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CE SHEET)

Date 27.06.1995

ETHICAL REVIEW COMMITTEE, ICDDR,B.

ipal Investigators Dr. Nigar S Shahid Trainee Investigator (if any)

lication No. 95-017 Dr. Mark C. Steinhoff Supporting Agency (if Non-ICDDR,B)

le of Study Study of the immunogenicity of conjugate pneumococcal vaccine in infants of mothers who have and who have been immunized with polysaccharide vaccine

Project status:
(✓) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

cle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
(a) Ill subjects — Yes No
(b) Non-ill subjects — (Yes) No
(c) Minors or persons under guardianship — (Yes) No

Does the study involve:
(a) Physical risks to the subjects — Yes (No)
(b) Social Risks — Yes (No)
(c) Psychological risks to subjects — Yes (No)
(d) Discomfort to subjects — (Yes) No Minor
(e) Invasion of privacy — Yes (No)
(f) Disclosure of information damaging to subject or others — Yes (No)

Does the study involve:
(a) Use of records, (hospital, medical, death, birth or other) — (Yes) No
(b) Use of fetal tissue or abortus — Yes (No)
(c) Use of organs or body fluids — (Yes) No

Are subjects clearly informed about:
(a) Nature and purposes of study — (Yes) No
(b) Procedures to be followed including alternatives used — (Yes) No
(c) Physical risks — Yes No
(d) Sensitive questions — Yes No NA
(e) Benefits to be derived — (Yes) No
(f) Right to refuse to participate or to withdraw from study — (Yes) No
(g) Confidential handling of data — (Yes) No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — NA Yes No

5. Will signed consent form be required:
(a) From subjects — (Yes) No
(b) From parent or guardian (if subjects are minors) — (Yes) No
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:

___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
✓ Protocol (Required)
✓ Abstract Summary (Required)
✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
✓ Informed consent form for subjects
✓ Informed consent form for parent or guardian
___ Procedure for maintaining confidentiality
✓ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

ree to obtain approval of the Ethical Review Committee for any changes ving the rights and welfare of subjects before making such change.

gr S. Shahid
Principal Investigator

Trainee

APPLICATION FOR PROJECT GRANT

1.	PRINCIPAL INVESTIGATIONS:	Dr. Nigar Shahid, ICDDR,B Dr. Mark C. Steinhoff, JHU
	CO-INVESTIGATORS:	Drs. F. Qadri, M. Rahman, SA Sarker, RG Hamilton, Shahla Khatoon, Kohinoor Begum and Roushan Ara
2.	TITLE OF THE PROJECT:	Study of the immunogenicity of conjugate pneumococcal vaccine in infants of mothers who have and who have not been immunized with polysaccharide vaccine
3.	INSTITUTIONS:	ICDDR,B and Johns Hopkins University
4.	STARTING DATE:	When funds are available
5.	COMPLETION DATE:	2.5 years from starting date
6.	TOTAL BUDGET REQUIRED:	US$ 207,636 (ICDDR,B part)
7.	FUNDING SOURCE:	Unknown
8.	HEAD OF PROGRAM:	Dr. K.M.A. Aziz Acting Divisional Director Community Health Division

Abstract Summary

Deaths due to Pneumonia are common in children. S. pneumoniae (Spn) has been identified as a major pathogen causing acute lower respiratory infection (ALRI). In Bangladesh, as in countries around the world ALRI mortality rates are highest in the first half of infancy. As antibiotic resistance to the organism is growing, a vaccine for its prevention has a high priority. The newly developed Spn polysaccharide vaccine has been shown to be non immunogenic if administered before 6 months of age. Recent studies at ICDDR,B have shown that there is substantial maternal response and placental transfer of Spn antibodies when Spn polysaccharide vaccine is given to pregnant women during their third trimester. Protective levels of antibodies were maintained up to 14 weeks. Since the new Spn conjugate vaccines given to infants is likely to be protective from 2-4 months onwards, it is thought that the immunization to combined mother and child pairs may have the potential of reducing early ALRI infant mortality.

The objective of this protocol is to determine the immunogenicity of conjugate pneumococcal vaccine in infants of mothers who have been immunized with Spn polysaccharide vaccine.

A double-blind controlled trail is planned on 160 mothers who will be vaccinated around 32nd weeks of pregnancy either with Spn or the licensed Meningococcal meningitidis (Nm) vaccine. Their infants will be randomized to be vaccinated either by the Spn conjugate vaccine or the Hib ~~Influenzae~~ vaccine. Gestational age,

neuromuscular and physical maturity of the infant will be assessed. Mothers will be requested to provide sera before and after immunization and at delivery. Infants will have serum collected at birth (cord) and 1.5,5 and 9 months of age. Similarly nasopharyngeal swabs of mothers will be obtained for Spn culture and serotyping at vaccination and at delivery infants will be cultured for S. pneumoniae at birth and at every contact i.e. at age 1.5,2.5,3.5,5 and 9 months.

The maternal-cord serum pairs will provide data on the transplacental transfer of antibody. The infant sera will be used to determine the passively acquired antibody and the antibodies developed against the conjugate Spn vaccine. Nasopharyngeal cultures will be correlated with the presence and level of infant serum antibody level.

If the vaccine proves to produce protective levels of antibody in infants, a larger field trial may be considered in Malilab.

General Aims

1. To assess whether the presence of maternally derive antibodies to pneumococcal polysaccharides or to the carrier proteins influences the immunogenicity of glycoprotein conjugate pneumococcal vaccine.

Specific Aims

The specific objectives of the project are:

1. To assess the effect of maternally acquired polysaccharide specific antibodies on the filial pneumococcal antibody response to glycoprotein conjugate pneumococcal vaccine.
2. To assess the effect of maternally-acquired carrier protein-specific antibodies on the filial antibody response to glycoprotein conjugate pneumococcal vaccine, and to active immunization with the carrier protein.
3. To determine the IgG antibody responses on Bangladeshi infants to the study glycoprotein conjugate pneumococcal vaccine given in the standard WHO schedule of 6, 10, and 14 weeks in combination with routine DPT and OPV vaccines.
4. To assess the effect of maternal immunization, infant immunization, or both, on nasopharyngeal colonization and carriage of pneumococci in Bangladeshi infants.

Significance

Bacterial pneumonia accounts for 1.5 million deaths annually in infants and children worldwide being highest in the first few months of life. A high percentage of this is caused by S. pneumoniae (Spn). Although antibiotic treatment for pneumococcal disease is available, its diagnosis is difficult and the organism is fast developing multiple resistance to common antimicrobials.

Spn is an important cause of bacterial sepsis in Dhaka and accounts for 11% of ICDDR,B blood isolates. Fifty-two percent of Spn blood cultures are in infants of 4-12 months. Recent studies at ICDDR,B have shown that there is substantial maternal response and placental transfer of Spn antibodies when Spn polysaccharide vaccine was given to pregnant women during their third trimester. Protective levels of antibodies (>1 mcg/ml) were maintained upto 14 weeks and the median half life was calculated at 35 days. (manuscript submitted for publication) Since the new Spn conjugate vaccines are likely to be protective from 2-4 months onward, it is thought that a combined maternal immunization with polysaccharide and infants with the new conjugate vaccine may have the potential of reducing ALRI infant mortality.

Ethical Implication

The drawing of blood samples is associated with temporary discomfort and occasional bruising at the site. Both vaccines have been licensed by the FDA and given to adults with no reports of serious systemic reactions and a low rate of local symptoms. Biological samples will be collected by appropriately trained personnel. Our recently completed study on 70 women showed that pneumococcal vaccine was free of immediate side-effects, was safe immunogenic and the half-life of the antibody had a median of 35 days. Surveillance for Spn in blood cultures have shown that 60% of Spn positive cases are in infants. (Shahid et al - manuscript submitted for publication).

Participants are likely to have higher antibody titers to Spn or (Nm) after vaccination. Mothers who receive Spn vaccines will thus be protected against invasive Spn disease. The Nm vaccine given to 35 mothers has been found to be immunogenic and there is transfer of appreciable levels of Nm antibodies both in the child's serum as well as mothers' breastmilk. Severe (Nm) disease in adults is rare; therefore the presence of an increased antibody titre will probably be of little benefit to the mother. However, vaccination of mothers with either of these vaccines has the potential to protect their infants from neonatal (Nm) or Spn disease.

INTRODUCTION

Background

Pneumococcal disease is among the most common severe bacterial diseases of infants and children in developing regions. It is estimated that 20-40% of the 4 million infant and child deaths due to acute lower respiratory infection (ALRI) or pneumonia are caused by S. pneumoniae (Spn), with H. influenzae and viral organisms contributing a smaller fraction (1). ALRI mortality rates are highest in the post-neonatal period (2), and Spn is known to be an important causative agent in this age group (3). In Bangladesh, 61% of all pneumonia deaths in children under 5 years of age occur in infants under 6 months of age (4). A recent study of pneumonia in infants from the Gambia showed that Spn caused 20% of all cases and at least 13% of the cases were among 0 to 5 month old children (5). In addition, rates of

septicemia, meningitis and other severe Spn infections are higher during early infancy. A recent surveillance project for bacterial meningitis in the U.S. showed that Spn. was an important cause of meningitis and other severe infections in the first 5 months of life, with annual incidence rates of 38-98/100,000 (6). Antibiotic treatment of pneumococcal disease is effective; however, in regions with high infant mortality rates, primary care services are often poorly developed or under utilized, especially for newborn infants, and pneumococcal resistance to antimicrobial therapy is an increasing problem (7). For these reasons, the National Academy of Sciences has listed S. pneumoniae vaccine as the highest priority candidate in their vaccine priority listing which considered potential health benefits for developing countries (1). Given the success of immunization programs worldwide in delivering vaccines, this is an opportune time to evaluate the concept of adding an existing vaccine which has the potential for mortality reduction.

Protection against pneumococcal disease is dependent on serum antibody to the bacterial capsular polysaccharide. A vaccine which contains the polysaccharides of the 23 most invasive serotypes of S. pneumoniae has been licensed in the U.S. since 1983 for use in high-risk populations. This product is safe, stable and of proven efficacy against invasive Spn disease in high-risk adults and children older than 2 years of age (8). However, studies of its immunogenicity in infants has shown that some serotypes are poorly immunogenic before the age of two (9-

12). Unfortunately, most childhood ALRI deaths in developing countries occur before two years of age (2). Recent studies at ICDDR,B have shown that invasive Spn disease is present in 40% of infants under 6 months of age with ALRI and 68% in infancy; (6% of all blood culture isolations) is high amongst admitted diarrhoeal children. Surveillance for Spn serotypes among under 5 children with pneumonia at ICDDR,B has shown that serotypes 6 and 19 are the two most frequent serotypes and also account for a third of the deaths due to x Ray confirmed pneumonia patients at ICDDR,B. In Papua New Guinea, 8% of all childhood pneumonia deaths are in newborn infants, and 35% occur in 1 to 5 month old infants (13). Newly developed Spn polysaccharide vaccines which are conjugated to proteins are immunogenic in infants (14). Because polysaccharides are recognized mainly by T-cell independant mechanisms, they do not effectively produce high-level, high affirity antibodies, particularly in young children, nor do they induce the T cell memory required for booster response. The immunogenicity of polysaccharides can be increased by covalently linking them to a carrier protein. This approach is thought to work through the recruitment of T-helper cells as in the case of the Haemophilus influenzae vaccines (15). As a result of conjugation to proteins the magnitude of the B cell response is increased. The protective efficacy of such vaccines may be dependent on the isotype and subclass pattern of the immune response. In addition pre-existing natural exposure to the capsuler polysaccharide may also influence these patterns and

hence have an effect on the immunogenicity of the conjugate vaccines (16). If proved to be safe and effective, these vaccines will be widely used for infant immunization. However, the proposed conjugate vaccine will be limited to 5-7 serotypes and thus will be limited in its potential efficacy in the highest risk groups. In addition, a conjugate vaccine is unlikely to afford significant protection until after the second dose received at 2-4 months of age.

Maternal immunization with the licensed 23-valent Spn polysaccharide vaccine has the potential to provide passive protection to infants during the vulnerable first few months of life. However the conjugate vaccines take 6-12 weeks to provide protection and vaccination at 6 weeks of age will not prevent over 1/3 of the deaths due to Spn disease. Results of maternal immunization studies at ICDDR,B have shown that the polysaccharide vaccine is immunogenic in this population and the antibody has a median half life for 35 days. There is 50% of the antibody levels transfered to the infant at birth from the mother. Surveillance for Spn colonization in the nasopharyngeal has shown that 80% are colonized by 22 weeks of age. An earlier study in Africa has shown that adult women respond to the licensed pneumococcal vaccine and transfer antibody to their infants (17). Maternal immunization to protect both mother and newborn infant against tetanus is an established part of the EPI program. This proposed project will assess the effect of maternally derived antibody to infants vaccinated with

pneumococcal conjugate vaccine and will provide the biological rationale for further field studies with ARI vaccine.

Data Review

Pneumococcal Vaccine

A few studies have investigated placental transfer of Spn antibody in unimmunized women. An assessment of maternal and cord blood levels of antibody to pneumococcal serotype 7F showed that cord levels were 55% of maternal levels in 30 unimmunized women (18). Grunebaum, et al, assessed antibody levels for 12 pneumococcal serotypes in 12 maternal cord serum pairs from unimmunized subjects. The ratio of cord to maternal antibody levels ranged from 9% to 97% for the 12 serotypes , with a mean of 56% (19). Vincent-Ballereau et al. immunized 37 women in Burkina Faso with pneumococcal vaccine and tetanus toxoid and 17 women with tetanus toxoid alone during their eight month of pregnancy (17). The pneumococcal antibody was determined by a non-standard ELISA assay, and the statistical analysis was incomplete. Women who received pneumococcal vaccine had slightly higher titers of pneumococcal antibody than those who had not received the vaccine. Anti-pneumococcal antibody levels in cord blood were similar to those in maternal blood for all maternal-infant pairs. Cord levels of pneumococcal antibody were 20-90% higher in the infants born to immunized women than in those born to women in the control group. Recently at ICDDR,B results of a prospective randomized controlled trial have shown that maternal immunization with Spn polysaccharide vaccine at 32.33 weeks of

gestation that the vaccine is safe with no side-effects on mothers. It is immunogenic and there is 4 fold rise in antibody titre in the delivery blood compared to the pre-immunization titre; about 1/2 the antibodies is transferred to the child at birth, it has a half life of 35 days and has no effect on the colonization rates of S. pneumoniae in the nasopharyn of the infant.

Surveillance for invasive serotypes of S. pneumonia strains from 1992-1994 have shown that serotype 6 and 9 are responsible for a third of invasive pneumococcal disease in this population and 6 serotypes were responsible for two thirds of the invasive infections.

As part of an evaluation of the 14-valent Spn vaccine in adults in Papua New Guinea, 354 pregnant women received vaccine or placebo (13). A retrospective analysis of the experience of the mothers and their infants showed no evidence of an adverse effect on pregnancy outcome; the frequency of abortions, stillbirths and congenital defects was similar in the two groups of women. There was a 35% reduction in pneumonia episodes among the infants of the vaccinated women, when compared to infants of placebo recipients. In addition, a reduction of 32% in pneumonia episodes was noted in children younger than 17 months at the time of their mother's immunization. The authors suggest this latter group was protected through breast feeding. Other vaccines consisting of bacterial capsular polysaccharide have been evaluated in pregnant women (20-25).

Vaccines

The USFDA licensed 23 valent pneumococcal polysaccharide vaccine contains 25 micrograms of each of 23 serotypes of pneumococci. We and others have shown that this licensed vaccine is safe (28), our previous study in Dhaka showed it produces high titers of specific antibody in mothers and their infants.

The USFDA licensed meningococcal polysaccharide vaccine used as a control vaccine contains 50 micrograms of the polysaccharides of meningococcal types A,C,Y, and W-135 (25). This vaccine has been administered to pregnant women in Brazil and other countries and has also been shown to be safe in our previous study of maternal immunization.

