Clinical Study Report

Study Title: **An open label randomised controlled study to evaluate the induction of immune memory following infant vaccination with a glyco-conjugate *Neisseria meningitidis* serogroup C vaccine and to assess the immune response to the concurrent infant routine immunisations administered in consistent versus alternating limbs**

Internal Reference No: OVG 2008/6

Ethics Ref: 10/H0604/7

Eudract Number: 2009-016579-31

Date and Version No: 29.09.2012 Version 11

Chief Investigator:	**Professor Andrew J Pollard**
Investigators:	**Dr Matthew Snape, Dr David Pace, Dr Ameneh Khatami, Dr Andrew Marshall, Professor Adam Finn, Dr Saul Faust, Dr Paul Heath**
Sponsor:	**University of Oxford**
Funder:	**NIHR Oxford Biomedical Research Centre**
Signature of Chief Investigator:	

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host NHS Trust (s), regulatory authorities, and members of the Research Ethics Committee.

TABLE OF CONTENTS

1. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2	05.02.10	A Khatami	PCV13 to replace PCV7 Menjugate Kit to replace NeisVac-C Delay criteria for vaccination
2	3	22.04.10	A Khatami	Addition of new study group Addition of 24 month persistence sample
3	4	12.05.10	D Pace A Khatami	Addition of new study sites, and site specific procedures. Clarification of statistical analyses, randomisation and blinding. Clarification of sample handling procedures. Clarification of concomitant vaccines permitted in the trial Addition of trial steering committee Correction of typographical error in Appendix C. Removal of repeated secondary endpoints.

				Addition of new objective and endpoint. Clarification of Group numbers
4	5	26.11.10	A Khatami	Clarification of recruitment procedures Clarification of adverse event reporting procedures
5	6	23.02.11	A Khatami	Clarification of recruitment procedures Inclusion of Varicella Vaccine to list of non-study vaccines permitted.
6	7	03.06.11	A Khatami	Inclusion of Hep A Vaccine to list of non-study vaccines permitted.
7	8	06.10.11	A Khatami	Inclusion of interim analysis
8	9	03.11.11	A Khatami and D Pace	Clarification of statistical analyses and inclusion of a second attempt to blood sampling if first is unsuccessful
	9	13.12.11	A Khatami	Correction of typing errors on page 45 and 57
9	10	30.05.2012	A Tajar	Re-wording of hypothesis tests

10	11	29.05.2012	A Khatami	Clarification of V5 timelines and elimination criteria

2. SYNOPSIS

Study Title	**An open label randomised controlled study to evaluate the induction of immune memory following infant vaccination with a glyco-conjugate *Neisseria meningitidis* serogroup C vaccine and to assess the immune response to the concurrent infant routine immunisations administered in consistent versus alternating limbs**
Internal ref. no.	OVG 2008/6
Clinical Phase	Phase IV
Trial Design	Open label randomised controlled trial
Trial Participants	6 - 12 week old infants
Planned Sample Size	498 participants
Follow-up duration	24 months
Planned Trial Period	2010 – 2013
Primary Objective	The primary objective of this study is to demonstrate non-inferiority of the geometric mean titres (GMTs) of meningococcal serogroup C (MenC) specific serum bactericidal antibodies, using rabbit complement (rSBA), 1 month after a 12 month dose of Hib-MenC vaccine in children receiving a single dose of MenC-CRM_{197} vaccine at 3 months of age (single dose priming, Group 1) compared with those receiving 2 doses at 3 and 4 months of age (2 dose priming, Group 2). Non inferiority of the MenC serum bactericidal antibody geometric mean titres (SBA GMTs) would imply that the reduced schedule of MenC immunisation would be a more cost effective method of providing sustained immunity against MenC disease through childhood.
Secondary Objectives	Reduced dose MenC component:

	1. To assess whether MenC SBA GMTs measured 1 month after a 12 month dose of Hib-MenC are higher in children previously receiving single dose MenC-CRM_{197} vaccine priming at 3 months of age (Group 1) than in those receiving no priming doses of MenC vaccine (Group 3), demonstrating whether single dose MenC priming offers any advantage over no priming in terms of antibody levels at 13 months, and whether a single infant dose of MenC vaccine induces immune memory. 2. In an exploratory analysis, to compare the MenC SBA GMTs at day 6 after the 12 month dose of vaccine in a subset of participants (64 participants from the Single dose MenC-CRM_{197} [Group 1], Two dose MenC [Group 2] and Single Dose MenC-TT [Group 4] groups and all 64 in the control group [Group 3]: n=256) from all four groups, as it has been proposed that assessment of specific antibody levels at this earlier time point will more effectively discriminate between 'primed' and 'unprimed' immune responses to vaccines. 3. To assess the MenC SBA GMTs 2 months after a dose of MenC-CRM_{197} vaccine at 3 months of age (Group 1) compared to the MenC SBA GMTs taken 1 month after a course of MenC-CRM_{197} vaccine at 3 and 4 months of age (Group 2), and to a control group (Group 3) receiving no infant MenC immunisation who would be sampled at 5 months of age.

	4. To assess MenC SBA GMTs 2 months after a dose of a MenC-TT vaccine at 3 months of age (Single Dose MenC-TT Group, Group 4) compared to a single dose of a MenC-CRM_{197} conjugate vaccine (Single Dose MenC-CRM_{197} Group, Group 1), on a blood sample taken at 5 months of age. 5. To assess MenC SBA GMTs measured 12 months after a 12 month dose of Hib-MenC in all groups receiving priming vaccines with MenC-CRM_{197}, or no priming (Groups 1, 2 and 3), to determine whether 2 dose MenC priming offers any advantage over single dose MenC priming or no priming in terms of antibody levels at 24 months. 6. To assess MenC SBA GMTs 12 months after a 12 month dose of Hib-MenC in children previously receiving a single dose of a MenC-TT vaccine at 3 months of age (Group 4) compared to a single dose of a MenC-CRM_{197} conjugate vaccine (Group 1). 7. It is intended to measure the numbers of MenC specific memory B cells in the blood at 5 months, 12 months, 6 days following the 12 month booster dose, 13 months and 24 months of age, on a subset of participants. Specific memory B cells against diphtheria and tetanus will also be measured and used as a control. As many participants as possible would be included in this subset but the number would be determined by the practicalities of getting the blood to the laboratory in time (before midday) for processing.

	8. To assess the local and systemic adverse reactions experienced by participants in the four different study groups after immunisation with each dose of the MenC-CRM_{197}, MenC-TT and Hib-MenC vaccines. Alternating limb component 1. To compare the *S. pneumonia*e IgG GMCs and percentage of infants with serum concentration of *S. pneumonia*e specific IgG ≥0.35 μg/ml for all 13 PCV13 serotypes at 5, 12, 13 and 24 months of age in children receiving PCV13 in a consistent limb (subgroups 'a') vs those receiving this in alternating limbs (subgroups 'b'). 2. To compare serotype specific pneumococcal B cell phenotype in a subset of participants at 5, 12, 13 and 24 months of age in children receiving PCV13 in a consistent limb (sub groups 'a') vs those receiving this in alternating limbs (subgroups 'b'). 3. To compare the anti-PRP IgG GMCs and percentage of infants with serum concentration of anti-PRP IgG ≥0.15μg/ml and ≥1.0 μg/ml and at 5, 12 13 and 24 months of age in children receiving DTaP-IPV-Hib in a consistent limb (subgroups 'a') vs those receiving this in alternating limbs (subgroups 'b'). 4. To compare the anti-tetanus toxoid GMCs and percentage of infants with anti-tetanus toxoid >0.1 IU/ml at 5, 12, 13 and 24 months of age

	in participants receiving DTaP-IPV-Hib in a consistent limb (sub groups 'a') vs those receiving this in alternating limbs (subgroups 'b'). 5. To compare the local or systemic vaccine reactions in subgroups a and b at each vaccination time point.
Primary Endpoint	The difference in the MenC rSBA GMTs between the participants primed with two doses of MenC-CRM_{197} (Two Dose MenC Group, Group 2) and with one dose of MenC-CRM_{197} (Single Dose MenC-CRM_{197} Group, Group 1), one month following the Hib-MenC booster dose at 12 months.
Secondary Endpoints	After administration of the Hib-MenC booster dose at 12 months of age the following comparisons would be performed in order to assess differences between the: 1) MenC rSBA GMTs between the Two Dose MenC Group and the Control group at 6 and 28 days and 12 months later (Group 2 vs Group 3) 2) MenC rSBA GMTs between the Single dose MenC-CRM_{197} group and the Control group at 6 and 28 days and 12 months later (Group 1 vs Group 3) 3) MenC rSBA GMTs between the Single Dose MenC-TT group and the Control group at 6 and 28 days and 12 months later (Group 4 vs Group 3) 4) MenC rSBA GMTs between the Single Dose MenC-CRM_{197} group and the Single Dose MenC-TT group at 6 and 28 days and 12 months later (Group 1 vs Group 4) 5) Percentage of participants with MenC rSBA $\geq$1:8 and $\geq$1:128 between the Two Dose MenC group and the Control group at 6 and 28 days and 12 months later (Group 2 vs Group 3)

	6) Percentage of participants with MenC rSBA ≥1:8 and ≥1:128 between the Single dose MenC-CRM_{197} group and the Control group, at 6 and 28 days and 12 months later (Group 1 vs Group 3) 7) Percentage of participants with MenC rSBA ≥1:8 and ≥1:128 between the Two Dose MenC Group and the Single Dose MenC-CRM_{197} group, at 6 and 28 days and 12 months later (Group 2 vs Group 1) 8) Percentage of participants with MenC rSBA ≥1:8 and ≥1:128 between the Single Dose MenC-TT group and the Control group at 6 and 28 days and 12 months later (Group 4 vs Group 3) 9) Percentage of participants with MenC rSBA ≥1:8 and ≥1:128 between the Single Dose MenC-CRM_{197} group and the Single Dose MenC-TT group at 6 and 28 days and 12 months later (Group 1 vs Group 4) 10) Percentage of participants with MenC rSBA GMTs >1000 between the Two Dose MenC Group and the Control Group, at 6 and 28 days and 12 months later (Group 2 vs Group 3) 11) Percentage of participants with MenC rSBA GMTs >1000 between the Single Dose MenC-CRM_{197} Group and the Control Group, at 6 and 28 days and 12 months later (Group 1 vs Group 3) 12) Percentage of participants with MenC rSBA GMTs >1000 between the Two Dose MenC Group and the Single Dose MenC-CRM_{197} Group, at 6 and 28 days and 12 months later (Group 2 vs Group 1) 13) Percentage of participants with MenC rSBA GMTs >1000 between the Single Dose MenC-TT group and the Control group at 6 and 28 days and 12 months later (Group 4 vs Group 3)

