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RIVM report 124001 004

# Safety and immunogenicity of the RIVM hexavalent meningococcal B vesicle vaccine for Rotterdam children aged 2-3 and 7-8

J. Labadie, E.D. de Kleijn, A.B. Lafeber, M.M.M. Mees,
K. Booy, R. de Groot, G.W. van Omme, H. van Dijken,
A.J. Kuipers, G. van den Dobbelsteen, R.E. Juttmann,
M. Wala, A.J.W. van Alphen, H.C. Rümke

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This investigation has been performed by order and for the account of the Chief Inspectorate of Health Care (IGZ), within the framework of project V/000012/03/AE, clinical studies with meningococcal B vaccine.

RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71

# Abstract Nederlands

In dit rapport wordt verslag gedaan van een gerandomiseerde gecontroleerd fase-II klinische studie naar de veiligheid en immunogeniciteit van het RIVM hexavalente MenB vesicle vaccin in 189 kinderen van 2-3 jaar en 168 kinderen van 7-8 jaar in de stad Rotterdam. Twee concentraties van het MenB vesicle vaccin zijn onderzocht, waarbij hepatitis B vaccin (HB-VAX® DNA) werd gebruikt als controle en toegediend volgens hetzelfde schema als het RIVM MenB vaccin. Het vaccinatie schema was gebaseerd op 3 doses gegeven op 0, 2 and 8 maanden.
Het MenB vaccin was meer reactogeen dan het controle vaccin, maar de reacties waren mild en er traden geen ernstige bijwerkingen op. Koorts, één van de meest voorkomende systemische bijwerkingen na vaccinatie, kwam niet vaker voor na toediening van het MenB vesicle vaccin dan na toediening van het controle vaccin.
De serum bactericide antistof (SBA) respons werd gemeten tegen zes isogene varianten van stam H44/76 waarin elk PorA eiwit van het hexavalente vaccin individueel tot expressie is gebracht. In de bloedmonsters verkregen één maand na vaccinatie, hadden peuters (2-3 jaar) statistisch significant hogere SBA titers dan schoolkinderen (7-8 jaar). Er was geen significant verschil tussen de lage en hoge vaccin dosis. De SBA respons was voornamelijk gericht tegen één van de drie PorA eiwitten op elk van de twee vesicles.
Het RIVM hexavalent MenB vesicle vaccin zal verder worden verbeterd met betrekking tot zowel productie methoden als immunogeniteit.

# Abstract English

This report documents the results of a randomised controlled phase-II clinical study into the safety and immunogenicity of the RIVM hexavalent MenB vesicle vaccine among 189 children aged 2-3 and 168 children aged 7-8 in the city of Rotterdam, the Netherlands. Two concentrations of the MenB vesicle were investigated where hepatitis B vaccine (HB-VAX® DNA) was used as a control and administered according to the same schedule as the RIVM MenB vaccine. The vaccination schedule was based on 3 doses given at 0, 2 and 8 months.
The meningococcal vaccine was found to be more reactogenic than the control vaccine but the reactions were mild, no serious reactions occurred. Fever, one of the most common systemic adverse reactions after vaccination, did not occur more often after administration of the MenB vesicle vaccines than after administration of the control vaccine.
The serum bactericidal antibody (SBA) response was assessed against isogenic variants of strain H44/76 in which each vaccine PorA protein was expressed individually. In the blood samples obtained one month after vaccination, toddlers (aged 2-3) were shown to have statistically significant higher SBA titres than school children (aged 7-8). There was no significant difference between the low and high vaccine doses. The SBA response was directed predominantly at one of the three PorA proteins in each of the two vesicles.
The RIVM hexavalent MenB vesicle vaccine will be further improved, both with regard to both production methods and to immunogenicity.

# Preface

**Participating organisations and investigators**

1. ROTTERDAM

- Sophia Kinderziekenhuis/Academisch Ziekenhuis Rotterdam
  R. de Groot MD PhD, paediatrician, principal investigator
  E.D. de Kleijn MD, investigator

- Stichting Thuiszorg Rotterdam
  R.E.Juttmann MD, child health consultant, investigator
  M.Wala MD, child health consultant, investigator

- GGD Rotterdam e.o.
  G.W. van Omme MD, school health consultant, investigator
  G.Oskamp, head immunisation administration

2. BILTHOVEN

- LVO: Laboratory for Clinical Vaccine Research [JL, RL, MM, KB, HR]
- LVR: Laboratory of Vaccine Research [HD, AK, GD, AA]

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- staff (doctors and nurses) of resort centres for enrolling participants and giving the vaccinations and communicating all study data through filling endless numbers of forms.
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- all RIVM laboratory technicians involved in antibody determinations
- all unnamed members of the participating organisations who helped in one or another way.
- all colleagues showing their interest in the progress of the investigation.

# Abbreviations

| | |
|---|---|
| °C | Degrees centigrade |
| CB | Baby clinic *(consultatiebureau)* |
| CRF | Case Report Form |
| ELISA | Enzyme Linked Immunosorbent Assay |
| GCP | Good Clinical Practice |
| GGD | Municipal Public Health Service Rotterdam *(GGD Rotterdam eo)* |
| HB-VAX® DNA | Hepatitis B vaccine |
| HepB | Hepatitis B |
| IU | International Units |
| JGZ | Child Health Care *(Jeugdgezondheidszorg)* |
| KRZ | Bureau for Quality and Regulatory Affairs *(Bureau Kwaliteits en Registratiezaken RIVM)* |
| LVR | Laboratory of Vaccine Research *(Laboratorium voor Vaccine Research)* |
| LVO | Laboratory for Clinical Vaccine Research *(Laboratorium voor Veldonderzoek vaccins)* |
| LVO-BI | LVO Bio- and Immunochemistry section *(LVO afdeling bio- en immunochemie)* |
| MenB6-10 | Meningococcal B hexavalent vesicle vaccine (100 µg class 1 OMP per dose ) |
| MenB6-5 | Meningococcal B hexavalent vesicle vaccine (50 µg class 1 OMP per dose ) |
| OMP | Outer Membrane Protein (of N. meningitidis) |
| OMV | Outer Membrane Vesicle (of N. meningitidis) |
| PEA | Immunisation Administration *(Provinciale Entadministratie)* |
| PorA | class 1 OMP porin protein |
| RC | Resort Centre of the School Health Service *(Resort Centrum Schoolartsendienst)* |
| RIVM | National Institute of Public Health and the Environment *(Rijksinstituut voor Volksgezondheid en Milieu*) |
| RVP | National Childhood Immunisation Programme *(Rijksvaccinatieprogamma)* |
| SBA | serum bactericidal antibody assay |
| SKZ | Sophia Kinderziekenhuis/Academisch Ziekenhuis Rotterdam |
| STR | Stichting Thuiszorg Rotterdam |
| UTN | Unique Trial Number |

# Contents

# Samenvatting

**Achtergrond**
Sinds vaccinatie tegen Haemophilus influenzae type b in 1993 is opgenomen in het Rijksvaccinatieprogramma wordt bacteriële meningitis in Nederland voornamelijk veroorzaakt door Neisseria meningitidis (meningococ). Meningococcen ziekten, zoals meningitis en/of sepsis, komen vooral voor bij kinderen, zowel in de eerste levensjaren als op school- en adolescenten leeftijd. In West Europa is de meningococcen B serogroep verantwoordelijk voor 70-75% van alle gevallen. In het RIVM is een vesicle vaccin ontwikkeld dat zes verschillende meningococcen buitenmembraan eiwitten bevat.

**Methode**
In Rotterdam werd een gecontroleerde, gerandomiseerde fase-II studie naar veiligheid en immunogeniteit uitgevoerd. Een totaal van 357 kinderen werden gerecruteerd uit de populatie die voor de reguliere preventieve zorg de Consultatiebureaus en de Schoolartsendienst bezochten. Vaccins werden in een schema van drie vaccinaties toegediend (2+1). MenB5 (lage dosis) werd toegediend aan 127 kinderen, MenB10 (hoge dosis) aan 126 kinderen (hoge dosis), HB-VAX® DNA aan 104 kinderen (HepB). Bloedmonsters voor onderzoek op antistoffen werden op 4 tijdstippen afgenomen: voor de eerste vaccinatie en de derde vaccinatie en 1 maand na de tweede en de derde vaccinatie. Bijwerkingen werden 18-30 uur na iedere vaccinatie geëvalueerd door een hiervoor getrainde onderzoeksverpleegkundige. De ouders hielden een dagboekje bij voor bijwerkingen die optraden in de rest van de week na toediening van het vaccin.

**Resultaten**
Het meningococcen vaccin bleek meer reactogeen dan het hepatitis B vaccin, maar de reacties waren mild. Er traden geen ernstige bijwerkingen op. In beide leeftijdsgroepen en voor beide doses kwamen de lokale reacties pijn en roodheid op de injectie plaats het meest frequent voor. Koorts, één van de meest voorkomende systemische bijwerkingen na vaccinatie, trad ongeveer even vaak op in de MenB groep als in de controle groep. Minder vaccin-specifieke systemische bijwerkingen als hangerigheid, maar ook hoofdpijn bij schoolkinderen werden vaker gerapporteerd na vaccinatie met het MenB vaccin.
De SBA respons bij kleuters was in het 2e en 4e bloedmonster (1 maand na vaccinatie) significant hoger dan bij schoolkinderen. Ook hadden meer kleuters een beschermende SBA titer ≥ 1:4. Wij vonden geen verschil tussen de twee vaccinconcentraties. De SBA respons was voornamelijk gericht tegen één van de drie PorA eiwitten per vesicle, namelijk het P1.5$^{c}$,10 eiwit van de vesicle PL10124 en het P1.5,2 eiwit van de vesicle PL16215.

**Discussie**
De aard en de ernst van de bijwerkingen na vaccinatie zijn acceptabel. Er zijn geen ernstige bijwerkingen opgetreden. Het vaccin is immunogeen in zowel kleuters als schoolkinderen, zonder dat er verschil is tussen de hoge en de lage dosis van het vaccin werd gevonden. De SBA respons is voornamelijk gericht tegen één eiwit per vesicle. Mogelijk leidt de trivalente expressie van de PorA eiwitten op één vesicle tot interferentie in immuunstimulatie. Ook is het mogelijk dat de PorA eiwitten verschillen in immunogene eigenschappen.
Het RIVM hexavalent vaccin zal verder worden geoptimaliseerd, zowel met betrekking tot productie methoden als tot de optimale immunogeniteit.

# Summary

**Background**
Bacterial meningitis in the Netherlands is predominantly caused by Neisseria meningitidis (meningococcus) since introduction of vaccination against Haemophilus influenzae type b in the National Childhood Immunization Program in 1993. Meningococcal disease (meningitis and septicemia) predominantly occur in childhood, both in young infants and at school- and adolescent age. Meningococcus serogroup B causes 70-75% of Meningococcal disease in Western Europe. The RIVM has developed a vesicle vaccine that contains outer membrane proteins of six strains of meningococci.

**Methods**
A controlled, randomised phase-II study investigating safety and immunogenicity was done in Rotterdam. In total, 357 children were recruited from preventive health care services. The vaccines were administered in a 3 dose schedule (2+1). MenB5 was given to 127 children (low dose), MenB10 was given to 126 children (high dose), and as a control 104 children (group HepB) were given HB-VAX® DNA. Blood for antibody assays was taken at four moments: before the first vaccination, before the third vaccination and 1 month after the second and the third vaccination. Adverse events were assessed by a trained research nurse 18-30 hours after each vaccination and symptoms occurring 2-7 days after vaccination were reported by the parents.

**Results**
Although the MenB vesicle vaccine showed to be more reactogenic than the HepB vaccine symptoms were mild. No serious adverse events occurred. In both age groups and with both doses of MenB vaccine the local reactions pain and redness occurred most frequently. Fever, one of the most common systemic adverse reactions after vaccination, did not occur more often in the MenB groups than in the control group. Less specific systemic symptoms like drowsiness, and in school children headache, were reported more frequently after immunisation with MenB vaccines.
In the second and fourth blood samples (1 month after vaccination) toddlers had statistically significant higher SBA titres than school children. Higher proportions of toddlers had protective SBA titres $\geq$ 1:4 as well. There was no significant difference between the low and high doses of MenB vaccine. The SBA response was predominantly directed against one of the three PorA proteins of each vesicle, with the highest titres against the P1.5$^{c}$,10 protein of the PL10124 vesicle and the P1.5,2 protein of the PL16215 vesicle.

**Discussion**
The frequency and nature of the adverse events after vaccination are acceptable. No serious adverse events occurred. The vaccine is immunogenic in toddlers and in school children without difference between the high and low dose MenB vaccine. The SBA response was predominantly directed against one of the three PorA proteins of each vesicle. This may be caused by interference with immune stimulation from the trivalent PorA expression on one vesicle, or by intrinsic differences in immunogenicity of PorA proteins.
The RIVM hexavalent vaccine will be further improved, with regard to production methods and immunogenicity.

# 1. Introduction

In the Netherlands bacterial meningitis is predominantly caused by the Neisseria meningitidis (meningococcus) since vaccination against Haemophilus influenzae type b (Hib) was included in the National Childhood Immunisation Program (RVP) in 1993[1]. Meningococcal disease, meningitis or septicemia, occurs predominantly in two age clusters: 0-4 year olds (24 cases per 100,000 inhabitants) and 15-19 year olds (8 cases per 100,000 inhabitants)[2]. Mortality and morbidity of meningococcal infections are high. Mortality in children is 5-15% (30-90 cases/year) and 20-30% (100-150 cases/year) suffer serious and often permanent neurological sequelae like hearing loss, convulsive disorders, paralysis or mental retardation. Other complications are arthritis, vasculitis, peripheral necrosis, myocarditis, hydrocephalus and cranial nerve damage[3].

Meningococci are classified into twelve serogroups on the basis of antigenic differences of the capsular polysaccharide. In the Netherlands, over 90% of the cases are caused by serogroups A, B and C, of which serogroup B is the most common. Serogroups can be further classified into sero- and subtypes on the basis of class 2/3 and class 1 outer membrane proteins (OMP's)[4,5].

Effective polysaccharide vaccines against the serogroups A and C are available, but the serogroup B polysaccharide vaccine is poorly immunogenic in humans. Besides, this vaccine has been discouraged because of the presence of closely related, and probably cross-reacting antigens in the human brain[6].

Therefore, vaccines based on meningococcal outer membrane proteins are developed. The estimated efficacy of the Norwegian vaccine in teenagers is 57%[7]. Another study showed that the efficacy of the Cuban vaccine varied by age: 74% in children aged 48 months or older, 47% in children aged 24 to 47 months, and 37% in children aged less than 24 months[8]. The Norwegian and the Cuban vaccines both contain only one subtype, B:15:P1.7,16 and B:4:P1.19,15 respectively. These subtypes represent only a small minority of the case isolates in the Netherlands (~10% of serogroup B isolates)[4].
In the RIVM a genetically engineered vaccine containing class 1 outer membrane proteins of six meningococcal B subtypes has been developed[9]. Two vesicles each contain three of these OMP's. These six subtypes represent 75-80% of case isolates of serogroup B in the Netherlands.

In a phase-I clinical trial with this hexavalent OMP vesicle vaccine safety and immunogenicity in adult volunteers were demonstrated[10].
The hexavalent OMP vesicle vaccine was tested a phase-II trial in the UK where infants were simultaneously immunised with routine childhood immunisations. Side effects found in these infants were infrequent and mild. Three doses of the vaccine induced modest rises in bactericidal titres against the six vaccine strains. The size of the immune response after a fourth dose (booster dose) suggested the generation of immunological memory[11].

This report describes the results of a controlled, randomised phase-II study to evaluate the safety and immunogenicity of the hexavalent OMP vesicle vaccine in Dutch toddlers and school children.

# 2. Materials and methods

The study protocol "Onderzoek naar immunogeniteit en bijwerkingen van hexavalent meningococcen B vesicle vaccin bij kleuters en schoolkinderen"[4] was approved by the Institutional Ethics Review Board of the Sophia Children's hospital and the University hospital in Rotterdam.

## 2.1 Vaccines

The hexavalent meningococcal OMP vesicle vaccine contains the class 1 OMP of six meningococcal subtypes. The subtypes are expressed on two trivalent vesicles.

| trivalent vesicle | meningococcal subtypes[#] |
|---|---|
| PL16215 | P1.7,16<br>P1.19,15<br>P1.5,2 |
| PL10124 | P1.5[c],10<br>P1.12,13<br>P1.7[h],4 |

# also expressed on group C meningococci

Under study are two dosages of the MenB vaccine, containing 50 µg (B5, lot E92-8-2) and 100 µg (B10, lot E92-8-1) protein, equal to ca. 7.5 and 15 µg class 1 protein per subtype per dose respectively. The remaining protein content (10%) consists of class 4 en 5 OMP. The vaccine contains no group B capsular polysaccharide and not more than 10% LPS (GalE type)[12]. A 0.5 ml dose of vaccine also contains aluminumphosphate (1.5 mg), thiomersal (0.05 mg) and sucrose (10 mg).
A hepatitis B vaccine 10 µg/ml (HB-VAX® DNA, Pasteur-Merieux MSD, Amstelveen, the Netherlands) was administered in the control group in a 0.5 ml dose. This vaccine is registered (RVG nr 17461) and commercially available in the Netherlands. One HB-VAX® DNA lot (lot HA 41120, expiration date June 8, 1997) was used in this study.

All vaccines were stored at the Immunisation Administration (PEA) at 4-8 °C throughout the study. Storage conditions (including facilities and temperature monitoring) for the trial vaccines were equal to those of the regular RVP vaccines. Transport of the vaccines from the PEA on the day of their administration was done by the study personnel using insulated containers. At the RC vaccines were transferred to the refrigerator used for RVP vaccines, during house calls the vaccines remained in the insulated containers.
Since the HB-VAX® DNA vaccine was used in its regular commercial vial, blinding of the study was not possible. However, the study personnel performing the antibody tests were blinded for the vaccine the participants had received.

## 2.2 Participants

Toddlers
Children born in 1993 (2-3 years of age) living in Rotterdam were invited to participate in the study during a regular visit to the CB. Direct mailing accelerated initially slow enrolment. During the subsequent visit to the CB additional information was given to the parents, both in writing as well as orally and parents questions were addressed.

School children
Children born in 1988 (7-8 years of age) living in Rotterdam were invited to participate by direct mailing. During the subsequent visit to the RC additional information was given to the parents, both in writing as well as orally and parents questions were addressed.

## 2.3 Study design and procedures

After evaluation of inclusion and exclusion criteria and signing of an informed consent form by the parents, the child was enrolled. A Unique Trial Number (UTN) was assigned in order of enrolment. The numbers 1-300 were reserved for toddlers and 400-700 were reserved for school children. Participants were randomised according to a computer-generated list, assigning them by UTN to one of the three study groups (low dose, high dose or control).

Participants were immunised according to a three-dose schedule with resp. 2 and 6 months interval between vaccinations. A trained observer between 18 and 30 hours assessed adverse events after administration of the vaccine. The parents in a diary recorded specific symptoms and signs occurring during the rest of the week after each vaccination.
This diary was used to complete the CRF at the next study visit. Study personnel filled out the CRF.
Toddlers were immunised and venipunctured at home and school children were immunised at the RC's and venipunctured at the RC's or at home.
HB-VAX® DNA vaccine serves as a control for the adverse event analysis. The antibody titres of participants in the HepB control group also give an indication of seroconversion due to intercurrent natural meningococcal exposure in the catchment population. This vaccine has a record of mild adverse reactions[13,14] and was preferred to a placebo because it offers the recipients immunised with it protection against Hepatitis B.

### 2.3.1 Study size calculation

A statistical power calculation was made using Epi Info version 6.0[15]. Under the assumption of a maximal seroconversion by intercurrent infections of 10% in the HB-VAX® DNA controlgroup and minimal 70% seroconversion after MenB vaccination a difference in seroconversion between HB-VAX® DNA and MenB could be established with 25 children in each group [confidence of 95%; power of 90%]. Fifty evaluable children in each group at conclusion of the study were calculated to be necessary to show seroconversion differences between both MenB doses and adverse event differences between HB-VAX® DNA and MenB.
Previous experience with vaccine trials in children showed that participation of 10% of the invited target population with a dropout rate of 10% could be expected. This would mean inclusion of 55-60 children after inviting approximately 500 children per vaccine group for each of both age groups.