An USFDA IND licensed glycoprotein conjugate pneumococcal vaccine which will have been evaluated in phase I/II trials in the United States will be administered to study infants. The pneumococcal conjugate vaccines contain polysaccharides of up to 9 different pneumococcal serotypes, chemically conjugated to a variety of carrier proteins. Our group has extensive experience with the Praxis/Lederie (now Wyeth-Lederle Vaccines and Pediatrics) product which has the polysaccharides of serotypes 6B, 14, 18C, 19F, 23F conjugated to a non-toxic mutant diphtheria toxin CRM197 (28). This product has been shown to be safe and immunogenic in 2 month old US infants. A similar nine valent conjugate vaccine (with serotypes 1,4,5,6B,9V,14,18C,19F,23F) has been developed, and will be evaluated in phase I/II studies by

Dr. Steinhoff in Baltimore infants at 2,4,6 months of age in July 1995.

A USFDA licensed recombinant Hepatitis B vaccine will be used as a control vaccine for the infants, and administered at 6, 10 and 14 weeks. This vaccine is licensed for universal infant use in the USA and recommended by some EPI programs of developing countries with high rates of infant and childhood infection (29). It is not known if hepatitis B infections of infants in Bangladesh are common, but the vaccine is safe and effective.

Meningococcal Vaccine

An evaluation of 51 pregnant Brazilian women immunized with the N. meningitidis type A and C polysaccharide vaccine (Nm) during an epidemic of meningococcal disease showed no adverse effects on the fetus or on pregnancy outcome (23). Moreover, immunization of the 51 infants with meningococcal vaccine at 6 months of age resulted in a normal antibody response, indicating that immune tolerance in the infants was not a consequence of maternal immunization (25). Similarly at ICDDR,B the (Nm) vaccine was found to be safe in 35 mothers who showed good antibody response, half of which was transferred to the infant.

A recent symposium reviewed the status of maternal immunization for protection of infants and concluded that immunization with polysaccharide vaccines was likely to result in infant protection (25-31). If maternal immunization provides protective antibody levels in early infancy, the effect on infant mortality of the addition of a licensed pneumococcal vaccine to

an established EPI program of maternal immunization with tetanus toxoid will be evaluated. If proven effective, the policy implications of maternal immunization are significant, even after protein conjugate Spn vaccines become available for routine use in infants, because maternal immunization has the potential to close the infant's window of vulnerability before the age when active immunization results in protective levels of antibody.

Study design

The study will be a prospective randomized double-blind controlled trial comparing Spn polysaccharide vaccine with Nm types A and C vaccine ((Nm)) in healthy pregnant women and on the immunogenicity of glycoprotein conjugate vaccine in their infants. No placebo immunization will be used. The vaccines to be used are all immunogenic and safe to pregnant mothers and infants.

Approximately 114 healthy pregnant women, aged 15-40 years and who are in third trimester of pregnancy will be randomized in a ratio of 1:2 to receive either the licensed 23 valent pneumococcal polysaccharide vaccine, or the licensed meningococcal polysaccharide vaccine along with tetanus toxoid in the opposite area as per EPI recommendation at 32±2 weeks of gestation.

Infants vaccinated mothers will be assigned in a 2:1 ratio to receive an USFDA IND licenced glycoprotein conjugate pneumococcal vaccine or the hepatitis B vaccine as a control as shown in Table I. Mothers will be requested to provide sera (2ml) from her vein before and after one month of immunization

and at delivery. Infants will have serum (1ml) collected at birth (cord) and 1.5m, 5m and 9 months of age from the infants' vein. Similarly nasopharyngeal swabs of mothers will be obtained for Spn culture and serotyping at vaccination and at delivery infants will be cultured for S. pneumoniae at birth and at every contact i.e.. at age 1.5,2.5,3.5,5 and 9 months.

The pneumococcal conjugate vaccine contains polysaccharides of upto 9 different pneumococcal serotypes, chemically conjugated to a variety of carrier proteins. The Praxis Lederle product which we will be using and in which our group has extensive experience has polysaccharides of serotypes 1,5,6,14,18,19,23 conjugated to a non-toxic mutant diptheria toxin CMR197 (28). Serotypes 6,14 and 19 have been found to be common serotypes circulatory in Bangladesh. All infants of enrolled mothers will receive 3 doses of study vaccines at EPI contact along with the WHO recommended standard immunization schedule in Bangladesh (OPV and DPT) will the Spn vaccine given in the other area.

Sample Size Estimate

In order to detect a difference of 50% in the antibody GMT of children of vaccinated mothers vs. unvaccinated mothers, at least 29 children in each group will be studied (using standard formulae and a one-sided alpha error level of 0.05, beta error of 0.20, power of 80%, and assuming that the GMT and standard deviation of the antibody titers are similar to or current data in Dhaka). Using similar error assumptions, 29 subjects will also provide an 80% power to detect a true correlation

coefficient of -0.50 or greater between the continuous variables, and to find a true reduction of pneumococcal colonization from 80% to 50%. Assuming a 30% dropout rate, we will recruit 38 mothers for each group, a total of 114 mother infant pairs.

Statistical Procedures and Analytic Plan

Because the distributions of antibody titers and of the increases in titer (post-immunization antibody level divided by pre-immunization antibody level) are usually positively skewed, the values will be log transformed to approximate a normal distribution for statistical analyses. The antilogarithms of the means of the log-transformed values are reported as geometric mean titers (GMT) or as geometric mean increases. Means of continuous variables will be compared by t test or Mann-Whitney U test; the relationship between continuous variables will be assessed by calculating Pearson or Spearman correlation coefficients. The chi square or Fisher's exact test will be used to compare proportions between groups.

Demographic and biological characteristics of the mothers in the pneumococcal and the meningococcal vaccine groups will be compared to assess the success of randomization. Pneumococcal antibody levels in the maternal serum specimen at delivery will be compared with infant cord antibody titers to determine the percent of transplacental transmission of maternal antibody for each pair. We will compare final infant antibody titer means and distributions of infants born to pneumococcal and meningococcal

vaccinated mothers to assess the differences in infants' antibody response related to maternal immunization experience.

The effect of specific maternal antibomococcal conjugate vaccine will be assessed by calculating the correlation coefficient of infant antibody titers at 18 weeks (one month after the third dose of vaccine) with the specific antibody titer in the cord specimen (predictor variable). A negative correlation between the two would imply that higher antibody titers in the cord specimen inhibit the infants production of antibodies after active immunization. To determine if maternal antibody to the carrier protein (diphtheria toxoid) influences the filial antibody response to the glycoprotein conjugate vaccine or diphtheria antitoxin antibody levels and final pneumococcal polysaccharide antibody would suggest inhibition related to maternal protein carrier antibodies. Similar analyses will be done using tetanus toxoid antibodies as a control, since tetanus vaccine is received by both mothers and infants. A multivariable regression technique will be used to assess the joint effects of maternal titers, birth weight, and other demographic variables on the final infant antibody titers to pneumococcal polysaccharide, tetanus and diphtheria toxoids.

The effect of maternal and/or infant immunization on nasopharyngeal colonization with pneumococci will be evaluated by comparing with the chi square test the rates of colonization with vaccine serotypes in vaccinated and unvaccinated infants. We will also compare mean serum antibody titers for the most common

colonizing serotypes between colonized and non-colonized infants. Mean breast milk antibody titers against common serotypes of those mothers who breast feed their infants will be compared in colonized infants.

Anticipated Outcome

If we show that maternal immunization does not have a negative effect on active immunization of the infant, then consideration of an evaluation of the effect of maternal immunization on early disease is appropriate. A field trial utilizing an established pneumococcal vaccine and an existing antenatal vaccine delivery system could be undertaken to determine the efficacy of maternal immunization in the reduction of early infant mortality and morbidity in Bangladesh. Over 80% of pregnant women in interferes with active immunization of the infant, such a trial could not be contemplated without further studies of the immunological mechanisms involved.

Time schedule

It is estimated that 3 years will be required to complete recruitment of 160 mothers, followup of infants for 1 year, laboratory studies and data analysis.

Local approval, hiring of field staff	Aug 95 - Oct 95
Recruitment of subjects	Nov 95 - July 96
Followup of infants	Feb 97 - Jan 98
Laboratory Analysis	July 97 - Feb 98
Report & publication writing	March 98 - Apr 98

Training of Personnel

Project personnel will be recruited during the first three months of the project. The PI and the coordinator have the experience in carrying out the last pneumococcal vaccine study. An effort will be taken to recruit the trained personnel who were also in the earlier project. Randomization of vaccine will be done using the permutted table of randomization and supervisors of each recruitment team will be provided the closed envelope containing the vaccine code to be used. Randomization will be checked on site and at ICDDR,B later for correctness and recording will be done. Data will be entered daily in duplicate and checked by supervisors and frequency printouts will be made weekly and checked by the PI.

Study Population and Recruitment of Participants

The study will be a prospective randomized double-masked controlled trial comparing Spn polysaccharide vaccine with Nm type A and C in healthy pregnant women and Spn conjugate vaccine with Nm vaccinated infants. Study participants will be recruited from mothers in Dhaka who are planning or registered to deliver in maternity units of our co-investigators. Vaccination will occur in the third trimester of pregnancy as per schedule noted above. Women who have had Spn or Nm immunization or known pneumococcal or meningeal disease within the past 5 years, who are <15 or >40 years of age, who are known to have had complicated pregnancies, or who are at risk for premature labor will be excluded. After giving witnessed written consent, women

will be randomized to receive one of the vaccines (Spn or Nm) using the permuted table of randomization. The code will be maintained with a disinterested third party and will be broken when the protocol is completed. Randomization will also be performed in case of vaccination of infants.

Post-Immunization Monitoring

Each participant will be given a questionnaire regarding possible local and systemic side effects at 24, 48 & 72 hours after immunization. Data on pregnancy outcome will be obtained on all women in both groups. Information regarding illness during the subsequent pregnancy and delivery, estimated gestational age (as described below) of the infant at delivery, complications of delivery, birth weight and neonatal illnesses will be recorded. We will arrange for follow-up of the mothers and infants to ensure collection of the specimens. Routine medical care and routine immunizations will be provided for the infants during the period of the study.

A weighing scale will be carried to the site of delivery and birth weight recorded at site within 24 hours of birth.

The gestational age of the infants will be estimated by two methods. We will ask the mothers the date of the first day of the last menstrual period and calculate the duration of gestation from that date to the date of birth. In addition, each child will be examined within 48 hours of age by a physician who will use the method of Ballard to assess the neuromuscular and physical maturity of the infant. The Ballard technique assigns a

standard score for neuromuscular and physical maturity and allows estimation of gestational age (32).

The dates of the last menstrual period, the dates of immunization of the mother and of delivery of the infant will be recorded and the gestational age and post-immunization interval will be calculated for each child. This will allow us to assess the effect of gestational age and interval from immunization to delivery on cord antibody levels.

Infants will be vaccinated by randomization with Spn conjugate vaccine and Hib vaccine during the EPI schedule for DPT vaccine.

Serum, and Culture Specimens (Table II)

All mothers will have serum collected immediately before and 1 month after immunization, and at the time of delivery. Infant cord blood will also be collected at the time of delivery. The infants will have serum samples collected at 6 weeks of age (at EPI scheduled) at 18-20 weeks and between 9 months and 1 year of age. Nasopharyngeal cultures for Spn will be obtained from mother at every immunization and delivery and the infant at each contact (birth, 1.5,2.5,3.5,5 weeks and anytime between 9-12 months of age. The maternal pre-and post-immunization sera will be used to assess the immunoglobulin isotype and subtype of the maternal antibody response. The maternal-cord serum pairs will provide data on the transplacental transfer of antibody and response to the conjugate vaccine. The infants' sera will be used to determine the half-life of the passively acquired

antibody. The results of the nasopharyngeal Spn cultures of the infants will be correlated with the presence and level of specific breast milk antibody and with infant serum antibody levels.

Specimen Management

Adhesive stickers are typed prior to sample collection and pasted on to the vials. They will then be transported to the appropriate labs in proper condition using the right transport media prope temperature and rightly recorded into forms. A register will be maintained by the project to hand over specimens to the respective labs. A computer system will be developed to enter data results directly. Regular checks on validity will be made. Alliquots of serum and breast milk samples will be stored in a -40o freezer. The computer system hee is already in place to store specimens such that they are easily retrievable when necessary and also placed back after use. Bacteriological Spn specimens will be lyophilized in duplicate and also maintained at -20oC. (Appendix I & II)

Antibody Assays

All serum specimens will be separated, aliquoted, frozen at -40oC in Dhaka to be analysed at ICDDR,B in accordance with methodology used by Dr. Hamilton, which is a method jointly developed with Dr. Carlone at CDC and is accepted methodology by ARI programe - WHO Geneva. (Appendix IV) Aliquotes of 20% of the samples will be shipped frozen to Dr. Hamilton laboratory for antibody assays to be run in parallel by him so as to provide an

independent evaluation of variability and standardization of the assay run at ICDDR,B immunology laboratory. Pneumococcal serotypes 6B and 19F will be used for antibody analysis because they are the most frequent serotypes isolated from blood cultures of ill children in Dhaka. Serum IgG and breast milk IgA antibodies to pneumococcal polysaccharides 6A and 19F are assayed by ELISA as previously described (33) with minor modifications. Serum specimens from each mother-infant pair will be assayed together in a single plate and all sera will be adsorbed with C polysaccharide. All pneumococcal assays will be performed by parallel titration with immune serum pool 89-SF (Dr. Carl Frasch, Center for Biologics Evaluation and Research, FDA) with the following antibody assignments: 13.4 µg/ml of IgG of IgG and 0.5 µg/ml of IgA anti-pneumococcus type 6B, 9.1 µg/ml of IgG and 0.6 µg/ml of IgA anti-type 19F. ELISAs for diphtheria and tetanus toxoid antibodies will be used, as previously described (33) with appropriate standards and controls.

Bacteriologic Methods for Colonization Study

The Nasopharyngeal swabs from the infants will be obtained in home or clinic using flexible Calgiswabs, immediately placed in Amies transport medium and shipped to the ICDDR,B microbiology laboratories and cultured with 8 hours (34,35). We have standardized these procedures in Dhaka and shown that experimentally inoculated pneumococci will survive for at least 2 days in local conditions in the Amies medium. The swabs will be inoculated in the laboratory on sheep blood agar with 0.5 µg/ml

Confidentiality

All participants will be assigned a study number at the time of recruitment, which will be used for all data analysis. Forms and records with personal identifiers will be kept in a secure file cabinet in Dhaka.

Collaborators

Obstetricians in charge of units where the deliveries will take place will be collaborators. Dr. Nigar Shahid is the P.I. at ICDDR,B, Dhaka and Dr. M. Steinhoff at Johns Hopkins University, Baltimore. Dr. Shahid will be responsible for all steps in the protocol. Which includes recruitment of women, follow-up, immunization, collection and processing of samples at ICDDR,B. Dr. R.C. Hamilton from the Department of Infection and Immunity, Johns Hopkins University School of Public Health will be collaborating for the laboratory assays. The Department of International Health at Johns Hopkins School of Public Health has a long history of successful collaboration with scientists at ICDDR,B.

Duties of Co-investigators

Dr. Firdausi Qadri will supervise the performance of the Elisa antibody assays.

Dr. Mahbubur Rahman will help supervise the bacterial isolation and characterization (serotyping) of the Spn strains.

Dr. S.A. Sarker and Dr..... will help in the followup and clinical assessment of subjects.

Professors Shahla Khatoon, Kohinoor Begum, Rowshan Ara will help recruit mothers for enrolling in to studies.