	14) Percentage of participants with MenC GMTs >1000 between the Single Dose MenC-CRM_{197} group and the Single Dose MenC-TT group at 6 and 28 days and 12 months later (Group 1 vs Group 4) After administration of the last MenC-CRM_{197} dose at 4 months of age the following comparisons would be performed to assess differences between: 15) MenC rSBA GMTs between the Two Dose MenC Group vs Single Dose MenC-CRM_{197} Group (Group 2 vs Group 1), Two Dose MenC Group vs Control Group (Group 2 vs Group 3), Single Dose MenC-CRM_{197} vs Control Group (Group 1 vs Group 3), Single Dose MenC-TT vs Control group (Group 4 vs Group 3), and Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT group (Group 1 vs Group 4) at 5 and 12 months of age 16) Percentage of participants with MenC rSBA ≥1:8 between the Two Dose MenC Group vs Single Dose MenC-CRM_{197} Group (Group 2 vs Group 1), Two Dose MenC Group vs Control Group (Group 2 vs Group 3), Single Dose MenC-CRM_{197} vs Control Group (Group 1 vs Group 3), Single Dose MenC-TT vs Control Group (Group 4 vs Group 3), Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT Group (Group 1 vs Group 4) at 5 and 12 months of age 17) Percentage of participants with MenC rSBA ≥1:128 between the Two Dose MenC Group vs Single Dose MenC-CRM_{197} Group (Group 2 vs Group 1), Two Dose MenC Group vs Control Group (Group 2 vs Group 3), Single Dose MenC-CRM_{197} vs Control Group (Group 1 vs Group 3), Single Dose MenC-TT vs Control Group (Group 4 vs Group 3), Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT group (Group 1 vs Group 4) at 5 and 12 months of age 18) Number of MenC memory B cells at 5 months, 12 months, 12 months+6 days, 13 months and 24 months (on a subset of participants) between the Two

	Dose MenC Group vs Single Dose MenC-CRM_{197} Group (Group 2 vs Group 1), Two Dose MenC Group vs Control Group (Group 2 vs Group 3), Single Dose MenC-CRM_{197} vs Control Group (Group 1 vs Group 3), Single Dose MenC-TT vs Control Group (Group 4 vs Group 3), Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT (Group 1 vs Group 4). **Alternating limb component** After administration of the DTaP-IPV-Hib and PCV-13 the following comparisons would be performed to assess differences between the: 1. Anti-*S. pneumoniae* IgG GMCs or percentage of infants with serum concentration of *S. pneumonia*e specific IgG ≥0.35 μg/ml for each of the 13 serotypes at 5, 12, 13 and 24 months of age in children receiving PCV13 in a consistent limb (sub groups 'a') vs those receiving this in alternating limbs (subgroups 'b'). 2. Serotype specific pneumococcal B cell phenotype in a subset of participants at 5, 12, 13 and 24 months of age in children receiving PCV13 in a consistent limb (sub groups 'a') vs those receiving this in alternating limbs (subgroups 'b'). 3. Anti-PRP IgG GMCs or percentage of infants with serum concentration of anti-PRP IgG ≥0.15μg/ml and ≥1.0 μg/ml at 5, 12, 13 and 24 months of age in children receiving DTaP-IPV-Hib in a consistent limb (sub groups 'a') vs those receiving this in alternating limbs (subgroups 'b'). 4. Anti-tetanus toxoid GMCs or percentage of participants with anti-tetanus toxoid >0.1IU/ml at 5, 12, 13 and 24 months of age in children

	receiving DTaP-IPV-Hib in a consistent limb (sub groups ‘a’) vs those receiving this in alternating limbs (subgroups ‘b’).
Investigational Medicinal Products	All vaccines to be used are licensed. MenC-CRM_{197} vaccine (*Menjugate*, Novartis Vaccines and Diagnostics) MenC-TT vaccine (*NeisVac-C*, Baxter Healthcare) DTaP-IPV-Hib (*Pediacel*, Sanofi Pasteur MSD) Hib-MenC-TT (*Menitorix*, GlaxoSmithKline Biologicals) PCV13 (*Prevenar-13*, Wyeth Vaccines)

3. ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
ATP	According to protocol
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Clinical Trials
CTA	Clinical Trials Authorisation
CTRG	Clinical Trials & Research Governance, University of Oxford
DTaP-IPV-Hib	Pediacel (Sanofi Pasteur, MSD)
EC	Ethics Committee (see REC)
GCP	Good Clinical Practice
Hib-MenC	Menitorix (GlaxoSmithKline Biologicals)
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Products
IRB	Independent Review Board
ITT	Intention to treat
MenC	Serogroup C meningococcal
MMR	Measles, mumps and rubella vaccine
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
NRES	National Research Ethics Service (previously known as COREC)

OVG	Oxford Vaccine Group
PCV13	Prevenar-13 (Wyeth Vaccines)
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SIL	Subject Information Leaflet (see PIL)
SmPC/SPC	Summary of Products Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TSG	Oxford Radcliffe Hospitals Trust / University of Oxford Trials Safety Group

4. BACKGROUND AND RATIONALE

BACKGROUND:

Neisseria meningitidis is a major cause of meningitis and septicaemia globally with the greatest burden of endemic disease occurring in infants, children below 4 years of age and in adolescents[1,2]. However, during epidemics older children and adults are also affected[3]. *N. meningitidis* serogroups A, B, C, Y and W-135 cause the majority of invasive meningococcal disease worldwide with serogroups B and C accounting for more than 90% of cases in Europe and the US[1,2]. Because of the fulminant nature of the disease the overall case fatality rate remains at 4-10%, in spite of the prompt initiation of effective antibiotics and advances in intensive care[4,5]. 11-19% of survivors often sustain permanent disabilities, including neurological and intellectual impairment, amputations and hearing loss[6]. Vaccination is the only rational strategy for prevention of meningococcal disease.

In the 1990s an increase in the number of cases caused by the ST11 hyperinvasive clone of serogroup C was observed in Europe and the US[7]. This led to the formulation and development of three protein-polysaccharide conjugate MenC vaccines; two CRM197 conjugates: *Menjugate* (Novartis Vaccines and Diagnostics, Siena, Italy), and *Meningitec* (Wyeth Vaccines, Pearl River, New York) and one utilising tetanus toxoid as a carrier protein: *Neisvac-C* (Baxter Vaccines Beltsville, MD). Pre-licensure clinical trials showed that in contrast to plain polysaccharide meningococcal vaccines, these MenC conjugate vaccines resulted in the production of bactericidal antibodies from infancy, due to their ability to recruit T-cell help and the subsequent stimulation of immune memory[8]. Because of the rise in MenC disease and the availability of safety and immunogenicity data, these three MenC vaccines were first licensed in the UK in 1999 and used for a mass immunisation campaign directed against children and adolescents, despite the lack of formal efficacy data. These vaccines are used interchangeably in the UK immunisation schedule and are given at 3 and 4 months of age, with a combined Hib-MenC vaccine given as a booster dose at 12 months of age (this schedule was introduced in 2006 but from 1999-2006 a schedule of 3 doses at 2, 3 and 4 months of age without a booster was used). Other countries adopting routine

immunisation against MenC disease have used alternative immunisation schedules, with many, such as Australia and The Netherlands, opting for a single dose of MenC at 12 months of age.[7] The MenC vaccine has been introduced in Malta in 2009 and although not yet part of the national immunisation schedule, because of budgetary restrictions, is available privately where it is administered according to the UK schedule.

The administration of conjugate vaccines during clinical trials is usually standardised in a way that sequential doses of the study vaccine are administered in the same limb. Such practice may theoretically result in a better immune response due to stimulation of a greater number of memory B and T cells resident in draining lymph nodes previously primed by the same vaccine antigens[9,10]. However, after licensure sequential doses of these vaccines are not usually administered as such and in many instances are administered in alternating limbs.

RATIONALE:

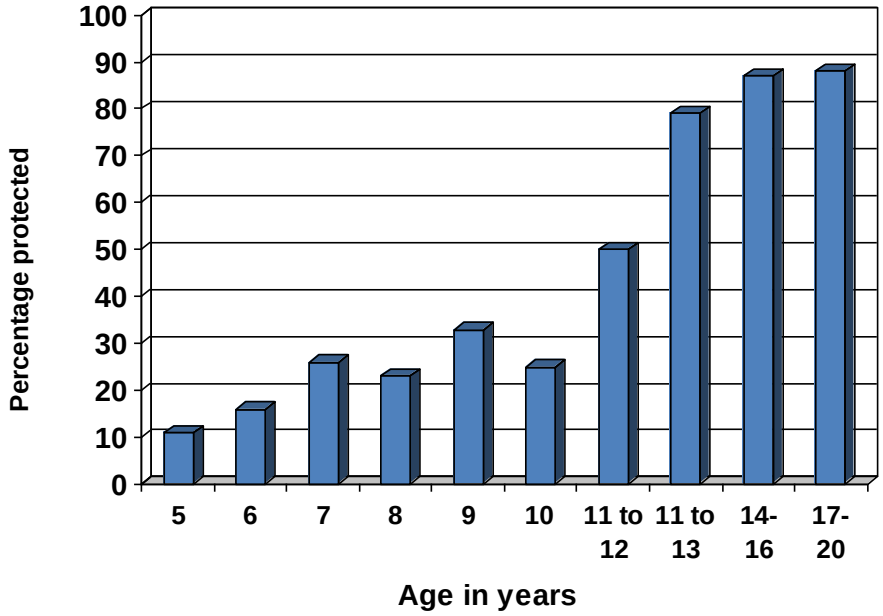
The purpose of this study is to evaluate the most appropriate immunisation schedule against MenC disease for UK children in the current era where the disease is under control. In Malta the MenC vaccine is not yet part of the national immunisation programme but has become available privately since late 2009. Malta has the highest incidence rate of invasive meningococcal disease (IMD) in Europe (crude incidence rate of 8.1 per 100,000 population) and epidemiological data from 1994-2006 have shown that although the majority are caused by MenB, 16% of all cases of microbiologically confirmed invasive meningococcal disease are caused by MenC[11,12]. In Malta children from 1-14 years of age suffer the majority of the total burden of IMD. Due to the lack of molecular techniques in identifying the meningococcus, 52% of cases of IMD which fit the clinical diagnostic criteria are unconfirmed which would suggest that the disease burden of MenC might be greater[11]. The introduction of routine MenC vaccination could have a modest reduction of IMD in Malta. This study would provide essential information on the most beneficial and cost-effective

MenC schedule and will be crucial in determining how the MenC vaccine would best fit in the current national immunisation programme, when introduced.

