187	6480-6800	162-170	324-340	7.0	9.0-10.0
86-90	189-198	6880-7200	172-180	344-360	7.0	9.0-10.0
91-95	200-209	7280-7600	182-190	364-380	7.0	9.0-10.0
96-100	211-220	7680-8000	192-200	384-400	7.0	9.0-10.0

Based on blood volume of:		
kg	**mL/kg**	
1-2	100	Pre-term infant
> 2	80	Term infant - adult

This information is similar to that used by the Committee on Clinical Investigations, Children's Hospital in Los Angeles, CA; Baylor College of Medicine, Dallas, TX; and Cincinnati Children's Hospital Institutional Review Board, OH. These charts were adapted by: Rhona Jack, Ph.D. Children's Hospital and Regional Medical Center Laboratory, Seattle, WA in August 2001.

APPENDIX #4:

INFORMED CONSENT (ENGLISH TRANSLATION)

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

TITLE: **Maternally Derived Anti-Dengue Antibodies and the Risk of Dengue Hemorrhagic Fever in Infants: a Case-Control Study**

PRINCIPAL INVESTIGATORS: Doris Salgado and Jairo Rodriguez DATE: _____

RESEARCH SUBJECT'S NAME: _____

You and your child are invited to participate in a research study so that researchers may learn more about how infants become sick from dengue infection. This form will tell you why the study is being done and what will be done if you decide to take part in the study.

Purpose of research

The objective of this study is to learn how previous infection with dengue virus in a mother can affect how ill the child becomes when he or she gets infected with dengue as an infant. The dengue virus is carried by mosquitoes and is very common in Colombia. Most people who get dengue become sick with fever, headache, weakness and muscle aches (dengue fever). However, some people become sicker, developing dengue hemorrhagic fever with complications such as bleeding or shock (low blood pressure). Your child is suspected to be sick from dengue infection, although certain tests will need to be done in order to confirm this.

Doctors at Universidad Surcolombiana (USCO) are working with scientists from the University of Massachusetts Medical School in the United States to study the influence of prior infections in mothers on infants who become infected with the dengue virus. Researchers want to compare mothers of infants who have dengue hemorrhagic fever with mothers of infants who have dengue fever. The researchers will perform special blood tests on mothers and infants of both groups.

Your rights

It is important for you to know that:
1) YOUR PARTICIPATION IS ENTIRELY VOLUNTARY.
2) YOU MAY DECIDE NOT TO TAKE PART OR DECIDE TO QUIT THE STUDY AT ANY TIME, WITHOUT ANY PENALTY.
3) YOU WILL BE TOLD ABOUT ANY NEW INFORMATION OR CHANGES IN THE STUDY THAT MIGHT AFFECT YOUR PARTICIPATION.

Study procedures

The first thing that will be done is to determine if you qualify for this study. You have been approached because your child is likely to be infected with the dengue virus; ***there is no reason to believe that you are ill from dengue infection at this time***. The doctor or nurse will collect information and take a blood sample from you, and a much smaller sample from your child today. No further blood samples will be taken from you, although the doctor or nurse will collect a second sample on your child. If you have brought your child to the clinic, you will be asked to return to the clinic two days from now. If your child is in the hospital, the doctor or nurse will collect the sample while your child is still in the hospital two days from now.

You are being asked to do the following:
-You will see the doctor or nurse conducting this study. This visit should last about 15 minutes.
-You will be asked some questions about your health.
-You will ***not*** be examined by the doctor or nurse in the clinic.
-A blood sample (about 12 ml, or the amount of blood in a vial a little larger than the size of your 3rd finger) will be drawn from your arm with a needle.
-A much smaller sample (about 2 ml) will be drawn from your child today, and the same amount will be drawn in two days.

All of your blood is being used strictly for research. Your child's blood is being used mostly for the research study. If your child were not in the research study, he or she might have some of these tests done anyway.

If the doctor thinks that your child needs additional medical care, you may be referred to the hospital. If your child is admitted to the hospital, members of the health care team conducting the study will visit you in the hospital during the scheduled visit days to obtain a blood sample. The results of these studies will be added to your hospital record.

Risks of the experimental procedures
Blood drawing can cause some symptoms. Some people may feel lightheaded or even faint. Blood drawing may also cause pain and bruising. The amount of blood collected in this study is very unlikely to cause anemia (low red blood count).

Benefits
You might benefit in one of the following ways:
1) The costs of the medical tests required for this research study will be paid by the researchers.
2) The information collected in this study may benefit you or others in the future by helping doctors find the best ways to treat patients with dengue, and also by helping doctors design an effective vaccine against dengue.
3) **However, it is likely that neither you nor your child will obtain any benefit from participating in this project.**

Costs
There will be no additional costs to you for participating in this study. Clinic visits and laboratory tests that are required for this research study will be free of charge.