References

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BUDGET

PERSONNEL (ICDDR.B)

Expno	Employee Name	Parent B.C.	Gr-St.	Year wise I 1st	2nd	3rd	4th	5th	Yearly Salary	Budgeted Amount 1st Year	2nd Year	3rd Year	4th Year	5th Year	Total
19695	DR.NIGAR SAYEM SHAHID	300111	HOD6	40	40	20			19,932	7,973	8,531	4,564	0	0	21,068
35709	DR. FIRDAUSI QADRI	204011	HOD5	0	5	0			21,324	0	1,141	0	0	0	1,141
16428	DR. MAHBUBUR RAHMAN	200111	HOC4	5	5	0			14,604	730	781	0	0	0	1,511
17475	DR. MD.S.ALAM SARKER	110110	HOC5	5	5	0			15,408	770	824	0	0	0	1,594
14266	MISS TAHMINA BEGUM	300111	C318	80	80	40			3,876	3,101	3,318	1,775	0	0	8,194
	Research Officer-CS5			100	100	50			4,548	4,548	4,866	2,604	0	0	12,018
	Medical Officer-HOA			100	100	0			8,148	8,148	8,718	0	0	0	16,866
	Programmer-HOA			25	25	12.5			8,148	2,037	2,180	1,166	0	0	5,383
	Admi. Asst.-CS3			100	100	50			2,904	2,904	3,107	1,662	0	0	7,674
	DET & Manag-CS4			100	100	50			3,480	3,480	3,724	1,992	0	0	9,196
	HA - CS3			100	100	0			2,904	2,904	3,107	0	0	0	6,011
	HA - CS3			100	100	0			2,904	2,904	3,107	0	0	0	6,011
	HA - CS3			100	100	0			2,904	2,904	3,107	0	0	0	6,011
	Field Worker			100	100	0			768	768	822	0	0	0	1,590
	Field Worker			100	100	0			768	768	822	0	0	0	1,590
	Field Worker			100	100	0			768	768	822	0	0	0	1,590
								Total--->		44,707	48,978	13,763	0	0	107,448

LABORATORY SUPPLIES

One dram vials	15 gross	400	400	200	1,000
10 ml screw vial	2 gross	100	150	50	300
Pasteur Pipette	15 gross	40	40	20	100
Vaccine syring, (other syring)	14 box	50	100	50	200
Disinfectant	-	-	50	-	50
Falcon tubes	15 bags	150	150	100	400
Pin tips	5 bags	-	50	-	50
Amies transport media	5 bags	100	50	50	200
Nasopharyngeal swabs	5 boxes	50	100	50	200
(Immuno-chemicals and plastics included in test)					
	Sub-Total:	890	1,090	520	2,500

LABORATORY TESTS

1. Spa isolates + sensitivity 160x10x$10	150	300	150	600
2. IgG estimate 114x3x$3.50x4 antigens	-	7,760	5,008	12,768
3. Lyophilization 600x$2.85x3 vials	1,000	2,500	1,630	5,130
4. Serotyping of Spa strain	1,000	2,500	1,500	5,000
5. Pathology tests - (For subject backup-grouping & Rh factor, diarr pathogen)	150	250	100	500
Sub-Total:	2,300	13,310	8,388	23,998

STOCK SUPPLIES				
Stationary, offices supplies files, clipboard	400	400	200	1,000
Sub-Total:				1,000
NON-STOCK SUPPLIES				
File cabinets, chair, table, umbrella, pocket calculator, gumboots, bags, computer diskette	700	200	100	1,000
Sub-Total:				1,000
OTHER COST				
Telephone, Fax	150	150	200	500
Shipping	250	150	100	500
EPI immunization	400	800	400	1,600
Xeroxing	150	150	100	400
Cost for collecting blood	260	100	-	360
Staff clinic facilities	200	200	100	500
Maintenance	150	150	100	400
Medical Illustration	100	200	100	400
Medicine & Other cost for field	100	300	100	500
Sub-Total:	1,760	2,200	1,200	5,160
CAPITAL ITEMS				
+ 4^{0} C refregerator (1)	600	-	-	600
Infant weighing scales (4)	1,200	-		1,200
Length board (5)	100	-	-	100
Sub-Total:	1,900	-	-	1,900
Cost of computer, power-pack with printer	4,000	-	-	4,000
TRAVEL (LOCAL) (Tk.2500/week for 2 1/4 yrs)	2,500	3,000	2,000	7,500
TRAVEL INTERNATIONAL (Dhaka-USA & perdiem) 1 trip to conference	-	-	4,000	4,000
Sub-Total:	6,500	3,000	6,000	15,500
TOTAL:	59,157	69,173	30,171	158,501
31% overhead	18,338	21,444	9,353	49,135
GRAND TOTAL:	77,495	90,617	39,524	207,636

1/7/91

International Centre for Diarrhoeal Disease Research, Bangladesh
P.O. Box 128, Mohakhali, Dhaka-1212, Bangladesh

Consent form (for pneumonia vaccine protocol)

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is conducting studies on the development of pneumonia vaccine strategies for Bangladesh. Deaths due to pneumonia are highest during infancy, diagnosis and treatment is getting more difficult due to fast development of antibiotic resistant strains. Mothers vaccinated during pregnancy with pneumonia vaccine maintain protective levels of immunity only upto 14 weeks of birth. This protective level has to be increased upto 1 year of age of the child. For the purpose of this result we shall collect relevant information from you through a questionnaire. We will ask you some questions related to your present pregnancy, past obstetric history and socio-economic condition which should take about 30 minutes of your time. Either the pneumonia or the meningitis vaccine will be administered to you during the end of eighth month of your pregnancy. 2ml blood will be drawn from your vein at the time of vaccination and one month later. You will be requested to give another sample of blood (2ml) from your vein when your child is delivered. You will be requested to provide throat swabs at the time of vaccination and at delivery.

Cord blood will be obtained at delivery by the clinic staff. We will perform a thorough physical examination of your child between day 1-2 of birth and collect nasopharyngeal swabs for culture. Either the pneumonia or the hepatitis vaccine will be given to your baby in 3 doses along with the DPT vaccines according to EPI schedule. 1ml of blood will be drawn from the baby's cubital vein when he/she is 1.5, 5 and 9 months of age. These body fluids are required for assessing the extent to which the vaccine may be effective. We shall follow-up your child upto 12 months of age and give the child appropriate medical care through our physicians whenever necessary.

All information related to you and your infant shall be kept confidential.

If you decide to participate in the study, please put your signature/left thumb impression. If at any stage of the study, you wish to withdraw, you may do so, and the same treatment shall continue even after your withdrawal.

---------- ----------
Signature of Interviewer Signature/of Subject/(LTI)

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Signature of Investigator Date

Abstract Summary for ERC:

1. Death rates with pneumonia is highest during the first six months of life and antibiotic resistance to the organisms caus childhood pneumonia is increasing rapidly. Various ways to combat infant deaths is being considered. It was observed tha vaccinating very young children with pneumonia vaccine did not produce acceptable levels of serum antibody so that infectio to the organisms may be avoided. However a recent study at ICDDR,B has shwon that mothers vaccinated with pneumonia vacci have protective levels of antibodies upto 35 days. This projec is aimed at examining whether vaccinating pregnants mothers and their infants against pneumonia provides protection to newborns in early infancy. As in the case of tetanus the thought of providing antibodies to infants from vaccinated mother-infant pair is being considered. This project will require the cooperations of pregnant mothers and in turn their new borns. Voluntary consent will be obtained both from mothers and their husbands.

2. Mothers and their infants will be vaccinated either with Streptococcal pneumonia or Meningicoccal meningitidis polysaccharide vaccines. Both vaccines have proven to be safe causing little or no side effects. Infants will be randomized receive either the S. pneumoniae conjugate vaccine or the Hepatitis B vaccine. The vaccines will be administered at the time of the EPI schedule.

3. Mothers suffering from headaches, common cold etc. will not be enrolled. Diabetics mothers with known history of difficult previous child birth will not be enrolled.

4. The study will be a randomized double blind trial. All information will be coded and kept under lock and key.

5. The project field staff coming in contact with mothers and infants will be females. Trained personnel will help in the collection of serum and nasopharyngeal swabs from mothers as we as from their infants.

   a. A written informed consent will be administered to both th mother and her husband.

   b. Other than the type of vaccination being administered no information will be withheld from the subjects.

   c. There is minimum potential risk to the subjects. Treatment will be given for respiratory infectious and diarrhoeal diseases.

6. The initial interview will take 15-20 min and will be done at the antinatal clinics where the mothers will been rolled. All subsequent interviews will be done at home and will take 5-10 minutes.

INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE
RESEARCH, BANGLADESH

সম্মতি পত্র (নিউমেনিয়া প্রকল্প)

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ (ICDDR,B) বাংলাদেশের জন্য উন্নত নিউমোনিয়া প্রতিষেধক ভ্যাকসিন (টিকা) এর গবেষণার কাজ হাতে নিয়েছে। নিউমোনিয়ার কারণে শিশু মৃত্যুর হার সবচাইতে বেশী। ব্যবহৃত এন্টিবায়োটিকের প্রতিরোধ ক্ষমতা দ্রুত কমে যাওয়ার কারণে নিউমোনিয়া রোগ নিরুপণ করাও খুব জটিল। দেখা গেছে গর্ভাবস্থায় নিউমোনিয়া টিকা গ্রহণকারী মা'দের সন্তান ভূমিষ্ট হওয়ার ১৪ সপ্তাহ পর্যন্ত শিশুদের নিউমোনিয়া জীবাণু প্রতিরোধ ক্ষমতা থাকে। এই প্রতিরোধ মাত্রা শিশুর ১২ মাস বয়স পর্যন্ত হওয়া উচিত। এই গবেষণার কারণে আমরা আপনার কাছে প্রশ্ন পত্রের মাধ্যমে কিছু প্রাসঙ্গিক তথ্যাদি জানতে চাইবো। আমরা আপনাকে আপনার বর্তমান গর্ভাবস্থা, পূর্বের গর্ভাবস্থা এবং আর্থ-সামাজিক অবস্থা সম্বন্ধে কিছু প্রশ্ন করবো। এতে সময় নেবে ৩০ মিনিটের মত। অষ্টম মাসের গর্ভের শেষে নিউমোনিয়া অথবা মেনেন্জাইটিস এ দু'টোর মধ্যে যে কোন একটি টিকা আপনাকে দেয়া হবে। টিকা দানের সময় এবং টিকা দানের ১ মাস পর আপনার হাতের শিরা হতে ২ মিঃ লিঃ (2ml চা চামচের ১/২ চামচের কম) করে রক্ত নেয়া হবে। সন্তান ভুমিষ্ট হওয়ার সময় আরো ২ মিঃ লিঃ (2ml)রক্ত আপনার শিরা হতে দেওয়ার জন্য অনুরোধ করবো। আমরা আপনাকে টিকাদানের সময় এবং সন্তান প্রসবকালে আপনার গলার লালা সংগ্রহ করবো।

ক্লিনিকের কর্মচারীদের মাধ্যমে সন্তান প্রসবের সময় নাড়ীর রক্ত সংগ্রহ করা হবে। শিশুর জন্মের ১-২ দিনের মধ্যে আমরা শিশুর সম্পূর্ন শারীরিক পরীক্ষা করবো এবং গলার লালা সংগ্রহ করে পরীক্ষা করবো। ই পি আই (EPI)নিয়ম অনুসারে ডিপিটি (DPT)টিকা দানের সময় নিউমোনিয়া অথবা হেপাটাইটিস টিকা আপনার শিশুকে মোট ৩ (তিন) ডোজ দেয়া হবে। শিশুর দেড় (১.৫) মাস, পাঁচ (৫) মাস ও নয় (৯) মাস বয়সের সময় শিশুর হাতের শিরা থেকে ১ মিঃ লিঃ (1ml চা চামচের ১/৪ চামুচ) করে রক্ত সংগ্রহ করা হবে। এটা নেয়া হবে শিশুর শরীরে টিকার কার্যকারীতা দেখার জন্য। আমরা ১২ মাস বয়স পর্যন্ত আপনার শিশুকে পর্যবেক্ষণ করবো এবং প্রয়োজন অনুসারে আমাদের ডাক্তারের মাধ্যমে আপনার শিশুকে সঠিক চিকিৎসা দেব। আপনার এবং আপনার শিশুর সব রকম তথ্যের গোপনীয়তা রক্ষা করা হবে।

যদি আপনি এ গবেষণায় অংশ গ্রহণ করতে সিদ্ধান্ত নেন তবে নীচে আপনার স্বাক্ষর/বৃদ্ধাঙ্গুলীর ছাপ দিন। গবেষণা চলাকালীন যে কোন সময়ে আপনি এবং আপনার শিশুকে গবেষণা থেকে প্রত্যাহার করে নিতে পারবেন, তার জন্য চিকিৎসার কোন তারতম্য হবে না।

অংশগ্রহণকারীর স্বাক্ষর ঃ---------- সাক্ষাতকারীর স্বাক্ষরঃ----------

তারিখ ----------

গবেষকের স্বাক্ষর ঃ----------

7. There is minimum risk involved and biological samples will be collected with appropriately trained personnel. This project will provide the information on the effect of maternally-derived antibody on the antibody response of infants to pneumonia vaccine and estimate the effect of mother-infant pair vaccination on colonization of infants by S. pneumoniae. This will provide biological rationale for further studies on the efficacy of maternal immunization.

8. Activities involve the use of obstetrical records, mother and infant sera, cord blood and nasopharyngeal swab specimen.

Table 1 Study groups for analysis

	Group		
Subject	A	B	C
mother	Spn	Mn	Mn
infants	Pcj	Pcj	HiB conj

Vaccines: Spn = pneumococcal polysaccharide
Pci = pneumococcal conjugate
Mn = meningococcal
Hiβ= *H.influenzea* β

Comparisons between infant groups

Group A vs B: effect of maternal antibodies on infant response to active immunization.

Group A,B, vs. C: 1) effect of maternal or infant immunization, or both, on carriage of pneumococci.

Group B vs C: Immunogenicity of glycoprotein conjugate vaccine in Dhaka infants using the 6,10,14 weeks routine EPI schedule.

Flow Chart

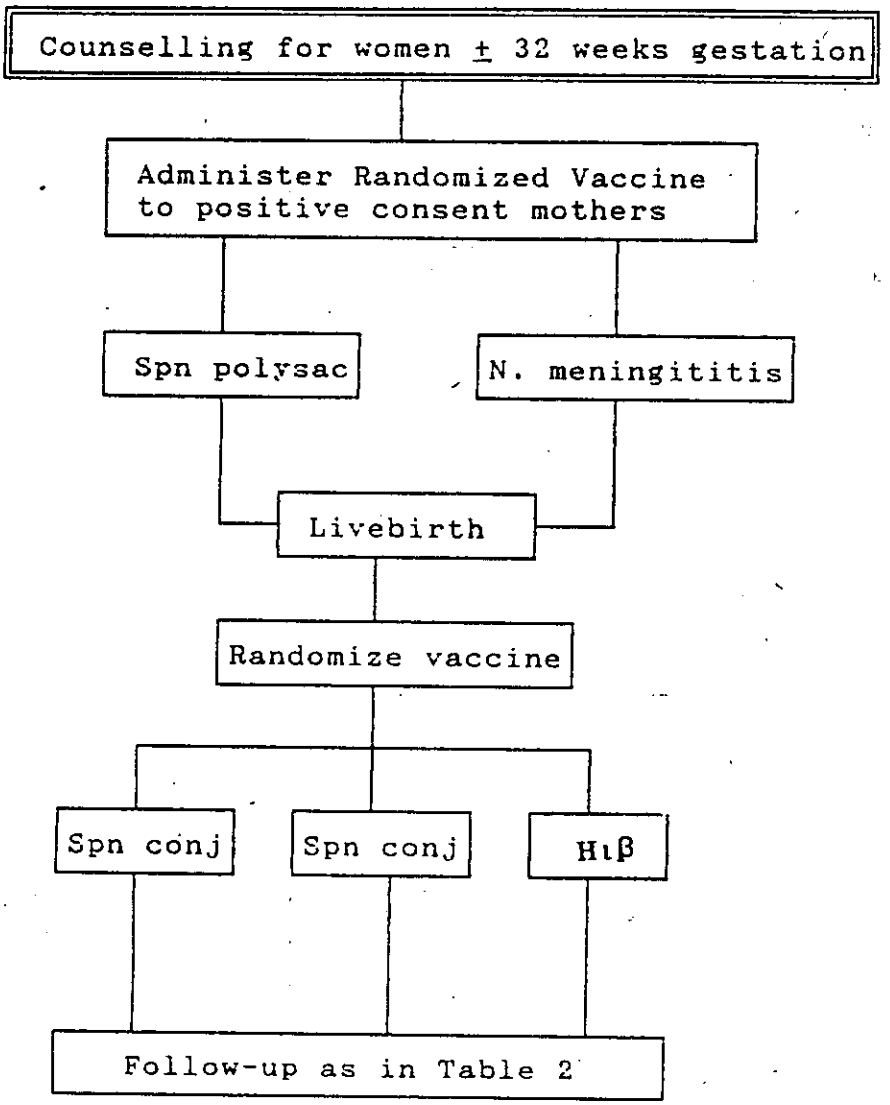
Counselling for women ± 32 weeks gestation
Administer Randomized Vaccine to positive consent mothers
Spn polysac
N. meningititis
Livebirth
Randomize vaccine
Spn conj
Spn conj
Hiβ
Follow-up as in Table 2

Table 2 Schedule of Procedures

Procedures wks	Maternal wks		Infant					
	32(±2)	del	0,	6,	10,	14,	22,	39
Vaccination	V[1]	---	--	Cj[2],	Cj,	Cj		
Blood	B	B		B		B	B	B
Culture	C	C	C	C	C	C	C	C

1. Mothers receive either Spn or Nm polysaccharide vaccine. (V[1])
2. Infants receive either the conjugate 9-valent Spn or the Hib vaccine. (Cj[2])
3. Cord blood will be collected at birth in addition to blood samples at these periods.(B)
4. Nasopharyngeal cultures at these ages.