This study will assess the impact that reducing the number of doses of MenC vaccine given in the first few months of life has on the height and duration of the antibody response and on the B-cell memory response to the Hib-MenC vaccine given at 12 months of age. Given the possibility that further immunisations will become available for use in the UK infant immunisation schedule (e.g. against serogroup B meningococcoccus[13]), a reduction in the number of MenC vaccines given in the first few months of life in the UK needs to be considered. The option of a single priming dose of MenC vaccine given in the first few months of life, followed by a booster dose of Hib-MenC at 12 months of age, may potentially enable both a reduction in infant MenC doses and the sustained protection afforded by 'prime-boost' immunisation schedules. It is therefore appropriate to compare this possibility with the current UK schedule (2 doses priming) and the option used in many other countries (single dose of MenC at 12 months of age with no priming).

There are, however, no published studies of a MenC immunisation programme using single infant priming followed by a booster dose of a MenC conjugate vaccine at 1 year of age. Several studies have assessed the immunogenicity of a single priming dose of MenC vaccine given at 2 months of age,[14,15] or the antibody response after the first MenC dose,[16,17] but none have assessed the impact of reducing the number of priming MenC vaccine doses on the response to the booster dose of Hib-MenC at 12 months of age. This is of particular relevance as it is likely that this booster dose response is of more importance in generating sustained population immunity against MenC disease than the response to the infant (priming) doses of MenC vaccine. Conversely, without the 12 month booster (the original UK schedule had no booster), antibody levels wane very rapidly and the majority of children have antibody levels below the protective threshold within a few years of immunisation. Indeed, recent studies have shown that the majority of UK primary school aged children (immunised with a single dose of MenC vaccine in the "catch up" campaign in 1999) have low antibody titres[18] (figure 1).

Figure 1: Rates of seroprotection (SBA titres ≥ 1:8) against MenC disease in UK children and adolescents (adapted from Perrett et al (unpublished data) and Snape et al, 2008[18] , showing that the vast majority of children under 10 years of age in the UK in 2008 are not protected against serogroup C meningococcal disease – these children received MenC either as a 3 dose infant schedule with no booster (children aged 5-10 years), or as a single dose of vaccine in the catch up campaign in 1999 (those now over 10 years of age).



Since MenC causes disease throughout childhood and adolescence[19], and the effectiveness of MenC vaccines are known to decline in populations whose SBA titres have waned post MenC immunisation[20], maintaining adequate antibody levels seems vital for individual immunity. Despite the central importance of this concept, there are no published systematically collected data on the persistence of immunity following immunisation in the current UK schedule.

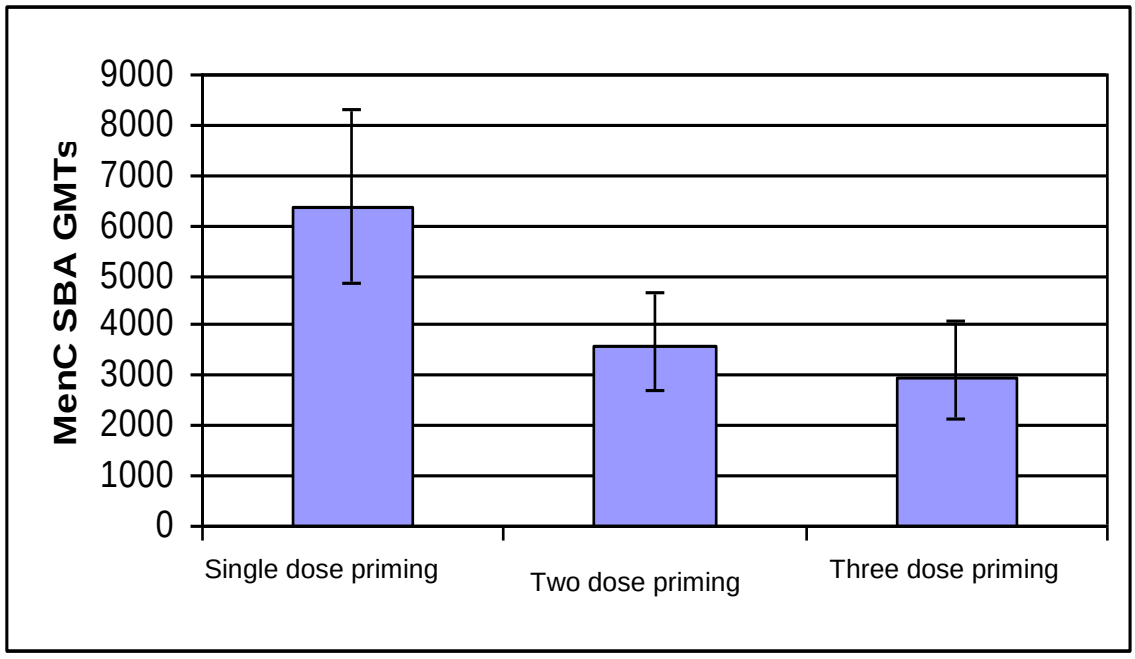
Since MenC antibody titres are low among UK children under 10 years of age, the low levels of MenC disease currently seen in the UK may be attributed to herd immunity, which is most likely due to reduced MenC nasopharyngeal carriage rates in adolescents and young adults[21]. This herd immune effect was induced by the catch up campaign when the vaccine was introduced in 1999 and is related to the high effectiveness and antibody persistence in the older vaccinated groups (over 10 years) that effectively blocks transmission of the organism in the population, presumably because teenagers and young adults drive transmission of this organism. However, whilst herd immunity means that in 2009 there is very little risk of MenC disease even amongst the unimmunised, it seems unlikely that herd immunity will be maintained indefinitely, especially now that most children have very low antibody levels. In the UK the future maintenance of herd immunity is critically dependent on the persistence of the immune response following the 12 month booster dose of Hib-MenC, since no further doses of the MenC vaccine are currently given in the schedule.

The impact of reducing the number of priming MenC vaccine doses on the response to the 12 month booster dose of Hib-MenC therefore needs to be specifically assessed in an appropriately designed clinical trial as proposed herein.

Intriguingly, Borrow et al. have suggested that reducing the number of MenC priming doses from 2 to 1 may actually enhance the response to the 12 month booster dose[14]. In this study children received a dose of a 'plain polysaccharide' meningococcal serogroup A and C vaccine, rather than a conjugated MenC vaccine, at 12 months of age[14]. Children immunised with a single priming dose of MenC vaccine mounted a greater response to the 12 month dose of plain polysaccharide vaccine than those immunised with 2 or 3 infant doses of MenC vaccine, such that the single dose priming MenC group had the highest SBA geometric mean titres at 13 months of age (figure 2). If similar results were observed in response to the Hib-MenC conjugate vaccine, this would suggest that reducing the number of priming MenC vaccine doses may actually enhance the maintenance of immune protection through late childhood.

The investigators are not aware of any studies addressing the comparison between the number of priming doses and the response to the booster dose at 12 months of age. One currently recruiting study being conducted by the Health Protection Agency (HPA) is examining the important question of 1 dose priming schedules with MenC vaccines from different manufacturers, but the design of this study does not include a comparison of different priming schedules, and will not provide information on long term persistence of antibodies[22].

Figure 2: MenC specific SBA geometric mean titres 1 month after immunisation with a MenAC plain polysaccharide vaccine at 12 months of age*, according to number of priming doses received in infancy (adapted from Borrrow et al[14]).



* MenAC plain polysaccharide vaccine used in this study as an immunological challenge to assess immune priming. The vaccine currently used as a booster dose in the UK schedule is a combined Hib-MenC conjugate vaccine, for which no studies have assessed the impact of altering the number of priming doses of MenC vaccine. (Note that polysaccharide vaccines are not used in routine infant immunisation but no similar data are available for boosting with a MenC conjugate vaccine).

In addition, several studies have indicated that the response to the Hib-MenC-TT booster may depend on the type of MenC conjugate vaccine that is used for priming in infancy[23]. These differences may be related to the type of carrier protein that is used for the priming doses, or may be an effect of using different carrier proteins for priming and boosting. These previous studies have assessed the differences in schedules that had 2 or 3 priming doses; whereas this current study will be designed to assess potential differences in a single dose priming schedule. The two MenC conjugate vaccines that will be addressed are the two which are most commonly used in the UK and Europe: MenC-CRM_{197} (conjugated to mutant diphtheria toxin) and MenC-TT (conjugated to tetanus toxoid).

Furthermore, this proposed study will evaluate a novel means of assessing the induction of immunological memory, a defining feature of a successful conjugate vaccine[24]. Immune memory has classically been assessed by the anamnestic response to a plain polysaccharide boost. There are, however, uncertainties regarding the appropriateness of administering meningococcal plain polysaccharide vaccines to children <2 years of age who are known to respond poorly to unconjugated polysaccharide antigens[25]. In addition the hyporesponsiveness observed following repeated doses of plain polysaccharide vaccines raises concerns that receipt of these vaccines could potentially hinder a child's ability to respond to natural infection with MenC. It has therefore recently been proposed that the WHO guidelines on the clinical evaluation of MenC vaccines be altered to recommend the use of booster doses of conjugate vaccines to assess immunologic memory[26]. However, no studies have provided data to allow distinction between primed and unprimed responses. One suggested means of doing this is to assess whether SBA titres increase above baseline more rapidly following a 'challenge' dose of MenC vaccine in those who have

previously been primed by prior immunisation with a MenC vaccine than in those that are vaccine naïve. No clinical trials have previously assessed this, and the design of this study affords an opportunity to generate novel data to explore this issue. If it can be shown that a rise above baseline antibody levels can be seen at day 6 in primed, but not in unprimed, participants, this will provide an important new measure of immune priming that will be relevant for the design of future studies of conjugate vaccines.