### 2.3.2 Study design by immunisation group

| Time (months) | -1 | 0 | 2 | 3 | 6 | 8 | 9 |
|---|---|---|---|---|---|---|---|
| Low dose | | B5 | B5 | | | B5 | |
| High dose | | B10 | B10 | | | B10 | |
| Control | | HB | HB | | | HB | |
| Activity | i | < b1 | | b2 | | < b3 | b4 |
| | | 0 > | 0 > | | | 0 > | |
| | | | e | e | | e | e |

**legend:**

| | |
|---|---|
| low dose: | received B5: meningococcal B vesicle vaccine, 50 µg class 1 OMP per dose |
| high dose: | received B10: meningococcal B vesicle vaccine, 100 µg class 1 OMP per dose |
| control: | received HB: HB-VAX® DNA |
| i: | intake |
| <b: | blood sample 0-14 days before vaccination |
| b: | blood sample 4-6 weeks after vaccination |
| o>: | observation of adverse reactions by trained observer (18-30 hrs after vaccination) |
| e: | evaluation of adverse reactions observed by parents (up to 7 days after vaccination) |

### 2.3.3 Blood sampling and storage

Blood was sampled by venipuncture after application of the local anaesthetic lidocain/prilocain [EMLA™] by physicians or trained research nurses at the participant's home or at the RC.
Blood samples were sent to the RIVM in Bilthoven by regular mail. Upon arrival serum was isolated and stored at -20°C at LVO-BI until distribution of aliquots for blinded specific antibody measurements by LVR. Consequently, tubes with serum specimens were marked with a code, which did not reveal the immunisation group.

### 2.3.4 Injections

Both MenB vesicle and HB-VAX® DNA vaccines were administered by intramuscular injection in the thigh (vastus lateralis muscle) or in the upper arm (deltoid or triceps muscle) depending on physician or research nurse preference. The date, site, time of injection and vaccine lot number was recorded in the CRF.

## 2.4 Adverse reactions evaluation

Specific systemic symptoms (fever [temperature ≥ 38.5 $^{o}$C], headache, drowsiness, less appetite, nausea, joint complaints, cutaneous symptoms and unusual crying and also sleepiness in toddlers and absenteeism from school in school children) and the occurrence of local symptoms (pain, redness, swelling, itching and reduced use of the arm or leg) were assessed by a trained observer at 18-30 hours after administration of the vaccine. Parents recorded occurrence of these specific symptoms and other possible adverse events in a 7-day

diary, which was used to complete the CRF at the next study visit. Serious adverse events were to be immediately communicated to the RIVM by the investigator.

## 2.5 Antibody tests

### 2.5.1 ELISA

Since a trivalent vaccine vesicle was used as the ELISA-coat, antibodies detected in the ELISA show immunogenicity against OMP from the two trivalent strains PL16215 and PL10124, which constitute the hexavalent vaccine[10].
In short after overnight coating of the microtitre plates at room temperature threefold serial dilutions of serum samples were incubated for 90 minutes at 37$^{o}$C. After incubation with peroxidase conjugated rabbit anti-human IgG (90 minutes at 37$^{o}$C) peroxidase substrate colouring reaction was read at 450 nm. IgG antibody titres are expressed as the dilution which gives 50% of ODmax-ODmin.

### 2.5.2 IgG subclass ELISA

ELISA plates were coated with whole cells of isogenic strains exclusively expressing the P1.5$^{c}$,10, P1.12,13 or P1.7$^{h}$,4 PorA protein of the PL10124 vesicle. Instead of rabbit anti-human IgG (see2.5.1) peroxidase conjugated mouse anti-human IgG-subclass specific monoclonal antibodies (CLB, Amsterdam) were used: IgG1 specific MH161-1 ME, IgG2 specific MH162-1ME, IgG3 specific MH163-1ME and IgG4 specific MH164-4ME. Antibody titres are expressed as the 10log reciprocal of the dilution giving 50% ODmax.

### 2.5.3 Serum Bactericidal Antibody (SBA) Assay

Bactericidal activity of antibodies against isogenic variants of strain H44/76 was determined as described by Peeters and Rouppe van der Voort[10,18].
In short 2-fold dilutions of heat inactivated sera (30 min at 56$^{o}$C), complement (final concentration 10% (v/v)) and 3.0 x $10^2$ c.f.u. bacteria were incubated in a microtitre plate for 60 minutes at 37 $^{o}$C and subsequently 7µl of this suspension was spotted onto GC agar plates. Six isogenic strains with the same set of alleles as present in the two trivalent vaccine strains ( H44/76, TR52, TR15 and TR10, TR1213, TR4) were used. After 18-20h incubation at 37$^{o}$C in 5% $CO_2$, the colonies from time zero were counted. The average number of c.f.u. at time zero was set at 100%. The serum bactericidal titre was reported as the reciprocal of the serum dilution yielding $\geq$ 90% killing.
Antibodies detected in the SBA Assay show class 1 OMP specific, functional (bactericidal) immunogenicity that is assumed to correlate with specific protective immunity. In earlier studies, SBA titres of 1:4 or more were associated with protection against clinical disease[16,17,23].

### 2.5.4 Protection

To assess the potential protection of the vaccine against systemic meningococcal disease, data about Neisseria meningitidis isolates from patients were used. The data were obtained from the Netherlands Reference Laboratory for Bacterial Meningitis (RLBM)[2]. The percentages isolates were estimated as the proportion isolates of all circulating N. meningitidis subtypes, as well as the proportion isolates of the subtypes represented by the hexavalent vesicle vaccine. Potential protection per subtype was estimated as:

(% participants with SBA titre ≥ 1:4) * (% isolates) * (1/100).

The total potential protection of vaccination was estimated by summation of the protection by the six subtypes present in the vaccine.

This total potential protection was assessed for the low and high dose group, for toddlers and school children, and for the period 1993-1996 and the year 1996. Because the subtype proteins are expressed also on group C meningococci, potential protection was estimated for the serogroup B as well as for the serogroup B+C.

## 2.6 Data handling and validation

All data of participants relevant for the study have been recorded on Case Report Forms (CRF's). Antibody titres were obtained later, but are an integral part of the final CRF. Clinical and serological data have been entered in a LVO database, for storage and analysis. CRF data have been entered in a computer by a company, specialised in data entry (UPC, Nieuwegein).

The first 192 serum samples measured in the SBA assay were decoded to verify the reliability of the titration procedure. Results of the antibody titrations have been handed over to LVO, on diskettes, after a formal verification of the integrity and plausibility of the data. For further statistical analysis the data have been exported to SPSS [version 7.0 for Windows[19]]. After each step, checks were made to ensure that the correct data were used for final reporting.

### 2.6.1 Study monitoring

During the clinical stages of the study, monitoring visits were made to each study facility (all RC's, PEA, SKZ) by the monitor (KB) assigned by the RIVM to guard protocol adherence. The monitor checked all of the CRF's. Study progress and operational quality was reviewed on a regular basis in meetings with executives of the participating organisations.

### 2.6.2 Study audit

An RIVM-KRZ GCP-audit was conducted on 26 May 1997 at the end of the clinical stages of the study, regarding the RIVM study monitoring and trial document filing activities.

## 2.7 Data editing

### 2.7.1 Default choices for serological values beyond measuring range

To facilitate statistical analyses, serological values below (<) or exceeding (>) the measuring ranges were recoded into default values according to the following figure.

| Antibody test | Serological value | Default value |
|---|---|---|
| ELISA | <100<br>>24300 | 99<br>25000 |
| SBA assay | <2<br>>128 | 1<br>256 |

*figure 1*

### 2.7.2 Protocol adherence

After entering all data in a computer database, a final assessment of protocol adherence was made. All data from a child have been excluded from analysis in the following situations:

- children with missing records of a correct intake procedure, including those on the informed consent.
- children with doubtful fulfilment of inclusion or exclusion criteria, specified in the study protocol.
- children with missing data forms, or apparently faulty filled forms.

In case of the situations listed below children were excluded for analysis from the moment that the protocol violation had occurred:

- children that did not adhere to the study regimen, i.e. switched from one group to another.
- children that did receive vaccines other than the lots specified in the study protocol.
- interval between vaccinations differed from the interval specified in the protocol: shorter than 7 weeks or longer than 13 weeks between the first and the second dose, and/or shorter than 5 months or longer than 9 months between the second and third dose.
- interval between vaccination and blood sampling differed from the interval specified in the protocol:
  0-14 days before vaccination 1 (blood sample 1) and vaccination 3 (blood sample 3)
  4-6 weeks after vaccination 2 (blood sample 2) and vaccination 3 (blood sample 4)
- interval between vaccination and observation of adverse events differed from the interval specified in the protocol:
  observation 1 → 8-30 hours after vaccination (by trained observer)
  observation 2 → 24-72 hours after vaccination (by the parents)
  observation 3 → 3-7 days after vaccination (by the parents).

## 2.8 Statistical analysis

The aim of the study was to assess the immunogenicity and safety of the RIVM hexavalent meningococcal B vesicle vaccine in healthy toddlers and school children.

Adverse events were assessed 18-30 hours after each vaccination (observation 1) by a study nurse. Symptoms occurring 2 to 3 days (observation 2) and 4 to 7 (observation 3) days after vaccination were reported by the parents. Numbers and percentages of local and systemic adverse events were assessed for each observation and each vaccination per study group and per age group. Chi square or Fisher's exact tests were used to compare the study groups per age group and per vaccination with respect to local and systemic adverse events at the first observation moment after each vaccination.

The data obtained are described by individual line listings of the serological results per UTN and order of blood sample. This list is not included in this report. In this report the antibody titres are described per meningococcal antigen by study group and by order of blood sample.

Elisa and SBA results were transformed to logarithmic values to calculate Geometric Mean Titres (GMT's) and 95% confidence intervals (95% CI's). Per study group and per age group, the antibody responses from blood sample 1 to 4 are shown in graphs. These graphs are made for ELISA and SBA responses. For each blood sample the percentages of participants with reciprocal bactericidal titres to each strain equal to, or greater than 4 were assessed per study group and per age under the assumption that this represents a protective titre[16,17,23]. Proportions of participants with a fourfold or greater rises in SBA titres (blood sample 1 to 2,

1 to 4, and 3 to 4) were assessed, because fourfold or greater rises in SBA titres are considered significant responses[20]. Mann-Whitney-U test was used to compare the low and the high dose group with respect to GMT's of Elisa and SBA (per age group). This test was also used to compare toddlers and school children with respect to GMT's in Elisa and SBA titres (per study group).

# 3. Results

## 3.1 Study population

A total of 357 children, 189 toddlers and 168 school children, were enrolled in the study and informed consent was obtained from all parents. These children have been recruited from an approximated total catchment population of 4240 (1840 toddlers and 2400 schoolchildren) in Rotterdam (Table 1). After the informed consent was signed, the participants were randomised to one of the three immunisation groups (Table 2). The study populations were comparable as to allocation of vaccination groups and male/female distribution. There were 171 female and 186 male participants (ratio: 0.92).
Table 3 shows the number of participants who dropped-out during the study. Table 4 shows the numbers of patients who were excluded for (part of) the analyses according to the criteria mentioned in paragraph 2.7.2. “Protocol adherence”. The numbers of evaluable participants for the serological analysis are given in Table 5A, and for the adverse event analysis in Table 5B.

## 3.2 Adverse reactions

No serious adverse events occurred after vaccination. Table 6 shows the numbers of systemic adverse reactions in toddlers and school children monitored for seven days after each vaccination. Results on the local adverse reactions are given in Table 7. Due to non-evaluable participants or to missing data for some adverse reactions the valid percentages are given. Local reactions were more common than systemic reactions. Most adverse events lasted for three days. There were no signs of increasing intensity of symptoms during subsequent days in the week after vaccination. However, after subsequent vaccinations an increasing percentage of toddlers reported pain at the injection site and an increasing percentage of school children reported swelling ≥ 2.5cm.
Chi-square or Fisher’s exact tests were used to compare the low and the high dose groups with respect to adverse events 18-30 hours after each vaccination (table 8A+B). In school children, drowsiness and less appetite occur significantly more often in the high dose group after the first vaccination. Overall, there were no statistically significant differences between the high and the low dose groups. Therefore, data of these two groups were pooled. The pooled MenB group and the HepB group were compared with respect to adverse events 18-30 hours after vaccination, using the Chi square test or Fisher’s exact test (table 9A+B). Local reactions are significantly more common in the MenB group in toddlers as well as in school children, especially pain and redness. Some systemic reactions were significantly more often seen in the MenB group after the second vaccination in school children and after the second and third vaccination in toddlers. The frequency of fever, one of the most common systemic adverse reactions after vaccination, did not differ between the MenB and the HepB group.
The occurrence of adverse reactions after vaccination with MenB in toddlers was compared with the occurrence in school children by Chi square test or Fisher’s exact test. Pain, redness, swelling, itching and headache occurred more often in school children (for all $p<=0.02$).

## 3.3 Antibody response

SBA GMT's and 95% CI's against the meningococcal strains are summarised in Table 10, for both MenB groups as well as for the HepB group. This table also shows the percentages of participants with reciprocal bactericidal titres equal to, or greater than 1:4.

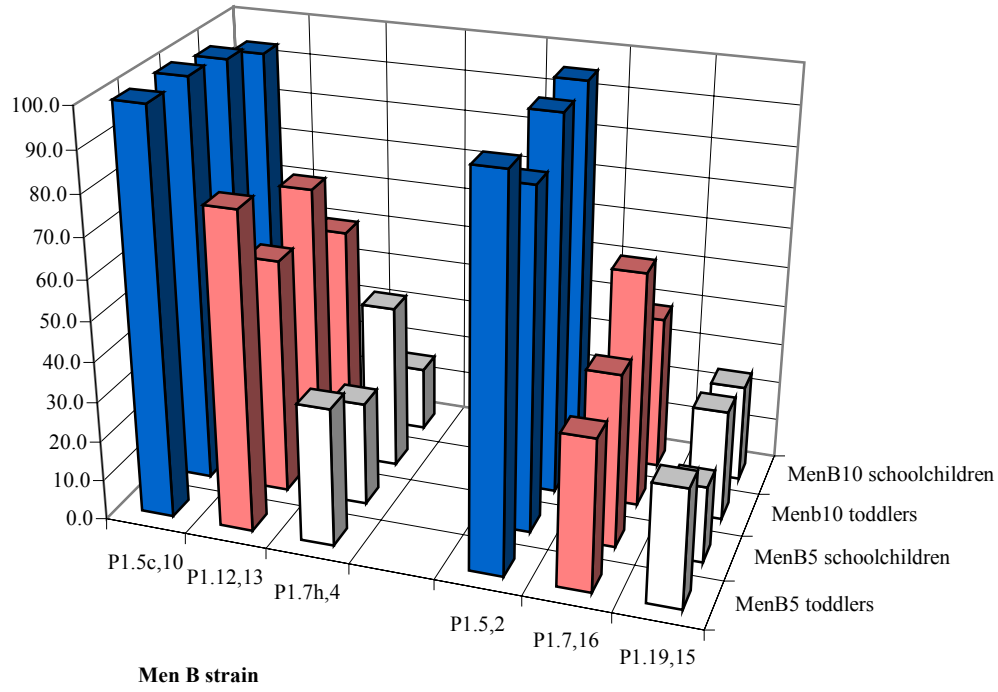


*Figure 1 Percentages of participants with reciprocal bactericidal titres ≥ 1:4*

Table 11 shows Elisa GMT's and 95% CI's against the two vesicles.Figure 1 shows the SBA responses to the vaccines from blood sample 1 to sample 4. Subtypes P1.7,16; P1.5,2 and P1.19,15 are expressed on vesicle PL16215 and the subtypes P1.5$^{c}$,10; P1.12,13 and P1.7$^{h}$,4 are expressed on vesicle PL10124. The SBA GMT's rise after the first two vaccinations. Before the third vaccination the titres have declined, and they rise again after the third vaccination. These SBA responses are seen for both MenB doses, for both age groups, and for all strains. No remarkable changes in SBA titres to any of the six isogenic strains were found in the HepB groups, indicating that contribution of natural circulation of meningococci did not play a major role in the induction of immunity during the study. Significantly higher SBA titres in blood sample 4 were found for the strains P1.5,2 and P1.5$^{c}$,10 as compared to P1.7,16; P1.19,15; P1.12,13; P1.7$^{h}$,4 ($p<0.01$; Wilcoxon signed rank test). In addition, a significant higher SBA titre in blood sample 4 was found for the strain P1.5$^{c}$,10 as compared to P1.5,2. ($p<0.01$;Wilcoxon signed rank test).

Apparent discrepancies in the totals of serological tests (tables 5A and 10) can be explained as follows:

- in some children, the size of blood samples obtained was too small to permit completion of all serological tests
- in the SBA assay three blood samples caused spontaneous lysis of the test bacteria which rendered them unfit for analysis of antibody-dependent complement-mediated lysis.

Figure 2 shows the ELISA responses from blood sample 1 to sample 4. Like the SBA titres, the ELISA titres rise after the first two vaccinations. Before the third vaccination the GMT's have declined, and they rise again after the third vaccination. These ELISA responses are seen for both MenB groups, for both age groups, and for both vesicles. As expected, no ELISA responses were found in the HepB groups.

The two MenB groups were compared with respect to the ELISA titres, using the Mann-Whitney-U test. For toddlers, the ELISA titres against PL16215 in the fourth blood sample were significant higher for the high dose group as compared to low dose (resp. 16244 and 13605; p=0.04). For school children, no significant differences were found.

The Mann-Whitney-U test was also used to compare the low and the high dose group with respect to SBA GMT's in each blood sample. In toddlers, the high dose group differed significantly from the low dose group only in the bactericidal titre against strain P1.7,16 in the fourth blood sample (p=0.02). For the high dose group the mean of the 2log-titre was 5.86 and for the low dose group it was 3.25 (Table 10). In school children, no statistically significant differences in bactericidal antibody titres were found for the two meningococcal groups. Because of the small differences in antibody titres between the low and the high dose group, these data were pooled.
The SBA GMT's in the pooled MenB group were compared with those in the HepB group. Before the first vaccination, the bactericidal titre/activity in toddlers against P1.5$^{c}$,10 and P1.7$^{h}$,4 is significantly higher in the HepB group as compared with the MenB group ($p<0.05$; Mann-Whitney-U). After the second vaccination, a significantly higher response to all strains was found in the MenB group, with exception of the response to P1.7$^{h}$,4 in school children. After three vaccinations, the GMT's against all 6 strains were significantly higher in the MenB group, for toddlers as well as for school children ($P<0.05$; data not shown).

The SBA response and the ELISA response between toddlers and school children were also compared using the Mann-Whitney-U test. In some blood samples the response was significantly higher in toddlers (Table 12 and 13).

Because SBA titres equal to, or greater than1:4 were associated with protection against clinical disease, percentages of participants with bactericidal titres ≥ 1:4 for all blood samples are shown in Figure 3. In the blood samples 2 and 4, the highest proportions are seen, especially for toddlers and against the strains P1.5,2 and P1.5$^{c}$,10. After the third vaccination, the proportion of participants with bactericidal titres ≥ 1:4 against the strains P1.5,2 and P1.5$^{c}$,10, are resp. 90% and 100% for the low dose group, and resp. 95% and 96% for the high dose group.
The percentages of participants showing a significant response to vaccination (fourfold or greater rises in bactericidal antibody titres) is shown in Figures 4 (blood sample 1 to 2, 1 to 4, and 3 to 4). The greatest percentages are seen for the strains P1.5,2 and P1.5$^{c}$,10, especially for toddlers. No important difference was found between the high and the low dose MenB. Most non-responders after two vaccinations are found against the strains expressed on the vesicle PL16215 as compared to PL10124. Most non-responders are school children. After the third vaccination (booster) most non-responders showed a titre rise ≥ 4 against one or

more strains. For some children the bactericidal titre after the third vaccination was lower than that after the second vaccination. Overall, in these particular children the SBA titre after two vaccinations was high as compared to that of the other children.