National Immunization Study
ICDDR,B Budget for subcontract for 30 months (REVISED 11/20/95)

Personnel	effort	months	THRASH	PRAXIS	ICDDRB	TOTAL	
Dr. Nigar Shahid - PI	25%	30	9,420	5,652		15,072	
Dr. Ferdousi Qadri - CI	5%	12	760			760	
Dr. Mahbubur Rahman -CI	5%	18			900	900	
Dr. S.A. Sarker-CI	5%	24			933	933	
Ms. Tahmina Begum, coordinator	90%	24	806		7,269	8,075	
Research Officer	100%	24	4,176		8,550	12,726	
Medical Officer	100%	24	7,620	7,620		15,240	
Programmer	25%	24	3,850	3,850		7,700	
Secretary	100%	24	3,666		3,667	7,333	
Data Manager	100%	30	7,954	7,954		15,908	
Health Assistant	100%	60	13,663	5,237		18,900	
Field Worker	100%	60	3,750	4,350		8,100	
Resch Off Assistant	100%	24	1,500		1200	2,700	
	Subtotal		57,165	34,663	22,519	114,347	11
Laboratory Supplies						0	
One dram vials	15 gross		1,000			1,000	
10 ml screw vial	2 gross		300			300	
Pasteur Pipette	15 gross		100			100	
Vaccine syringe	14 box		200				
Disinfectant	–		50				
Falcon tubes	15 bags		400				
Pipe tips	5 bags		50				
Amies transport media	5 bags		200				
Nasopharyngeal swabs	5 boxes		200				
	Subtotal		2,500	0	0		
Supplies							
Stock Supplies: office and computer items			1,000				
Non-Stock Supplies: umbrella, gumboots filecabinets, etc			1,000		1		
			2,000		1		
other cost							
Telephone, fax, email			900				
Shipping			960				
Xeroxing			400		100		
Medical Illustration			150		100		
Medicines, honoraria , other cost for field			300				
	Subtotal		2,710	0	2,		
Laboratory Tests							
1. IgG estimate 237x12x$3.75 (ELISA pneumo)			10,665	5,000	10, 00		
2. Pathology tests - (For clinical care, cultures, Rh factor etc			1,000	0			
3. Cultures 150x10x$5+ sensitivity			0	600	6, 00		
4.Serotyping (Spn + Hib)			1,000	4,000	1, 00		
5. vaccination costs			0	2,750	0		
	Subtotal		12,665	12,350	18,		
Capital Items							
Computer for data-entry			0	3,500	500		
+4 degree C refrigerator (1)			640			640	
Infant weighing scales (1)			300		900	1,200	
Length board (1)			20		80	100	
	Subtotal		960	3,500	1,480	5,940	
Travel						0	
local (Tk 2500/wk)			4,700	0	5,000	9,700	
International (Dhaka to USA)			0	4,000	2,000	6,000	
	Subtotal		4,700	4,000	7,000	15,700	
	Grand Total		82,700	54,513	52,599	189,812	
	7% overhead		5,789	0	0	5,789	
	Total plus overhead		$88,488	$54,513	$52,599	195,000	1

THRASH PRAXIS ICDDRB TOTAL

1st draft June 1

EFFECT C

Budget St

1. Effect
on res

E,6
7/8/13 b

EFFECT OF MATERNAL IMMUNIZATION ON INFANT RESPONSE TO PNEUMOCOCCAL VACCIN

M. C. Steinhoff, N. Shahid

Johns Hopkins University
ICDDR,B

Budget Summary (revised 11/17/95)

Goal	Sponsor	Budget ICDDR,B
1. Effect of maternal pneumococcal antibodies on response of infants to 9vPncJ	Thrasher Fund	88,488
2. Effect of maternal diphtheria antibodies on response of infants to 9vPncJ	Thrasher Fund	(see above)
3. Immunogenicity of 9vPncJ in Dhaka infants	Praxis Lederle	54,513
4. Immunization and colonization with pneumococci in Dhaka infants	ICDDR,B	52,599
		195,600

9vPncJ = 9-valent pneumococcal-CRM197 conjugate vaccine

APPENDIX - I

I Serum sample

Subject	Time	No.	Where drawn
a. Mothers (2 ml-IV)	At vaccination	114	Antenatal clinic
	1M Post Vacc	114	"
	At delivery	114	Hospital
	Total samples	342	
b. Infants (1ml-IV)	Cord	114	Hospital
	1.5m	114	ICDDR,B Clinic
	5m	114	" "
	9m	114	" "
		456	

Total - 798 serum samples

II Nasopharyngeal (NP) cultures of mothers and infants

Mothers

Intervals - immunization + delivery 114 x 2 = 228

Infants interval - birth, 1.5,2.5,3.5,5 and 9 months
i.e. 114 x 6 = 684

Total NP cultures = 912

APPENDIX - IIA

Assays to be done

798 Sera Elisa ICDDR,B & 6,14,19 plus 2 other common Bangladesh serotypes
IgG to Spn
IgG to (Nm) polysaccharide -

912 infants nasopharyngeal swabs - ICDDR,B
Indentification and serotyping of Spn strains.

APPENDIX - III

The method of Kronvall (Kronvall, G. 1973) will be used to prepare the coagglutination reagents as described below.

S. aureus Cowan 1 will be grown overnight in Trypticase Soy Broth (TSB) at 37° C on a shaker.

The bacterial cells will be washed three times with phosphate-buffered saline (PBS; 0.03 M phosphate, 0.12m Nacl, pH 7.4) and suspended in 0.5% formaldehyde in PBS. Then the suspension will be incubated at room temperature for 3h.

Following incubation formaldehyde treated bacterial cells will be washed three times with PBS and diluted ten times in PBS.

The cell suspension will then be heated at 80° C in a water bath for 1h and cooled quickly.

Finally cells will be washed with PBS and diluted ten times in PBS which will then be stored at 4° C.

To coat the cells with antiserum 1 ml of the stabilized, heat-treated S. aureus cell suspension will be added to 0.2 ml of high-titre S. pneumoniae omniserum. (obtained from statuns serum institution Copenhagen) For 9 different pools (A,B.....I) separate and frequently isolated serotypes (6,14,19) of the 83 serotypes separate preparations will be made.

The suspension will be incubated at room temperature for 3h with gentle hand shaking at 0.5h intervals.

Following centrifugation and washing with PBS, the cells will be diluted finally to a 2% (vol/vol) suspension in PBS. These cells designated as SPN.COAG reagent will be stored at 4° C until used and prepared every month.

A 2% (vol/vol) formaldehyde-stabilized, heat-treated S. aureus cell suspension in PBS which will not be coated with antiserum, will be used as a negative control.

As a positive antigen control S. pneumoniae of known serotype will be used. If emulsified with SPN. COAG reagent visible agglutination will be observed.

Pneumococcal ELISA Protocol (unamplified) Version of 5/94

(Dr. G. Siber)

Reagents:

Pneumococcal C polysaccharide (Porter Anderson, University of Rochester)
Pneumococcal type specific PS (ATCC)
89-SF Standard Serum (Carl Frasch, CBER)

10X PBS — 81.0 g NaCl, 1.57 g $NaH_2PO_4 \cdot H_2O$, 6.89 g Na_2HPO_4 (anhydrous). Make up to 1 liter with distilled water and adjust pH to 7.2 with 10 N NaOH. Dilute 1/10 for use as 1X PBS.

PBSTween — To 1X PBS add 0.05% Tween 20 (Sigma #P 1379)

DEA buffer — To 400 ml distilled water add 0.05 g $MgCl_2 \cdot 6H_2O$. Add 48.5 ml Diethanolamine (Sigma #D 8885) with stirring. Bring pH to 9.8 with 12 N HCl and adjust final volume to 500 ml.

Detecting Ab — We use alk phos conjugated antibodies from Caltag Laboratories, South San Francisco CA. New lots are checked for isotype specificity using human IgG, IgM and IgA.

Substrate — Sigma 104® Phosphatase Substrate

Plates — Nunc® Immuno plate Polysorp U96, Cat #475434 or Polysorp F96, Cat #475094 (for **antigen** coating)
Any inexpensive microtiter plate which holds at least 320 µl/well (for **dilutions**)

Method:

Coat plates: Checker to determine optimal coating concentration of pneumococcal polysaccharide, usually about 5 µg/ml. Dilute to that concentration in 1X PBS, 100 µl/well, seal and incubate at 37°C for 5 hrs in the dark. Seal in Ziploc bags and store at 4°C up to one month. These are your **antigen plates.**

One day assay: Prepare **dilution plate.** One dilution plate will fill three antigen-coated plates. Plates will be set up as follows:

	1	2	3	4	5	6	7	8	9	10	11	12
A							PT	PT				
B												
C												
D	Unk 4		Unk 5		Unk 6				Unk 8		Unk 9	
E												
F												
G												
H	Unk 1		Unk 2		Unk 3		89-SF		Unk 7		G141A-3	

Prepare C Ps diluent by diluting C Ps to 2.5 µg/ml in PBSTween. Dilute Unknowns and Standard in C Ps diluent directly in first wells of **dilution plate.** Unknowns and standard are diluted starting at 1/50 by adding 294 µl of C Ps diluent to first dilution wells, then adding 6 µl of sample directly to the

wells. Mix these wells 5 times by drawing up and down with a 12 channel pipet set at 100 µl. Let dilutions sit at room temperature for 30 minutes.

Just before end of 30 minute incubation, wash coated **antigen plates** 3 times with PBSTween. Leave last wash in plates until ready to fill.

Add PBSTween (no C Ps) to remaining wells of **dilution plate**, 200 µl/well.

At end of 30 minute incubation, mix row H of **dilution plate** 5 times by drawing up and down with a 12 channel pipet set at 100 µl. Transfer 100 µl to row G, Mix 5 times, transfer to row F, mix, transfer to row E, Mix. Do not discard extra volume, but eject pipet tips. Using a regular single channel pipetman transfer 100 µl from each of the standard's row E wells to row D (E7 → D7, E8 → D8).

Using new pipet tips continue diluting second half of **dilution plate**. Starting at row D, Mix 5 times, transfer 100 µl to row C, mix, transfer to row B, mix, IF DESIRED, remove tips 7 AND 8 for PBSTween controls, and transfer to row A, mix.

Empty last wash from antigen plates. Transfer contents of **dilution plate** to **antigen plates** using 12-channel pipet, 50 µl/well. Remember to start with most dilute sample in row A and work towards row D. DISCARD TIPS and with new tips transfer beginning at row E and work towards row H. Cover plates with plastic wrap or sealers and incubate at room temperature for 2 hours.

Wash plates 3 times with PBSTween, add detecting antibody diluted in PBSTween (again checkered to determine optimal concentration), 50 µl/well and incubate at RT 2 hours.

Wash plate 2 times with PBSTween, 1 time with DEA buffer, add substrate diluted to 1 mg/ml in DEA buffer, 100 µl/well, and develop at room temp for 30 minutes. If desired stop reaction with 50 µl/well of 3N NaOH and read at 405 nm.

Meningococcus A or C specific IgG, IgM or IgA ELISA

Reagents:

Methylated human serum albumin
Meningococcal group A or C polysaccharide

Plates	Immulon® 2, Dynatech Laboratories #011-010-3455
10X PBS	81.0 g NaCl, 1.57 g $NaH_2PO_4 \cdot H_2O$, 6.89 g Na_2HPO_4 (anhydrous). Make up to 1 liter with distilled water and adjust pH to 7.2 with 10 N NaOH. Dilute 1/10 for use as 1X PBS.
PBSTween	To 1X PBS add 0.05% Tween 20 (Sigma #P 1379)
Blocking buffer	To 1X PBS-Tween add 5% Fetal Calf Serum and filter.
Detecting Ab	Alkaline phosphatase conjugated antibodies from Caltag Laboratories, South San Francisco CA. New lots are checked for isotype specificity using human IgG, IgM and IgA.
DEA buffer	To 400 ml distilled water add 0.05 g $MgCl_2 \cdot 6H_2O$. Add 48.5 ml Diethanolamine (Sigma #D 8885) with stirring. Bring pH to 9.8 with 12 N HCl and adjust final volume to 500 ml.
Substrate	Sigma 104® Phosphatase Substrate
Stopper	3N NaOH

Method: All reagents are used at 100 µl/well, washes at 150 µl/well, except as noted.

Day 1

Coat plates: Mix equal volumes of mHSA and meningococcal polysaccharide at a final concentration of 5 µg/ml each in 1X PBS: Make up solutions of 10 µg/ml each. Add meningococcal polysaccharide dropwise to the mHSA while stirring. Add 100 µl/well, seal and incubate at 4° overnight. Plates can be sealed in a ziploc bag and used for up to a month if no signs of evaporation.

Day 2

Wash wells 3 times with PBSTween. Block plates for 1 hour at room temperature with 150 µl/well of blocking buffer.

Plate will be set up as follows:

	1	2	3	4	5	6	7	8	9	10	11	12
A							PT	PT				
B												
C												
D	Unk 4		Unk 5		Unk 6				Unk 8		Unk 9	
E												
F												
G												
H	Unk 1		Unk 2		Unk 3		CDC 1992		Unk 7		G141A-3	

Remove blocking solution and dilute samples and standard directly in plate:

Add 142.5 µl of blocking buffer to rows D and H (except D7 and D8). Add 7.5 µl of sample directly to these wells. Fill remainder of plate with 100 µl/well of blocking buffer. Mix row H five times by drawing up and down with a 12 channel pipet set at 50 µl. Transfer 50 µl to row G, mix 5 times, transfer to row F, mix, transfer to row E, mix. Remove tips 7 and 8 (for standard curve) and discard extra volume from remainder of wells. Eject pipet tips. Using a regular single channel pipetman transfer 50 µl from each of the standard's row E wells to row D (E7 → D7, E8 → D8).

Using new pipet tips continue diluting second half of plate. Starting at row D, mix 5 times, transfer 50 µl to row C, mix, transfer to row B, mix. IF DESIRED, remove tips 7 AND 8 for PBSTween controls, and transfer to row A, mix. Discard extra 50 µl from row A.

Seal and incubate overnight at 4° C.

Day 3

Wash plate 3 times with PBSTween, add detecting antibody diluted in blocking buffer (checkered to determine optimal concentration) and incubate at RT for 2 hours.

Wash plate 3 times with PBSTween, add substrate diluted to 1 mg/ml in DEA buffer and develop at room temp for 30 min. If desired stop reaction with 50 µl/well of stopper solution and read at 405 nm.