Alternating limb component

This study would also provide the opportunity to investigate whether the immunogenicity of the Hib, tetanus and pneumococcal components, within the routinely recommended vaccines (Pediacel and Prevenar-13), that are administered concurrently with the MenC vaccine would be affected if sequential doses are administered within the same limb or in the alternating limb. Protection against vaccine preventable diseases is dependent on the generation of sustained and functional disease-specific antibody concentrations or a timely 'secondary' antibody response on exposure to the relevant disease which in turn are dependent on the generation of mature B cells in lymph nodes draining the site of vaccination. After priming, a significant proportion of mature B cells remain within these nodes[9], accompanied by retained antigen and memory CD4 T-cells that can aid antibody responses[10]. Memory B cells appear to preferentially home to lymph nodes that have been primed with their cognate antigen[27]. Sequential immunisations, as performed in clinical trials, may therefore be more effective if given into the same limb since vaccine antigens should reach more memory B and T-cells, resident in draining lymph nodes. Post licensure sequential doses of vaccines are not usually administered in the same limb, a practice that was shown to result in reduced response rates to an intradermal rabies vaccine[28]. No further studies have been performed to assess the effect that alternating limbs with sequential immunisations has on the immunogenicity of conjugate vaccines.

Immuno-genetics

An additional aspect to be assessed in this study is the impact of genetic factors influencing the response to immunisation. Host immuno-genetics are likely to play a critical role in modulating the responses to paediatric vaccines. Twin studies on several vaccines including measles, mumps and rubella, have shown high heritability of vaccine antibody responses[29]. Some genetic associations have already been identified between genes of the adaptive and innate immune response and some vaccines, for example human leukocyte antigen (HLA) alleles and measles antibody responses[30,31], and IL-1β polymorphisms and hepatitis B vaccine responses[32]. These studies have been small scale and based on single candidate genes and the extent to which genetic variation contributes to vaccine responses remains poorly understood. Insight into which genetic variants affect responses to specific vaccines will be of value for 2 main reasons:

1. it will help identify the critical immune pathways leading to protection after vaccination and lead to the production of more effective vaccines
2. it will help identify genes that may play important roles in wild-type infection and lead to better understanding of disease pathogenesis, which in turn may lead to the development of novel therapies

The blood samples obtained in this study provide an opportunity to extract DNA from the cellular plug remaining after serum centrifugation. The DNA samples obtained in this study can then contribute to a DNA bank pooling samples from multiple different Oxford Vaccine Group studies. These DNA samples can then be used for genome wide analysis of the genetic factors influencing the host response to the vaccines received in the relevant studies. This DNA extraction and storage will only occur with the specific consent of participants, and DNA will not be analysed for any other purpose than to assess factors influencing the immune response to vaccines.

5. OBJECTIVES

The overall objective of this study is to determine the relative immunogenicity of different schedules of serogroup C meningococcal (MenC) vaccine in the first year of life and to determine whether a reduced dose schedule could be used to save costs for the NHS.

5.1 Primary Objective

The primary objective of this study is to demonstrate non-inferiority of the geometric mean titres (GMTs) of meningococcal serogroup C (MenC) specific serum bactericidal antibodies, using rabbit complement (rSBA), 1 month after a 12 month dose of Hib-MenC vaccine in children receiving a single dose of MenC-CRM_{197}vaccine at 3 months of age (Single Dose priming) compared with those receiving 2 doses at 3 and 4 months of age (Two Dose priming). Non-inferiority of the MenC serum bactericidal antibody geometric mean titres (SBA GMTs) would imply that the reduced schedule of MenC immunisation would be a more cost effective method of providing sustained immunity against MenC disease through childhood.

5.2 Secondary Objectives

Reduced dose MenC component:

1. To assess whether MenC SBA GMTs measured 1 month after a 12 month dose of Hib-MenC are higher in children previously receiving Single Dose MenC-CRM_{197} vaccine priming at 3 months of age (Single Dose MenC-CRM_{197} Group) than in those receiving no priming doses of MenC vaccine (Control Group), demonstrating whether single dose MenC priming offers any advantage over no priming in terms of antibody levels at 13 months, and whether a single infant dose of MenC vaccine induces immune memory.

2. In an exploratory analysis, to compare the MenC SBA GMTs at day 6 after the 12 month dose of vaccine in a subset of participants (64 participants each from the Single Dose MenC-

CRM_{197}, Single Dose MenC-TT and Two Dose MenC Groups, and all 64 in the control group: n=256) from all four groups, as it has been proposed that assessment of specific antibody levels at this earlier time point will more effectively discriminate between 'primed' and 'unprimed' immune responses to vaccines.

3. To assess the MenC SBA GMTs 2 months after a dose of MenC-CRM_{197} vaccine at 3 months of age (Single Dose MenC-CRM CRM_{197} Group) compared to the MenC SBA GMTs taken 1 month after a course of MenC-CRM_{197} vaccine at 3 and 4 months of age (Two Dose MenC Group), and to a Control Group receiving no infant MenC immunisation who would be sampled at 5 months of age.

4. To assess MenC SBA GMTs 2 months after a dose of a MenC-TT vaccine at 3 months of age (Single Dose MenC-TT Group) compared to a Single Dose of a MenC-CRM_{197} conjugate vaccine (Single Dose MenC-CRM_{197} Group), and to the Control Group, on a blood sample taken at 5 months of age.

5. To assess MenC SBA GMTs measured 12 months after a 12 month dose of Hib-MenC in all groups receiving priming vaccines with MenC-CRM_{197}, or no priming, to determine whether 2 dose MenC priming offers any advantage over single dose MenC priming or no priming in terms of antibody levels at 24 months.

6. To assess MenC SBA GMTs 12 months after a 12 month dose of Hib-MenC in children previously receiving a single dose of a MenC-TT vaccine at 3 months of age (Single Dose MenC-TT Group) compared to a single dose of a MenC-CRM_{197} conjugate vaccine (Single Dose MenC-CRM_{197} Group).

7. It is intended to measure the numbers of MenC specific memory B cells in the blood at 5 months, 12 months, 6 days following the 12 month booster dose, at 13 months and 24 months on a subset of participants. Specific memory B cells against diphtheria and tetanus will also be measured and used as a control. As many participants as possible would be included in this subset but the number would be determined by the practicalities of getting the blood to the laboratory in time (before midday) for processing.

8. To assess the local and systemic adverse reactions experienced by participants in the four different study groups after immunisation with each dose of the MenC-CRM_{197}, MenC-TT and Hib-MenC vaccines.

Alternating limb component

1. To compare the *S. pneumonia*e IgG GMCs and percentage of infants with serum concentration of *S. pneumonia*e specific IgG ≥0.35 μg/ml for all 13 PCV13 serotypes at 5, 12, 13 and 24 months of age in children receiving PCV13 in a consistent limb (subgroups 'a') vs those receiving this in alternating limbs (subgroups 'b').
2. To compare serotype specific pneumococcal B cell phenotype in a subset of participants at 5, 12, 13 and 24 months of age in children receiving PCV13 in a consistent limb (sub groups 'a') vs those receiving this in alternating limbs (subgroups 'b').

3. To compare the anti-PRP IgG GMCs and percentage of infants with serum concentration of anti-PRP IgG ≥0.15μg/ml and ≥1.0 μg/ml and at 5, 12, 13 and 24 months of age in children receiving DTaP-IPV-Hib in a consistent limb (sub groups 'a') vs those receiving this in alternating limbs (subgroups 'b').

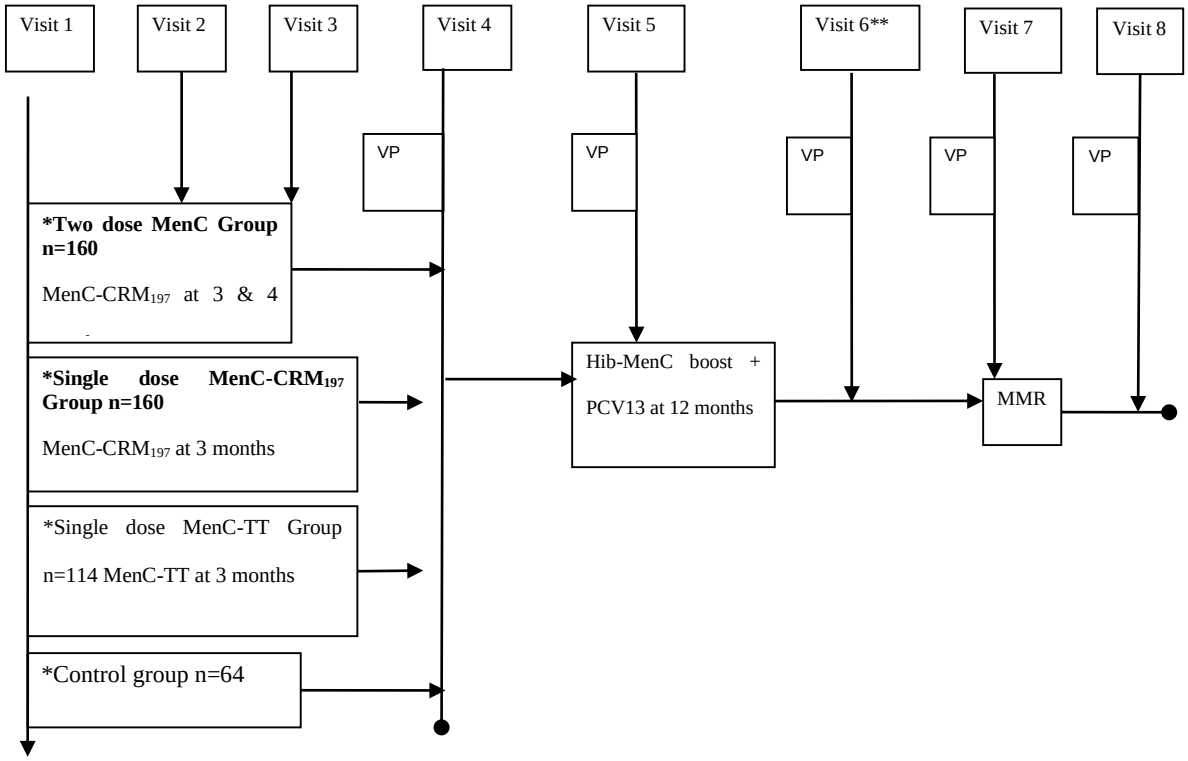
4. To compare the anti-tetanus toxoid GMCs and percentage of infants with anti-tetanus toxoid >0.1 IU/ml at 5,12, 13 and 24 months of age in children receiving DTaP-IPV-Hib in a consistent limb (sub groups ‘a’) vs those receiving this in alternating limbs (subgroups ‘b’)