Compensation.
You will receive financial compensation each day you come into the clinic to defray the cost of traveling to the clinic. When you complete the study, you will also receive a gift of less than US $10 (or the local currency equivalent).

Alternatives
If you choose not to participate, your child will still receive treatment for your illness and undergo the standard blood tests and other tests that you need.

Confidentiality
Yours and your child's research records will be kept confidential. In all study records you and your child will be identified by a code number and your names will be known only to the researchers. Your names will not be used in any reports or publications of this study.

Withdrawal from the study
You may withdraw from this study at any time. You may also be withdrawn from this research study by the study physician if he/she feels that it is not in you or your child's interest to continue to participate.

The quality of care you receive at this hospital or clinic will NOT be affected in any way if you decide not to participate or if you withdraw from the study.

Research injury/compensation
If you or your child are injured or have any harmful effects as a direct result of you or your child's participation in this research, treatment will be made available to you or your child. Only necessary medical treatment will be offered; neither you nor your child will receive any additional compensation.

Use of samples
The blood samples that will be taken from you will be used to test for past dengue infection. Samples will be stored at USCO or at the University of Massachusetts. As part of this study, the investigators plan to collect and store extra samples. These samples may be used for future research to learn more about dengue and other diseases. Samples may also be shared with investigators at other institutions. In some research, your samples may enable researchers to develop medical tests or treatments that have commercial value. You will not receive any money that may result from any such commercial tests or treatments. Genetic research may be performed on your samples to study more about the nature of dengue disease; however, no genetic information obtained from this research will be placed in your child's medical records. For any such studies, the samples will be identified only by codes so that you or your child's name cannot be easily identified.

Any additional research studies using your samples will be reviewed by the investigators' Institutional Review Board, a committee that oversees medical research to protect the rights and welfare of the volunteers.

You can change your mind at any time about allowing your identifiable samples to be used for future research. If you do, contact the investigators and let them know. Then your samples will no longer be made available for research.

Researchers may want to know more about you in the future. If you agree to be re-contacted, you may still change your mind about providing information in the future.

(Please check below to indicate whether or not you may be contacted in the future.)

__ I may be re-contacted for information.
__ I may not be re-contacted for information.

Subject initials _____ Date _____

Questions
Please feel free to ask any questions you may have about the study or about your rights as a research subject. If other questions occur to you later, you may ask:

At USCO—Dr. Doris Salgado or Dr. Jairo Rodriguez, telephone ***(telephone number inserted here).***
If at any time during or after the study, you would like to discuss the study or your research rights with someone who is not associated with the research study, you may contact ***(additional contact information inserted here)***.

The purpose and procedures of this research project have been explained to me and I understand them. I have been told about all of the predictable discomfort, risks, and benefits that might result, and I understand them. I have been told that unforeseen events may occur. I agree to participate as a subject in this research project. I understand that I may end my participation at any time.

Subject's signature: _____ DATE: _____

NAME:_____

Witness signature: _____ DATE: _____

NAME:_____

INVESTIGATOR'S DECLARATION

I have explained to the above-named subject the nature and purpose of the procedures described above and the foreseeable risks, discomforts, and benefits that may result. I have asked the subject if any questions have arisen regarding the procedures and have answered these questions to the best of my ability. I have considered and rejected alternative procedures for answering this research question.

_____DATE:_____

APPENDIX #5: Informed Consent, Spanish Translation

CONSENTIMIENTO INFORMADO

CONSENTIMIENTO PARA PARTICIPAR EN UN ESTUDIO DE INVESTIGACIÓN

TÍTULO: **Anticuerpos contra el dengue derivados de la madre y riesgo de fiebre del dengue hemorrágico en bebés: Estudio de casos y controles**

INVESTIGADORES PRINCIPALES: Doris Salgado y Jairo Rodriguez FECHA: _____

NOMBRE DEL SUJETO DE INVESTIGACIÓN: _____

Estamos invitando a usted y su niño a participar en un estudio de investigación para que los investigadores puedan aprender más sobre la forma en que los bebés se enferman por la infección por dengue. Este formulario le explica por qué estamos llevando a cabo el estudio y lo que se hará si usted decide participar.

Propósito de la investigación

El objetivo de este estudio es averiguar de qué forma una infección anterior con un virus de dengue en la madre puede afectar la severidad de la enfermedad en el niño cuando éste se infecta con dengue siendo bebé. En Colombia, los virus de dengue son portados por los mosquitos y son muy comunes. La mayoría de las personas infectadas por el dengue se enferman con fiebre, dolores de cabeza, debilidad y dolores musculares (fiebre del dengue). Sin embargo, algunas personas se enferman más y desarrollan fiebre del dengue hemorrágico, con complicaciones como sangrado o shock (presión arterial baja). Se sospecha que su niño está enfermo por una infección con dengue, aunque para confirmarlo será necesario hacer ciertas pruebas.