ELISA for diphtheria toxoid will be conducted using standard methods as in Barington et al Infect and Immu, 1993; 61(3):432-438

SPN VACCINE STUDY
Form I (ENROLMENT)

1. Subject#.....:_____
2. Date of Interview.....:_____dd/mm/yy
3. Age of the subject.....:_____years
4. Place of Enrolment.....:TAC------> 1
KB-------> 2
AM-------> 3
Other----> 7
5. Address_____ _____ _____

6. Husband's age.....:_____years

7. Head of the family.....:Husband--> 1
Self-----> 2
Son------> 3

8. Obstetric History:
a. LMP Date.....:_____dd/mm/yy
b. Gravida.....:_____no.
c. Para.....:_____
d. Previous history
1. Live birth.....: Yes--> 1 No--> 2
2. Precipitated labor.....: Yes--> 1 No--> 2
3. Abortion.....: Yes--> 1 No--> 2

9. Age of the last child (0=No child).....:_____months
10. Blood Group of the subject:
a. Group.....: A----> 1
B----> 2
AB---> 3
O----> 4

b. Rh type.....: Positive--> 1
Negative--> 2

11. Blood Group of the husband
a. Group.....: A----> 1
B----> 2
AB---> 3
O----> 4

b. Rh type.....: Positive--> 1
Negative--> 2

12. Has TT been given during this pregnancy.....: Yes---> 1 No--->
13. Date TT given (to be given in right arm).....:_____dd/mm/yy
14. Date of vaccine given (to be given in left arm)....:_____dd/mm/yy
15. Vaccine code.....:_____
16. Blood collected.....: Yes---> 1 No---> 2
17. Has previous child got breastfeeding.....: Yes-------> 1
No--------> 2
No child--> 9

18. Mother's Weight (nearest .1kg).....:___/___/___/ kg.
19. Mother's Height (nearest .1cm).....:___/___/___/___/ cm
20. Interviewer's ID #.....:_____

CHD:SPN\DANI\F1
DOI:6/1995

SPN VACCINE STUDY
Form II (SOCIOECONOMIC STATUS)

1. Study #..... : _____
2. Date of Interview..... : _____dd/mm/yy
3. Any side effect of vaccine..... : Yes---> 1 No---> 2
4. Reaction to the vaccine immediately after given vaccine (24-36 hrs.):
   a. Rash..... : Yes---> 1 No---> 2
   b. Itching..... : Yes---> 1 No---> 2
   c. Erythema..... : Yes---> 1 No---> 2
   d. Pain..... : Yes---> 1 No---> 2
   e. Others _____ : Yes---> 1 No---> 2
   f. Body temperature:
      - 8 hrs after vaccination..... : _____ ^{0}C
      - in the evening..... : _____ ^{0}C

5. Date side effect..... : _____dd/mm/yy

==========

6. Subject's years of schooling..... : _____yrs.
7. Husband's years of schooling..... : _____yrs.

8. Is subject an earning member..... : Yes---> 1 No---> 2
9. No. of the members eating together..... : _____no.
10. No. of the rooms in the house..... : _____no.
11. Does family own:
    a. AC..... : Yes---> 1 No---> 2
    b. Fan..... : Yes---> 1 No---> 2
    c. TV..... : Yes---> 1 No---> 2
    d. VCP/VCR..... : Yes---> 1 No---> 2
    e. Refrigerator..... : Yes---> 1 No---> 2
    f. Luxery Cot..... : Yes---> 1 No---> 2

12. Type of fuel used for cooking..... : Gas----------> 1
    Electricity-----> 2
    Firewood--------> 3
    Cowdung/leaves--> 4
    Misc. scraps----> 5

13. Light source at night..... : Electricity-----> 1
    Kerosine lamp---> 2
14. Husband's occupation..... : _____
15. Wife's occupation..... : _____

16. Disposal site of garbage..... : Courtyard---------->1
    Outside the house->2

17. Husband smokes..... : _____# sticks/day
18. Subject smokes..... : _____# sticks/day
19. Caretaker smokes..... : _____# sticks/day
20. Other member smokes..... : _____# sticks/day
21. Is husband a Zarda/Tobacco taker..... : Yes---> 1 No---> 2
22. Is subject a Zarda/Tobacco taker..... : Yes---> 1 No---> 2
23. Is caretaker a Zarda/Tobacco taker..... : Yes---> 1 No---> 2
24. Is other member a Zarda/Tobacco taker..... : Yes---> 1 No---> 2

Interviewer's ID #..... : _____

CHD:SPN\DANI\F2
DOI:6/1995

SPN VACCINE STUDY
Form III (DELIVERY RELATED INFORMATION)

1. Subject #.....: _____
2. Date of Interview.....: _____dd/mm/yy
3. Where was child delivered.....: Clinic-----> 1
   Hospital---> 2
   Home-------> 3
   Other------> 4

4. Date of delivery.....: _____dd/mm/yy
5. Time of delivery (in 24 hrs).....: _____dd/mm/yy

6. Who delivered.....: Consultant---> 1
   Physician----> 2
   Trained TBA--> 3
   Untrained TBA->4
   Other--------> 5

7. Mode of delivery.....: Normal-------> 1
   C/S----------> 2
   Forceps------> 3
   Episiotomy---> 4
   Other--------> 5

8. Was the mother given anaesthesia.....: None---------> 1
   GA-----------> 2
   EA-----------> 3

9. Complications (in detail) of delivery:

   _____: _____
   _____: _____
   _____: _____
   _____: _____

10. APGAR score.....: 0 minutes----> 1
    1 minutes----> 2

11. Birth weight.....: _____Kgm
12. Birth length (nearest .1 cm).....: ____/____/____/

13. When was the baby put to the breast.....: _____hrs after delivery

14. Is cord blood collected.....: Yes---> 1 No---> 2
15. Is mother's blood collected.....: Yes---> 1 No---> 2
16. Is colostrom collected.....: Yes---> 1 No---> 2
17. Is nasal swab collected.....: Yes---> 1 No---> 2

18. Mother's Height.....: _____cm

19. Interviewer:s ID #.....: _____

CHD:SPN\DANI\F3.
DOI:6/1995

SPN VACCINE STUDY
Form II (SOCIOECONOMIC STATUS)

1. Study #: _____
2. Date of Interview: _____dd/mm/yy
3. Any side effect of vaccine: Yes---> 1 No---> 2
4. Reaction to the vaccine immediately after given vaccine (24-36 hrs.):
   a. Rash: Yes---> 1 No---> 2
   b. Itching: Yes---> 1 No---> 2
   c. Erythema: Yes---> 1 No---> 2
   d. Pain: Yes---> 1 No---> 2
   e. Others_____: Yes---> 1 No---> 2
   f. Body temperature:
      - 8 hrs after vaccination: _____ ^{0}C
      - in the evening: _____ ^{0}C

5. Date side effect: _____dd/mm/yy

==========

6. Subject's years of schooling: _____yrs.
7. Husband's years of schooling: _____yrs.

8. Is subject an earning member: Yes---> 1 No---> 2
9. No. of the members eating together: _____no.
10. No. of the rooms in the house: _____no.
11. Does family own:
    a. AC: Yes---> 1 No---> 2
    b. Fan: Yes---> 1 No---> 2
    c. TV: Yes---> 1 No---> 2
    d. VCP/VCR: Yes---> 1 No---> 2
    e. Refrigerator: Yes---> 1 No---> 2
    f. Luxery Cot: Yes---> 1 No---> 2

12. Type of fuel used for cooking: Gas----------> 1
    Electricity-----> 2
    Firewood--------> 3
    Cowdung/leaves--> 4
    Misc. scraps----> 5

13. Light source at night: Electricity-----> 1
    Kerosine lamp---> 2

14. Husband's occupation: _____
15. Wife's occupation: _____

16. Disposal site of garbage: Courtyard---------->1
    Outside the house->2

17. Husband smokes: _____# sticks/day
18. Subject smokes: _____# sticks/day
19. Caretaker smokes: _____# sticks/day
20. Other member smokes: _____# sticks/day
21. Is husband a Zarda/Tobacco taker: Yes---> 1 No---> 2
22. Is subject a Zarda/Tobacco taker: Yes---> 1 No---> 2
23. Is caretaker a Zarda/Tobacco taker: Yes---> 1 No---> 2
24. Is other member a Zarda/Tobacco taker: Yes---> 1 No---> 2

Interviewer's ID #: _____

CHD:SPN\DANI\F2
DOI:6/1995

SPN VACCINE STUDY
Form III (DELIVERY RELATED INFORMATION)

1. Subject #.....: _____
2. Date of Interview.....: _____dd/mm/yy
3. Where was child delivered.....: Clinic-----> 1
   Hospital---> 2
   Home-------> 3
   Other------> 4

4. Date of delivery.....: _____dd/mm/yy
5. Time of delivery (in 24 hrs).....: _____dd/mm/yy

6. Who delivered.....: Consultant---> 1
   Physician----> 2
   Trained TBA--> 3
   Untrained TBA->4
   Other--------> 5

7. Mode of delivery.....: Normal-------> 1
   C/S----------> 2
   Forceps------> 3
   Episiotomy---> 4
   Other--------> 5

8. Was the mother given anaesthesia.....: None---------> 1
   GA-----------> 2
   EA-----------> 3

9. Complications (in detail) of delivery:

   _____: _____
   _____: _____
   _____: _____
   _____: _____

10. APGAR score.....: 0 minutes----> 1
    1 minutes----> 2

11. Birth weight.....: _____Kgm
12. Birth length (nearest .1 cm).....: ____/____/____/

13. When was the baby put to the breast.....: _____hrs after delivery

14. Is cord blood collected.....: Yes---> 1 No---> 2
15. Is mother's blood collected.....: Yes---> 1 No---> 2
16. Is colostrom collected.....: Yes---> 1 No---> 2
17. Is nasal swab collected.....: Yes---> 1 No---> 2

18. Mother's Height.....: _____cm

19. Interviewer:s ID #.....: _____

CHD:SPN\DANI\F3
DOI:6/1995

SPN VACCINE STUDY
Form IV (ASSESSMENT OF FETAL MATURATION: PAGE-1

1. Subject #.....:_____

2. Date of Interview (dd/mm/yy).....:_____

3. Neuremuscular Maturity:

a. Posture.....:0=Arms and legs extended
1=Beginning of flexion of hips and knees. arms extended
2=Stronger flexion of legs. arms extended
3=Arms slightly flexed & legs are flexed and abducted
4=Full flexion of arms & legs

b. Square Window.....:0=90° 1 = 60° 2 = 45°
3=30° 4=0°

c. Arm Recoil.....:0=180° 2=100-180°
3=90-100° 4<90°

d. Popliteal Angle.....:0=180° 1=160° 2=130°
3=110° 4=90° 5=<90°

e. Scarf Sign.....:0=Elbow reaches opposite side
1=Elbow between midline & opposite axillary line
2=Elbow reaches midline
3=Elbow will not reach midline

f. Heel to ear.....:0=According to the sampled diagram
1=According to the sampled diagram
2=According to the sampled diagram
3=According to the sampled diagram
4=According to the sampled diagram

4. Physical Maturity:

a. Skin.....:0=Gelatious red. transparent
1=Smooth pink. visible veins
2=Superficial peeling. &/or rash. few veins
3=Cracking pale area. rare veins
4=Parchment deep cracking. no vessels
5=Leathery cracked wrinkled

b. Lanugo.....:0=None
1=Abundant
2=Thinning
3=Bald areas
4=Mostly bald

SPN VACCINE STUDY
Form IV (ASSESSMENT OF FETAL MATURATION): PAGE-2

1. Subject #.....:_____

2. Physical Maturity (cont.):

c. Plantar Crease.....:0=No crease
1=Faint red marks
2=Anterior tranverse crease only
3=Creases ant. 2/3
4=Creases cover entire sole

d. Breast.....:0=Barely percept
1=Flat areola no bud
2=Stippled areola 1-2mm bud
3=Raised areola 3-4mm bud
4=Full areola 5-10mm bud

e. Ear.....:0=Pinna flat, stays folded
1=Slightly curved pinna: soft with slow recoil
2=Weel-curve pinna:soft but ready coil
3=Formed & firm with instant recoil
4=Thick cartige ear stiff

f. Genitals (in males).....:0=Scrotom empty no rugae
2=Testes descending, few rugae
3=Testes down, good rugae
4=Testes pendulous, deep rugae

g. Genitals (in females).....:0=Prominant clitoris & labia minora
2=Majora & minora equally prominant
3=Majora large, minora small
4=Clitoris & monora completely covered

5. Interviewer's ID #.....:_____

CHD:SPN\DANI\F4
DOI:6/1995

SPN & OTHERS VACCINE RECORD FORM--IV A

CHILD NO :

(CHECK THE VACCINE CARD & UPDATE IT)

Name of vaccine	IST DOSE	2ND DOSE	3RD DOSE
	Date	Date	Date
BCG		*	*
POLIO			
DPT			
MEASLES			

RECORD ANY REACTION AFTER THE IMMUNIZATION:-

Record any reaction after vaccination	IST DOSE (6 week)	2ND DOSE (10 week)	3RD DOSE (14 week)
	DATE	DATE	DATE
24 hrs			
48 hrs			
72 hrs			

Reactions:-

1. Pain
2. Redness
3. Erythema
4. Itching
5. Cough
6. Suppuration (injecti site)
7. Others (specify)
8. Fever (Record the body temperature)

SPN VACCINE STUDY

Form V (VISIT FORM)

1. Subject #.....:_____

2. Date of Interview.....:_____dd/mm/yy

3. Nasal swab taken.....: Yes---> 1 No---> 2

4. Feeding of the child in the last 14 days.....: BM only------> 1
BM+Water-----> 2
BM+Food------> 3
No BM--------> 4

5. Morbidity of this child in past 14 days:

a. Fever.....: Yes---> 1 No---> 2

If fever:
Was temperature taken.....: Yes---> 1 No---> 2

If temperature taken:
Body temperature.....:_____°C

b. Cough/sneezing/running nose.....: Yes---> 1 No---> 2

c. Rapid respiration/breathing difficulty.....: Yes---> 1 No---> 2

d. Any other complaints.....: Yes---> 1 No---> 2

e. Diarrhea.....: Yes---> 1 No---> 2

f. Was physician consulted for any complaint...: Yes---> 1 No---> 2

If yes for which complain(s):

_____ :_____
_____ :_____
_____ :_____

Drugs prescribed:

_____ :_____
_____ :_____
_____ :_____

6. Interviewer's ID #.....:_____

CHD:SPN\DANI\F5
DOI:6/1995

RECORD OF NP SWAB FORM - V A

CHILD NO : _____

SL #	ID #	VACC. TYPE	WK.NS COLL.	DATE NS COLL.	RESULT SPN	AB	GRO	TYPE

CHILD MO
SPN STUDY
SCHEDULE PROCEDURES

PROCEDURE

MATERNAL | INFANT

	28 months (32 W)	PV (36W)	DELIVERY	[illegible]	[illegible]	10W (2.3m	14W (3.5m)	[illegible]	[illegible]
Vaccin	V	-	-	-	V	V	V	-	Mes:
Serum	S	S	S	S	S	S	-	S	S
Culture	C	-	C	C	C	C	C	C	C

1. Date of enrollment :

2. Date of delivery :

BREAST MILK COLLECTION : Date

CALS. dAY	6 w	10 w	[illegible]	5 months	6 months
3					
7					
15					

SPN VACCINE STUDY
Form 1 (ENROLMENT)