## 3.4 Estimated protection

Table 14 shows the proportions of participants potentially protected after three vaccinations against subtypes of Neisseria meningitidis circulating in the Netherlands and represented in the hexavalent vaccine.
First, the proportion of participants with a protective bactericidal titre ≥ 1:4 in the fourth blood sample was assessed per subtype[16,17,23]. Then, the percentage of isolates in patients was assessed for these subtypes. For example: 43.1% of the school children in the MenB5 group showed a bactericidal titre ≥ 1:4 against P1.7,16. In the period 1993-1996 14.7% of the isolates were of this subtype. The protection against P1.7,16 after three vaccinations is (43.1) * (14.7) * (1/100) = 6.3%.
The protection against the six vaccine subtypes is (6.3+5.2+1.1+7.2+0.5+11.9) = 32.3%
In the same manner, the protection was estimated for toddlers, for the high dose group, and for the serogroup B+C (because the subtypes are also expressed on group C meningococci).
To see if the estimated potential protection changed in the time, protection was estimated for 1996 as well as for the period 1993-96.
The average estimated protection against the subtypes of Neisseria meningitidis circulating in 1996 that are covered by the vaccine after three vaccinations is 31-45%. The average protection against all circulating subtypes is about 24-36%, since the vaccine covers only 80% all circulating subtypes.

## 3.5 IgG subclass response

The unexpected difference in bactericidal antibody responses between toddlers and school children could possibly have been caused by an age-dependent difference in IgG-subclass response. IgG-subclasses differ in complement activating properties with the IgG1 and IgG3 subclasses of specific relevance for this vaccine. Post vaccination sera (4$^{th}$ sample) of 44 toddlers and 46 school children were analysed for PorA-specific IgG content and IgG-subclass distribution against the proteins of the PL10124 vesicle (Table 15). The IgGtotal and IgG1 response to P1.7$^{h}$,4 was lower compared to the P1.5$^{c}$,10 and P1.12,13. Irrespective of the PorA, IgG titres were significantly higher in toddlers than in school children. No correlation could be established between the SBA titres and the PorA-specific IgG or IgG-subclass titres.

# 4. Discussion

This study showed that the hexavalent OMP vesicle vaccine was well tolerated; the rate and severity of the observed adverse reactions are acceptable. The vaccine is moderately immunogenic in toddlers and school children. Some of the PorA components of the vaccine, including the P1.7$^{h}$,4 PorA, were observed to be weaker immunogens. The SBA response was predominantly directed against one of the three PorA proteins of each vesicle, with the highest titres against the P1.5,2 protein of the PL16215 vesicle and against the P1.5$^{c}$,10 protein of the PL10124 vesicle.

## 4.1 Adverse reactions

Observation of specific systemic and local symptoms by a trained observer at 18-30 hours after administration of the vaccine gives the most objective results despite the lack of blinding. Observed frequencies of adverse reactions between low and high dose MenB vesicle vaccines were comparable. In both age groups, pain and redness at the injection site were the most frequent local reactions; with occurrence of any local reaction in about half the participants. Local reactions were significantly less often observed in the hepatitis B vaccine control group. Fever, one of the most common systemic adverse reactions after vaccination in children, did not occur more often after administration of the MenB vesicle vaccines than after the hepatitis B control vaccine. Less vaccine-specific systemic symptoms like drowsiness were reported more frequently after administration of the MenB vesicle vaccines. The nature and severity of the symptoms did not influence vaccine acceptability to the parents or the participants. The main proportion of dropouts occurred after the first blood sampling but before the first vaccination, mainly amongst the toddlers.
In Gloucestershire UK the same two concentrations of the hexavalent RIVM vesicle vaccine were tested in infants[11]. The vaccines were also well tolerated in this age group. There were no significant differences in rates of local or systemic reactions between low dose and high dose groups. The reported local reactions during 7 days post-immunisation observation were mild, no serious adverse events occurred.
The frequency and severity of the adverse events in Rotterdam and in Gloucestershire indicate that the high dose of the vesicle vaccine also is an acceptable vaccine candidate.
In Norway a monovalent OMV vaccine was prepared in a similar way as the RIVM hexavalent vaccine: also a vesicle presentation but additionally expressing non-PorA proteins. The Norwegian vaccine strain 44/76 is characterised as B:15:P1.7,16:L3,7[21]. Trials were conducted in Norway, Chile and Iceland[6,22,23]. In total more than 185.000 doses of this OMV vaccine were administered to military recruits, students, school children, toddlers and infants. Generally, local side effects were more common after vaccination with the OMV vaccine than after a control vaccination; no significant difference was observed for the systemic reactions. Comparison of different trials with different vaccines in various populations is difficult. However, the frequency of local redness and nausea are comparable between the Rotterdam and the Chile studies and local pain between the Rotterdam and the Norway phase II studies[22,24]. During the largest Norwegian trial eleven serious adverse events were observed, of which seven in the vaccine group[25]. Four of these were serious neurological events, all observed in the vaccine group (acute transverse myelitis, myelopathy, myalgic encephalomyelitis and demyelinating disease). However, no causal relationship has been established. There are no reports of any neurological or permanent adverse events in the studies with our MenB vesicle vaccine.

## 4.2 Immunogenicity

The SBA response in this study was assessed against six isogenic variants of strain H44/76 in which each vaccine PorA-protein was expressed individually. Antibodies detected in the SBA assay show class 1 OMP specific, functional (bactericidal) immunogenicity that is assumed to correlate with specific protective immunity. There was no important difference between the low and high doses of the MenB vaccine. The MenB specific SBA response among the control children (HB-VAX® DNA) was negligible. This indicates that the contribution of natural exposure to meningococci did not play a major role in the induction of immunity in the study children. A significant difference was found between the two age groups: in the second and fourth blood samples, both obtained one month after vaccination, toddlers had statistically significant higher SBA GMT's than school children. In earlier studies, SBA titres of 1:4 or more were associated with protection against clinical disease[16,17,23]. A higher proportion of toddlers had protective titres ≥ 1:4 as compared to school children.
This seems to be in conflict with the age-specific incidence of group B meningococcal disease[2]. Even more so SBA titres obtained from the UK infants[11] (before and after vaccination) revealed to be structurally higher (two to four fold) than those obtained from the older children in Rotterdam. The RIVM and UK SBA assays appeared to differ in several aspects: different salt concentrations were used during the test; in the UK 25% complement was used, as opposed to 10% in the RIVM (both of human origin). In the UK 50% killing was the cut-off criterion, in RIVM this was 90% killing. But in both assay systems, the same isogenic test strains were used. These differences need to be taken into account when the data obtained in the two studies with the same vaccines are compared. By defining responses as a rise in post vs. pre vaccination SBA titres ≥4, the obvious differences between the RIVM and the UK assay may be reduced. However, the same age-dependent difference is still seen. It could possibly have been caused by an age-dependent proportional difference in IgG-subclass response. IgG-subclasses differ in complement activating properties with the IgG1 and IgG3 subclasses of specific relevance for this vaccine[30]. But contrary to findings in adults[31] we could not established a correlation between the SBA titres and the PorA-specific IgG or IgG-subclass titres in the 4$^{th}$ blood sample of a selection of toddlers and school children.
Also in a trial in Chile, infant recipients of the Norwegian and the Cuban monovalent meningococcal B OMP vaccine (resp. strain B:15:P1.7,16:L3,7 and CU385/83 B:4:P1.15) demonstrated higher response rates than children and adults [22]. However, results of a study with the Cuban meningococcal B OMP vaccine in Brazil (age: 3-83 months) showed that there were significantly lower bactericidal titres in children less than 24 months of age and also a significantly lower percentage of these children with bactericidal titre ≥1:4 after vaccination as compared with the older children[27].
Immunogenicity was also assessed by measurement of antibodies directed against the vesicles in an ELISA. Results are of limited importance because analysis on the level of the individual PorA proteins is not possible since three of them are expressed on each vesicle. Overall, no differences of relevance were found in ELISA titres when the two dosages of the MenB vesicle vaccine were compared. However, toddlers respond with statistically significant higher mean ELISA titres after each vaccination as compared to school children. The overall correlation between ELISA titres and SBA titres was poor.

In this study and in Gloucestershire, high SBA titres were found against one of the three PorA proteins of each vesicle, P1.5,2 and P1.5$^{c}$,10. The anti-P1.7$^{h}$,4 and P1.19,15 SBA responses were the weakest, each on a different vesicle. The same phenomenon was observed in baby cynomolgus monkeys[29]. Two reasons may account for this lower response:

- The trivalent expression together with two other PorA's on one vesicle may cause interference with immune stimulation,
- The P1.7$^{h}$, 4 and P1.19,15 PorA may be less immunogenic than other PorA's.

Most non-responders after two priming vaccinations are found against the PorA's expressed on the vesicle PL16215. After the third (booster) vaccination most non-responders showed a titre rise ≥4 against at least one or more PorA's. For some children the bactericidal titre after the third vaccination was lower than after the second vaccination. Overall, the SBA titre in the second blood sample in those children was high as compared to that of the other children.

In the first blood sample (before vaccination) in both toddlers and school children SBA-titres ≥ 1:4 are most frequently detected against P1.5,2 and P1.5$^{c}$,10. This might seem surprising, because in the Netherlands P1.5,2 and P1.5$^{c}$,10 are less frequently isolated meningococcal group B subtypes[2]. However P1.5 is one the most prevalent meningococcal group C subtype[2]. Antibody titres against P1.5$^{c}$,10 remain highest after six months following the primary series of two vaccinations as compared to the other PorA proteins. Overall, the SBA-response is highest in children who have pre-vaccination SBA-titres. SBA-titres ≥ 1:4 against the most frequently isolated P1.7$^{h}$,4 subtype are detected in only a low proportion of participants before vaccination (<2%) but unfortunately also after a series of three vaccinations (29.4%). It may be speculated that just because of its low immunogenicity P1.7$^{h}$,4 is the most prevalent isolate from patients with invasive disease.

The calculated potential protection in 2-7 year old children against the circulating subtypes of Neisseria meningitidis in the Netherlands covered by the vaccine is about 31-45% after three vaccinations. The potential protection to all circulating subtypes is about 24-36%. This calculation is based on the assumption that a bactericidal antibody titre ≥ 1:4 one month after vaccination is a correlate of protection. An efficacy study in Norway, including 171.800 13-21 year olds, showed an overall protective efficacy of 57.4% after two vaccine doses. Efficacy decreased during that study, which lasted 29 months: in the first 10 months it was 86.7%, the last 10 months it was 30.3% [32]. Information about the persistence of immunity induced by our vaccine is lacking. Therefore participants of the study in UK infants are being addressed for a blood sampling approximately two years after the last vaccination. Also, to assess antibody persistence and the booster response to a monovalent P1.7$^{h}$, 4 OMP vesicle vaccine, the toddlers and school children who participated in this study Rotterdam will be investigated approximately 2.5 years after their last vaccination.

# 5. Conclusions and recommendations

The RIVM hexavalent vaccine has an acceptable reactogenicity profile, also in the highest tested dose, and is immunogenic.

The RIVM hexavalent vaccine will need to be further optimised, both with regard to production methods, and immunogenicity: adjuvans alternatives, specific enhancement of (individual) PorA protein immunogenicity, notably P1.7$^{h}$,4!

Serum bactericidal assays should be standardised to make data obtained in different laboratories better comparable.

# References


1. Netherlands Reference Laboratory for Bacterial Meningitis (AMC/RIVM). Bacterial meningitis in the Netherlands; annual report 1994. Amsterdam: University of Amsterdam, 1995.
2. Netherlands Reference Laboratory for Bacterial Meningitis (AMC/RIVM). Bacterial meningitis in the Netherlands; annual report 1996. Amsterdam: University of Amsterdam, 1997.
3. Steven N, Wood M. The clinical spectrum of meningococcal disease. In: Meningococcal Disease. Ed. By K. Cartwright. Publ.: John Wiley & Sons, England 1995: 177-205.
4. Rümke HC. Onderzoek naar immunogeniteit en bijwerkingen van hexavalent meningococcen B vesicle vaccin bij kleuters en schoolkinderen. Protocol versie 2.1, 1995.
5. Scholten RJPM. General introduction. In: The increased incidence of meningococcal disease in the Netherlands 1980-1990; an attempt at an epidemiological explanation. 1993: 4-16.
6. Bjune G, Høiby EE, Grønnesby JK, Arnesen O, Fredriksen JHO, Haltstensen A, Holten E. Lindbak AK, Nokleby H, Rosenqvist E, Solberg LK, Closs O, Froholm LO, Lystad A, Bakketeig LS, Hareide B. Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. Lancet 1991; 338: 1093-1096.
7. Bjune G, Grønnesby JK, Høiby EA, Closs O, Nøkleby H. Results of an efficacy trial with an outer membrane vesicle vaccine against systemic serogroup B meningococcal disease in Norway. In: NIPH Annals; 1991: 125-132.
8. Cassio de Moraes J, Perkins B, Camargo MCC, Rosetto Hidaldo NT, Aparecida Barbosa H, Tavares Sacchi C, Gattas VL, Plikaytis BD and Broome CV. Protective efficacy of a serogroup B meningococcal vaccine in Sao Paulo, Brazil. Lancet 1992; 340: 1074-1078.
9. Van der Ley P, Poolman JT. Construction of a multivalent meningococcal vaccine strain based on the class 1 outer membrane protein. Infection and Immunity 1992; 60: 3156-3161.
10. Peeters, CCAM, Rümke HC, Sundermann LC, Rouppe van der Voort EM, Meulenbelt J, Schuller M, Kuipers AJ, van der Ley P, Poolman JT. Phase I clinical trial with a hexavalent PorA containing meningococcal outer membrane vesicle vaccine. Vaccine 1996; 14: 1008-1015.
11. Cartwright K, Morris R, Rümke H, Fox A, Borrow R, Begg N, Richmond P, Poolman J. Immunogenicity and reactogenicity in UK infants of a novel meningococcal vesicle vaccine containing multiple class 1 (PorA) outer membrane proteins. Vaccine, accepted Jan. 1999.
12. Claassen I., Meylis J., Van der Ley P., Peeters C., Brons H., Robert J., Borsboom D., Van der Ark A., Van Straaten I., Roholl P., Kuipers B., Poolman J. Production, characterization and control of a Neisseria meningitidis hexavalent class 1 outer membrane protein containing vesicle vaccine. Vaccine 1996; 10: 1001-1008.
13. Institute of Medicine. Adverse events associated with childhood vaccines. Evidence bearing on causality. National Academy Press, ed. by Stratton KR, Howe CJ and Johnston Jr RB. Washington DC, 1994.
14. West DJ Clinical experience with hepatitis B vaccines. American Journal of Infection Control 1989; 17: 172-180.
15. Dean AG, Dean JA, Coulombier D, Brendel KA, Smith DC, Burton AH, Dicker RC, Sullivan K, Fagan RF, Arner TG. Epi Info, version 6: a word processing, database, and

statistics program for epidemiology on microcomputers. Atlanta; Centers for Disease Control and Prevention, 1994.
16. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus I. The role of humoral antibodies. J Exp Med 1969; 129: 1307-1326.
17. Frasch CE. Meningococcal vaccines: past, present and future. In: Meningococcal Disease. Ed. By K. Cartwright. Publ.: John Wiley & Sons, England 1995: 245-283.
18. Rouppe van der Voort EM, van der Ley P, van der Biezen J, George S, Tunnela O, van Dijken H, Kuipers B, and Poolman JT. Specificity of human bactericidal antibodies against PorA P1.7,16 induced with a hexavalent outer membrane vesicle vaccine. Infect Immun1996; 64: 2745 - 2751.
19. SPSS for Windows. Release 7.0 (Dec 19 1995) SPSS Inc.; 1989-1995
20. Rosenqvist E, Høiby EA, Wedege E, Bryn K, Kolberg J, Klem A, Rønnild E, Bjune G and Nøkleby H. Human antibody reponses to meningococcal outer membrane antigens after three doses of the Norwegian group B. Infect Immun1995; 63: 4642 - 4652.
21. Fredriksen JH, Rosenqvist E, Wedege E, Bryn K, Bjune G, Frøholm LO, Lindbak AK, Møgster B, Namork E, Rye U, Stabbetorp G, Winsnes R, Aase B, Closs O. Production, characterization ans control of MenB-vaccine <Folkehelsa>: an outer membrane vesicle vaccine against group B meningococcal disease. In: NIPH annals; 1991: 67-79.
22. Tappero JW, Lagos R, Ballesteros AM, Plikaytis B, Williams D, Dykes J, Gheesling LL, Carlone GM, Hoiby EA, Holst J, Nokleby H, Rosenqvist E, Sierra G, Campa C, Sotolongo F, Vega J, Garcia J, Herrera P, Poolman JT,Perkins BA. Immunogenicity of 2 serogroup B outer-membrane protein meningococcal vaccines - A randomized controlled trial in Chile. JAMA 1999; 281 (16) : 1520-1527.
23. Perkins BA, Jonsdottir K, Briem H, Griffiths E, Plikaytis BD, Høiby EA, Rosenqvist E, Holst J, Nøkleby H, Sotolongo F, Sierra G, Campa HC, Carlone GM, Williams D, Dykes J, Kapczynski D, Tikhomirov E, Wenger JD, Broome CV. Immunogenicity of two efficacious outer membrane protein-based serogroup B meningococcal vaccines among young adults in Iceland. J Infect Dis 1998; 177: 683-691.
24. Nøkleby H., Feiring B. The Norwegian meningococcal group B outer membrane vescicle vaccine: side effects in phase II trials. In: NIPH annals; 1991: 95-101
25. Aavitsland P, Bjune G, Aasen S, Halvorsen S. Adverse events following vaccine or placebo injection in an efficacy trial of an outer membrane vesicle vaccine against group B meningococcal disease in Norwegian secondary schools 1988-1991. In: NIPH annals; 1991: 133-134.
26. De Moraes JC, Perkins BA, Camargo MCC, Hidalgo NTR, Barbosa HA, Sacchi CT, Land Gral IM, Gattas VL, Vasconcelos HDG, Plikaytis BD, Wenger JD, Broome CV. Protective efficacy of a serogroup B meningococcal vaccine in Sao Paulo, Brasil. Lancet 1992;340:1074-78.
27. Milagres LG, Ramos SR, Sacchi CT, Melles CEA, Vieira VSD, Sato H, Brito GS, Moraes JC, Frasch CE. Immune response of Brazilian children to a Neisseria meningitidis serogroup B outer membrane protein vaccine: comparison with efficacy. Infection and Immunity 1994;62:4419-4424.
28. Boslego J, Garcia J, Cruz C, Zollinger W, Brandt B, Ruiz S, Martinez M, Arthur J, Underwood P, Silva W, Moran E, Hankins W, Gilly J, Mays J and the Chilean National Committee for Meningococcal Disease. Efficacy, safety, and immunogenicity of a meningococcal group B (15:P1.3) outer membrane protein vaccine in Iquique, Chile. Vaccine 1995;13: 821-829.
29. Rouppe van der Voort E.M., Schuller M., De Vries P., Van den Dobbelsteen G., Van der Ley P., Poolman J. Immunogenicity studies with eperimental meningococcal group B outer membrane vaccines in baby cynomolgus monkeys; a comparison between the Dutch

and the Norwegian H44/76 derived vesicle vaccines. Thesis, Amsterdam 1998 Ch.6: 89-111.
30. Naess LM., Rosenqvist E., Hoiby EA., Michaelsen TE. Quantitation of IgG subclass antibody responses after immunization with a group B meningococcal outer membrane vesicle vaccine, using monoclonal mouse-human chimeric antibodies as standards.J Immunol Methods. 1996; 196(1): 41-9
31. Naess LM, Aarvak T, Aase A, Oftung F, Hoiby EA, Sandin R, Michaelsen TE. Human IgG subclass responses in relation to serum bactericidal and opsonic activities after immunization with three doses of the Norwegian serogroup B meningococcal outer membrane vesicle vaccine. Vaccine 1999; 17 (7-8) : 754-764
32. Rosenqvist E., Høiby E.E. Bjune G. Bryn K., Caugant D.A., Closs O., Fredriksen J.H., Grønnesby J.K., Nøkleby H., Wedege E., Frøholm L.O. Meningococcal group B outer membrane vesicle vaccine trial among teenagers in Norway. Advances in Modern Biotechnology. La Habana, Cuba. Symposium Vol. 1: 5.2.1992

# Declaration of quality control

Undersigned states herewith that the research presented in this report has been carried out according to the OECD principles of Good Clinical Practice (GCP) and that this report reflects a complete, correct and reliable overview of the results obtained.