5. To compare the local or systemic vaccine reactions in subgroups a and b at each vaccination time point.

6 TRIAL DESIGN

6.1 Summary of Trial Design

This is a phase IV open label randomised controlled trial enrolling 498 participants. Study participation would be for 23-24 months during which there will be 8 study visits as outlined in the attached figure and as detailed in section 6.4.2. A list of the study procedures and the interval between study visits are shown in Appendices A, B and C. Study visit 6 would be performed in all participants in the control group, and an equal number (i.e. 64) of participants in the Two Dose MenC, Single Dose MenC-CRM_{197} and Single Dose MenC-TT groups (as determined by randomisation at enrolment).



*Participants would be randomised to receive MenC-CRM_{197} at 3 and 4 months (Two Dose MenC Group), or a single dose at 3 months (Single Dose MenC-CRM_{197} Group) or a single dose of MenC-TT at 3 months (Single dose MenC-TT Group) or no priming doses (Control Group). All participants will receive the DTaP-IPV-Hib vaccine at 2, 3 and 4 months and the PCV13 at 2 and 4 months according to the UK immunisation schedule. All participants would be boosted with the

Hib-MenC vaccine and the booster dose of PCV13 at 12 months and will receive the MMR vaccine at 13 months.

Venepuncture (VP), taking 5 ml of blood, will be performed at 5 months (Visit 4), and taking 7.5ml of blood will be performed at 12 months (Visit 5), 12 months+6 days (Visit 6), at 13 months (Visit 7) and at 24 months (Visit 8). Two attempts may be made to obtain a blood sample at each visit, if the parent gives verbal consent at the time of the procedure for a second attempt. If no blood is obtained, a second visit may be made if the parent agrees to this.

** Visit 6 will be performed on all participants in the control group and a subset of 64 participants in the Two Dose MenC, Single Dose MenC-CRM_{197} and Single Dose MenC-TT groups (as determined by randomisation at enrolment, see section 6.4.4)

In addition, participants will be randomised at enrolment to receive PCV13 and DTaP-IPV-Hib in either consistent limbs (subgroup a) or alternating limbs (subgroup b) as follows

Subgroup a (Consistent limbs)	**Subgroup b** (Alternating limbs)
DTaP-IPV-Hib in right leg at 2, 3 and 4 months PCV13 in right leg at 2, 4and 12 months	DTaP-IPV-Hib in left leg at 2 months and in right leg at 3 and 4 months* PCV13 in left leg at 2 months, right leg at 4 months and left arm at 12 months

* DTaP-IPV-Hib given in same limb at 3 and 4 months to maintain consistency of co-administration of vaccines in the same limb (i.e. MenC-CRM_{197} or MenC-TT always given by itself and DTaP-IPV-Hib co-administered with PCV13 at 2 and 4 months – see Appendix A).

6.2 Primary and Secondary Endpoints/Outcome Measures

Primary endpoint

The difference in the MenC rSBA GMTs between the participants primed with two doses of MenC-CRM_{197} (Two Dose MenC Group) and with one dose of MenC-CRM_{197} (Single Dose MenC-CRM_{197} Group), one month following the Hib-MenC booster dose at 12 months.

Secondary endpoints

Reduced MenC component

After administration of the Hib-MenC booster dose at 12 months of age the following comparisons would be performed in order to assess differences between the:

1) MenC rSBA GMTs between the Two Dose MenC Group and the Control group at 6 and 28 days and 12 months later

2) MenC rSBA GMTs between the Single dose MenC-CRM_{197} group and the Control group at 6 and 28 days and 12 months later

3) MenC rSBA GMTs between the Single Dose MenC-TT group and the Control group at 6 and 28 days and 12 months later

4) MenC rSBA GMTs between the Single Dose MenC-CRM_{197} group and the Single Dose MenC-TT group at 6 and 28 days and 12 months later

5) Percentage of participants with MenC rSBA ≥1:8 and ≥1:128 between the Two Dose MenC group and the Control group at 6 and 28 days and 12 months later

6) Percentage of participants with MenC rSBA ≥1:8 and ≥1:128 between the Single dose MenC-CRM_{197} group and the Control group, at 6 and 28 days and 12 months later

7) Percentage of participants with MenC rSBA ≥1:8 and ≥1:128 between the Single dose MenC-TT group and the Control group, at 6 and 28 days and 12 months later

8) Percentage of participants with MenC rSBA ≥1:8 and ≥1:128 between the Single Dose MenC-TT group and the Control group at 6 and 28 days and 12 months later (Group 4 vs Group 3)

9) Percentage of participants with MenC rSBA ≥1:8 and ≥1:128 between the Single Dose MenC-CRM_{197} group and the Single Dose MenC-TT group at 6 and 28 days and 12 months later

10) Percentage of participants with MenC rSBA GMTs >1000 between the Two Dose MenC Group and the Control Group, at 6 and 28 days and 12 months later.

11) Percentage of participants with MenC rSBA GMTs >1000 between the Single Dose MenC-CRM_{197} Group and the Control Group, at 6 and 28 days and 12 months later.

12) Percentage of participants with MenC rSBA GMTs >1000 between the Two Dose MenC Group and the Single Dose MenC-CRM_{197} Group, at 6 and 28 days and 12 months later.

13) Percentage of participants with MenC rSBA GMTs >1000 between the Single Dose MenC-TT group and the Control group at 6 and 28 days and 12 months later (Group 4 vs Group 3)

14) Percentage of participants with MenC rSBA GMTs >1000 between the Single Dose MenC-TT Group and the Control Group, at 6 and 28 days and 12 months later.

After administration of the last MenC-CRM_{197} dose at 4 months of age the following comparisons would be performed to assess differences between:

15) MenC rSBA GMTs between the Two Dose MenC Group vs Single Dose MenC-CRM_{197} Group, Two Dose MenC Group vs Control Group, Single Dose MenC-CRM_{197} vs Control Group, Single Dose MenC-TT vs Control group, and Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT group at 5 and 12 months of age.

16) Percentage of participants with MenC rSBA ≥1:8 between the Two Dose MenC Group vs Single Dose MenC-CRM_{197} Group, Two Dose MenC Group vs Control Group, Single Dose MenC-

CRM_{197} vs Control Group, Single Dose MenC-TT vs Control Group, Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT Group at 5 and 12 months of age.

17) Percentage of participants with MenC rSBA ≥1:128 between the Two Dose MenC Group vs Single Dose MenC-CRM_{197} Group, Two Dose MenC Group vs Control Group, Single Dose MenC-CRM_{197} vs Control Group, Single Dose MenC-TT vs Control Group, Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT group at 5 and 12 months of age.

18) Number of MenC memory B cells at 5 months, 12 months, 12 months+6 days, 13 months and 24 months (on a subset of participants) between the Two Dose MenC Group vs Single Dose MenC-CRM_{197} Group, Two Dose MenC Group vs Control Group, Single Dose MenC-CRM_{197} vs Control Group, Single Dose MenC-TT vs Control Group, Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT.

Alternating limb component

After administration of the DTaP-IPV-Hib and PCV-13 the following comparisons would be performed to assess differences between the:

1. Anti-*S. pneumoniae* IgG GMCs or percentage of infants with serum concentration of *S. pneumonia*e specific IgG ≥0.35 μg/ml for each of the 13 serotypes at 5, 12, 13 and 24 months of age in children receiving PCV13 in a consistent limb (sub groups 'a') vs those receiving this in alternating limbs (subgroups 'b').

2. Serotype specific pneumococcal B cell phenotype in a subset of participants at 5, 12, 13 and 24 months of age in children receiving PCV13 in a consistent limb (sub groups 'a') vs those receiving this in alternating limbs (subgroups 'b').

3. Anti-PRP IgG GMCs or percentage of infants with serum concentration of anti-PRP IgG ≥ 0.15 μg/mL and ≥1.0 μg/ml at 5, 12, 13 and 24 months of age in children receiving DTaP-

IPV-Hib in a consistent limb (sub groups ‘a’) vs those receiving this in alternating limbs (subgroups ‘b’).

4. Anti-tetanus toxoid GMCs or percentage of participants with anti-tetanus toxoid >0.1IU/ml at 5, 12, 13 and 24 months of age in participants in receiving DTaP-IPV-Hib in a consistent limb (sub groups ‘a’) vs those receiving this in alternating limbs (subgroups ‘b’).

Reactogenicity

The following comparisons would be performed to assess differences between:

Local adverse events

1) The percentage of infants with each type of adverse event, and at least one adverse event for local adverse events after one dose of MenC-CRM_{197} at 3 months (Single Dose MenC-CRM_{197} Group) vs the second dose of MenC-CRM_{197} at 4 months (Two Dose MenC Group).

2) The percentage of infants with each type of adverse event, and at least one adverse event for local adverse events after one dose of MenC-CRM_{197} at 3 months (Single Dose MenC-CRM_{197} Group) vs one dose of MenC-TT at 3 months (Single Dose MenC-TT Group).