1. Subject#.....: _____
2. Date of Interview.....: _____dd/mm/yy
3. Age of the subject.....: _____years
4. Place of Enrolment.....: TAC------> 1
   KB-------> 2
   AM-------> 3
   Other----> 7
5. Address _____
6. Husband's age.....: _____years
7. Head of the family.....: Husband--> 1
   Self-----> 2
   Son------> 3
8. Obstetric History:
   a. LMP Date.....: _____dd/mm/yy
   b. Gravida.....: _____no.
   c. Para.....: _____
   d. Previous history
      1. Live birth.....: Yes--> 1 No--> 2
      2. Precipitated labor.....: Yes--> 1 No--> 2
      3. Abortion.....: Yes--> 1 No--> 2
9. Age of the last child (0=No child).....: _____ months
10. Blood Group of the subject:
    a. Group.....: A----> 1
       B----> 2
       AB---> 3
       O----> 4
    b. Rh type.....: Positive--> 1
       Negative--> 2
11. Blood Group of the husband
    a. Group.....: A----> 1
       B----> 2
       AB---> 3
       O----> 4
    b. Rh type.....: Positive--> 1
       Negative--> 2
12. Has TT been given during this pregnancy.....: Yes---> 1 No--->
13. Date TT given (to be given in right arm).....: _____dd/mm/yy
14. Date of vaccine given (to be given in left arm)....: _____dd/mm/yy
15. Vaccine code.....:
16. Blood collected.....: Yes---> 1 No---> 2
17. Has previous child got breastfeeding.....: Yes-------> 1
    No--------> 2
    No child--> 9
18. Mother's Weight (nearest .1kg).....: ___/___/___/ kg.
19. Mother's Height (nearest .1cm).....: ___/___/___/___/ cm
20. Interviewer's ID #.....: _____

CRD:SPN\DANI\F1
DOI:6/1995

Form II (SOCIOECONOMIC STATUS)

1. Study #.....: _____
2. Date of Interview.....: _____dd/mm/yy
3. Any side effect of vaccine.....: Yes---> 1 No---> 2
4. Reaction to the vaccine immediately after given vaccine (24-36 hrs.):
   a. Rash.....: Yes---> 1 No---> 2
   b. Itching.....: Yes---> 1 No---> 2
   c. Erythema.....: Yes---> 1 No---> 2
   d. Pain.....: Yes---> 1 No---> 2
   e. Others _____: Yes---> 1 No---> 2
   f. Body temperature:
      - 8 hrs after vaccination.....: _____ ^{0}C
      - in the evening.....: _____ ^{0}C

5. Date side effect.....: _____dd/mm/yy

---

6. Subject's years of schooling.....: _____yrs.
7. Husband's years of schooling.....: _____yrs.

8. Is subject an earning member.....: Yes---> 1 No---> 2
9. No. of the members eating together.....: _____no.
10. No. of the rooms in the house.....: _____no.
11. Does family own:
    a. AC.....: Yes---> 1 No---> 2
    b. Fan.....: Yes---> 1 No---> 2
    c. TV.....: Yes---> 1 No---> 2
    d. VCP/VCR.....: Yes---> 1 No---> 2
    e. Refrigerator.....: Yes---> 1 No---> 2
    f. Luxury Cot.....: Yes---> 1 No---> 2

12. Type of fuel used for cooking.....: Gas----------> 1
    Electricity-----> 2
    Firewood--------> 3
    Cowdung/leaves--> 4
    Misc. scraps----> 5

13. Light source at night.....: Electricity------> 1
    Kerosine lamp---> 2

14. Husband's occupation.....: _____
15. Wife's occupation.....: _____

16. Disposal site of garbage.....: Courtyard---------->1
    Outside the house->2

17. Husband smokes.....: _____ # sticks/day
18. Subject smokes.....: _____ # sticks/day
19. Caretaker smokes.....: _____ # sticks/day
20. Other member smokes.....: _____ # sticks/day
21. Is husband a Zarda/Tobacco taker.....: Yes---> 1 No---> 2
22. Is subject a Zarda/Tobacco taker.....: Yes---> 1 No---> 2
23. Is caretaker a Zarda/Tobacco taker.....: Yes---> 1 No---> 2
24. Is other member a Zarda/Tobacco taker.....: Yes---> 1 No---> 2

Interviewer's ID #.....: _____

CHD:SPN\DANI\F2
DOI:6/1995

SPN VACCINE STUDY
Form III (DELIVERY RELATED INFORMATION)

1. Subject #.....: _____
2. Date of Interview.....: _____dd/mm/yy
3. Where was child delivered.....: Clinic-----> 1
   Hospital---> 2
   Home-------> 3
   Other------> 4

4. Date of delivery.....: _____dd/mm/yy
5. Time of delivery (in 24 hrs).....: _____dd/mm/yy

6. Who delivered.....: Consultant---> 1
   Physician----> 2
   Trained TBA--> 3
   Untrained TBA->4
   Other--------> 5

7. Mode of delivery.....: Normal-------> 1
   C/S----------> 2
   Forceps------> 3
   Episiotomy---> 4
   Other--------> 5

8. Was the mother given anaesthesia.....: None---------> 1
   GA-----------> 2
   EA-----------> 3

9. Complications (in detail) of delivery:

   ----------: ----------
   ----------: ----------
   ----------: ----------
   ----------: ----------

10. APGAR score.....: 0 minutes----> 1
    1 minutes----> 2

11. Birth weight.....: _____Kgm
12. Birth length (nearest .1 cm).....: ___/___/___/

13. When was the baby put to the breast.....: _____hrs after delivery

14. Is cord blood collected.....: Yes---> 1 No---> 2
15. Is mother's blood collected.....: Yes---> 1 No---> 2
16. Is colostrom collected.....: Yes---> 1 No---> 2
17. Is nasal swab collected.....: Yes---> 1 No---> 2

18. Mother's Height.....: _____ cm

19. Interviewer:s ID #.....: _____

CRD:SPN\DANI\F3
DOI:6/1995

SPN VACCINE STUDY
Form IV (ASSESSMENT OF FETAL MATURATION: PAGE-1

1. Subject #.....:_____

2. Date of Interview (dd/mm/yy).....:_____

3. Neuromuscular Maturity:

a. Posture.....:0=Arms and legs extended
1=Beginning of flexion of hips and knees, arms extended
2=Stronger flexion of legs, arms extended
3=Arms slightly flexed & legs are flexed and abducted
4=Full flexion of arms & legs

b. Square Window.....:0=90° 1 = 60° 2 = 45°
3=30° 4=0°

c. Arm Recoil.....:0=180° 2=100-180°
3=90-100° 4<90°

d. Popliteal Angle.....:0=180° 1=160° 2=130°
3=110° 4=90° 5=<90°

e. Scarf Sign.....:0=Elbow reaches opposite side
1=Elbow between midline & opposite axillary line
2=Elbow reaches midline
3=Elbow will not reach midline

f. Heel to ear.....:0=According to the sampled diagram
1=According to the sampled diagram
2=According to the sampled diagram
3=According to the sampled diagram
4=According to the sampled diagram

4. Physical Maturity:

a. Skin.....:0=Gelatious red, transparent
1=Smooth pink, visible veins
2=Superficial peeling, &/or rash, few veins
3=Cracking pale area, rare veins
4=Parchment deep cracking, no vessels
5=Leathery cracked wrinkled

b. Lanugo.....:0=None
1=Abundant
2=Thinning
3=Bald areas
4=Mostly bald

SPN VACCINE STUDY
Form IV (ASSESSMENT OF FETAL MATURATION): PAGE-2

1. Subject #.....:_____

2. Physical Maturity (cont.):

c. Plantar Crease.....:0=No crease
1=Faint red marks
2=Anterior tranverse crease only
3=Creases ant. 2/3
4=Creases cover entire sole

d. Breast.....:0=Barely percept
1=Flat areola no bud
2=Stippled areola 1-2mm bud
3=Raised areola 3-4mm bud
4=Full areola 5-10mm bud

e. Ear.....:0=Pinna flat, stays folded
1=Slightly curved pinna: soft with slow recoil
2=Well-curve pinna;soft but ready coil
3=Formed & firm with instant recoil
4=Thick cartige ear stiff

f. Genitals (in males).....:0=Scrotom empty no ruqae
2=Testes descending, few ruqae
3=Testes down, good ruqae
4=Testes pendulous, deep ruqae

g. Genitals (in females).....:0=Prominant clitoris & labia minora'
2=Maiora & minora equally prominant
3=Maiora large, minora small
4=Clitoris & monora completely covered

5. Interviewer's ID #.....:_____

CHD:SPN\DANI\F4
DOI:6/1995

SPN & OTHERS VACCINE RECORD FORM—IVA

CHILD NO :

(CHECK THE VACCINE CARD & UPDATE IT)

Name of vaccine	IST DOSE	2ND DOSE	3RD DOSE
	Date	Date	Date
BCG		*	*
POLIO			
DPT			
MEASLES			

RECORD ANY REACTION AFTER THE IMMUNIZATION:-

Record any reaction after vaccination	IST DOSE (6 week)	2ND DOSE (10 week)	3RD DOSE (14 week)
	DATE	DATE	DATE
24 hrs			
48 hrs			
72 hrs			

Reactions:-

1. Pain
2. Redness
3. Erythema
4. Itching
5. Cough
6. Suppuration (injectio site)
7. Others (specify)
8. Fever (Record the body temperature)

DATE OF COLS.& BREAST MILK COLLECTED:-

DAYS	Cols. Date	BM IST	2ND	3RD	4TH	5Th	6TH	7Th	8TH	9TH
3day	1									
7day	2									
15day	3									

SPN VACCINE STUDY
Form V -(VISIT FORM.)

1. Subject #.....: _____

2. Date of Interview.....: _____dd/mm/yy

3. Nasal swab taken.....: Yes---> 1 No---> 2

4. Feeding of the child in the last 14 days.....: BM only------> 1
BM+Water-----> 2
BM+Food-------> 3
No BM--------> 4

5. Morbidity of this child in past 14 days:

a. Fever.....: Yes---> 1 No---> 2

If fever:
Was temperature taken.....: Yes---> 1 No---> 2

If temperature taken:
Body temperature.....: ____ °C

b. Cough/sneezing/running nose.....: Yes---> 1 No---> 2

c. Rapid respiration/breathing difficulty.....: Yes---> 1 No---> 2

d. Any other complaints.....: Yes---> 1 No---> 2

e. Diarrhea.....: Yes---> 1 No---> 2

f. Was physician consulted for any complaint...: Yes---> 1 No---> 2

If yes for which complain(s):

_____ : _____
_____ : _____
_____ : _____

Drugs prescribed:

_____ : _____
_____ : _____
_____ : _____

6. Interviewer's ID #.....: _____

CHD:SPN\DANI\FS
DOI:6/1995

RECORD OF NASAL SWAB FORM - V A

CHILD NO : ____

SL #	ID #	VACC. TYPE	WK.NS COLL.	DATE NS COLL.	RESULT SPN	AB	GRO	TYPE

ment 1.

Date 27-12-98

CE SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B.

cipal Investigator Dr. Nigar S. Shahid Trainee Investigator (if any) _____

ication No. _____ Supporting Agency (if Non-ICDDR,B) _____

le of Study Safety and Immunogenicity of Early Neonatal Immunization with ~~Conjugate Pneumococcal Vaccine (Addendum Dec. 95-017)~~

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

cle the appropriate answer to each of the following (if Not Applicable write NA).

Source of Population:
(a) ICI subjects — Yes No
(b) Non-ICI subjects — (Yes) No
(c) Minors or persons under guardianship — (Yes) No

Does the study involve:
(a) Physical risks to the subjects — Yes (No)
(b) Social Risks — Yes (No)
(c) Psychological risks to subjects — Yes (No)
(d) Discomfort to subjects — (Yes) No
(e) Invasion of privacy — Yes (No)
(f) Disclosure of information damaging to subject or others — Yes (No)

Does the study involve:
(a) Use of records, (hospital, medical, death, birth or other) — (Yes) No
(b) Use of fetal tissue or abortus — Yes No
(c) Use of organs or body fluids. Placenta & Serum Samples — (Yes) No

Are subjects clearly informed about:
(a) Nature and purposes of study — (Yes) No
(b) Procedures to be followed including alternatives used — Yes (No)
(c) Physical risks — Yes (No)
(d) Sensitive questions — Yes (No)
(e) Benefits to be derived — (Yes) No
(f) Right to refuse to participate or to withdraw from study — (Yes) No
(g) Confidential handling of data — (Yes) No
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes (No)

5. Will signed consent form be required:
(a) From subjects — (Yes) No
(b) From parent or guardian (if subjects are minors) — (Yes) No
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:

___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
___ Protocol (Required)
✓ Abstract Summary (Required)
✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
___ Informed consent form for subjects
✓ Informed consent form for parent or guardian
✓ Procedure for maintaining confidentiality
___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

gree to obtain approval of the Ethical Review Committee for any changes lving the rights and welfare of subjects before making such change.

Nigar S. Shahid
Principal Investigator

Trainee

Addendum to Project (95-017) : Study of the immunogenicity of Conjugate Pneumococcal Vaccine in infants of mothers who have and who have not been immunized with polysaccharide Pneumococcal vaccines

Title of addendum : Safety and Immunogenicity Trial of Early Neonatal Immunization with the Conjugate Pneumococcal Vaccine

Project Description :

Background and Justification :

In Bangladesh as in other developing countries, it is estimated that a quarter of the ALRI specific morbidity in children 0-4 years of age occurs in the first six months of life (1) and that 40-50% of the resulting ALRI mortality is due to pneumococcal infections (2). Current vaccine strategies and schedules to prevent infections in infants generally initiate immunization in the second month of life (3 infant doses given in the second, third and fourth month) and protective levels of antibody may not appear until 4-5 months of age when the highest proportions of the invasive pneumococcal disease and deaths occur (3). Polysaccharide vaccines have been found to be non-immunogenic if administered to infants before two years of age (4), however, the new conjugates vaccines are immunogenic in the very young. The glycoprotein conjugate pneumococcal vaccines contain polysaccharides of 9 different pneumococcal serotypes conjugated to a variety of carrier proteins. Clinical trials of the 2, 5, & 7-valent glycoprotein conjugate pneumococcal vaccine have been conducted in young children both in developing and in developed countries and found to be safe and immunogenic when given along with other routine vaccines (5-7). In addition, conjugate pneumococcal vaccines reduces colonizing of the bacteria in the nasopharyx (8).

Maternal and neonatal vaccine strategies have been used successfully throughout the world to prevent infections in the very young. Maternal immunization with tetanus toxoid (TT) induces an antibody response in the mother, which protects the newborn by transplacental passage of the antibody. We have conducted an immunogenicity study of maternal immunization with the 23-valent polysaccharide pneumococcal vaccine. The vaccine was found to be safe and immunogenic in mothers and protective levels of antibodies (>0.15μg/mL) were observed in the infants blood up to 22 weeks of age (9). Maternal immunization do not produce impaired responses in their infants either with maternal TT (a protein antigen) nor with polysaccharides. However, in developing countries mothers may come to a health facility for delivery and not for antenatal care. In addition, many women may not like to take a vaccine during pregnancy. In the original study (95-017),we proposed to examine the effect of both passive and active immunization to pneumococcal vaccines. There were 3 study groups in the original study. Mothers were to receive their vaccine doses along with the antenatal tetanus toxoid (TT) dose and the infants the vaccines at the EPI contacts. In Group 1 both the mother and her infant were to receive pneumococcal vaccine, in group 2, infants were to receive three doses of the conjugate pneumococcal vaccine, in group 3 neither the mother nor her infant receive the pneumococcal vaccine. The project (95-017) aims to assess whether the presence of maternally derived (passive) antibodies pneumococcal polysaccharide or the carrier- protein affects infants' responses to (active) immunization with the conjugate pnuemococcal vaccine and correlate nasopharyngeal pneumococcal carriage with the presence and levels of infant serum antibodies.