GCP inspections of the experiments and reports submitted to the management research team leader took place on:

| Inspection Date | Type of Inspection |
|---|---|
| 31-10-96 | Transfer Sera |

This report was inspected on 17 October 2000
Inspection of report no. 124001.003

Quality control officer:

| | | |
|---|---|---|
| name | : | M.C. Jongerius |
| laboratory | : | Laboratory for Clinical Vaccine Research |

# Appendix 1 Mailing list

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|---|---|
| 1 | Hoofdinspecteur Preventieve en Curatieve Gezondheidszorg |
| 2 | Directeur-Generaal Volksgezondheid |
| 3 | Inspectie Gezondheidszorg, Inspecteur Infectieziekten |
| 4 | Gezondheidsraad, Den Haag voorzitter |
| 5 | Gezondheidsraad, Den Haag secretaris werkgroep RVP |
| 6 | Medisch Ethische Commissie AZR/EUR, Rotterdam |
| 7-9 | Prof. Dr R. de Groot |
| 10-17 | GGD Rotterdam en omstreken |
| 18-21 | Stichting Thuiszorg Rotterdam |
| 22 | Nationaal Referentie Laboratorium Bacteriële Meningitis AMC/RIVM, Amsterdam |
| 23 | Depot Nederlandse Publikaties en Nederlandse Bibliografie |
| 24 | Directie RIVM |
| 25 | Directeur sector Vaccins |
| 26 | Directeur sector Volksgezondheidsonderzoek |
| 27-29 | Hoofd LVO |
| 30-31 | Hoofd LCB |
| 32-33 | Hoofd LPO |
| 34-35 | Hoofd LVR |
| 36-37 | Hoofd KRZ |
| 38 | Hoofd CIE |
| 39 | Hoofd LIS |
| 40 | Hoofd LIO |
| 41 | Prof. Juhani Eskola, Nat. Inst. Public Health, Helsinki Finland |
| 42 | Prof. Keith Cartwright, Public Health Laboratory Gloucester, UK |
| 43 | Dr. Ray Borrow, Public Health Laboratory, Manchester, UK |
| 44 | Dr. David Salisbury, Dept. of Health, London, UK |
| 45 | Dr M.A.E Conyn-van Spaendonck |
| 46 | H.E. de Melker |
| 47-58 | Leden IGZ infectieziektenoverleg |
| 59-73 | Auteurs |
| 74 | SBD/Voorlichting en Public Relations |
| 75 | Bureau Rapportenregistratie |
| 76 | Bibliotheek RIVM |
| 77-91 | Bureau Rapportenbeheer |
| 92-125 | Reserve |

# Appendix 2 Participants

*Table 1. Number of recruited participants*

| | toddlers | school children |
|---|---|---|
| CB visit | 640 | - |
| direct mailing | 1200 | 2400 |
| enrolment | nk* | 270 |
| intake | nk | 227 |
| Inform. Consent | 189 | 168 |

*nk = not known

*Table 2. Participant randomisation*

| | toddlers | | school children | | total | |
|---|---|---|---|---|---|---|
| low dose | 65 | | 62 | | 127 | |
| high dose | 62 | | 64 | | 126 | |
| control | 62 | | 42 | | 104 | |
| total | 189 | | 168 | | 357 | |
| sex | f=90 | m=99 | f=81 | m=87 | f=171 | m=186 |

*Table 3. Participant dropout*

| | toddlers | | school children | | total | |
|---|---|---|---|---|---|---|
| | < v1 | > v1 | < v1 | > v1 | < v1 | > v1 |
| low dose | 3 | 1 | 1 | 0 | 4 | 1 |
| high dose | 8 | 1 | 0 | 2 | 8 | 3 |
| control | 6 | 0 | 2 | 1 | 8 | 1 |
| total | 17 | 2 | 3 | 3 | 20 | 5 |

< v1 before first vaccination
> v1 after first vaccination

*Table 4. Exclusion of participants for protocol violations*

| | toddlers | school children | total |
|---|---|---|---|
| interval vaccination 1- observation | 8 | 3 | 11 |
| interval vaccination 2- observation | 2 | 0 | 2 |
| interval vaccination 3- observation | 2 | 0 | 2 |
| interval between vaccinations | 1 | 0 | 1 |
| interval vaccination - blood sampling | 0 | 1 | 1 |
| received wrong study vaccine | 0 | 2 | 2 |
| received other vaccine during the study | 1 | 1 | 2 |
| total | 14 | 7 | 21 |

*Table 5A. Number of evaluable participants - antibody response*

| | toddlers | school children |
|---|---|---|
| Informed Consent | 189 | 168 |
| blood sample 1 | 172 | 165 |
| blood sample 2 | 168 | 161 |
| blood sample 3 | 167 | 161 |
| blood sample 4 | 167 | 158 |

*Table 5B. Number of evaluable participants - adverse events*

| | toddlers | school children |
|---|---|---|
| Informed Consent | 189 | 168 |
| vaccination 1 | 164 | 162 |
| vaccination 2 | 170 | 161 |
| vaccination 3 | 167 | 159 |

# Appendix 3 Systemic adverse events

*Table 6. Systemic adverse events*

**Low dose MenB - toddlers**

Vaccination 1 N=58

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 15 | 25.9 | 8 | 12.9 | 7 | 11.3 |
| fever | 3 | 5.2 | 3 | 4.8 | 2 | 3.2 |
| headache | 1 | 1.7 | 0 | | 0 | |
| drowsiness | 9 | 15.5 | 6 | 9.7 | 2 | 3.2 |
| sleepy | 1 | 1.7 | 0 | | 0 | |
| unusual crying | 7 | 12.1 | 3 | 4.8 | 1 | 1.6 |
| less appetite | 7 | 12.1 | 4 | 6.5 | 1 | 1.6 |
| nausea | 1 | 1.7 | 1 | 1.6 | 0 | |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 0 | | 0 | | 2 | 3.2 |
| illness in family | 2 | 3.4 | 1 | 1.6 | 0 | |
| doctor or hospital visit | 0 | | 0 | | 0 | |

Vaccination 2 N=60

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 11 | 18.3 | 9 | 14.5 | 1 | 1.6 |
| fever | 3 | 5.0 | 2 | 3.2 | 0 | |
| headache | 1 | 1.7 | 0 | | 0 | |
| drowsiness | 9 | 15.0 | 7 | 11.3 | 1 | 1.6 |
| sleepy | 0 | | 0 | | 0 | |
| unusual crying | 7 | 11.7 | 1 | 1.6 | 1 | 1.6 |
| less appetite | 5 | 8.3 | 3 | 4.8 | 0 | |
| nausea | 1 | 1.7 | 1 | 1.6 | 0 | |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 0 | | 1 | 1.6 | 0 | |
| illness in family | 1 | 1.7 | 0 | | 0 | |
| doctor or hospital visit | 2 | 3.3 | 0 | | 0 | |

Vaccination 3 N=60

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 12 | 20.0 | 3 | 4.9 | 1 | 1.6 |
| fever | 1 | 1.7 | 1 | 1.6 | 1 | 1.6 |
| headache | 0 | | 0 | | 0 | |
| drowsiness | 9 | 15.0 | 3 | 4.9 | 1 | 1.6 |
| sleepy | 1 | 1.7 | 0 | | 0 | |
| unusual crying | 2 | 3.3 | 0 | | 0 | |
| less appetite | 2 | 3.3 | 2 | 3.3 | 1 | 1.6 |
| nausea | 0 | | 0 | | 1 | 1.6 |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 0 | | 1 | 1.6 | 1 | 1.6 |
| illness in family | 3 | 5.0 | 1 | 1.6 | 1 | 1.6 |
| doctor or hospital visit | 1 | 1.7 | 1 | 1.6 | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
  Observation 2 : 24 - 72 hours after vaccination
  Observation 3 : 3 - 7 days after vaccination

*Table 6. Systemic adverse events (continued)*

**High dose MenB - toddlers**

Vaccination 1 N=53

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 13 | 24.5 | 4 | 7.4 | 1 | 1.9 |
| fever | 2 | 3.8 | 0 | | 0 | |
| headache | 0 | | 0 | | 0 | |
| drowsiness | 7 | 13.2 | 3 | 5.6 | 1 | 1.9 |
| sleepy | 0 | | 0 | | 0 | |
| unusual crying | 6 | 11.3 | 2 | 3.7 | 1 | 1.9 |
| less appetite | 3 | 5.7 | 1 | 1.9 | 1 | 1.9 |
| nausea | 2 | 3.8 | 0 | | 0 | |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 0 | | 0 | | 0 | |
| illness in family | 2 | 3.8 | 0 | | 0 | |
| doctor or hospital visit | 0 | | 0 | | 0 | |

Vaccination 2 N=54

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 15 | 27.8 | 7 | 13.0 | 5 | 9.3 |
| fever | 2 | 3.7 | 2 | 3.7 | 2 | 3.7 |
| headache | 1 | 1.9 | 0 | | 0 | |
| drowsiness | 11 | 20.4 | 6 | 11.1 | 3 | 5.6 |
| sleepy | 0 | | 1 | 1.9 | 0 | |
| unusual crying | 6 | 11.1 | 2 | 3.7 | 1 | 1.9 |
| less appetite | 2 | 3.7 | 4 | 7.4 | 3 | 5.6 |
| nausea | 1 | 1.9 | 1 | 1.9 | 0 | |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 1 | 1.9 | 1 | 1.9 | 1 | 1.9 |
| illness in family | 1 | 1.9 | 0 | | 0 | |
| doctor or hospital visit | 0 | | 1 | 1.9 | 0 | |

Vaccination 3 N=52

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 14 | 26.9 | 6 | 11.3 | 4 | 7.5 |
| fever | 1 | 1.9 | 5 | 9.4 | 3 | 5.7 |
| headache | 0 | | 0 | | 0 | |
| drowsiness | 11 | 21.2 | 5 | 9.4 | 4 | 7.5 |
| sleepy | 3 | 5.8 | 0 | | 0 | |
| unusual crying | 6 | 11.5 | 3 | 5.7 | 2 | 3.8 |
| less appetite | 7 | 13.5 | 4 | 7.5 | 3 | 5.7 |
| nausea | 3 | 5.8 | 1 | 1.9 | 1 | 1.9 |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 0 | | 0 | | 0 | |
| illness in family | 3 | 5.8 | 2 | 3.8 | 1 | 1.9 |
| doctor or hospital visit | 0 | | 1 | 1.9 | 2 | 3.8 |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

*Table 6. Systemic adverse events (continued)*

**Control group - toddlers**

Vaccination 1 N=53

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 7 | 13.2 | 8 | 14.3 | 4 | 7.1 |
| fever | 2 | 3.8 | 3 | 5.4 | 3 | 5.4 |
| headache | 1 | 1.9 | 0 | | 0 | |
| drowsiness | 5 | 9.4 | 7 | 12.5 | 3 | 5.4 |
| sleepy | 1 | 1.9 | 0 | | 0 | |
| unusual crying | 2 | 3.8 | 2 | 3.6 | 1 | 1.8 |
| less appetite | 2 | 3.8 | 3 | 5.4 | 2 | 3.6 |
| nausea | 0 | | 3 | 5.4 | 1 | 1.8 |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 1 | 1.9 | 1 | 1.8 | 1 | 1.8 |
| illness in family | 0 | | 0 | | 1 | 1.8 |
| doctor or hospital visit | 0 | | 1 | 1.8 | 0 | |

Vaccination 2 N=56

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 8 | 14.3 | 2 | 3.6 | 2 | 3.6 |
| fever | 1 | 1.8 | 0 | | 2 | 3.6 |
| headache | 0 | | 0 | | 0 | |
| drowsiness | 2 | 3.6 | 1 | 1.8 | 2 | 3.6 |
| sleepy | 0 | | 0 | | 0 | |
| unusual crying | 5 | 8.9 | 0 | | 0 | |
| less appetite | 1 | 1.8 | 0 | | 2 | 3.6 |
| nausea | 0 | | 0 | | 1 | 1.8 |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 0 | | 1 | 1.8 | 0 | |
| illness in family | 0 | | 0 | | 0 | |
| doctor or hospital visit | 0 | | 1 | 1.8 | 0 | |

Vaccination 3 N=55

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 4 | 7.3 | 4 | 7.3 | 2 | 3.6 |
| fever | 1 | 1.8 | 3 | 5.5 | 1 | 1.8 |
| headache | 0 | | 0 | | 0 | |
| drowsiness | 4 | 7.3 | 4 | 7.3 | 2 | 3.6 |
| sleepy | 0 | | 0 | | 0 | |
| unusual crying | 0 | | 1 | 1.8 | 1 | 1.8 |
| less appetite | 2 | 3.6 | 4 | 7.3 | 1 | 1.8 |
| nausea | 0 | | 0 | | 0 | |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 0 | | 0 | | 0 | |
| illness in family | 1 | 1.8 | 2 | 3.6 | 1 | 1.8 |
| doctor or hospital visit | 1 | 1.8 | 2 | 3.6 | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

*Table 6. Systemic adverse events (continued)*

**Low dose MenB - school children**

Vaccination 1 N=60

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 17 | 28.3 | 7 | 11.5 | 6 | 9.8 |
| fever | 0 | | 1 | 1.6 | 3 | 4.9 |
| headache | 12 | 20.0 | 3 | 4.9 | 3 | 4.9 |
| drowsiness | 5 | 8.3 | 3 | 4.9 | 2 | 3.3 |
| less appetite | 1 | 1.7 | 1 | 1.6 | 1 | 1.6 |
| nausea | 2 | 3.3 | 0 | | 0 | |
| joint complaints | 1 | 1.7 | 1 | 1.6 | 2 | 3.3 |
| cutaneous symptoms | 2 | 3.3 | 1 | 1.6 | 1 | 1.6 |
| absence school/crèche | 1 | 1.7 | 0 | | 1 | 1.6 |
| illness in family | 5 | 8.3 | 0 | | 0 | |
| doctor or hospital visit | 0 | | 2 | 3.3 | 1 | 1.6 |

Vaccination 2 N=60

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 20 | 33.3 | 9 | 15.0 | 4 | 6.7 |
| fever | 2 | 3.3 | 1 | 1.7 | 2 | 3.3 |
| headache | 11 | 18.3 | 5 | 8.3 | 3 | 5.0 |
| drowsiness | 12 | 20.0 | 7 | 11.7 | 3 | 5.0 |
| less appetite | 5 | 8.3 | 2 | 3.3 | 1 | 1.7 |
| nausea | 2 | 3.3 | 2 | 3.3 | 2 | 3.3 |
| joint complaints | 1 | 1.7 | 0 | | 0 | |
| cutaneous symptoms | 1 | 1.7 | 0 | | 0 | |
| absence school/crèche | 3 | 5.0 | 2 | 3.3 | 2 | 3.3 |
| illness in family | 4 | 6.7 | 4 | 6.7 | 0 | |
| doctor or hospital visit | 1 | 1.7 | 1 | 1.7 | 1 | 1.7 |

Vaccination 3 N=58

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 12 | 20.7 | 4 | 6.9 | 0 | |
| fever | 1 | 1.7 | 0 | | 0 | |
| headache | 8 | 13.8 | 2 | 3.4 | 0 | |
| drowsiness | 7 | 12.1 | 2 | 3.4 | 0 | |
| less appetite | 4 | 6.9 | 0 | | 0 | |
| nausea | 2 | 3.4 | 0 | | 0 | |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 0 | | 0 | | 0 | |
| absence school/crèche | 3 | 5.2 | 1 | 1.7 | 0 | |
| illness in family | 2 | 3.4 | 0 | | 0 | |
| doctor or hospital visit | 0 | | 0 | | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

*Table 6. Systemic adverse events (continued)*

**High dose MenB - school children**

Vaccination 1 N=63

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 26 | 41.3 | 11 | 17.7 | 3 | 4.8 |
| fever | 0 | | 1 | 1.6 | 1 | 1.6 |
| headache | 8 | 12.7 | 4 | 6.5 | 0 | |
| drowsiness | 16 | 25.4 | 7 | 11.3 | 2 | 3.2 |
| less appetite | 8 | 12.7 | 1 | 1.6 | 0 | |
| nausea | 5 | 7.9 | 2 | 3.2 | 0 | |
| joint complaints | 5 | 7.9 | 0 | | 0 | |
| cutaneous symptoms | 0 | | 1 | 1.6 | 1 | 1.6 |
| absence school/crèche | 1 | 1.6 | 1 | 1.6 | 2 | 3.2 |
| illness in family | 7 | 11.1 | 2 | 3.2 | 1 | 1.6 |
| doctor or hospital visit | 0 | | 0 | | 1 | 1.6 |

Vaccination 2 N=62

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 24 | 38.7 | 7 | 11.3 | 2 | 3.2 |
| fever | 1 | 1.6 | 0 | | 1 | 1.6 |
| headache | 9 | 14.5 | 2 | 3.2 | 1 | 1.6 |
| drowsiness | 10 | 16.1 | 4 | 6.5 | 1 | 1.6 |
| less appetite | 7 | 11.3 | 0 | | 0 | |
| nausea | 4 | 6.5 | 0 | | 0 | |
| joint complaints | 1 | 1.6 | 0 | | 0 | |
| cutaneous symptoms | 4 | 6.5 | 2 | 3.2 | 1 | 1.6 |
| absence school/crèche | 11 | 17.7 | 3 | 4.8 | 1 | 1.6 |
| illness in family | 6 | 9.7 | 1 | 1.6 | 1 | 1.6 |
| doctor or hospital visit | 0 | | 0 | | 1 | 1.6 |

Vaccination 3 N=62

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 15 | 24.2 | 3 | 4.8 | 1 | 1.6 |
| fever | 1 | 1.6 | 0 | | 0 | |
| headache | 9 | 14.5 | 2 | 3.2 | 1 | 1.6 |
| drowsiness | 6 | 9.7 | 2 | 3.2 | 0 | |
| less appetite | 5 | 8.1 | 2 | 3.2 | 0 | |
| nausea | 5 | 8.1 | 0 | | 0 | |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 0 | | 0 | | 0 | |
| absence school/crèche | 5 | 8.1 | 2 | 3.2 | 0 | |
| illness in family | 1 | 1.6 | 0 | | 0 | |
| doctor or hospital visit | 1 | 1.6 | 0 | | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

*Table 6. Systemic adverse events (continued)*

**Control group - school children**

Vaccination 1 N=39

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 9 | 23.1 | 3 | 7.7 | 3 | 7.7 |
| fever | 0 | | 0 | | 0 | |
| headache | 2 | 5.1 | 0 | | 2 | 5.1 |
| drowsiness | 2 | 5.1 | 1 | 2.6 | 1 | 2.6 |
| less appetite | 1 | 2.6 | 1 | 2.6 | 2 | 5.1 |
| nausea | 2 | 5.1 | 1 | 2.6 | 0 | |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 3 | 7.7 | 1 | 2.6 | 0 | |
| absence school/crèche | 1 | 2.6 | 0 | | 0 | |
| illness in family | 5 | 12.8 | 2 | 5.1 | 1 | 2.6 |
| doctor or hospital visit | 0 | | 0 | | 0 | |

Vaccination 2 N=39

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 5 | 12.8 | 3 | 7.7 | 3 | 7.7 |
| fever | 0 | | 2 | 5.1 | 2 | 5.1 |
| headache | 2 | 5.1 | 1 | 2.6 | 2 | 5.1 |
| drowsiness | 1 | 2.6 | 0 | | 1 | 2.6 |
| less appetite | 1 | 2.6 | 2 | 5.1 | 1 | 2.6 |
| nausea | 1 | 2.6 | 1 | 2.6 | 0 | |
| joint complaints | 0 | | 0 | | 0 | |
| cutaneous symptoms | 1 | 2.6 | 0 | | 0 | |
| absence school/crèche | 1 | 2.6 | 2 | 5.1 | 2 | 5.1 |
| illness in family | 2 | 5.1 | 0 | | 0 | |
| doctor or hospital visit | 0 | | 0 | | 0 | |