3) The percentage of infants with each type of adverse event, and at least one adverse event for local adverse events after each dose of DTaP-IPV-Hib (at 2, 3 and 4 months) and PCV13 (at 2 and 4 months) in the consistent limb group (sub groups ‘a’) vs. the alternating limb group (subgroups ‘b’).

4) The percentage of infants with each type of adverse event, and at least one adverse event for local adverse events after the 12 month booster MenC and PCV13 vaccination in the

   a. Two Dose MenC Group vs 0 Dose Control Group
   b. Single Dose MenC-CRM_{197} Group vs. 0 Dose Control Group
   c. Single Dose MenC-TT Group vs. 0 Dose Control Group
   d. Single Dose MenC-CRM_{197} Group vs. Two Dose MenC Group
   e. Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT Group
   f. the consistent limb group vs. the alternating limb group

Systemic adverse events

1) The percentage of infants with each type of adverse event, and at least one adverse event for systemic adverse events after MenC (3 and 4 months), DTaP-IPV-Hib (2,3 and 4 months) and PCV13 (2 and 4 months) vaccination in the:

   a. Two Dose MenC Group vs. 0 Dose Control Group
   b. Single Dose MenC-CRM_{197} Group vs. 0 Dose Control Group
   c. Single Dose MenC-TT Group vs. 0 Dose Control Group
   d. Single Dose MenC-CRM_{197} Group vs. Two Dose MenC Group
   e. Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT Group
   f. The consistent limb group vs. the alternating limb group

2) The percentage of infants with each type of adverse event, and at least one adverse event for systemic adverse events after the 12 month booster MenC and PCV13 vaccination in the:

a. Two dose MenC Group vs. 0 dose control Group

b. Single Dose MenC-CRM_{197} Group vs. 0 Dose Control Group

c. Single Dose MenC-TT Group vs. 0 Dose Control Group

d. Single Dose MenC-CRM_{197} Group vs. Two Dose MenC Group

e. Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT Group

f. The consistent limb group vs. the alternating limb group

6.3 Trial Participants

6.3.1 Overall Description of Trial Participants

Healthy 6-12 week old male and female infants born between 37 and 42 weeks of gestation would be recruited in four centres in the United Kingdom (Oxford, London, Bristol and Southampton) and one centre in Malta.

6.3.2 Inclusion Criteria

- Healthy male or female infants aged 6-12 weeks at the time of the first vaccination and who were born between 37 and 42 weeks of gestation
- Infants who are known to be free from medical problems as determined by a medical history and clinical examination
- Parents or guardians who are willing for their child to participate and who would be expected to comply with the requirements of the protocol
- Parents/guardians who have given informed consent for their child's participation in the study

6.3.3 Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- History of invasive meningococcal C disease
- Previous vaccination against meningococcal serogroup C disease
- Planned administration/administration of vaccines, since birth, other than the study vaccines (with the exception of oral rotavirus vaccine, Hepatitis B vaccine, Hepatitis A vaccine, Influenza vaccines and BCG, that can be administered 14 days before or after study vaccines and Varicella vaccine that can be administered 14 days before or after study vaccines or 4 weeks before or after other live vaccines, i.e. MMR. Varicella vaccine can also be given in the form of the combined Measles Mumps Rubella Varicella vaccine from 13 months of age).
- Receipt of investigational vaccines/drugs, other than the vaccines used in the study, within 30 days prior to receiving the first dose of the vaccines or their planned use during the study period, until 1 month after the administration of the final study vaccine (ie at 12 months of age).
- Confirmed or suspected immunosuppressive or immunodeficient conditions, including human immunodeficiency virus (HIV) infection.
- A family history of congenital or hereditary immunodeficiency.
- Receipt of more than 2 weeks of immunosuppressants or immune modifying drugs, (e.g. prednisolone >0.5mg/kg/day)
- History of allergy to any component of the vaccines.
- Major congenital defects or serious chronic illness.
- History of any neurologic disorders or seizures
- Acute disease at the time of recruitment as defined by the presence of a moderate or severe illness with or without fever (with the exception of minor illnesses such as diarrhoea, mild upper respiratory infection without fever). In such situations enrolment should be postponed until the participant has recovered.
- Administration of immunoglobulins and/or any blood products since birth or planned administration during the study period

- Parents who plan to move out of the geographical area where the study would be conducted.

6.3.4 Elimination criteria during the study

The following criteria will be checked at each visit subsequent to the first visit and if any become applicable during the study, it will not require withdrawal of the participant from the study but may determine the participant's evaluability in the completer's population (CP) analysis, in which case the data would be included in the Intention to Treat (ITT) analysis.

- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccine(s) during the study period, until 1 month after the administration of the final study vaccine (ie at 12 months of age).
- Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs during the study period. (For corticosteroids, this will mean prednisone, or equivalent, ≥ 0.5 mg/kg/day. Inhaled and topical steroids are allowed.)
- Administration of a vaccine not foreseen by the study protocol during the period starting from 30 days before each dose of vaccine(s) and ending 30 days after.
- Administration of immunoglobulins and/or any blood products during the study period.
- Administration of any of the vaccines used in the study outside of the stipulated time period
- Administration of any of the vaccines used in the study in an incorrect limb

6.3.5 Delaying criteria during the study visits

Participants with an acute illness would be recruited after the illness has resolved.

Vaccine administration will be delayed in case of acute illness or axillary temperature >38^{o}C.

Venesampling will be delayed for 1 week after the stopping of antibiotics in order to avoid interference with the MenC rSBA assays.

6.3.6 Study Procedures

The schedule of the following procedures has been listed in Appendix B.

Detailed description of study visits

i) Visit 1: Study Day 0: First vaccination visit (6-12 weeks)

- Written informed consent is obtained from the participant's parent/guardian.
- Inclusion/exclusion criteria will be checked prior to enrolment.
- A medical and vaccination history will be taken and recorded
- Any concomitant medications which are not listed in the exclusion criteria and thus allowed by the study protocol will be recorded.
- Pre-vaccination assessment of body temperature
- Randomisation
- Vaccination: intramuscular administration of one dose of the vaccines used in the study (according to group) will be administered as described in section 7.
- Explanation of diary cards to assess local and systemic adverse events for 4 days following vaccination

The vaccinees will be observed closely for at least 15 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.

The subjects' parents/guardians will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

ii) Visit 2: Study Month 1: Vaccination visit, at approximately 3 months of age (28-42 days after visit 1)

- Reporting of SAEs that might have occurred since the last visit.

- Collection of diary card
- Check of elimination criteria.
- Check of contraindications.
- Recording of any concomitant immunosuppressive medication or vaccines not foreseen by the study protocol
- Pre-vaccination assessment of axillary body temperature.
- Vaccination: intramuscular administration of one dose of the vaccines used in the study (according to group) will be administered.
- Explanation of diary card to assess local and systemic adverse events for 4 days following vaccination

The vaccinees will be observed closely for at least 15 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.

The subjects' parents/guardians will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

iii) Visit 3: Study Month 2: Vaccination visit, at approximately 4 months of age (28-42 days after Visit 2)

- Reporting of SAEs that might have occurred since the last visit.
- Collection of diary card
- Check of elimination criteria.
- Check of contraindications.
- Recording of any concomitant immunosuppressive medication or vaccines not foreseen by the study protocol
- Pre-vaccination assessment of axillary body temperature
- Vaccination: intramuscular administration of one dose of the vaccines used in the study (according to group) will be administered.

- Explanation of diary card to assess local and systemic adverse events for 4 days following vaccination

The vaccinees will be observed closely for at least 15 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.

The subjects' parents/guardians will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

iv) Visit 4: Study Month 3: Blood sampling visit, at approximately 5 months of age (28-42 days after visit 3)

- Reporting of SAEs that might have occurred since the last visit.
- Collection of diary card
- Check of elimination criteria.
- Recording of any concomitant immunosuppressive medication or vaccines not foreseen by the study protocol
- Collection of blood for serology (and B cells studies on a subset of participants): 5.0 ml of whole blood. If less than 4mL of blood is collected, the whole sample will be used serology assays. If 4-5mL of blood is collected the sample will be split in half and shared evenly between serology assays and B cell studies. 2-2.5mL whole blood will provide about 1mL of serum for serology.

The subjects' parents/guardians will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

v) Visit 5: Study Month 10: Vaccination visit, at 12 months of age (51 – 58 weeks)

- Check of exclusion criteria.
- Check of elimination criteria.
- Check of contraindications.

- Assessment and recording of medical history and vaccination history.
- Recording of any concomitant medication or vaccines not foreseen by the study protocol
- Retrospectively reporting of SAEs that might have occurred since the last visit of the primary phase.
- Pre-vaccination assessment of body temperature
- Collection of blood for serology (and B cell studies on a subset of participants): 7.5 ml of whole blood. If less than 4mL of blood is collected, the whole sample will be used serology assays. If 4-6mL of blood is collected the sample will be split in half and shared evenly between serology assays and B cell studies. If >6mL of blood is collected, 3mL will be used to provide at least 1mL of serum for serology assays, and the remainder will be used for B cell studies.
- Vaccination: intramuscular administration of one dose of the Hib-MenC vaccine and one dose of PCV13
- Explanation of diary card to assess local and systemic adverse events for 4 days following vaccination

The vaccinees will be observed closely for at least 15 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.

The subjects' parents/guardians will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

vi) Visit 6: Study month 10: Blood sampling visit, at 6 days following Hib-MenC booster vaccination (Performed on 256 participants; all participants in the control group and 64 participants each from the Two Dose MenC, Single Dose MenC-CRM$_{197}$ and Single Dose MenC-TT Groups as determined by randomisation at enrolment).