In South Asia, around 50% newborns are low birth weight babies (10). In urban Dhaka, it was observed that 42.9% of births were LBWs and that 21.2% were pre-mature as measured by the last menstrual period (11). This is of particular concern as premature infants receive lower levels of passive maternal antibodies and the newborn may not be adequately protected regardless of appreciable levels of specific antibodies in the mother (12). Studies with maternal TT have shown that transplacental passage of IgG correlate with gestational age (13). An infant of 32 weeks gestation has approximately half the IgG serum level of a full-term infant (14). We have also observed a positive correlation with the interval between maternal immunization (with the 23-valent polysaccharide pneumococcal vaccine) and the birth of the infant (9). Since a minimum of 21 days is required for a 4-fold rise in antibody titres, a maternal vaccine dose may not produce appreciable transplacental transmission of passive antibodies in the event of pre-scheduled births (15). This may be an added risk factor for acquiring ALRI among infants in developing countries. Since T-cells are competent at birth (16) and since conjugate vaccines are T-cell mediated, conjugate vaccines could induce priming at birth. This may contribute to early protection and perhaps inhibition of colonization. As such, immunizing with the conjugate pneumococcal vaccine at birth may speed up the process of developing active antibodies in the very young. Immunity would be both specific and would cover the period of highest risk. The World Health Organization's suggestion of immunization with pneumococcal polysaccharide conjugate vaccines in the newborn period needs to be evaluated. (17). Concerns of the effect of infant responses to vaccines in the presence of maternal antibodies are raised. Cord anti-pneumococcal IgG of 7.5 and 6.8 μg/ml were observed in infants of pneumococcal vaccinees by us (9). These levels were three times higher than cord levels of infants whose mothers did not receive the pneumococcal vaccines during pregnancy. These levels are unlikely to inhibit response after 3 infant doses of pneumococcal vaccines. High antibody responses have been observed after 3 doses of active immunization in infants against tetanus toxoid (TT) (18) and respiratory syncytial virus (RRV) (19). Experience with neonatal hepatitis B immunization has been very positive and has resulted in the drastic reduction in vertical transmission of

HBsAg chronic carriers and a 85% reduction in the incidence of infant infections (20). BCG administration in neonates reduces the risk of tuberculosis by 50% (21). A study in which Hib vaccines was administered to neonates at 2 days of age showed that the neonatal dose induced immunogenic response >3.5 fold in infants when the maternal antibodies were <3.0 µg/mL. Although a lower response to the neonatal dose was observed in infants with higher maternal antibody levels (>3.0µg/mL), maternal antibodies appeared to have little influence on the antibody concentration reached after the second and third vaccine dose (22). Eskola and Kayhty also observed that adverse events in the early immunization schedule in neonates and premature babies was mild and spontaneously resolving (23). To-date, there are no similar studies for the conjugate pneumococcal vaccine during the early neonatal period. We propose, therefore, to incorporate within the approved project on maternal and infant immunization trial with the 9-valent pneumococcal vaccine (95-017) trial an additional study group where the mother will receive the control vaccine during pregnancy and her newborn will receive the same 9-valent conjugate pneumococcal (vaccine conjugated to a non-toxic mutant diphtheria toxin CRM 197) at an early schedule, first dose given by 5 days of age and the next 2 doses at 6 and 10 weeks. This addendum will help us study the antibody responses to conjugate vaccines when the first dose is administered to the infant soon after birth. If it can be demonstrated that early neonatal immunization with conjugate pneumococcal vaccine could produce significant levels of anti body, this may have a substantial effect in reducing both the ALRI incidence and mortality in children under 5 years of age.

Recently, there have been concerns on the safety issues related to immunization during pregnancy (17,24). Maternal immunization with bacterial (25) and viral vaccines(26) have been found to be safe with no apparent side effects. A recent meting of WHO examined 8 studies conducted worldwide on maternal immunization with pneumococcal vaccine. These studies were quite dissimilar as regards study design and population, length and intensity of follow-up and monitoring of vaccine side-effects. An increase in perinatal deaths was observed in two studies but no biological plausible mechanism could be attributable to the casualty. In an effort to address this issue it was suggested that studies be designed to investigate the possible mechanism (17). There was a suggestion that placental samples be retained for pathological examination and the newborns urine be collected for the presence of polysaccharide antigen in the foetus. As a secondary objective we are proposing to examine the placentas for deposition of immuno-complex and evidence of acute plecental damage in case of premature deliveries or stillbirths. Serological tests to examine the pneumococcal antibody titre, undegraded vaccine antigen and circulating immune complexes could be estimated from maternal serum to examine the paradox of how a vaccine could lead to excess perinatal deaths. At each delivery, placenta will be preserved in screw cap containers in formaldehyde. In case of a neonatal death pathological examination will be done for immune complex deposition .The placental samples of infants crossing one month of age will be discarded.

Safety and data monitoring committee: A 3 member safety and data monitoring group comprising of ICDDR,B scientists will be formed. They will review all available data and make decisions on the progress of the study based on adverse events both in mothers and in infants. These adverse events include any adverse reactions due to vaccines, spontaneous abortions, premature delivery, still birth, perinatal or late neonatal deaths, maternal deaths or serious delivery related sequel.

Aim:

The aim of this addendum would be to compare the immunogenicity and safety of the 9-valent conjugate pneumococcal vaccine when the first dose is administered to infants in the early neonatal period in comparison to the maternal and infant immunization schedule as proposed in the original protocol.

Specific Objectives:

1. To measure the specific antibody response in the young infant at 6 weeks of age after the administration of a dose of the conjugate pneumococcal vaccine in the early neonatal period ie by 5 days of age.

2. To determine the effect of maternal antibodies on the antibody responses of the infants to the first neonatal dose of the 9-valent conjugate pneumococcal vaccine.

3 To compare the vaccine antibody responses after 3 doses of the vaccine between infants receiving the first dose in the early neonatal period and those receiving it at 6 weeks of age with or without a maternal polysaccharide dose.

4 To look for safety of routine and early neonatal immunization in infancy and evidence on severe side-effects following maternal immunization with polysaccharide pneumococcal vaccine.

Study Design:

This is an addendum to the already approved randomized double-blind controlled immunogenicity trial of maternal and infant immunization (95-017) which will be conducted in Dhaka in collaborating with private and public maternity care facilities. In the original project there are 3 study groups. Pregnant women are randomized to receives a dose of the polysaccharide pneumococcal or the control vaccine at 32 ± 2 weeks of gestation . Their infants receive either the 3 doses of the conjugate pneumococcal or control vaccines at the EPI contact at 6, 10 and 14 weeks. Therefore, in Group 1 both mothers and infants receive pneumococcal vaccine. In Group 2, only the infants and in Group 3 neither the mother, nor their infants receive any pneumococcal vaccine. In this addendum we are proposing to include another study group, Group 4, i.e. the early neonatal immunization group, in which infants will receive the first dose of the conjugate 9-valent pneumococcal vaccine within the first 5 days of life. The second and third doses will be administered at 6 and 10 weeks of age. The mothers receive a control vaccine during gestation.

Enrollment and randomization Procedure:

After a mother consents to join the study she will be given an unique identification number which will indicate the study group she is assigned to. This will involve a change in the randomization procedure and design from two step to one step randomization. This change adds strength to the study design. The numbers assigned will be from a pre-prepared list of numbers. Randomization will be a one step block design of 4 to ensure that in each block all 4 groups are equally represented. This will also entail that infants born to mothers in groups 1-3 will receive a placebo at day 5. Mothers in group 4 will receive the control vaccine and their infants a placebo at week 14. The control vaccines will be the HiB polysaccharide for the mother and conjugate for the infants. An appropriate placebo will have to be decided upon. Blood samples will be drawn as shown in the schedule from both mother-infant pairs to examine antibody responses to the vaccination doses and compare with the non-immunized groups. Serum antibodies will again be evaluated at 9 months of age of the infant and a dose of the 23- valent pneumococcal will be administered at this point of contact. Antibodies to this vaccine dose will again be evaluated 4 weeks later.

In all, we now have 4 groups
Group 1—maternal Spn + infant Spn regular,
Group 2—Only infant Spn regular,
Group 3—*no* maternal *no* infant Spn
Group 4-- early infant Spn

Study Schedule:

Groups	Mother (32 wk)	1 mth	Birth (0wks)	6wks	10wks	14wks	18wks	39wks (9M)	*43wks (+1M)
1. (M+I)	Ps,M	M	M,Plc. B	CjSp,B	CjSp	CjSp,B	B	Ps,B	B
2. (routine I)	Ph,M	M	M,Plc. B	CjSp,B	CjSp	CjSp,B	B	Ps,B	B
3. (M-I-)	Ph, M	M	M, Plc,B	CjH,B	CjH	CjH,B	B	Ps,B	B
4. (early I)	Ph,M	M	M,CjSp B	CjSp,B	CjSp	Plc,B	B	Ps,B	B

NP cultures at each contact.

Ps-23-valent polysaccharide pneumococcal vaccine, CjSp- pneumococcal conjugate vaccine
Ph-polysaccharide HiB vaccine, CjH-Conjugate HiB, Plc- Placebo
M - Maternal blood specimens, B - Infant blood specimens (cord blood at birth) .
NP- nasopharyngeal cultures for *S. pneumoniae* isolation and serotyping

Groups 1-3 as per original project (95-017), Group 4- addendum

Comparison between study groups:

Group 1 vs 2 : Effect of maternally acquired polysaccharide specific antibodies on infant response to active immunization. (study objective 1 and 2 of protocol # 95-017)
Group 2 vs 3: Safety and immunogenicity of glycoprotein conjugate vaccine administered at routine EPI schedule. (Study objective 3 of protocol # 95-017)
Groups 1, 2, and 3 : Effect of maternal or infant immunization , or both, on carriage of pneumococci.

Addendum:

Group 1 vs 4: Comparison between maternal + infant immunization to early immunization strategy.
Group 2 vs 4: Comparison of early infant and routine infant immunization.

Selection criteria: The selection criteria will be followed as per the original protocol.

Inclusion criteria:

1. Women between 15 and 40 years of age, in the third trimester of pregnancy with a previous history of uncomplicated live birth.
2. Plan to deliver in Dhaka and have no plans to leave Dhaka in the next 12 months.

Exclusion criteria:

1. Women who are at known risk for pre-mature labour (hypertensive and/or diabetic).

Follow-up of study subjects:

As per the parent protocol (95-017) mothers will be retained for two hours in the clinic after immunization for immediate adverse-effects and later followed up at 8, 24, 48 and 72 hours for fever, local and systemic reactions. A similar routine will also be followed for each infants dose to register adverse effects. Home visits for morbidity surveillance will be conducted weekly and mothers will be requested to contact the project for any treatment required both for themselves and their infants. Blood samples will be collected at birth (cord blood) and at 6, 14, 18, 39 and 41 weeks from the infant. In addition a serum sample will be collected from the mother soon after delivery.

Laboratory assessment:

As per the original project, serum samples will be analysed for selected serotype specific IgG antibodies to *S. pneumoniae* and specific antibodies to 5 selected serotypes and Hib polysaccharides will be measured by ELISA. Ninety-six well microtitre ELISA plates will be coated with the appropriate concentration of antigen and blocked. Appropriate serial dilutions of serum samples will be prepared in duplicates and incubate on the coated plates. Serum from negative and positive reference individuals will be run on the sam plate to reduce variability of the assay. For isotype determinations, the antigen- specific plate bound antibody will be detected by HRP-conjugated isotype-specific reagents recognizing human IgM, IgA, IgG1,IgG2, IgG3 and IgG4. ELISA will be developed with TMB substrate and read with an automated ELISA reader.

The qualitative or idiotypic response to vaccination with Hib will be assessed through use of anti-idiotypic monoclonal antibodies to Hib-Id1 and Hib-Id2 idiotypes (27) . Idiotypic profiles for individual pneumococcal serotypes will be assessed by determining the idiotype-specific spectrotypes using serotype-specific affinity purification and isoelectric focusing (28). A 10% of subsample of the serum collected for antibody estimation will be sent to a standard laboratory for opsonization, standardization and quality control.

Bacteriological methods for colonization study: Nasopharygeal swabs will be collected for Spn isolation and serotyping at each contact as per methods laid down in the protocol (95-017). Results of colonization will be compared to antibody levels at each point of specimen collection in each vaccine group.

Study Outcomes:

Main outcomes will be antibody levels at 6, 18 and 39 weeks. These levels will be compared with those in cord blood at birth. Adverse effects after each dose of the conjugate vaccines will also be monitored.

Sample Size:

The sample size for the original project (95-017) was estimated using data from our first study on titre variability and calculated as follows: In order to detect a difference of 50% in the antibody GMT of infants of vaccinated mothers vs unvaccinated mothers, at least 29 children in each group will be studied (using standard formulae and a one-sided alpha error level of 0.05, beta error of 0.20, power of 80%, and assuming that the GMT and standard deviation of the antibody titers are similar to or current data). Using similar error assumptions, 29 subjects will also provide an 80% power to detect a true correlation coefficient of -0.50 or greater between the continuous variables, and to find a true reduction of pneumococcal colonisation from 80% to 50%. We will use a similar sample size for the fourth group. Assuming a 30% dropout rate, we will recruit 38 mothers in each group ie a total of 160 for the project.

Study Design:

This is an addendum to the already approved randomized double-blind controlled immunogenicity trial of maternal and infant immunization (95-017) which will be conducted in Dhaka in collaborating with private and public maternity care facilities. In the original project there are 3 study groups. Pregnant women are randomized to receives a dose of the polysaccharide pneumococcal or the control vaccine at 32 ± 2 weeks of gestation . Their infants receive either the 3 doses of the conjugate pneumococcal or control vaccines at the EPI contact at 6, 10 and 14 weeks. Therefore, in Group 1 both mothers and infants receive pneumococcal vaccine. In Group 2, only the infants and in Group 3 neither the mother, nor their infants receive any pneumococcal vaccine. In this addendum we are proposing to include another study group, Group 4, i.e. the early neonatal immunization group, in which infants will receive the first dose of the conjugate 9-valent pneumococcal vaccine within the first 5 days of life. The second and third doses will be administered at 6 and 10 weeks of age. The mothers receive a control vaccine during gestation.

Enrollment and randomization Procedure:

After a mother consents to join the study she will be given an unique identification number which will indicate the study group she is assigned to. This will involve a change in the randomization procedure and design from two step to one step randomization. This change adds strength to the study design. The numbers assigned will be from a pre-prepared list of numbers. Randomization will be a one step block design of 4 to ensure that in each block all 4 groups are equally represented. This will also entail that infants born to mothers in groups 1-3 will receive a placebo at day 5. Mothers in group 4 will receive the control vaccine and their infants a placebo at week 14. The control vaccines will be the HiB polysaccharide for the mother and conjugate for the infants. An appropriate placebo will have to be decided upon. Blood samples will be drawn as shown in the schedule from both mother-infant pairs to examine antibody responses to the vaccination doses and compare with the non-immunized groups. Serum antibodies will again be evaluated at 9 months of age of the infant and a dose of the 23- valent pneumococcal will be administered at this point of contact. Antibodies to this vaccine dose will again be evaluated 4 weeks later.

In all, we now have 4 groups
Group 1—maternal Spn + infant Spn regular,
Group 2—Only infant Spn regular,
Group 3—*no* maternal *no* infant Spn
Group 4-- early infant Spn

Study Schedule:

Groups	Mother (32 wk)	1 mth	Birth (0wks)	6wks	10wks	14wks.	18wks	39wks (9M)	*43wks (+1M)
1. (M+I)	Ps,M	M	M.Plc. B	CjSp.B	CjSp	CjSp.B	B	Ps,B	B
2. (routine I)	Ph,M	M	M.Plc. B	CjSp.B	CjSp	CjSp.B	B	Ps,B	B
3. (M-I-)	Ph, M	M	M. Plc.B	CjH.B	CjH	CjH.B	B	Ps,B	B
4. (early I)	Ph,M	M	M.CjSp B	CjSp.B	CjSp	Plc.B	B	Ps.B	B

NP cultures at each contact.

Ps-23-valent polysaccharide pneumococcal vaccine, CjSp- pneumococcal conjugate vaccine
Ph-polysaccharide HiB vaccine, CjH-Conjugate HiB, Plc- Placebo
M - Maternal blood specimens, B - Infant blood specimens (cord blood at birth) ,
NP- nasopharyngeal cultures for *S. pneumoniae* isolation and serotyping

Groups 1-3 as per original project (95-017), Group 4- addendum

Comparison between study groups:

Group 1 vs 2 : Effect of maternally acquired polysaccharide specific antibodies on infant response to active immunization. (study objective 1 and 2 of protocol # 95-017)
Group 2 vs 3: Safety and immunogenicity of glycoprotein conjugate vaccine administered at routine EPI schedule. (Study objective 3 of protocol # 95-017)
Groups 1, 2, and 3 : Effect of maternal or infant immunization , or both, on carriage of pneumococci.