Vaccination 3 N=39

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any systemic reaction | 5 | 12.8 | 2 | 5.1 | 1 | 2.6 |
| fever | 0 | | 0 | | 0 | |
| headache | 4 | 10.3 | 2 | 5.1 | 0 | |
| unusual crying | 1 | 2.6 | 0 | | 0 | |
| less appetite | 0 | | 1 | 2.6 | 1 | 2.6 |
| nausea | 0 | | 0 | | 0 | |
| joint complaints | 1 | 2.6 | 0 | | 0 | |
| cutaneous symptoms | 1 | 2.6 | 1 | 2.6 | 1 | 2.6 |
| absence school/crèche | 3 | 7.7 | 1 | 2.6 | 1 | 2.6 |
| illness in family | 2 | 5.1 | 1 | 2.6 | 1 | 2.6 |
| doctor or hospital visit | 0 | | 1 | 2.6 | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

# Appendix 4 Local adverse events

*Table 7. Local adverse events*

**Low dose MenB - toddlers**

Vaccination 1 N=58

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 34 | 58.6 | 8 | 12.9 | 1 | 1.6 |
| pain | 25 | 43.1 | 7 | 11.3 | 1 | 1.6 |
| redness | 18 | 31.0 | 0 | | 0 | |
| redness >2.5cm | 0 | | 0 | | 0 | |
| swelling >2.5cm | 1 | 1.7 | 1 | 1.6 | 0 | |
| not using arm/leg | 3 | 5.2 | 1 | 1.6 | 0 | |
| itching | 0 | | 0 | | 0 | |

Vaccination 2 N=60

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 34 | 56.7 | 13 | 21.0 | 3 | 4.8 |
| pain | 27 | 45.0 | 11 | 17.7 | 3 | 4.8 |
| redness | 11 | 18.3 | 4 | 6.5 | 0 | |
| redness >2.5cm | 0 | | 1 | 1.6 | 0 | |
| swelling >2.5cm | 2 | 3.3 | 0 | | 0 | |
| not using arm/leg | 5 | 8.3 | 4 | 6.5 | 1 | 1.6 |
| itching | 0 | | 0 | | 0 | |

Vaccination 3 N=60

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 33 | 55.0 | 11 | 18.0 | 2 | 3.3 |
| pain | 31 | 51.7 | 9 | 14.8 | 2 | 3.3 |
| redness | 11 | 18.3 | 2 | 3.3 | 0 | |
| redness >2.5cm | 0 | | 0 | | 0 | |
| swelling >2.5cm | 0 | | 0 | | 0 | |
| not using arm/leg | 1 | 1.7 | 1 | 1.6 | 0 | |
| itching | 0 | | 0 | | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

*Table 7. Local adverse events (continued)*

**High dose MenB - toddlers**

Vaccination 1 N=53

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 29 | 54.7 | 11 | 20.4 | 1 | 1.9 |
| pain | 24 | 45.3 | 9 | 16.7 | 0 | |
| redness | 13 | 24.5 | 2 | 3.7 | 1 | 1.9 |
| redness >2.5cm | 1 | 1.9 | 1 | 1.9 | 1 | 1.9 |
| swelling >2.5cm | 1 | 1.9 | 1 | 1.9 | 0 | |
| not using arm/leg | 7 | 13.2 | 1 | 1.9 | 0 | |
| itching | 0 | | 0 | | 0 | |

Vaccination 2 N=54

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 36 | 66.7 | 13 | 24.1 | 6 | 11.1 |
| pain | 29 | 53.7 | 13 | 24.1 | 6 | 11.1 |
| redness | 13 | 24.1 | 1 | 1.9 | 1 | 1.9 |
| redness >2.5cm | 2 | 3.7 | 1 | 1.9 | 1 | 1.9 |
| swelling >2.5cm | 0 | | 1 | 1.9 | 1 | 1.9 |
| not using arm/leg | 9 | 16.7 | 4 | 7.4 | 2 | 3.7 |
| itching | 0 | | 0 | | 0 | |

Vaccination 3 N=52

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 39 | 75.0 | 19 | 35.8 | 4 | 7.5 |
| pain | 36 | 69.2 | 19 | 35.8 | 4 | 7.5 |
| redness | 13 | 25.0 | 5 | 9.4 | 2 | 3.8 |
| redness >2.5cm | 2 | 3.8 | 4 | 7.5 | 1 | 1.9 |
| swelling >2.5cm | 2 | 3.8 | 0 | | 0 | |
| not using arm/leg | 5 | 9.6 | 1 | 1.9 | 0 | |
| itching | 0 | | 0 | | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

*Table 7. Local adverse events (continued)*

**Control group - toddlers**

Vaccination 1 N=53

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 5 | 9.4 | 1 | 1.8 | 0 | |
| pain | 5 | 9.4 | 1 | 1.8 | 0 | |
| redness | 0 | | 0 | | 0 | |
| redness >2.5cm | 0 | | 0 | | 0 | |
| swelling >2.5cm | 0 | | 0 | | 0 | |
| not using arm/leg | 0 | | 0 | | 0 | |
| itching | 0 | | 0 | | 0 | |

Vaccination 2 N=56

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 7 | 12.5 | 0 | | 0 | |
| pain | 7 | 12.5 | 0 | | 0 | |
| redness | 0 | | 0 | | 0 | |
| redness >2.5cm | 0 | | 0 | | 0 | |
| swelling >2.5cm | 0 | | 0 | | 0 | |
| not using arm/leg | 1 | 1.8 | 0 | | 0 | |
| itching | 0 | | 0 | | 0 | |

Vaccination 3 N=55

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 5 | 9.1 | 1 | 1.8 | 1 | 1.8 |
| pain | 4 | 7.3 | 1 | 1.8 | 1 | 1.8 |
| redness | 1 | 1.8 | 1 | 1.8 | 1 | 1.8 |
| redness >2.5cm | 0 | | 1 | 1.8 | 1 | 1.8 |
| swelling >2.5cm | 0 | | 1 | 1.8 | 1 | 1.8 |
| not using arm/leg | 2 | 3.6 | 1 | 1.8 | 0 | |
| itching | 0 | | 0 | | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

*Table 7. Local adverse events (continued)*

**Low dose MenB - school children**

Vaccination 1 N=60

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 52 | 86.7 | 33 | 54.1 | 12 | 19.7 |
| pain | 51 | 85.0 | 31 | 50.8 | 10 | 16.4 |
| redness | 24 | 40.0 | 19 | 31.1 | 6 | 9.8 |
| redness >2.5cm | 7 | 11.7 | 9 | 14.8 | 5 | 8.2 |
| swelling >2.5cm | 8 | 13.3 | 9 | 15.0 | 6 | 9.8 |
| not using arm/leg | 5 | 8.5 | 4 | 6.6 | 2 | 3.3 |
| itching | 2 | 3.3 | 4 | 6.6 | 2 | 3.3 |

Vaccination 2 N=60

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 56 | 93.3 | 21 | 35.0 | 3 | 5.0 |
| pain | 53 | 88.3 | 19 | 31.7 | 2 | 3.3 |
| redness | 30 | 50.0 | 11 | 18.3 | 0 | |
| redness >2.5cm | 11 | 18.3 | 6 | 10.0 | 0 | |
| swelling >2.5cm | 8 | 13.3 | 8 | 13.3 | 2 | 3.3 |
| not using arm/leg | 4 | 6.7 | 3 | 5.0 | 0 | |
| itching | 5 | 8.3 | 1 | 1.7 | 0 | |

Vaccination 3 N=58

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 43 | 74.1 | 18 | 31.0 | 3 | 5.2 |
| pain | 40 | 69.0 | 15 | 25.9 | 3 | 5.2 |
| redness | 22 | 37.9 | 6 | 10.3 | 1 | 1.7 |
| redness >2.5cm | 6 | 10.3 | 3 | 5.2 | 1 | 1.7 |
| swelling >2.5cm | 13 | 22.4 | 5 | 8.6 | 1 | 1.7 |
| not using arm/leg | 5 | 8.6 | 0 | | 0 | |
| itching | 4 | 6.9 | 0 | | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

*Table 7. Local adverse events (continued)*

**High dose MenB - school children**

Vaccination 1 N=63

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 58 | 92.1 | 32 | 51.6 | 10 | 16.1 |
| pain | 56 | 88.9 | 30 | 48.4 | 9 | 14.5 |
| redness | 23 | 36.5 | 12 | 19.7 | 3 | 4.8 |
| redness >2.5cm | 6 | 9.5 | 6 | 9.8 | 1 | 1.6 |
| swelling >2.5cm | 7 | 11.1 | 6 | 9.8 | 3 | 4.8 |
| not using arm/leg | 4 | 6.3 | 2 | 3.3 | 1 | 1.6 |
| itching | 7 | 11.1 | 2 | 3.3 | 0 | |

Vaccination 2 N=62

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 54 | 87.1 | 26 | 41.9 | 7 | 11.3 |
| pain | 52 | 83.9 | 25 | 40.3 | 7 | 11.3 |
| redness | 29 | 46.8 | 8 | 12.9 | 2 | 3.2 |
| redness >2.5cm | 14 | 22.6 | 5 | 8.1 | 2 | 3.2 |
| swelling >2.5cm | 17 | 27.4 | 7 | 11.3 | 2 | 3.2 |
| not using arm/leg | 8 | 12.9 | 13 | 21.0 | 3 | 4.8 |
| itching | 1 | 1.6 | 1 | 1.6 | 1 | 1.6 |

Vaccination 3 N=62

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 54 | 87.1 | 28 | 45.2 | 5 | 8.1 |
| pain | 48 | 77.4 | 26 | 41.9 | 4 | 6.5 |
| redness | 24 | 38.7 | 10 | 16.1 | 2 | 3.2 |
| redness >2.5cm | 8 | 12.9 | 3 | 4.8 | 2 | 3.2 |
| swelling >2.5cm | 23 | 37.1 | 8 | 12.9 | 4 | 6.5 |
| not using arm/leg | 6 | 9.7 | 0 | | 0 | |
| itching | 1 | 1.6 | 0 | | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

*Table 7. Local adverse events (continued)*

**Control group - school children**

Vaccination 1 N=39

| | Observation 1* | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 12 | 30.8 | 1 | 2.6 | 1 | 2.6 |
| pain | 7 | 17.9 | 0 | | 0 | |
| redness | 2 | 5.1 | 1 | 2.6 | 1 | 2.6 |
| redness >2.5cm | 0 | | 0 | | 0 | |
| swelling >2.5cm | 3 | 7.7 | 0 | | 0 | |
| not using arm/leg | 1 | 2.6 | 0 | | 0 | |
| itching | 0 | | 0 | | 1 | 2.6 |

Vaccination 2 N=39

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 13 | 33.3 | 4 | 10.3 | 0 | |
| pain | 12 | 30.8 | 4 | 10.3 | 0 | |
| redness | 0 | | 1 | 2.6 | 0 | |
| redness >2.5cm | 0 | | 0 | | 0 | |
| swelling >2.5cm | 0 | | 0 | | 0 | |
| not using arm/leg | 2 | 5.1 | 1 | 2.6 | 0 | |
| itching | 1 | 2.6 | 0 | | 0 | |

Vaccination 3 N=39

| | Observation 1 | | Observation 2 | | Observation 3 | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| any local reaction | 9 | 23.1 | 5 | 12.8 | 1 | 2.6 |
| pain | 7 | 17.9 | 4 | 10.3 | 1 | 2.6 |
| redness | 3 | 7.7 | 1 | 2.6 | 0 | |
| redness >2.5cm | 0 | | 0 | | 0 | |
| swelling >2.5cm | 0 | | 1 | 2.6 | 0 | |
| not using arm/leg | 0 | | 0 | | 0 | |
| itching | 1 | 2.6 | 0 | | 0 | |

* Observation 1 : 18 - 30 hours after vaccination
Observation 2 : 24 - 72 hours after vaccination
Observation 3 : 3 - 7 days after vaccination

# Appendix 5 Adverse events low vs. high dose MenB

*Table 8A. Adverse events low vs. high dose MenB - toddlers*

**Vaccination 1** Low dose: N=58 High dose: N=53

| Reaction | Low dose | | High dose | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *34* | *58.6* | *29* | *54.7* | *0.82* |
| pain | 25 | 43.1 | 24 | 45.3 | 0.97 |
| redness | 18 | 31.0 | 13 | 24.5 | 0.58 |
| redness >2.5cm | 0 | | 1 | 1.9 | 0.48 |
| swelling >2.5cm | 1 | 1.7 | 1 | 1.9 | 1.00 |
| not using arm/leg | 3 | 5.2 | 7 | 13.2 | 0.19 |
| itching | 0 | | 0 | | |
| *Any systemic reaction* | *15* | *25.9* | *13* | *24.5* | *1.00* |
| fever | 3 | 5.2 | 2 | 3.8 | 1.00 |
| headache | 1 | 1.7 | 0 | | 1.00 |
| drowsiness | 9 | 15.5 | 7 | 13.2 | 0.94 |
| sleepy | 1 | 1.7 | 0 | | 1.00 |
| unusual crying | 7 | 12.1 | 6 | 11.3 | 1.00 |
| less appetite | 7 | 12.1 | 3 | 5.7 | 0.33 |
| nausea | 1 | 1.7 | 2 | 3.8 | 0.61 |
| joint complaints | 0 | | 0 | | |
| cutaneous symptoms | 0 | | 0 | | |

**Vaccination 2** Low dose: N=60 High dose: N=54

| Reaction | Low dose | | High dose | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *34* | *56.7* | *36* | *66.7* | *0.37* |
| pain | 27 | 45.0 | 29 | 53.7 | 0.46 |
| redness | 11 | 18.3 | 13 | 24.1 | 0.60 |
| redness >2.5cm | 0 | | 2 | 3.7 | 0.22 |
| swelling >2.5cm | 2 | 3.3 | 0 | | 0.50 |
| not using arm/leg | 5 | 8.3 | 9 | 16.7 | 0.29 |
| itching | 0 | | 0 | | |
| *Any systemic reaction* | *11* | *18.3* | *15* | *27.8* | *0.33* |
| fever | 3 | 5.0 | 2 | 3.7 | 1.00 |
| headache | 1 | 1.7 | 1 | 1.9 | 1.00 |
| drowsiness | 9 | 15.0 | 11 | 20.4 | 0.61 |
| sleepy | 0 | | 0 | | |
| unusual crying | 7 | 11.7 | 6 | 11.1 | 1.00 |
| less appetite | 5 | 8.3 | 2 | 3.7 | 0.44 |
| nausea | 1 | 1.7 | 1 | 1.9 | 1.00 |
| joint complaints | 0 | | 0 | | |
| cutaneous symptoms | 0 | | 1 | 1.9 | 0.47 |

*Table 8A. Adverse events low vs. high dose MenB - toddlers (continued)*

**Vaccination 3** Low dose: N=60 High dose: N=52

| Reaction | Low dose | | High dose | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *33* | *55.0* | *39* | *75.0* | *0.05* |
| pain | 31 | 51.7 | 36 | 69.2 | 0.09 |
| redness | 11 | 18.3 | 13 | 25.0 | 0.53 |
| redness >2.5cm | 0 | | 2 | 3.8 | 0.21 |
| swelling >2.5cm | 0 | | 2 | 3.8 | 0.21 |
| not using arm/leg | 1 | 1.7 | 5 | 9.6 | 0.10 |
| itching | 0 | | 0 | | |
| *Any systemic reaction* | *12* | *18.3* | *14* | *26.9* | *0.52* |
| fever | 1 | 1.7 | 1 | 1.9 | 1.00 |
| headache | 0 | | 0 | | |
| drowsiness | 9 | 15.0 | 11 | 21.2 | 0.55 |
| sleepy | 1 | 1.7 | 3 | 5.8 | 0.34 |
| unusual crying | 2 | 3.3 | 6 | 11.5 | 0.14 |
| less appetite | 2 | 3.3 | 7 | 13.5 | 0.08 |
| nausea | 0 | | 3 | 5.8 | 0.10 |
| joint complaints | 0 | | 0 | | |
| cutaneous symptoms | 0 | | 0 | | |

*Table 8B. Adverse events low vs. high dose MenB - school children*

**Vaccination 1** Low dose: N=60 High dose: N=63

| Reaction | Low dose | | High dose | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *52* | *86.7* | *58* | *92.1* | *0.50* |
| pain | 51 | 85.0 | 56 | 88.9 | 0.71 |
| redness | 24 | 40.0 | 23 | 36.5 | 0.83 |
| redness >2.5cm | 7 | 11.7 | 6 | 9.5 | 0.93 |
| swelling >2.5cm | 8 | 13.3 | 7 | 11.1 | 0.92 |
| not using arm/leg | 5 | 8.5 | 4 | 6.3 | 0.74 |
| itching | 2 | 3.3 | 7 | 11.1 | 0.17 |
| *Any systemic reaction* | *17* | *28.3* | *26* | *41.3* | *0.19* |
| fever | 0 | | 0 | | |
| headache | 12 | 20.0 | 8 | 12.7 | 0.39 |
| drowsiness | 5 | 8.3 | 16 | 25.4 | 0.02 |
| less appetite | 1 | 1.7 | 8 | 12.7 | 0.03 |
| nausea | 2 | 3.3 | 5 | 7.9 | 0.44 |
| joint complaints | 1 | 1.7 | 5 | 7.9 | 0.21 |
| cutaneous symptoms | 2 | 3.3 | 0 | | 0.24 |
| absence school | 1 | 1.7 | 1 | 1.6 | 1.00 |

**Vaccination 2** Low dose: N=60 High dose: N=62

| Reaction | Low dose | | High dose | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *56* | *93.3* | *54* | *87.1* | *0.39* |
| pain | 53 | 88.3 | 52 | 83.9 | 0.65 |
| redness | 30 | 50.0 | 29 | 46.8 | 0.86 |
| redness >2.5cm | 11 | 18.3 | 14 | 22.6 | 0.72 |
| swelling >2.5cm | 8 | 13.3 | 17 | 27.4 | 0.09 |
| not using arm/leg | 4 | 6.7 | 8 | 12.9 | 0.39 |
| itching | 5 | 8.3 | 1 | 1.6 | 0.17 |
| *Any systemic reaction* | *20* | *33.3* | *24* | *38.7* | *0.67* |
| fever | 2 | 3.3 | 1 | 1.6 | 0.62 |
| headache | 11 | 18.3 | 9 | 14.5 | 0.75 |
| drowsiness | 12 | 20.0 | 10 | 16.1 | 0.75 |
| less appetite | 5 | 8.3 | 7 | 11.3 | 0.81 |
| nausea | 2 | 3.3 | 4 | 6.5 | 0.68 |
| joint complaints | 1 | 1.7 | 1 | 1.6 | 1.00 |
| cutaneous symptoms | 1 | 1.7 | 4 | 6.5 | 0.37 |
| absence school | 3 | 5.0 | 11 | 17.7 | 0.05 |

*Table 8B. Adverse events low vs. high dose MenB - school children (continued)*

**Vaccination 3** Low dose: N=58 High dose: N=62

| Reaction | Low dose | | High dose | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *43* | *74.1* | *54* | *87.1* | *0.12* |
| pain | 40 | 69.0 | 48 | 77.4 | 0.40 |
| redness | 22 | 37.9 | 24 | 38.7 | 1.00 |
| redness >2.5cm | 6 | 10.3 | 8 | 12.9 | 0.78 |
| swelling >2.5cm | 13 | 22.4 | 23 | 37.1 | 0.12 |
| not using arm/leg | 5 | 8.6 | 6 | 9.7 | 1.00 |
| itching | 4 | 6.9 | 1 | 1.6 | 0.20 |
| *Any systemic reaction* | *12* | *20.7* | *15* | *24.2* | *0.81* |
| fever | 1 | 1.7 | 1 | 1.6 | 1.00 |
| headache | 8 | 13.8 | 9 | 14.5 | 1.00 |
| drowsiness | 7 | 12.1 | 6 | 9.7 | 0.90 |
| less appetite | 4 | 6.9 | 5 | 8.1 | 1.00 |
| nausea | 2 | 3.4 | 5 | 8.1 | 0.44 |
| joint complaints | 0 | | 0 | | |
| cutaneous symptoms | 0 | | 0 | | |
| absence school | 3 | 5.2 | 5 | 8.1 | 0.72 |