- Reporting of SAEs that might have occurred since the last visit.
- Collection of diary card

- Collection of blood for serology (and B cell studies on a subset of participants): 7.5 ml of whole blood. If less than 4mL of blood is collected, the whole sample will be used serology assays. If 4-6mL of blood is collected the sample will be split in half and shared evenly between serology assays and B cell studies (if applicable). If >6mL of blood is collected, 3mL will be used to provide at least 1mL of serum for serology assays, and the remainder will be used for B cell studies (if applicable).

vii) Visit 7: Study month 11: Blood sampling visit at approximately 13 to 14 months, 28-42 days after booster vaccination

- Reporting of SAEs that might have occurred since the Visit 5.
- Recording of any concomitant immunosuppressive medication or vaccines not foreseen by the study protocol
- Collection of blood for serology (and B cell studies on a subset of participants): 7.5 ml of whole blood. If less than 4mL of blood is collected, the whole sample will be used serology assays. If 4-6mL of blood is collected the sample will be split in half and shared evenly between serology assays and B cell studies. If >6mL of blood is collected, 3mL will be used to provide at least 1mL of serum for serology assays, and the remainder will be used for B cell studies.
- Administration of MMR

The vaccinees will be observed closely for at least 15 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.

viii) **Visit 8: Study month 22: Blood sampling visit at approximately 23 to 24 months, 11-12 months after booster vaccination**

- Reporting of SAEs that might have occurred since the Visit 7.
- Recording of any concomitant immunosuppressive medication or vaccines not foreseen by the study protocol
- Collection of blood for serology (and B cell studies on a subset of participants): 7.5 ml of whole blood. If less than 4mL of blood is collected, the whole sample will be used serology assays. If 4-6mL of blood is collected the sample will be split in half and shared evenly between serology assays and B cell studies. If >6mL of blood is collected, 3mL will be used

to provide at least 1mL of serum for serology assays, and the remainder will be used for B cell studies.

- Study conclusion

6.4 Informed Consent

The parents/legal guardians of the participants must personally sign and date the informed consent form before any study specific procedures are performed. Written and verbal versions of the participant information and Informed consent will be presented to the participants detailing the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the parent/legal guardian is free to withdraw his/her child from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. Specific consent will be obtained for the genetic analysis to be performed in the study; consent for this may be refused and the participant will still be eligible to participate in the other aspects of the study.

Parents/legal guardians will be allowed at least 24 hours to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of a parent/guardian dated signature and dated signature of the person who presented and obtained the informed consent. The person who obtained the consent would be suitably qualified and trained doctor or research nurse, and has been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participants. The original signed form will be retained at the study site.

6.4.1 Screening and Eligibility Assessment

This is a multi- centre study which is planned to be conducted by the Oxford Vaccine Group, Bristol Children's Vaccine Centre, Southampton University Hospital, and St George's Vaccine Institute in the UK and the Malta Children's Vaccine Group in Malta. An invitation letter, which describes

the study and which includes a reply form, would be sent to parents. Parents who are happy to take part are requested to contact the local study team and would then be given the opportunity to discuss the study. An appointment would then be set up for parents willing for their child to take part in the study.

In the UK, eligible participants will be identified through the child health computers of the Primary Care Trusts. Investigators or other allied health professionals at the research sites may also approach parents/carers opportunistically on post-natal wards. Investigators may also ask GPs and Health Visitors within their local network to identify and approach potential parents/carers, who have children of suitable age to participate in the study during the enrolment period. Children of parents interested in participating in the study would then be visited at their homes and recruited to the study after obtaining informed consent.

In Malta eligible participant will be identified through the Birth Register held at Mater Dei Hospital. Children of parents interested in taking part in the study would be given an appointment at the Paediatric Day Care Unit in Mater Dei Hospital and recruited after obtaining informed consent.

Inclusion and exclusion criteria (specified in section 6.3.2 and 6.3.3) will be checked on the first visit during which enrolment and randomisation will take place. Screening procedures are detailed in section 6.4.

The following data will be documented on the CRFs:

Demographics

The date of birth, gender, and ethnicity will be recorded.

Medical History

Details of any history of disease or surgical interventions in all systems will be recorded:

Physical Examination

Axillary temperature will be recorded.

6.4.2 Baseline Assessments

Measurement of axillary temperature together with a check of the inclusion and exclusion criteria to determine if the infant is healthy will be performed.

6.4.3 Randomisation and Code-breaking

Randomisation for all UK and Maltese participants will take place at the Oxford Vaccine Group in the UK on a 10:10:7:4 basis to the four study groups (Two Dose MenC, Single Dose MenC-CRM_{197}, Single Dose MenC-TT and Control Group). Each study group would then be randomised on a 1:1 basis to the consistent or alternate limb subgroup (a and b). 64 children from each of the Two Dose MenC, Single Dose MenC-CRM_{197} and Single Dose MenC-TT groups will also be randomly selected, as well as all the children in the Control group, to have the 12 month + 6 day blood visit, . Randomisation will occur by means of opening in a sequential manner a sealed envelope containing the study group the child is to be randomised to. These envelopes will be prepared by the study statistician and the list held by the statistician and the study sites.

Due to the nature of the study, the study is unblinded, therefore no unblinding procedure is required. Analysis of immunogenicity and safety data will be performed by study staff and laboratory staff in linked-anonymised form such that lab samples and diary cards will only be labelled with participant initials/number. In the event of twins being enrolled from the same family, the diary card may be labelled with the participant's first initial or name.

6.4.4 Subsequent assessments

A detailed description of subsequent assessments may be found in section 6.3.6.

6.5 Definition of End of Trial

The end of trial is the date of completion of all study assays and after administration of any booster doses of vaccines that may be necessary for participants who have sub-optimal results.

6.6 Discontinuation/ Withdrawal of Participants from Study Treatment

Each participant has the right to withdraw from the study at any time. In addition, the investigator may discontinue a participant from the study at any time if the investigator considers it necessary for any reason including:

Ineligibility (either arising during the study or retrospective having been overlooked at screening)

Significant protocol deviation

Significant non-compliance with study requirements

An adverse event which requires discontinuation of the study medication or results in inability to continue to comply with study procedures

Consent withdrawal

Lost to follow up

If a participant is withdrawn from the study the blood samples and any safety data already collected would be included in the analysis.

The reason for withdrawal will be recorded in the CRF.

If the participant is withdrawn due to an adverse event, the investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

6.7 Source Data

In this study the CRF will be used as the source document for collection of demographic data, documentation of inclusion and exclusion criteria, medical and vaccination history and the findings on physical examination. If a participant sustains a SAE then hospital records would be accessed after obtaining parental/guardian consent. Identifying information would be stored separately from the CRF.

All documents will be stored safely in locked cupboards during the study and archived for 3 years after the child turns 18 years of age. Access to these documents would only be available to the investigators, monitors and auditors directly involved in the study. On all study-specific documents, other than the signed consent, the participant will be referred to by the study participant number/initials, not by name.

7. TREATMENT OF TRIAL PARTICIPANTS

7.1 Description of Study Treatment

This is a Phase IV clinical trial and all vaccines used in this study are licensed.

a) **MenC-CRM$_{197}$: (*Menjugate*, Novartis Vaccines and Diagnositics):** conjugate *Neisseria meningitidis* serogorup C polysaccharide-protein conjugate vaccine[33].

b) **MenC-TT: (*NeisVac-C*, Baxter Vaccines):** conjugate *Neisseria meningitidis* serogorup C polysaccharide-protein conjugate vaccine[34].

c) **DTaP-IPV-Hib: (*Pediacel*, Sanofi Pasteur, MSD):** combined diphtheria, tetanus, acellular pertussis, inactivated polio, *Haemophilus influenzae* type b (DTPa-IPV-Hib) vaccine[35].

d) **PCV13: (*Prevenar-13*, Wyeth Vaccines):** thirteen valent pneumococcal polysaccharide-protein conjugate vaccine[36].

e) **Hib-MenC: (*Menitorix*, GlaxoSmithKline Biologicals):** combined *Haemophilus influenzae* type b and *Neisseria meningitidis* serogroup C polysaccharide-protein conjugate vaccine[37].

A measles mumps and rubella (MMR) vaccine will be administered during visit 7 but will not form part of the study evaluation.

7.1.1 Dosage and administration

Details on the dosage and administration of each vaccine are given in the study flow chart in Appendix A.

The vaccinees will be observed closely for 15 minutes following the administration of vaccines, with appropriate medical treatment readily available in case of a rare anaphylactic reaction.

Vaccination will be performed by a registered doctor or nurse.

Injection technique

In order to ensure proper intramuscular injection of the study vaccines, a 23G (0.5mm in diameter) needle of at least 1 inch (2.54 cm) length will be used. All vaccines will be administered intramuscularly. The following injection technique will be used[38].

Injections in the thigh

The needle is inserted in the anterolateral aspect of the middle third of the thigh in the vastus lateralis muscle at an angle of 90º to the long axis of the leg with the subject supine or whilst held in the 'cuddle'. If more than 1 injection is to be given in to the thigh then they will be given at least 2.5 cm apart.

Injections in the deltoid

The injection in the deltoid will be done using a 25 mm (1 inch), 23 gauge needle. The needle is inserted in the deltoid at 90º to the long axis of the arm.

7.2 Storage of Study Treatment

UK

Vaccines are kept in a locked room in each of the study centres. The refrigerator temperature is regularly monitored and any temperature deviation outside the +2ºC to +8ºC will be communicated to a designated person via a pager system. In the case of refrigerator failure all vaccines will be transferred to a backup vaccine fridge.

Malta

Vaccines are kept locked in a fridge in a locked room at the Paediatric Day Care Unit in Mater Dei Hospital. The refrigerator temperature is regularly monitored and any temperature deviation outside the +2ºC to +8ºC will be communicated to the chief engineer in hospital via the Building Management System. The chief engineer would then inform a designated study member via a

pager system. In the case of refrigerator failure all vaccines will be transferred to a backup vaccine fridge.

7.3 Compliance with Study Treatment

Participants are vaccinated during the designated vaccine visits.

7.4 Accountability of the Study Treatment

Menjugate, NeisVac-C, Pediacel, Prevenar-13, Menitorix and the MMR vaccine will be supplied by Movianto to the each of the UK study sites. The Oxford Vaccine Group would supply all vaccines to the Malta site. All unused vaccines and returned used vials will be retrieved at the end of the study. The vaccines will be collected from the vaccine fridge at the beginning of each day scheduled for study visits. All used vaccine boxes will be returned to OVG at the end of each study day.