Los médicos de la Universidad Surcolombiana (USCO) están colaborando con científicos de University of Massachusetts Medical School de Estados Unidos para estudiar la influencia de infecciones previas en las madres, en bebés que contraen una infección con virus de dengue. Los investigadores quieren comparar a madres de bebés que tienen fiebre del dengue hemorrágico con madres de bebés que tienen fiebre del dengue. Para ello, les harán análisis de sangre especiales a madres y niños de ambos grupos.

Sus derechos

Es importante que usted sepa que:
1) SU PARTICIPACIÓN ES TOTALMENTE VOLUNTARIA.
2) USTED PUEDE DECIDIR QUE NO PARTICIPARÁ O QUE SE RETIRARÁ DEL ESTUDIO EN CUALQUIER MOMENTO, SIN NINGUNA PENALIDAD.
3) LA MANTENDREMOS AL TANTO DE CUALQUIER INFORMACIÓN NUEVA O CAMBIO EN EL ESTUDIO QUE PUEDA AFECTAR SU PARTICIPACIÓN.

Procedimientos del estudio

Lo primero que se hará es determinar si usted reúne los requisitos para participar en este estudio. A usted la han invitado a participar porque es probable que su niño esté infectado con un virus de dengue; ***en este momento no hay motivos para creer que usted esté enferma por infección con un virus de dengue***. Hoy, el médico o la enfermera obtendrán información y le extraerán una muestra de sangre a usted y otra muestra de sangre mucho más pequeña a su niño. A usted no le extraerán ninguna otra muestra de sangre, pero a su niño le extraerán

una segunda muestra. Si usted ha traído a su niño a la clínica, le pedirán que vuelva a la clínica en dos días. Si su niño está hospitalizado, el médico o la enfermera obtendrán la muestra en dos días mientras su niño esté todavía en el hospital.

A usted se le pide que haga lo siguiente:
- Ver al médico o la enfermera que lleva a cabo este estudio. Esta visita debería durar aproximadamente 15 minutos.
- Contestar algunas preguntas sobre su salud.
- El médico o la enfermera ***no*** la examinará en la clínica.
- Con una aguja, le extraerán una muestra de sangre de aproximadamente 12 mililitros (menos de tres cucharaditas) de un brazo.
- A su niño se le extraerá una muestra de sangre mucho más pequeña hoy (aproximadamente 2 mililitros, menos de media cucharadita) y la misma cantidad en dos días.

Toda su sangre se usará exclusivamente para la investigación. La sangre de su niño se usará en su mayor parte para el estudio de investigación. Si su niño no estuviera en el estudio de investigación, posiblemente le harían algunas de estas pruebas de todos modos.

Si el médico opina que su niño necesita atención médica adicional, tal vez los envíen al hospital. Si su niño es hospitalizado, los miembros del equipo de atención médica que realiza el estudio los visitarán en el hospital durante los días planificados para obtener la muestra de sangre. Los resultados de estos estudios se incorporarán a su historial médico.

Riesgos de los procedimientos experimentales
La extracción de sangre puede causar algunos síntomas. Algunas personas pueden sentir mareos o desmayarse. La extracción de sangre también puede causar dolor y hacer que se formen moretones. Es muy improbable que la cantidad de sangre extraída en este estudio cause anemia (un recuento bajo de glóbulos rojos).

Beneficios
Usted podría beneficiarse de una de las siguientes maneras:
1) Los costos de las pruebas médicas necesarias para este estudio de investigación serán pagados por los investigadores.
2) La información obtenida mediante este estudio tal vez beneficie a ustedes o a otros en el futuro al ayudar a los médicos a encontrar mejores maneras de tratar a los pacientes con dengue y a elaborar una vacuna eficaz contra esta enfermedad.
3) **Sin embargo, es probable que ni usted ni su niño obtengan ningún beneficio por participar en este proyecto.**

Costos
La participación en este estudio no implicará ningún costo adicional para usted. Las visitas a la clínica y las pruebas de laboratorio requeridas para este estudio de investigación serán gratuitas.

Compensación.
Usted recibirá compensación económica cada día que venga a la clínica para cubrir sus gastos de viaje a la clínica. Cuando complete el estudio, también recibirá un regalo de menos que $10 dólares estadounidenses (o el equivalente en moneda local).

Alternativas
Su niño recibirá tratamiento para su enfermedad y se le harán los análisis de sangre estándar y otras pruebas que necesite aun si usted decide no participar en el estudio.