# Appendix 6 Adverse events MenB vs. HepB

*Table 9A. Adverse events MenB vs. HepB - toddlers*

**Vaccination 1** MenB: N=111 HepB: N=53

| Reaction | MenB | | HB | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *63* | *56.8* | *5* | *9.4* | *<0.01* |
| pain | 49 | 44.1 | 5 | 9.4 | <0.01 |
| redness | 31 | 27.9 | 0 | | <0.01 |
| redness >2.5cm | 1 | 0.9 | 0 | | 1.00 |
| swelling >2.5cm | 2 | 1.8 | 0 | | 1.00 |
| not using arm/leg | 10 | 9.0 | 0 | | 0.03 |
| itching | 0 | | 0 | | |
| *Any systemic reaction* | *28* | *25.2* | *7* | *13.2* | *0.12* |
| fever | 5 | 4.5 | 2 | 3.8 | 1.00 |
| headache | 1 | 0.9 | 1 | 1.9 | 0.54 |
| drowsiness | 16 | 14.4 | 5 | 9.4 | 0.52 |
| sleepy | 1 | 0.9 | 1 | 1.9 | 0.54 |
| unusual crying | 13 | 11.7 | 2 | 3.8 | 0.15 |
| less appetite | 10 | 9.0 | 2 | 3.8 | 0.34 |
| nausea | 3 | 2.7 | 0 | | 0.55 |
| joint complaints | 0 | | 0 | | |
| cutaneous symptoms | 0 | | 1 | 1.9 | 0.32 |

**Vaccination 2** MenB: N=114 HepB: N=56

| Reaction | MenB | | HB | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *70* | *61.4* | *7* | *12.5* | *<0.01* |
| pain | 56 | 49.1 | 7 | 12.5 | <0.01 |
| redness | 24 | 21.1 | 0 | | <0.01 |
| redness >2.5cm | 2 | 1.8 | 0 | | 1.00 |
| swelling >2.5cm | 2 | 1.8 | 0 | | 1.00 |
| not using arm/leg | 14 | 12.3 | 1 | 1.8 | 0.02 |
| itching | 0 | | 0 | | |
| *Any systemic reaction* | *26* | *22.8* | *8* | *14.3* | *0.27* |
| fever | 5 | 4.4 | 1 | 1.8 | 0.67 |
| headache | 2 | 1.8 | 0 | | 1.00 |
| drowsiness | 20 | 17.5 | 2 | 3.6 | 0.02 |
| sleepy | 0 | | 0 | | |
| unusual crying | 13 | 11.4 | 5 | 8.9 | 0.82 |
| less appetite | 7 | 6.1 | 1 | 1.8 | 0.27 |
| nausea | 2 | 1.8 | 0 | | 1.00 |
| joint complaints | 0 | | 0 | | |
| cutaneous symptoms | 1 | 0.9 | 0 | | 1.00 |

*Table 9A. Adverse events MenB vs. HepB - toddlers (continued)*

**Vaccination 3** MenB: N=112 HepB: N=55

| Reaction | MenB | | HB | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *72* | *64.3* | *5* | *9.1* | *<0.01* |
| pain | 67 | 59.8 | 4 | 7.3 | <0.01 |
| redness | 24 | 21.4 | 1 | 1.8 | <0.01 |
| redness >2.5cm | 2 | 1.8 | 0 | | 1.00 |
| swelling >2.5cm | 2 | 1.8 | 0 | | 1.00 |
| not using arm/leg | 6 | 5.4 | 2 | 3.6 | 1.00 |
| itching | 0 | | 0 | | |
| *Any systemic reaction* | *26* | *23.2* | *4* | *7.3* | *0.02* |
| fever | 2 | 1.8 | 1 | 1.8 | 1.00 |
| headache | 0 | | 0 | | |
| drowsiness | 20 | 17.9 | 4 | 7.3 | 0.11 |
| sleepy | 4 | 3.6 | 0 | | 0.30 |
| unusual crying | 8 | 7.1 | 0 | | 0.05 |
| less appetite | 9 | 8.0 | 2 | 3.6 | 0.34 |
| nausea | 3 | 2.7 | 0 | | 0.55 |
| joint complaints | 0 | | 0 | | |
| cutaneous symptoms | 0 | | 0 | | |

*Table 9B. Adverse events MenB vs. HepB - school children*

**Vaccination 1** MenB: N=123 HepB: N=39

| Reaction | MenB | | HB | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *110* | *89.4* | *12* | *30.8* | *<0.01* |
| pain | 107 | 87.0 | 7 | 17.9 | <0.01 |
| redness | 47 | 38.2 | 2 | 5.1 | <0.01 |
| redness >2.5cm | 13 | 10.6 | 0 | | 0.04 |
| swelling >2.5cm | 15 | 12.2 | 3 | 7.7 | 0.57 |
| not using arm/leg | 9 | 7.4 | 1 | 2.6 | 0.45 |
| itching | 9 | 7.3 | 0 | | 0.12 |
| *Any systemic reaction* | *43* | *35.0* | *9* | *23.1* | *0.24* |
| fever | 0 | | 0 | | |
| headache | 20 | 16.3 | 2 | 5.1 | 0.13 |
| drowsiness | 21 | 17.1 | 2 | 5.1 | 0.11 |
| less appetite | 9 | 7.3 | 1 | 2.6 | 0.45 |
| nausea | 7 | 5.7 | 2 | 5.1 | 1.00 |
| joint complaints | 6 | 4.9 | 0 | | 0.34 |
| cutaneous symptoms | 2 | 1.6 | 3 | 7.7 | 0.09 |
| absence school | 2 | 1.6 | 1 | 2.6 | 0.57 |

**Vaccination 2** MenB: N=122 HepB: N=39

| Reaction | MenB | | HB | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *110* | *90.2* | *13* | *33.3* | *<0.01* |
| pain | 105 | 86.1 | 12 | 30.8 | <0.01 |
| redness | 59 | 48.4 | 0 | | <0.01 |
| redness >2.5cm | 25 | 20.5 | 0 | | 0.01 |
| swelling >2.5cm | 25 | 20.5 | 0 | | 0.01 |
| not using arm/leg | 12 | 9.8 | 2 | 5.1 | 0.52 |
| itching | 6 | 4.9 | 1 | 2.6 | 1.00 |
| *Any systemic reaction* | *44* | *36.1* | *5* | *12.8* | *0.01* |
| fever | 3 | 2.5 | 0 | | 1.00 |
| headache | 20 | 16.4 | 2 | 5.1 | 0.13 |
| drowsiness | 22 | 18.0 | 1 | 2.6 | 0.03 |
| less appetite | 12 | 9.8 | 1 | 2.6 | 0.19 |
| nausea | 6 | 4.9 | 1 | 2.6 | 1.00 |
| joint complaints | 2 | 1.6 | 0 | | 1.00 |
| cutaneous symptoms | 5 | 4.1 | 1 | 2.6 | 1.00 |
| absence school | 14 | 11.5 | 1 | 2.6 | 0.12 |

*Table 9B. Adverse events MenB vs. HepB - school children (continued)*

**Vaccination 3** MenB: N=120 HepB: N=39

| Reaction | MenB | | HB | | |
|---|---|---|---|---|---|
| | N | % | N | % | p-value |
| *Any local reaction* | *97* | *80.8* | *9* | *23.1* | *<0.01* |
| pain | 88 | 73.3 | 7 | 17.9 | <0.01 |
| redness | 46 | 38.3 | 3 | 7.7 | <0.01 |
| redness >2.5cm | 14 | 11.7 | 0 | | 0.02 |
| swelling >2.5cm | 36 | 30.0 | 0 | | <0.01 |
| not using arm/leg | 11 | 9.2 | 0 | | 0.07 |
| itching | 5 | 4.2 | 1 | 2.6 | 1.00 |
| *Any systemic reaction* | *27* | *22.5* | *5* | *12.8* | *0.28* |
| fever | 2 | 1.7 | 0 | | 1.00 |
| headache | 17 | 14.2 | 4 | 10.3 | 0.71 |
| drowsiness | 13 | 10.8 | 3 | 7.7 | 0.76 |
| less appetite | 9 | 7.5 | 0 | | 0.11 |
| nausea | 7 | 5.8 | 0 | | 0.20 |
| joint complaints | 0 | | 1 | 2.6 | 0.25 |
| cutaneous symptoms | 0 | | 1 | 2.6 | 0.24 |
| absence school | 8 | 6.7 | 3 | 7.7 | 0.72 |

# Appendix 7 Serum Bactericidal Antibody response

*Table 10. Serum Bactericidal Antibody response*

**Toddlers**

Vesicle PL16215

| | strain→ | P1.7,16 | | P1.5,2 | | P1.19,15 | |
|---|---|---|---|---|---|---|---|
| blood-sample | study group→ | Low dose | High dose | Low dose | High dose | Low dose | High dose |
| 1 | GMT | 1.00 | 1.00 | 1.01 | 1.04 | 1.00 | 1.08 |
| | 95%CI | - - | - - | [0.99 - 1.03] | [1.0 - 1.1] | - - | [0.9 - 1.3] |
| | %=1:4 | 0 | 0 | 0 | 1.9 | 0 | 1.9 |
| | N | 61 | 54 | 62 | 54 | 62 | 54 |
| 2 | GMT | 1.56 | 1.85 | 10.34 | 12.64 | 1.44 | 1.65 |
| | 95%CI | [1.2 - 2.0] | [1.4 - 2.5] | [7.2 - 14.8] | [8.6 - 18.6] | [1.2 - 1.7] | [1.2 - 2.3] |
| | %=1:4 | 20.3 | 24.1 | 83.3 | 84.9 | 18.3 | 14.8 |
| | N | 59 | 54 | 60 | 53 | 60 | 54 |
| 3 | GMT | 1.13 | 1.09 | 1.59 | 1.43 | 1.16 | 1.24 |
| | 95%CI | [0.9 - 1.4] | [1.0 - 1.2] | [1.2 - 2.1] | [1.1 - 1.9] | [1.0 - 1.4] | [0.7 - 1.6] |
| | %=1:4 | 3.3 | 3.8 | 15.0 | 11.5 | 5.0 | 5.8 |
| | N | 60 | 52 | 60 | 52 | 60 | 52 |
| 4 | GMT | 3.25* | 5.86 | 35.51 | 50.21 | 2.14 | 1.95 |
| | 95%CI | [2.1 - 5.0] | [3.7 - 9.3] | [24.9 - 50.2] | [34.3 - 73.5] | [1.6 - 2.9] | [1.4 - 2.7] |
| | %=1:4 | 37.7 | 58.8 | 95.1 | 94.1 | 29.5 | 27.5 |
| | N | 61 | 51 | 61 | 51 | 61 | 51 |

Vesicle PL10124

| | strain→ | P1.5[c],10 | | P1.12,13 | | P1.7[h],4 | |
|---|---|---|---|---|---|---|---|
| blood-sample | study group→ | Low dose | High dose | Low dose | High dose | Low dose | High dose |
| 1 | GMT | 1.09 | 1.01 | 1.05 | 1.01 | 1.00 | 1.00 |
| | 95%CI | [0.9 - 1.3] | [0.99 - 1.04] | [1.0 - 1.1] | [0.99 - 1.04] | - - | - - |
| | %=1:4 | 1.6 | 0 | 1.6 | 0 | 0 | 0 |
| | N | 62 | 54 | 62 | 54 | 61 | 54 |
| 2 | GMT | 128.00 | 136.24 | 5.39 | 5.82 | 1.40 | 1.48 |
| | 95%CI | [95.0 - 172.4] | [104.0 - 179.8] | [3.6 - 8.1] | [4.1 - 8.2] | [1.1 - 1.8] | [1.2 - 1.9] |
| | %=1:4 | 98.3 | 100 | 60.0 | 70.4 | 15.3 | 11.1 |
| | N | 60 | 53 | 60 | 54 | 59 | 54 |
| 3 | GMT | 9.51 | 11.79 | 1.21 | 1.17 | 1.13 | 1.04 |
| | 95%CI | [6.2 - 14.6] | [7.7 - 18.0] | [1.0 - 1.5] | [1.0 - 1.4] | [0.9 - 1.4] | [1.0 - 1.1] |
| | %=1:4 | 73.3 | 80.8 | 5.0 | 3.8 | 3.3 | 1.9 |
| | N | 60 | 52 | 60 | 52 | 60 | 52 |
| 4 | GMT | 203.66 | 192.67 | 8.94 | 6.54 | 2.69 | 2.97 |
| | 95%CI | [176.1 - 235.6] | [150.1 - 247.3] | [6.2 - 12.9] | [4.4 - 9.8] | [1.9 - 3.8] | [2.0 - 4.4] |
| | %=1:4 | 100 | 98.0 | 78.7 | 68.6 | 34.4 | 41.2 |
| | N | 61 | 51 | 61 | 51 | 61 | 51 |

* significant difference in GMT between concentrations ($p<0.05$; Mann-Whitney U)

GMT = Geometric Mean Titre
95%CI = 95% confidence interval
strain = class 1 OMP of the six meningococcal B subtypes
Low dose= received meningococcal B vesicle vaccine, 50µg class 1 OMP per dose
High dose= received meningococcal B vesicle vaccine, 100µg class 1 OMP per dose

*Table 10. Serum Bactericidal Antibody response (continued)*

**School children**

Vesicle PL16215

| | strain→ | P1.7,16 | | P1.5,2 | | P1.19,15 | |
|---|---|---|---|---|---|---|---|
| blood-sample | study group→ | Low dose | High dose | Low dose | High dose | Low dose | High dose |
| 1 | GMT | 1.00 | 1.02 | 1.15 | 1.06 | 1.00 | 1.13 |
| | 95%CI | - - | [1.0 - 1.1] | [1.0 - 1.4] | [1.0 - 1.1] | - - | [1.0 - 1.3] |
| | %=1:4 | 0 | 1.6 | 4.9 | 1.6 | 0 | 3.1 |
| | N | 61 | 64 | 61 | 64 | 61 | 64 |
| 2 | GMT | 1.55 | 1.52 | 5.78 | 5.03 | 1.37 | 1.83 |
| | 95%CI | [1.2 - 2.1] | [1.1 - 2.0] | [3.8 - 8.9] | [3.4 - 7.3] | [1.1 - 1.8] | [1.3 - 2.6] |
| | %=1:4 | 16.7 | 12.9 | 63.3 | 62.3 | 10.0 | 16.1 |
| | N | 60 | 62 | 60 | 61 | 60 | 62 |
| 3 | GMT | 1.29 | 1.21 | 1.93 | 1.57 | 1.21 | 1.25 |
| | 95%CI | [1.0 - 1.7] | [1.0 - 1.5] | [1.4 - 2.7] | [1.2 - 2.0] | [0.9 - 1.6] | [1.0 - 1.5] |
| | %=1:4 | 6.7 | 6.5 | 23.3 | 17.7 | 3.3 | 9.7 |
| | N | 60 | 62 | 60 | 62 | 60 | 62 |
| 4 | GMT | 3.03 | 3.58 | 20.82 | 27.10 | 1.65 | 2.11 |
| | 95%CI | [2.1 - 4.3] | [2.4 - 5.4] | [13.4 - 32.4] | [18.9 - 38.6] | [1.2 - 2.2] | [1.5 - 3.1] |
| | %=1:4 | 43.1 | 38.7 | 84.5 | 95.2 | 19.0 | 24.2 |
| | N | 58 | 62 | 58 | 62 | 58 | 62 |

Vesicle PL10124

| | strain→ | P1.5[c],10 | | P1.12,13 | | P1.7[h],4 | |
|---|---|---|---|---|---|---|---|
| blood-sample | study group→ | Low dose | High dose | Low dose | High dose | Low dose | High dose |
| 1 | GMT | 1.13 | 1.07 | 1.08 | 1.04 | 1.03 | 1.02 |
| | 95%CI | [1.0 - 1.3] | [1.0 - 1.2] | [1.0 - 1.2] | [1.0 - 1.1] | [1.0 - 1.1] | [1.0 - 1.1] |
| | %=1:4 | 4.9 | 3.1 | 3.3 | 1.6 | 1.6 | 1.6 |
| | N | 61 | 64 | 61 | 64 | 61 | 64 |
| 2 | GMT | 32.45 | 40.22 | 2.83 | 3.84 | 1.48 | 1.24 |
| | 95%CI | [22.0 - 47.5] | [26.4 - 61.4] | [2.0 - 4.1] | [2.7 - 5.4] | [1.1 - 2.0] | [1.1 - 1.4] |
| | %=1:4 | 93.3 | 93.4 | 40.0 | 50.0 | 10.0 | 9.7 |
| | N | 60 | 61 | 60 | 62 | 60 | 62 |
| 3 | GMT | 5.46 | 4.53 | 1.23 | 1.22 | 1.21 | 1.11 |
| | 95%CI | [3.6 - 8.2] | [3.0 - 6.7] | [1.0 - 1.5] | [1.1 - 1.4] | [1.0 - 1.5] | [1.0 - 1.3] |
| | %=1:4 | 65.0 | 53.2 | 6.7 | 11.3 | 8.3 | 4.8 |
| | N | 60 | 62 | 60 | 62 | 60 | 62 |
| 4 | GMT | 110.66 | 112.99 | 4.41 | 3.71 | 2.13 | 1.61 |
| | 95%CI | [82.1 - 149.1] | [76.1 - 167.7] | [2.9 - 6.7] | [2.6 - 5.3] | [1.5 - 3.0] | [1.2 - 2.2] |
| | %=1:4 | 100 | 93.5 | 58.6 | 50.0 | 25.9 | 16.1 |
| | N | 58 | 62 | 58 | 62 | 58 | 62 |

GMT = Geometric Mean Titre
95%CI = 95% confidence interval
strain = class 1 OMP of the six meningococcal B subtypes
Low dose= received meningococcal B vesicle vaccine, 50µg class 1 OMP per dose
High dose= received meningococcal B vesicle vaccine, 100µg class 1 OMP per dose

*Table 10. Serum Bactericidal Antibody response (continued)*

## Control group

## Toddlers

| | | vesicle PL16215 | | | vesicle PL10124 | | |
|---|---|---|---|---|---|---|---|
| blood-sample | strain→ | P1.7,16 | P1.5,2 | P1.19,15 | P1.5[c],10 | P1.12,13 | P1.7[h],4 |
| 1 | GMT | 1.03 | 1.16 | 1.04 | 1.21 | 1.03 | 1.08 |
| | 95%CI | [1.0-1.1] | [1.0-1.4] | [1.0-1.1] | [1.0-1.5] | [1.0-1.1] | [1.0-1.2] |
| | %≥1:4 | 1.8 | 5.4 | 1.8 | 7.1 | 0 | 3.6 |
| | N | 56 | 56 | 56 | 56 | 56 | 56 |
| 2 | GMT | 1.00 | 1.11 | 1.00 | 1.23 | 1.01 | 1.07 |
| | 95%CI | - - | [1.0-1.3] | - - | [1.0-1.5] | [0.99-1.04] | [1.0-1.2] |
| | %≥1:4 | 0 | 3.7 | 0 | 7.4 | 0 | 3.7 |
| | N | 54 | 54 | 54 | 54 | 54 | 54 |
| 3 | GMT | 1.01 | 1.09 | 1.00 | 1.22 | 1.01 | 1.11 |
| | 95%CI | [0.99-1.04] | [1.0-1.2] | - - | [1.0-1.5] | [0.99-1.04] | [1.0-1.3] |
| | %≥1:4 | 0 | 5.6 | 0 | 7.4 | 0 | 3.7 |
| | N | 55 | 55 | 55 | 55 | 55 | 55 |
| 4 | GMT | 1.00 | 1.08 | 1.00 | 1.22 | 1.04 | 1.07 |
| | 95%CI | - - | [1.0-1.2] | - - | [1.0-1.5] | [1.0-1.10] | [1.0-1.2] |
| | %≥1:4 | 0 | 3.8 | 0 | 7.5 | 1.8 | 3.8 |
| | N | 54 | 54 | 54 | 55 | 55 | 54 |