Addendum:

Group 1 vs 4: Comparison between maternal + infant immunization to early immunization strategy.
Group 2 vs 4: Comparison of early infant and routine infant immunization.

Selection criteria: The selection criteria will be followed as per the original protocol.

Inclusion criteria:

1. Women between 15 and 40 years of age, in the third trimester of pregnancy with a previous history of uncomplicated live birth.
2. Plan to deliver in Dhaka and have no plans to leave Dhaka in the next 12 months.

Exclusion criteria:

1. Women who are at known risk for pre-mature labour (hypertensive and/or diabetic).

Follow-up of study subjects:

As per the parent protocol (95-017) mothers will be retained for two hours in the clinic after immunization for immediate adverse-effects and later followed up at 8, 24, 48 and 72 hours for fever, local and systemic reactions. A similar routine will also be followed for each infants dose to register adverse effects. Home visits for morbidity surveillance will be conducted weekly and mothers will be requested to contact the project for any treatment required both for themselves and their infants. Blood samples will be collected at birth (cord blood) and at 6, 14, 18, 39 and 41 weeks from the infant. In addition a serum sample will be collected from the mother soon after delivery.

Laboratory assessment:

As per the original project, serum samples will be analysed for selected serotype specific IgG antibodies to *S. pneumoniae* and specific antibodies to 5 selected serotypes and Hib polysaccharides will be measured by ELISA. Ninety-six well microtitre ELISA plates will be coated with the appropriate concentration of antigen and blocked. Appropriate serial dilutions of serum samples will be prepared in duplicates and incubate on the coated plates. Serum from negative and positive reference individuals will be run on the sam plate to reduce variability of the assay. For isotype determinations, the antigen- specific plate bound antibody will be detected by HRP-conjugated isotype-specific reagents recognizing human IgM, IgA, IgG1,IgG2, IgG3 and IgG4. ELISA will be developed with TMB substrate and read with an automated ELISA reader.

The qualitative or idiotypic response to vaccination with Hib will be assessed through use of anti-idiotypic monoclonal antibodies to Hib-Id1 and Hib-Id2 idiotypes (27) . Idiotypic profiles for individual pneumococcal serotypes will be assessed by determining the idiotype-specific spectrotypes using serotype-specific affinity purification and isoelectric focusing (28). A 10% of subsample of the serum collected for antibody estimation will be sent to a standard laboratory for opsonization, standardization and quality control.

Bacteriological methods for colonization study: Nasopharygeal swabs will be collected for Spn isolation and serotyping at each contact as per methods laid down in the protocol (95-017). Results of colonization will be compared to antibody levels at each point of specimen collection in each vaccine group.

Study Outcomes:

Main outcomes will be antibody levels at 6, 18 and 39 weeks. These levels will be compared with those in cord blood at birth. Adverse effects after each dose of the conjugate vaccines will also be monitored.

Sample Size:

The sample size for the original project (95-017) was estimated using data from our first study on titre variability and calculated as follows: In order to detect a difference of 50% in the antibody GMT of infants of vaccinated mothers vs unvaccinated mothers, at least 29 children in each group will be studied (using standard formulae and a one-sided alpha error level of 0.05, beta error of 0.20, power of 80%, and assuming that the GMT and standard deviation of the antibody titers are similar to or current data). Using similar error assumptions, 29 subjects will also provide an 80% power to detect a true correlation coefficient of -0.50 or greater between the continuous variables, and to find a true reduction of pneumococcal colonisation from 80% to 50%. We will use a similar sample size for the fourth group. Assuming a 30% dropout rate, we will recruit 38 mothers in each group ie a total of 160 for the project.

Data Analysis :

The groups will be compared with respect to key characteristics to assess randomization. Proportions with adverse events and proportions seroconverting will be compared using chi-square tests.

The proportions of infants having long-term (>1.0 µg/mL) and short term (>0.15 µg/mL) protective levels at the various serum samples will be compared. Geometric mean titres will be analysed and compared based on parametric and non-parametric (Wilcoxon rank-sum test) methods.

The comparisons will also be made in the proportion seroconverting between the groups 1 and 5 to the serotypes of interest. Seroconversion will be defined as levels of anti-pneumococcal antibody which is 4-fold higher. The natural decay will be calculated from groups 3 and 4 in the original protocol, using data from our previous maternal immunization study, and other published studies on maternal and infant immunization. The expected levels will be estimated based on rate of natural decay per week.

Stratified analysis will be performed to assess risk factors for response using Cochrane-Mantel Hanzel tests and multi variate regression.

Total Number of Years of Project Anticipated :

We expect to conduct all activities of this addendum along with the original (95-017) protocol. The project is estimated to take 30 months to be completed.

Total Number of Years of Funding Anticipated : 30 Months.

Anticipated Benefit From Funding This Project :

This addendum in the trial will determine if the first dose of the newly developed conjugate 9-valent pneumococcal vaccine can safely be given soon after birth and whether it will provide induction of memory and prime for induction of response to two doses of the conjugate vaccine given at 6 and 10 weeks of life. This strategy may be useful when vaccination during pregnancy is not possible as many women only have contact with health care services at delivery or when maternal immunization may not be acceptable, although, there is no convincing evidence of risk to the foetus by immunizing pregnant women with bacterial vaccines, toxoids or inactive viral vaccines. It is expected that this regime of 3 doses will provide protective levels of anti-pneumococcal antibodies of 0.15 µg/mL up to 6 months of age. This level should be effective in preventing pneumococcal disease from the second month of life.

Policy Implication:

This trial will determine if the first dose of the newly developed conjugate 9 valent pneumococcal (Spn) vaccine given soon after birth will provide induction of memory and prime for induction of response to two doses of the conjugate vaccine given at 6 and 10 weeks of life. It is expected that this regime of 3 doses will provide short term anti- pneumococcal antibodies of 0.15 µg/mL up to 6 months of age thus preventing pneumococcal disease at the period of highest risk.

Relation of Project to Goals/Objectives of the CHR :

CHR has placed high priority to the study of vaccines to prevent diseases of global public health importance. Pneumococcus invasive disease has high mortality in the very young infants in developing countries. We are proposing to compare the early neonatal schedule as a new vaccine strategy for the pneumococcus vaccine to prevent disease in the very young along with the infant immunization schedule with or without the maternal dose. Estimation of vaccine antibody titres in the different immunization groups may provide information on the best/ appropriate vaccination strategy to prevent pneumococcal ARI disease in the very young and high risk group. This study thus meets CHR goals and objectives.

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International Centre for Diarrhoeal Disease Research, Bangladesh
P.O. Box 128, Mohakhali, Dhaka -1212, Bangladesh

Consent form (for pneumonia vaccine protocol)

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is conducting studies on the development of pneumonia vaccine strategies for Bangladesh. Deaths due to pneumonia are highest during infancy. diagnosis and treatment is getting more difficult due to fast development of antibiotic resistant strains. *Streptococcus pneumoniae* and *Haemophilus influenzae* are the two important bacterial causes of serious childhood pneumonia. Mothers vaccinated during pregnancy with pneumonia vaccine maintain protective levels of immunity up to 14 weeks of birth. This protective level has to be increased up to 1 year of age of the child. For the purpose of this result we shall collect relevant information from you through a questionnaire. We will ask you some questions related to your present pregnancy, past obstetric history and socio-economic condition which should take about 30 minutes of your time. Either one of the pneumonia vaccines (pneumococcal or Haemophilus influenzae b vaccine) will be administered to you during the third trimester after randomisation. 2ml of blood will be drawn from your vein at the time of vaccination and one month later. You will also be requested to give 2 ml of venous blood when your child will be delivered. You will be requested to provide nasopharyngeal swabs at the time of vaccination , one month later and at delivery.

Cord blood will be collected at delivery by the clinic staff. The project physician will perform a thorough physical examination of your child between 1-3 days of birth. Either one of the pneumonia vaccines.(pneumococcal or Hib vaccine) will be given to your baby in 3 doses in different schedules which may start as early as 5 days after birth or along with the DPT vaccines according to EPI schedules. Pneumococcal vaccine will be given at 9 months of age along with the Measles vaccine. 1ml of venous blood will be drawn at 6 weeks (1.5 months), 14 weeks (3.5 months), 20 weeks (5 months), 39 weeks (9 months) and 43 weeks(10 months) age. Nasopharyngeal swab will be collected from your baby at birth and at 6weeks, 10 weeks, 14 weeks, 18 weeks , 39 weeks and 43 weeks. The body fluids will be required for assessing the extent to which the vaccines may be effective. We shall follow up your child up to 12 months of age and give the child appropriate medical care through our project physician whenever necessary.

All information related to you and your child shall be kept confidential.

If you decide to participate in the study, please put your signature. If at any stage of the study , you wish to withdraw, you may do so, you will be entitled to the general treatment offered at the ICCDR,B hospital.

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Signature of Interviewer	Signature of Mother
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Signature of Investigator	Date

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka 1000, Bangladesh
Phone : 600171-78, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 880-2-886050, Cable : Cholera Dhaka

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

Date : June 30, 1998

To : Chairman, RRC

Through : Acting Director, PHSD

From : Dr. Nigar S. Shahid
Scientist, PHSD
P.I. Protocol # 95-017

Sub : **Commencement of Activities in Protocol # 95-017 entitled "Study of the immunogenicity of conjugate pneumococcal vaccine in infants of mothers who have and who not been immunized with polysaccharide vaccine". Budget Codes # 309741 & 309761**

We have started the activities of the protocol on June 8, 1998 . The delay was caused due to non- availability of the study vaccines The protocol length will be 30 months from this date. Please allow me this new starting date.

Thank You

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 871751-60, Telex . 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871686, Cable : Cholera Dhaka

>Memorandum<

Date : July 14, 1998

To : Dr. Nigar Shahid

From : Prof. V.I. Mathan
Chairperson, RRC

Subject : **Time Extension of your protocol # 95-017**

The Committee met on 13th July 1998 and considered your application for a fresh start of your protocol # 95-017 entitled "Study of the immunogenicity of conjugate pneumococcal vaccine in infants of mothers who have and who have not been immunized with polysaccharide vaccine."

After reviewing the justification of your application, the Committee allowed you for a fresh start of your protocol from June 8, 1998 for a period of 30 months.

Thank you.

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 600171-78, Telex : 675612 ICDD BJ,
Fax : 880-2-883116, 880-2-886050, Cable : Cholera Dhaka

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

To : Dr.Nigar S.Shahid, CHD

July 13,1995

From : Demissie Habte, MD
Chairman, RRC

Subject : Protocol No.95-017 entitled "Study of the immunogenicity of conjugate pneumococcal vaccine in infants of mothers who have and who have not been immunized with polysaccharide vaccine".

The Research Review Committee(RRC) met on Wednesday, the 12th July 1995 to consider your protocol referred to above.

I am pleased to inform you that the protocol is approved.

CC: Division Director, CHD

DH:zbmb

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 600171-78, Telex : 675612 ICDD BJ,
Fax : 880-2-883116, 880-2-886050, Cable : Cholera Dhaka

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

To : Dr.Nigar S. Shahid, CHD

August 23,1995

From : Prof.S.A.R. Chowdhury
Chairman, ERC

Subject : Protocol No.95-017 entitled "Study of the immunogenicity of conjugate pneumococcal vaccine in infants of mothers who have and who have not been immunized with polysaccharide vaccine".

Reference your memo dated August 02,1995 enclosing a modified copy of the above mentioned protocol, incorporating the recommendations of the Committee.

I am pleased to inform you that the modified version of the protocol is approved.

CC: Acting Division Director, CHD
Director

SARC.zbmb

ICDDR,B, DHAKA (AN INCOMPLETE FORM WILL NOT BE ACCEPTED: SL.NOS.9 & 13 MUST)

ETHICAL REVIEW COMMITTEE: PROTOCOL E X T E N S I O N/T E R M I N A T I O N FORM
RESEARCH REVIEW COMMITTEE:

PRINCIPAL INVESTIGATOR : Dr NIGAR SHAHID Protocol No: 95-017

Title:

Starting dates of Protocol: Original planned: 30 months from Starting date, Actual: 8.6.98.

(1) Status of Protocol :
Was/will be completed as scheduled ☐ Will require an extension ☑

(2) Results achieved :
Publication(s) : Yes ☐ No ☐
Manuscript(s) : Yes ☐ No ☐
Protocol could not be started due to non-availability of vaccine
If "Yes", please provide one copy each of papers, abstracts &/or manuscripts.
If no article or manuscript is available, please provide brief summary achieved:

(3) If an extension is needed, state length of extension required and reason extension needed (e.g. 6 months, field work completed but analysis work outstanding). CALCULATE EXTENSION FROM ORIGINAL EXPECTED COMPLETION DATE.

30 months/year Reasons: Protocol could not be started on time due to non-availibility of the study vaccines & also due to technical ground
Original expected completion date : 30 months from starting date (date not mentioned)

(4) State if you have added any methods or procedures not originally in the protocol:
none

(5) Any adverse effects in cases where the study involves human subjects:
none

(6) Additional data analysis/procedures not originally in the protocol is planned:
Yes ☐ No ☑

(7) If "Yes", give details :

(8) Name of Division involved P-H S&B

(9) Date on which plans for extension was approved in the Division: 1.6.98.

(10) Additional budget needed: Yes ☑ No ☐

(11) If yes, give details: US $ 76,000 approved by USAID-CHR funds

(12) Signature of the Principal Investigator: Nigar S. Shahid Date:

(13) Signature of Associate Director of the Division: Date:

* If space is insufficient to a particular question, please use seperate sheet.

Principal Investigator: Last, first, middle : Shahid, Nigar, Sayem

International Centre for Diarrhoeal Disease Research, Bangladesh **FOR OFFICE USE ONLY**

RESEARCH PROTOCOL

Protocol No: 95-017 Date: July 01, 1995

RRCApproval: Yes/ No Date: July 13, 1995

ERC Approval: Yes/No Date: August 23, 1995

1. Title of Project :

STUDY OF THE IMMUNOGENICITY OF CONJUGATE PNEUMOCOCCAL VACCINE IN INFANTS OF MOTHERS WHO HAVE AND WHO HAVE NOT BEEN IMMUNIZED WITH POLYSACCHARIDE VACCINE.

2a. Name of the Principal Investigator(s) (Last, Middle, First).	2b. Position / Title	2c. Qualifications
SHAHID, SAYEM, NIGAR	SCIENTISt /DR	MBBS, MSc, MPH
STEINHOFF, C, MARK	PROFESSOR/DR	MD

3. Name of the Division/ Branch / Programme of ICDDR,B under which the study will be carried out.
PUBLIC HEALTH SCIENCES DIVISION

4. Contact Address of the Principal Investigator	4b. Fax No: 880-2-886050/872529/883116
4a. Office Location: International Centre for Diarrheal Disease Research , Bangladesh (ICDDR,B) GPO BOX # 128, Dhaka-1000 Bangladesh	4c. E-mail: nshahid@icddrb.org 4d. Phone / Ext: 880-2-871751-60, 2213, 2217

5. Use of Human Subjects	5a. Use of Live Animal	5b. If Yes, Specify Animal Species
Yes ☑	Yes ☐	
No ☐	No ☐	

6. Dates of Proposed Period of Support (Day, Month, Year - DD/MM/YY)	7. Cost Required for the Budget Period		
	7a. 1st Year ($):	2nd Year ($):	3rd Year:
08 June'98 - 07 Dec'2000	81,485	108,647	54,323
	7b. Direct Cost ($) 208,521	Total Cost ($) 244,458	

8. Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Dr. K.M.A. Aziz (Acting) — Name of the Division Director

K.M.A. Aziz — Signature

July 1995 — Date of Approval

9. Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

10. Signature of PI

Nigar S. Shahid

Date: 16/6/95