Confidencialidad
Los archivos de investigación de usted y de su niño se mantendrán confidenciales. En todos los archivos del estudio, usted y su niño serán identificados mediante un código numérico y los únicos que sabrán sus nombres serán los investigadores. Los nombres de usted y su niño no se usarán en ningún informe o publicación que surja de este estudio.

Retirarse del estudio
Usted puede retirarse del estudio en cualquier momento. Además, el médico investigador también puede retirarlos de este estudio de investigación si opina que seguir participando no es lo más conveniente para su niño.

La calidad de la atención médica que usted reciba en este hospital o clínica NO se verá afectada de ninguna manera si usted decide no participar o se retira del estudio.

Compensación por lesiones relacionadas con la investigación
Si usted o su niño se lesionan o sufren algún efecto perjudicial como resultado directo de su participación en esta investigación, se les ofrecerá tratamiento. Solamente se les ofrecerá el tratamiento médico necesario; ni usted ni su niño recibirán ninguna compensación adicional.

Uso de las muestras
Las muestras de sangre extraídas se analizarán para detectar infección previa por dengue. Las muestras se almacenarán en USCO o en la Universidad de Massachusetts *(University of Massachusetts)*. Como parte de este estudio, los investigadores planean obtener y almacenar muestras adicionales. Estas muestras pueden usarse para investigaciones futuras con el fin de aprender más sobre el dengue y otras enfermedades. Las muestras también pueden compartirse con investigadores de otras instituciones. En algunos estudios de investigación, sus muestras podrían permitir que los investigadores desarrollen pruebas o tratamientos médicos que tienen valor comercial. Usted no recibirá ningún dinero que surja a raíz de cualquiera de estas pruebas o tratamientos comerciales. Es posible que se haga investigación genética con sus muestras para estudiar más a fondo la naturaleza de la enfermedad de dengue; sin embargo, en los historiales médicos de su niño no se colocará ninguna información genética obtenida a través de esta investigación. Para cualquiera de estos estudios, las muestras se identificarán solamente por códigos para que el nombre de usted y su niño no puedan ser identificados fácilmente.

Cualquier estudio de investigación adicional en el que se use sus muestras será inspeccionado por la Junta de Revisión Institucional de los investigadores. Una Junta de Revisión Institucional es un comité que supervisa las investigaciones médicas para proteger los derechos y el bienestar de los participantes voluntarios.

Usted puede cambiar de parecer en cualquier momento con respecto a su permiso para que sus muestras identificables se usen en investigaciones futuras. Si así lo hace, comuníquese con los investigadores y hágaselo saber. A partir de ese momento, sus muestras ya no estarán disponibles para la investigación.

Los investigadores tal vez quieran saber más sobre usted en el futuro. Aun si acepta que vuelvan a comunicarse con usted, puede cambiar de opinión con respecto a ofrecer información en el futuro.

(Por favor, marque a continuación para indicar si acepta o no que se vuelvan a comunicar con usted en el futuro).

__ Acepto que vuelvan a comunicarse conmigo para pedir más información.
__ No acepto que vuelvan a comunicarse conmigo para pedir más información.

Iniciales del sujeto _____ Fecha _____

Preguntas

Por favor siéntase en libertad de hacer cualquier pregunta que tenga sobre el estudio o sobre sus derechos como un sujeto de investigación. Si se le ocurren otras preguntas más adelante, puede dirigirlas a:
En USCO, Dra. Doris Salgado o Dr. Jairo Rodriguez, teléfono: ***(insertar número de teléfono aquí).***
Si en cualquier momento, durante o después del estudio, usted desea hablar sobre el mismo o sobre sus derechos como participante en una investigación con alguien no relacionado con este estudio, puede ponerse en contacto con ***(insertar información adicional de contacto aquí)***.

Me han explicado el propósito y los procedimientos de este proyecto de investigación y los comprendo. Me han informado de todas las molestias, riesgos y beneficios previsibles que podrían ocurrir, y los comprendo. Me han informado que pueden ocurrir acontecimientos imprevistos. Acepto participar como sujeto en este proyecto de investigación. Entiendo que puedo dejar de participar en cualquier momento.

Nombre del sujeto: _____ FECHA: _____

NOMBRE: _____

Firma del testigo: _____ FECHA: _____

NOMBRE: _____

DECLARACIÓN DEL INVESTIGADOR

He explicado al sujeto mencionado anteriormente la naturaleza y el propósito de los procedimientos descritos más arriba y los riesgos, molestias y beneficios previsibles que podrían ocurrir. Le he preguntado al sujeto si tenía preguntas con respecto a los procedimientos y he contestado estas preguntas a mi leal saber y entender. He considerado y rechazado procedimientos alternativos para contestar esta pregunta de investigación.

_____ FECHA: _____