## School children

| | | vesicle PL16215 | | | vesicle PL10124 | | |
|---|---|---|---|---|---|---|---|
| blood-sample | strain→ | P1.7,16 | P1.5,2 | P1.19,15 | P1.5[c],10 | P1.12,13 | P1.7[h],4 |
| 1 | GMT | 1.05 | 1.04 | 1.11 | 1.04 | 1.02 | 1.05 |
| | 95%CI | [1.0-1.1] | [1.0-1.1] | [1.0-1.3] | [1.0-1.1] | [1.0-1.1] | [1.0-1.1] |
| | %≥1:4 | 2.5 | 2.5 | 5.0 | 2.5 | 0 | 2.5 |
| | N | 40 | 40 | 40 | 40 | 40 | 40 |
| 2 | GMT | 1.07 | 1.09 | 1.11 | 1.16 | 1.07 | 1.09 |
| | 95%CI | [1.0-1.2] | [1.0-1.2] | [1.0-1.3] | [1.0-1.4] | [0.9-1.2] | [1.0-1.2] |
| | %≥1:4 | 5.1 | 2.6 | 5.1 | 7.7 | 2.6 | 5.2 |
| | N | 39 | 39 | 39 | 39 | 39 | 39 |
| 3 | GMT | 1.02 | 1.16 | 1.00 | 1.16 | 1.00 | 1.07 |
| | 95%CI | [1.0-1.01] | [1.0-1.4] | - - | [1.0-1.4] | - - | [1.0-1.2] |
| | %≥1:4 | 0 | 7.7 | 0 | 7.7 | 0 | 2.6 |
| | N | 39 | 39 | 39 | 39 | 39 | 39 |
| 4 | GMT | 1.06 | 1.08 | 1.06 | 1.33 | 1.04 | 1.16 |
| | 95%CI | [0.9-1.2] | [1.0-1.2] | [0.9-1.2] | [1.0-1.8] | [1.0-1.1] | [0.9-1.4] |
| | %≥1:4 | 2.7 | 2.7 | 2.7 | 10.8 | 2.7 | 5.4 |
| | N | 37 | 37 | 37 | 37 | 37 | 37 |

GMT = Geometric Mean Titre
95%CI = 95% confidence interval
strain = class 1 OMP of the six meningococcal B subtypes

# Appendix 8 ELISA Response

*Table 11. Elisa Response*

## Toddlers

| blood-sample | PL16215 | | | PL10124 | | |
|---|---|---|---|---|---|---|
| | Low dose | High dose | Control | Low dose | High dose | Control |
| 1 | 116 [102-131] | 119 [103-138] | 128 [108-152] | 161 [128-204] | 157 [125-197] | 168 [137-207] |
| 2 | 8849 [7442-10522] | 9920 [8316-11830] | 129 [107-155] | 9064 [7677-10703] | 9676 [8069-11601] | 155 [126-192] |
| 3 | 657 [505-854] | 792 [571-1098] | 113 [100-128] | 790 [625-999] | 840 [622-1135] | 125 [105-150] |
| 4 | 13605 [11937-15502] | 16244 [14145-18660] | 115 [102-131] | 12963 [11231-14904] | 14518 [12674-16634] | 121 [105-141] |

## School children

| blood-sample | PL16215 | | | PL10124 | | |
|---|---|---|---|---|---|---|
| | Low dose | High dose | Control | Low dose | High dose | Control |
| 1 | 124 [105-148] | 135 [117-156] | 130 [107-158] | 187 [156-224] | 159 [135-188] | 182 [148-223] |
| 2 | 4616 [3743-5695] | 5078 [4151-6213] | 129 104-159] | 4161 [3370-5139] | 4141 [3364-5097] | 157 [125-196] |
| 3 | 624 [467-834] | 556 [418-738] | 118 [101-136] | 592 [450-780] | 476 [375-605] | 116 [104-128] |
| 4 | 10294 [8750-12109] | 10116 [8490-12053] | 125 [102-153] | 8792 [7374-10483] | 8352 [6949-10039] | 122 [102-147] |

# Appendix 9 Bactericidal titres and ELISA titres

*Table 12. Bactericidal titres in Toddlers vs. School children (statistically significant differences shown only)*

| blood sample | strain | GMT toddlers | GMT school children | p# |
|---|---|---|---|---|
| *Low dose* | | | | |
| 2 | P1.5,2 | 10.34 | 5.78 | 0.02 |
| 2 | P1.5$^{c}$,10 | 128.00 | 9.51 | <0.01 |
| 3 | | 9.51 | 5.46 | 0.03 |
| 4 | | 203.66 | 110.66 | <0.01 |
| 2 | P1.12,13 | 5.39 | 2.83 | <0.01 |
| 4 | | 8.94 | 4.41 | <0.01 |
| *High dose* | | | | |
| 2 | P1.5,2 | 12.64 | 5.03 | <0.01 |
| 4 | | 50.21 | 27.10 | 0.01 |
| 2 | P1.5$^{c}$,10 | 136.24 | 40.22 | <0.01 |
| 3 | | 11.79 | 4.53 | <0.01 |
| 4 | | 192.67 | 112.99 | 0.04 |
| 4 | P1.12,13 | 6.54 | 3.71 | 0.03 |
| 4 | P1.7$^{h}$,4 | 2.97 | 1.61 | <0.01 |

# Mann-Whitney-U test

*Table 13. ELISA titres in Toddlers vs. School children (statistically significant differences shown only)*

| blood sample | vesicle | titre toddlers | titre school children | p# |
|---|---|---|---|---|
| *Low dose* | | | | |
| 2 | PL16215* | 8849 | 4616 | <0.01 |
| 4 | PL16215 | 13605 | 10294 | 0.01 |
| 1 | PL10124** | 161 | 187 | 0.03 |
| 2 | PL10124 | 9064 | 4161 | <0.01 |
| 4 | PL10124 | 12963 | 8792 | <0.01 |
| *High dose* | | | | |
| 2 | PL16215 | 9920 | 5078 | <0.01 |
| 4 | PL16215 | 16244 | 10116 | <0.01 |
| 2 | PL10124 | 9676 | 4141 | <0.01 |
| 3 | PL10124 | 840 | 476 | <0.01 |
| 4 | PL10124 | 14518 | 8352 | <0.01 |

* subtypes P1.7,16; P1.5,2 and P1.19,15
** subtypes P1.5$^{c}$,10; P1.12,13 and P1.7$^{h}$,4
# Mann-Whitney-U test

# Appendix 10 Proportion of participants potentially protected after vaccination

**TODDLERS - GROUP B**

| | strain | P1.7,16 | P1.5,2 | P1.19,15 | P1.5[c],10 | P1.12,13 | P1.7[h],4 | TOTAL |
|---|---|---|---|---|---|---|---|---|
| **MenB5** | %bact.titre>=4 | 37.7 | 95.1 | 29.5 | 100.0 | 78.7 | 34.4 | |
| **1993-1996** | % isolates | 14.7 | 6.1 | 6.0 | 7.2 | 0.9 | 46.1 | |
| **groupB** | % protected | 5.5 | 5.8 | 1.8 | 7.2 | 0.7 | 15.8 | **36.8** |
| **MenB5** | %bact.titre>=4 | 37.7 | 95.1 | 29.5 | 100.0 | 78.7 | 34.4 | |
| **1996** | % isolates | 11.7 | 5.0 | 6.8 | 7.3 | 1.0 | 46.8 | |
| **groupB** | % protected | 4.4 | 4.7 | 2.0 | 7.3 | 0.8 | 16.1 | **35.4** |
| **MenB10** | %bact.titre>=4 | 58.8 | 94.1 | 27.5 | 98.0 | 68.6 | 41.2 | |
| **1993-1996** | % isolates | 14.7 | 6.1 | 6.0 | 7.2 | 0.9 | 46.1 | |
| **groupB** | % protected | 8.6 | 5.8 | 1.7 | 7.0 | 0.6 | 19.0 | **42.7** |
| **MenB10** | %bact.titre>=4 | 58.8 | 94.1 | 27.5 | 98.0 | 68.6 | 41.2 | |
| **1996** | % isolates | 11.7 | 5.0 | 6.8 | 7.3 | 1.0 | 46.8 | |
| **groupB** | % protected | 6.9 | 4.7 | 1.9 | 7.2 | 0.7 | 19.3 | **40.6** |

**SCHOOLCHILDREN - GROUP B**

| | strain | P1.7,16 | P1.5,2 | P1.19,15 | P1.5[c],10 | P1.12,13 | P1.7[h],4 | TOTAL |
|---|---|---|---|---|---|---|---|---|
| **MenB5** | %bact.titre>=4 | 43.1 | 84.5 | 19.0 | 100.0 | 58.6 | 25.9 | |
| **1993-1996** | % isolates | 14.7 | 6.1 | 6.0 | 7.2 | 0.9 | 46.1 | |
| **groupB** | % protected | 6.3 | 5.2 | 1.1 | 7.2 | 0.5 | 11.9 | **32.3** |
| **MenB5** | %bact.titre>=4 | 43.1 | 84.5 | 19.0 | 100.0 | 58.6 | 25.9 | |
| **1996** | % isolates | 11.7 | 5.0 | 6.8 | 7.3 | 1.0 | 46.8 | |
| **groupB** | % protected | 5.0 | 4.2 | 1.3 | 7.3 | 0.6 | 12.1 | **30.6** |
| **MenB10** | %bact.titre>=4 | 38.7 | 95.2 | 24.2 | 93.5 | 50.0 | 16.1 | |
| **1993-1996** | % isolates | 14.7 | 6.1 | 6.0 | 7.2 | 0.9 | 46.1 | |
| **groupB** | % protected | 5.7 | 5.8 | 1.5 | 6.7 | 0.4 | 7.4 | **27.5** |
| **MenB10** | %bact.titre>=4 | 38.7 | 95.2 | 24.2 | 93.5 | 50.0 | 16.1 | |
| **1996** | % isolates | 11.7 | 5.0 | 6.8 | 7.3 | 1.0 | 46.8 | |
| **groupB** | % protected | 4.5 | 4.7 | 1.6 | 6.9 | 0.5 | 7.5 | **25.8** |

*Table 14. Proportion of participants potentially protected after vaccination*

*Table 14. Proportion of participants potentially protected after vaccination (continued)*

**TODDLERS - GROUP B+C**

| | strain | P1.7,16 | P1.5,2 | P1.19,15 | P1.5[c],10 | P1.12,13 | P1.7[h],4 | TOTAL |
|---|---|---|---|---|---|---|---|---|
| **MenB5** | %bact.titre>=4 | 37.7 | 95.1 | 29.5 | 100.0 | 78.7 | 34.4 | |
| **1993-1996** | % isolates | 13.9 | 12.6 | 5.5 | 6.7 | 0.9 | 41.1 | |
| **groupB+C** | % protected | 5.2 | 11.9 | 1.6 | 6.7 | 0.7 | 14.1 | **40.3** |
| **MenB5** | %bact.titre>=4 | 37.7 | 95.1 | 29.5 | 100.0 | 78.7 | 34.4 | |
| **1996** | % isolates | 11.1 | 11.3 | 6.1 | 8.4 | 0.9 | 42.4 | |
| **groupB+C** | % protected | 4.2 | 10.7 | 1.8 | 8.4 | 0.7 | 14.6 | **40.4** |
| **MenB10** | %bact.titre>=4 | 58.8 | 94.1 | 27.5 | 98.0 | 68.6 | 41.2 | |
| **1993-1996** | % isolates | 13.9 | 12.6 | 5.5 | 6.7 | 0.9 | 41.1 | |
| **groupB+C** | % protected | 8.2 | 11.8 | 1.5 | 6.6 | 0.6 | 16.9 | **45.6** |
| **MenB10** | %bact.titre>=4 | 58.8 | 94.1 | 27.5 | 98.0 | 68.6 | 41.2 | |
| **1996** | % isolates | 11.1 | 11.3 | 6.1 | 8.4 | 0.9 | 42.4 | |
| **groupB+C** | % protected | 6.5 | 10.6 | 1.7 | 8.2 | 0.6 | 17.5 | **45.1** |

**SCHOOLCHILDREN - GROUP B+C**

| | strain | P1.7,16 | P1.5,2 | P1.19,15 | P1.5[c],10 | P1.12,13 | P1.7[h],4 | TOTAL |
|---|---|---|---|---|---|---|---|---|
| **MenB5** | %bact.titre>=4 | 43.1 | 84.5 | 19.0 | 100.0 | 58.6 | 25.9 | |
| **1993-1996** | % isolates | 13.9 | 12.6 | 5.5 | 6.7 | 0.9 | 41.1 | |
| **groupB+C** | % protected | 6.0 | 10.6 | 1.0 | 6.7 | 0.5 | 10.7 | **35.5** |
| **MenB5** | %bact.titre>=4 | 43.1 | 84.5 | 19.0 | 100.0 | 58.6 | 25.9 | |
| **1996** | % isolates | 11.1 | 11.3 | 6.1 | 8.4 | 0.9 | 42.4 | |
| **groupB+C** | % protected | 4.8 | 9.5 | 1.2 | 8.4 | 0.5 | 11.0 | **35.4** |
| **MenB10** | %bact.titre>=4 | 38.7 | 95.2 | 24.2 | 93.5 | 50.0 | 16.1 | |
| **1993-1996** | % isolates | 13.9 | 12.6 | 5.5 | 6.7 | 0.9 | 41.1 | |
| **groupB+C** | % protected | 5.4 | 12.0 | 1.3 | 6.3 | 0.4 | 6.6 | **32.0** |
| **MenB10** | %bact.titre>=4 | 38.7 | 95.2 | 24.2 | 93.5 | 50.0 | 16.1 | |
| **1996** | % isolates | 11.1 | 11.3 | 6.1 | 8.4 | 0.9 | 42.4 | |
| **groupB+C** | % protected | 4.3 | 10.7 | 1.5 | 7.9 | 0.4 | 6.8 | **31.6** |

# Appendix 11 IgG subclass distribution in the 4th blood sample

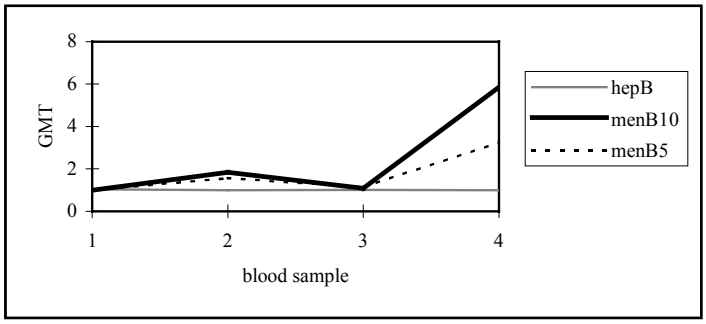
*Table 15. IgG subclass distribution in the 4th blood sample of Toddlers and School children (mean 10log ELISA titres ± SD)*

| Toddlers (n=44) | IgGtotal | IgG1 | IgG2 | IgG3 | IgG4 |
|---|---|---|---|---|---|
| P1.7[h],4 | 3.82 ± 0.31 | 3.64 ± 0.34 | 1.90 ± 0.83 | 2.77 ± 0.55 | 1.05 ± 0.22 |
| P1.5[c],10 | 3.96 ± 0.26 | 3.98 ± 0.31 | 2.02 ± 0.79 | 2.96 ± 0.59 | 1.11 ± 0.36 |
| P1.12,13 | 4.02 ± 0.25 | 3.77 ± 0.31 | 2.21 ± 0.72 | 2.71 ± 0.53 | 1.08 ± 0.32 |

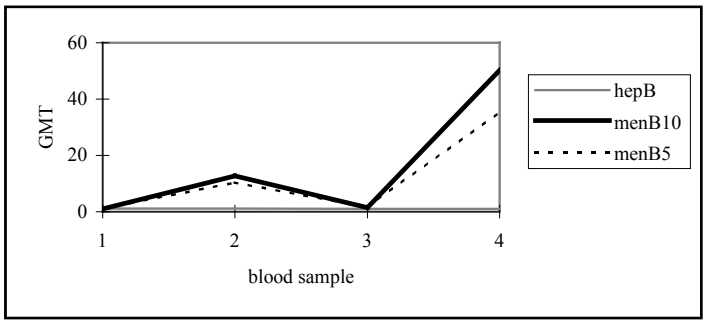
| Schoolchildren (n=46) | IgGtotal | IgG1 | IgG2 | IgG3 | IgG4 |
|---|---|---|---|---|---|
| P1.7[h],4 | 3.61 ± 0.49 | 3.45 ± 0.4 | 1.85 ± 0.70 | 2.47 ± 0.72 | 1.03 ± 0.18 |
| P1.5[c],10 | 3.82 ± 0.35 | 3.77 ± 0.42 | 1.88 ± 0.77 | 2.64 ± 0.82 | 1.13 ± 0.45 |
| P1.12,13 | 3.86 ± 0.29 | 3.61 ± 0.36 | 2.07 ± 0.64 | 2.42 ± 0.75 | 1.05 ± 0.24 |

# Appendix 12 SBA response

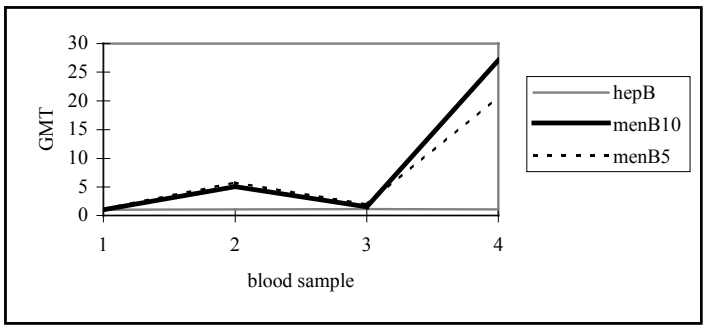
**Toddlers - strain P1.7,16**




**School children - strain P1.7,16**




**Toddlers - strain P1.5,2**




**School children - strain P1.5,2**

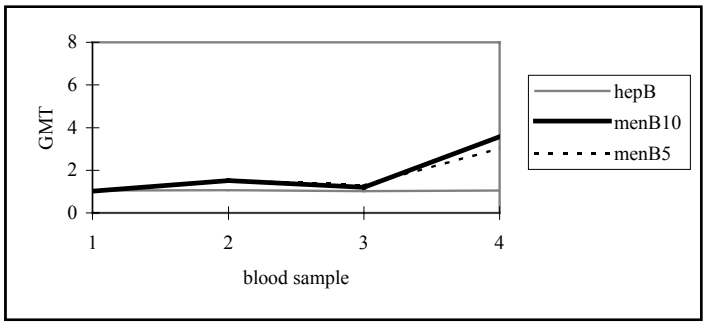
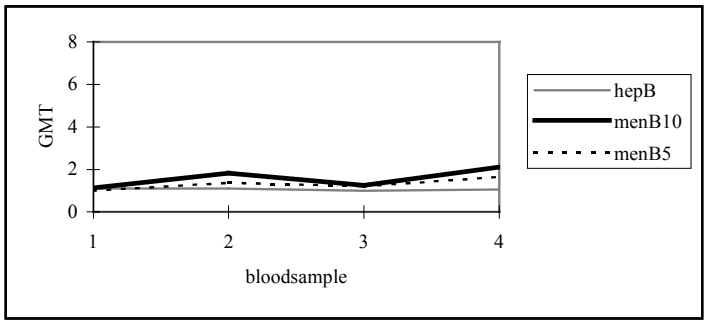
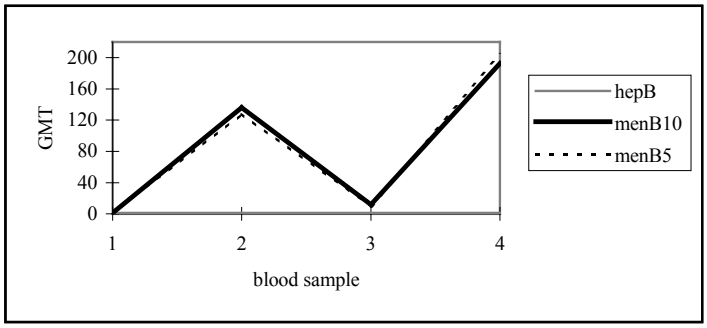



*Figure 2 SBA response*

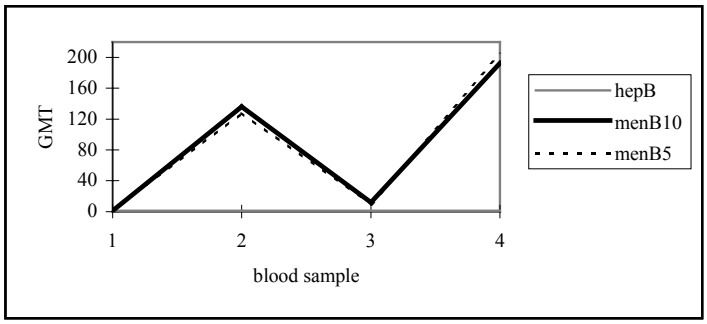
**Toddlers - strain P1.19,15**




**School children - strain P1.19,15**




**Toddlers - strain P1.5$^{c}$,10**




**School children – strain P1.5$^{c}$,10**

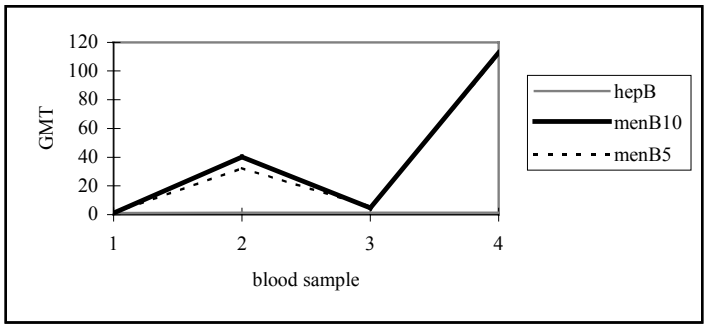
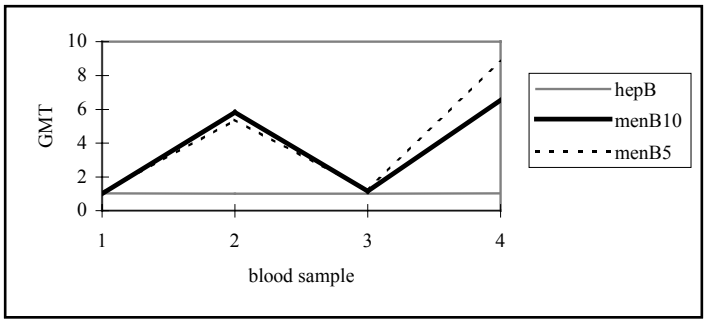
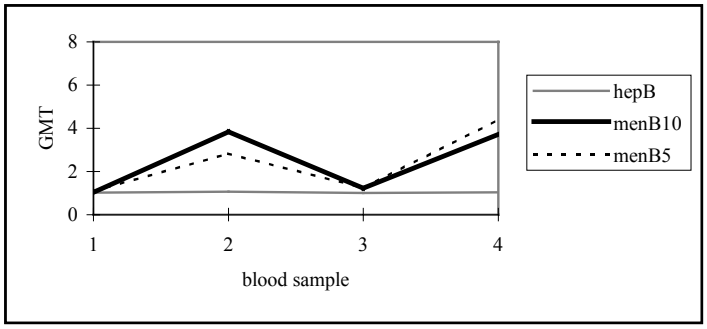



*Figure 2 SBA response (continued)*

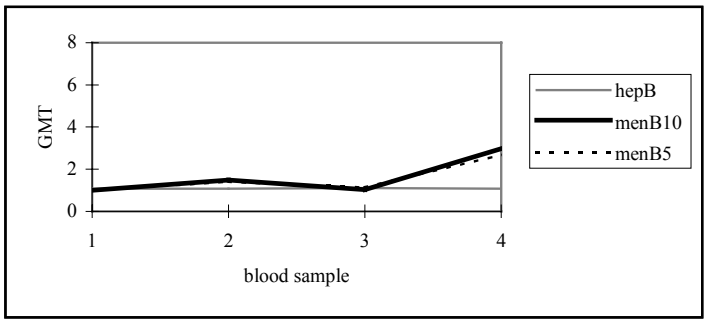
**Toddlers - strain P1.12,13**




**School children - strain P1.12,13**




**Toddlers - strain P1.7$^{h}$,4**




**School children - strain P1.7$^{h}$,4**

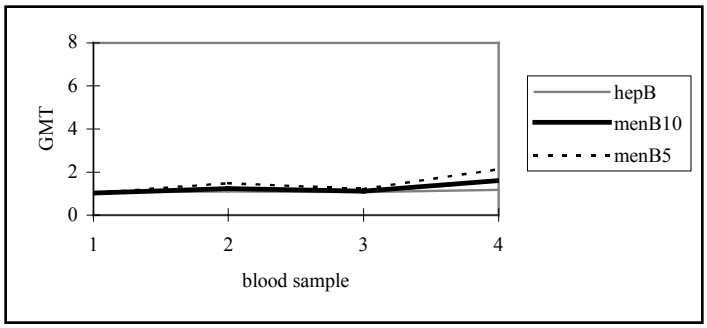
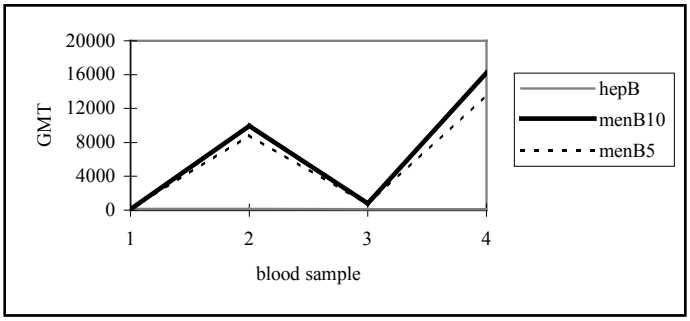


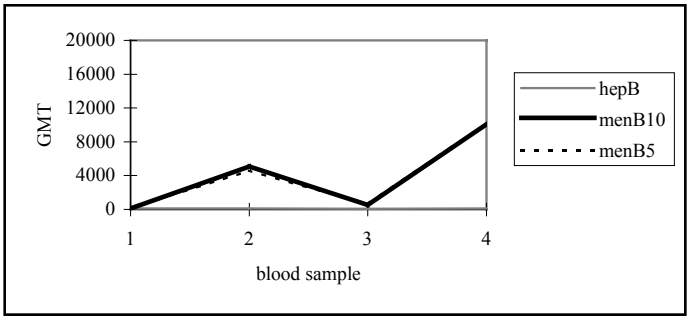

*Figure 2 SBA response (continued)*

# Appendix 13 ELISA response

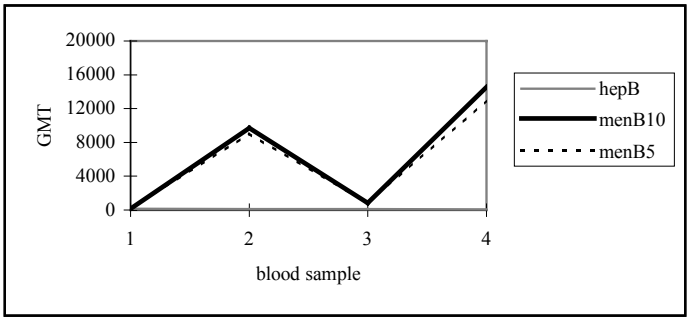
**Toddlers - vesicle PL16215**




**School children – vesicle PL16215**




**Toddlers - vesicle PL10124**




**School children – vesicle PL10124**

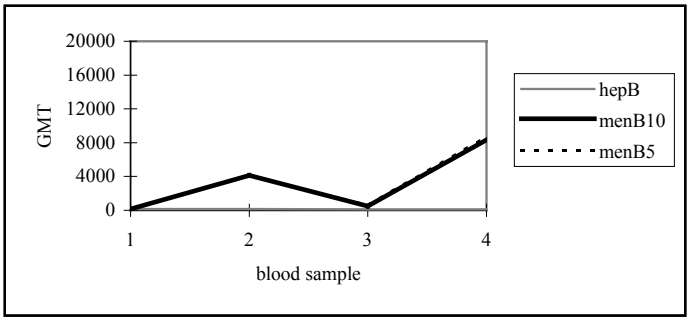
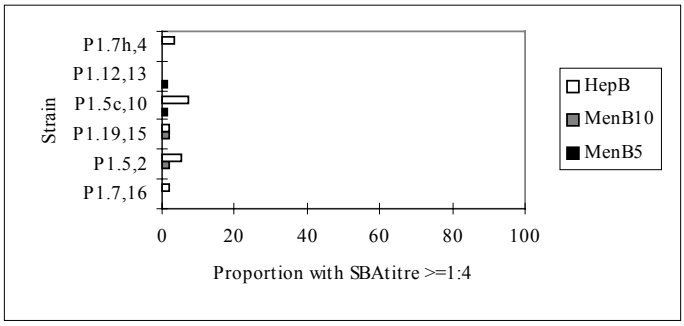


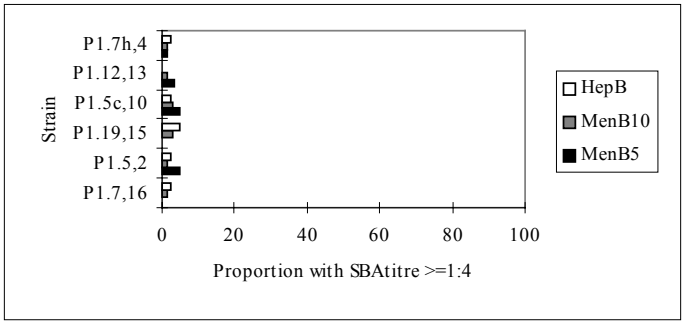

*Figure 3 ELISA response*

# Appendix 14 Proportion of participants with SBA titre ≥ 1:4

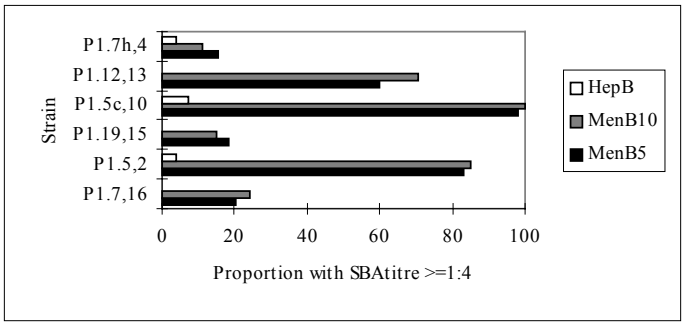
**Toddlers - blood sample 1**




**School children - blood sample 1**




**Toddlers - blood sample 2**




**School children - blood sample 2**

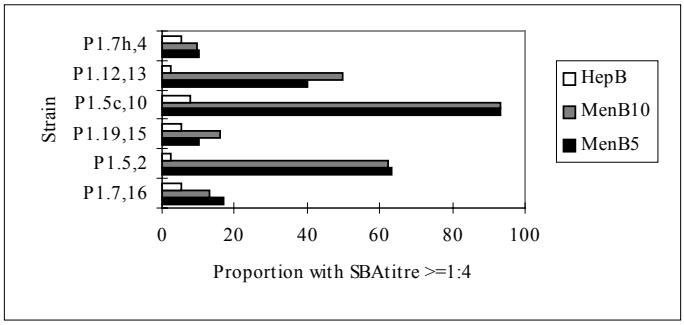
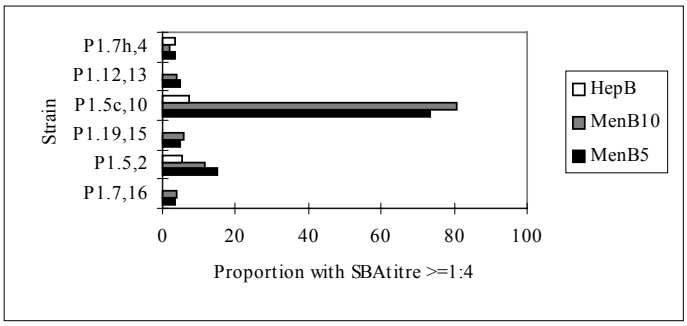
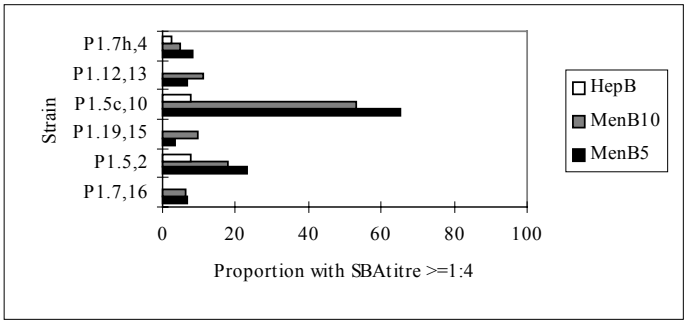



*Figure 4. Proportion of participants with SBA titre ≥ 1: 4*

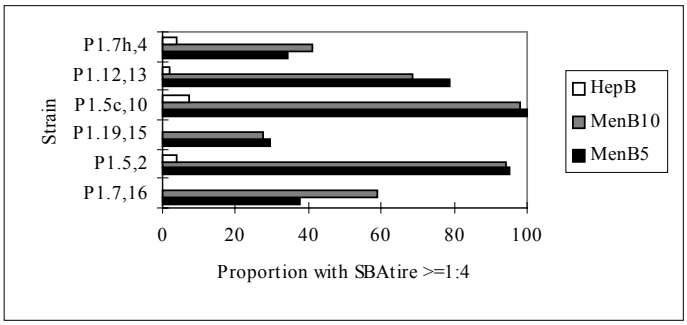
**Toddlers - blood sample 3**




**School children - blood sample 3**




**Toddlers -blood sample 4**




**School children - blood sample 4**

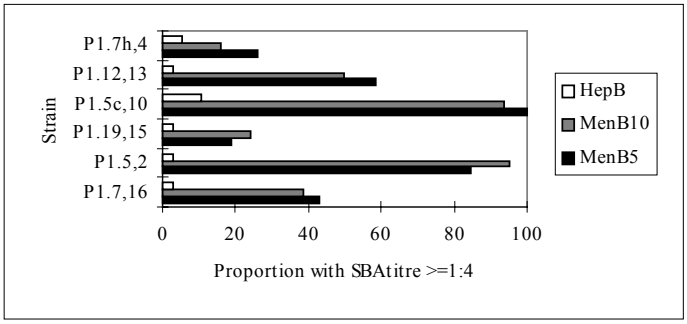
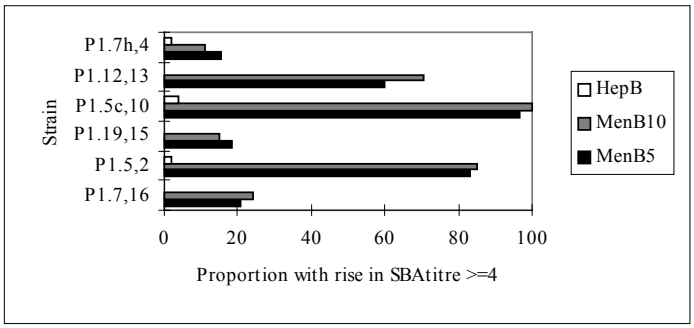


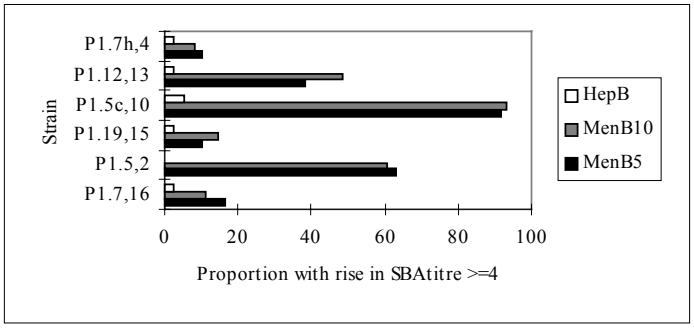

*Figure 4. Proportion of participants with SBA titre ≥ 4 (continued)*

# Appendix 15 Proportion of participants with rise in SBA titre ≥ 4

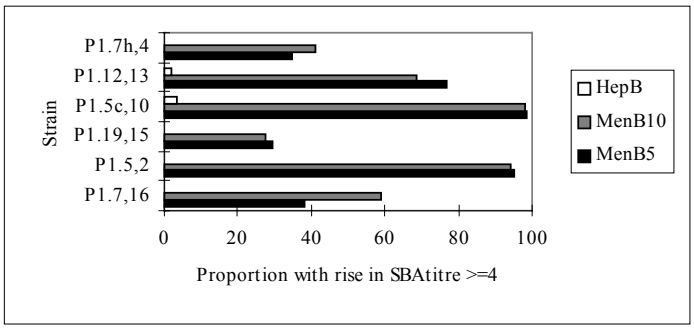
**Toddlers - blood sample 1 to 2**




**School children - blood sample 1 to 2**




**Toddlers - blood sample 1 to 4**




**School children - blood sample 1 to 4**

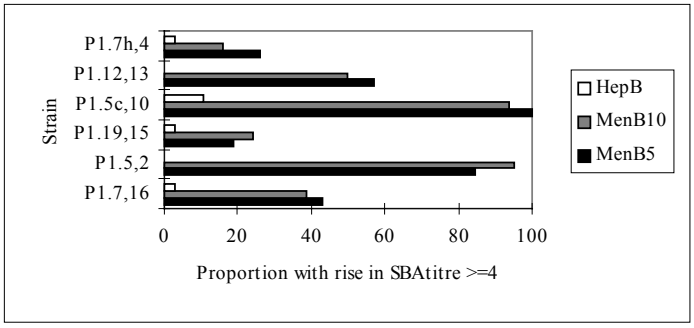
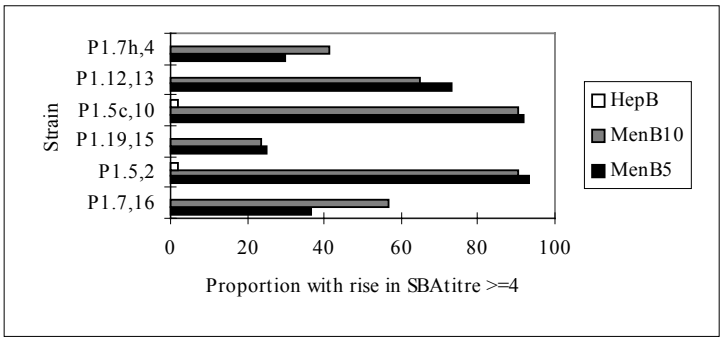



*Figure 5. Proportion of participants with rise in SBA titre ≥4*

**Toddlers - blood sample 3 to 4**




**School children - blood sample 3 to 4**

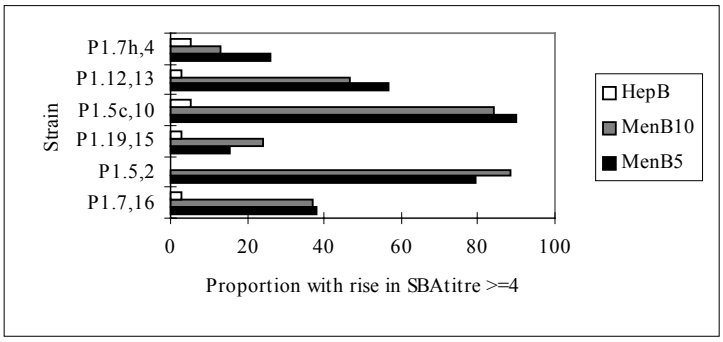



*Figure 5. Proportion of participants with rise in SBA titre ≥ 4 (continued)*