7.5 Concomitant Medication

Throughout the study the investigators will not prescribe any concomitant medications (with the possible exception of local anaesthetic cream if required for venepuncture). Only healthy infants will be recruited in the study and if the participant receives any of the vaccines/medications included in the exclusion criteria then the participant will not be enrolled but if already enrolled would be included in the ITT analysis but excluded from the according to protocol (ATP) analysis.

The following medications are contraindicated:

1) Concomitant vaccinations, except the study vaccines, rotavirus vaccine, the Hepatitis A and B vaccines, Influenza vaccines, BCG and the Varicella vaccine, that can be administered 14 days before or after study vaccines. (The Varicella vaccine can also be administered 4 weeks before or after other live vaccines, i.e. MMR. Varicella vaccine can also be given in the form of the combined Measles Mumps Rubella Varicella vaccine from 13 months of age).

2) Immunosuppressants e.g. azathioprine, cyclosporine, or prednisolone at a dose of >0.5mg/kg./day

or >20mg/day for more than 2 weeks

3) Immunoglobulins and/or any blood product

8 SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event (AE)

An AE or adverse experience is:

Any untoward medical occurrence in a patient or clinical investigation participant administered a medicinal product, which does not necessarily have to have a causal relationship with this treatment (the study medication). Since the medicinal products used in this study are vaccines the definition of the specific adverse events of fever and local reactions will follow the Brighton collaboration guidelines[39-42].

An AE can be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study medication, whether or not considered related to the study medication.

8.1.2 Adverse Reaction (AR)

All untoward and unintended responses to a medicinal product related to any dose.

The phrase "responses to a medicinal product" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.

All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

8.1.3 Severe Adverse Events

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe", which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based

on patient/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.1.4 Serious Adverse Event or Serious Adverse Reaction

A serious adverse event or reaction is any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Other important medical events*

*Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.1.5 Expected Serious Adverse Events/Reactions

No serious adverse events are expected from the vaccines administered in this study. All vaccines used are already licensed and used in several European countries.

8.1.6 Suspected Unexpected Serious Adverse Reactions

A serious adverse reaction, the nature or severity of which is not consistent with the summary of product characteristics.

8.1.7 Medically Significant adverse events

An adverse event that results in a consultation with a physician or other health professional

8.2 Reporting Procedures for All Adverse Events

All AEs attributed to the administration of DTaP-IPV-Hib, MenC-CRM$_{197}$, MenC-TT, PCV13 and Hib-MenC observed by the investigator or reported by the participant, will be recorded on the CRF. All solicited and unsolicited adverse events will be recorded for 4 days following immunisation on diary cards. Unsolicited medically significant adverse events occurring between visit 1 and 4 will be recorded on diary cards, as will those occurring between visit 5 and 7.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to study medication, other suspect drug or device and action taken. The relationship of AEs to the study medication will be assessed by a medically qualified investigator.

All SAEs will be recorded through the whole study. Follow-up information should be provided as necessary.

These AEs considered to be related to the study medication as judged by a medically qualified investigator or the sponsor will be followed until resolution or the event is considered stable. All related AEs that result in a participant's withdrawal from the study or are present at the end of the study, should be followed up until a satisfactory resolution occurs.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from the study (see section 6.6). A participant may also be voluntarily withdrawn from the study by a parent/guardian due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of study assessment

and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable.

8.3 Reporting Procedures for Serious Adverse Events

The Oxford Radcliffe Hospitals Trust / University of Oxford Trials Safety Group (TSG) will undertake to review immediately reported SAEs for the study. They will meet at regular intervals and consider:

- Occurrence and nature of adverse events
- Whether additional information on adverse events is required
- Consider taking appropriate action where necessary to halt trials
- Act / advise on incidents occurring between meetings that require rapid assessment (eg SUSARs)

All SAEs (from all sites) must be reported to the University of Oxford CTRG within one working day of discovery or notification of the event. CTRG will perform an initial check of the information and ensure that it is reviewed at the next TSG meeting. All SAE information must be recorded on an SAE form and faxed to CTRG. Additional information received for a case (follow-up or corrections to the original case) need to be detailed on a new SAE form and faxed to CTRG.

The CI will report all SUSARs to the Competent Authorities (MHRA in the UK and MA in Malta) and the Research Ethics Committee concerned. SUSARs occurring in the UK will only be reported to the MHRA, whereas SUSARs occurring in Malta will be reported to the MHRA and the MA. All SUSARs reported to the MHRA and MA will be reported to EudraVigilance. Fatal or life-threatening SUSARs must be reported within 7 days and all other SUSARs within 15 days. The CI will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants. The CI will also report SAEs that are considered related to the administration of study vaccines, or to study procedures, in an annual safety report to the

Competent Authorities (MHRA in the UK and MA in Malta) and the Research Ethics Committee concerned. All safety reports to the MHRA must be made electronically from the 1st of September 2010.

In addition to the expedited reporting above, the CI shall submit once a year throughout the clinical trial or on request a safety report to the Competent Authority (MHRA in the UK and MA in Malta) and Ethics Committee.

9 STATISTICS

Endpoint analysis will be carried out after all participants have completed visit 7 (13 month blood sample). An additional analysis will be carried out after the final follow-up visit at 24 months of age (visit 8). An interim analysis will be carried out after all participants have completed visit 4 (5 month blood sample).

9.1 Description of Statistical Methods

A) ***Description of demographics***

Baseline demographic characteristics (age in weeks, gender, race) of each study dose group will be tabulated.

The number of subjects, mean age (plus range and standard deviation) by gender of the enrolled subjects, as a whole, and per study dose group, will be reported.

The trial completion rate will be reported, together with the time and number of withdrawals and the reason for withdrawal, for each study dose group.

B) ***Analysis of Immunogenicity***

Descriptive analysis:

Summary statistics will be calculated for the following outcomes assessed at 1 month after completion of the primary immunisation, at 12 months plus 6 days and at 12 months plus 28 days and 24 months after the Hib-MenC booster dose for each study dose group. Continuous variables will be reported as means and standard deviations, and categorical variables will be reported as counts and percentages, together with the numbers of observations in all cases.

- MenC rSBA GMT
- Number of MenC specific memory B cells in the blood
- *S. pneumoniae* IgG GMC response for each of the 13 serotypes
- Pneumococcal serotype specific B cell phenotype
- Anti-PRP IgG GMC response
- Anti-tetanus toxoid GMC response
- Percentages of subjects with MenC rSBA ≥1:8 and ≥ 1:128
- Percentages of subjects with MenC rSBA GMT ≥ 1000
- Percentages of subjects with *S. pneumoniae* IgG ≥ 0.35 μg/ml for each of the 13 serotypes
- Percentages of subjects with anti-PRP IgG ≥0.15 μg/ml and ≥ 1.0 μg/ml
- Percentages of subjects with anti-tetanus toxoid > 0.1 IU/ml

Statistical Analysis

The analysis of the outcome variables will be performed on the intention–to-treat (ITT) population for the reduced dose MenC objectives and the completers population for the alternating limb objectives. A subject is included in the ITT population if they have at least one dose and at least one post-baseline assessment, and in the completers population if they receive all doses of vaccine and have all planned assessments.

An analysis of variance will be performed when the outcome variable is continuous. The model will contain the terms dose group (4 levels) and alternating limb group (2 levels). A term for centre will be included in the model. The results of a comparison between any two levels of a factor will be reported as a treatment effect with 95% confidence interval.

For the analysis of variables relating only to 0 dose control group the term relating to dose group will be omitted.

The following outcomes will be analysed (comparisons of interest are listed):

1. MenC rSBA GMT response variable assessed 28 days after the Hib-MenC 12 month booster dose
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT Group
2. MenC rSBA GMT response variable assessed 6 days after the Hib-MenC 12 month booster dose
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

3. MenC rSBA GMT response variable assessed 12 months after the Hib-MenC 12 month booster dose
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group

c. Single dose MenC-TT Group vs 0 dose control Group

d. Two dose MenC Group vs. 0 dose control Group

e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

3. MenC rSBA GMT response variable (persistence) assessed at 12 months of age

a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group

b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group

c. Single dose MenC-TT Group vs 0 dose control Group

d. Two dose MenC Group vs. 0 dose control Group

e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

4. MenC rSBA GMT response variable assessed at 5 months of age

a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group

b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group

c. Single dose MenC-TT Group vs 0 dose control Group

d. Two dose MenC Group vs. 0 dose control Group

e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

5. the numbers of MenC specific memory B cells in the blood at 5 months of age

a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group

b. Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT Group

6. the numbers of MenC specific memory B cells in the blood at 12 months of age

a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group

b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group

c. Single dose MenC-TT Group vs 0 dose control Group

d. Two dose MenC Group vs. 0 dose control Group

e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

7. the numbers of MenC specific memory B cells in the blood at 6 days after the Hib-MenC 12 month booster dose
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

8. The numbers of specific memory B cells in the blood at 28 days after the Hib-MenC 12 month booster dose
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group
9. The numbers of specific memory B cells in the blood at 11-12 months after the Hib-MenC booster dose
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

10. The *S. pneumoniae* IgG GMC response variable for each of the thirteen serotypes separately at 5 months of age
    a. the consistent limb group vs. the alternating limb group

11. the *S. pneumoniae* IgG GMC response variable for each of the thirteen serotypes separately, (persistence) at 12 months of age
    a. the consistent limb group vs. the alternating limb group

12. the *S. pneumoniae* IgG GMC response variable for each of the thirteen serotypes separately at 13 months of age after the PCV-13 booster dose
    a. the consistent limb group vs. the alternating limb group

13. 12. the *S. pneumoniae* IgG GMC response variable for each of the 13 serotypes separately at 24 months of age.
    a. the consistent limb group vs. the alternating limb group

14. the anti-PRP IgG GMC response variable at 5 months of age
    a. the consistent limb group vs. the alternating limb group

15. the anti-PRP IgG GMC response variable (persistence) at 12 months of age
    a. the consistent limb group vs. the alternating limb group

16. the anti-PRP IgG GMC response variable at 13 months of age, after the Hib-MenC booster dose
    a. the consistent limb group vs. the alternating limb group

17. the anti-PRP IgG GMC response variable at 24 months of age
    a. the consistent limb group vs. the alternating limb group

18. the anti-tetanus toxoid GMC response variable at 5 months of age ,

a. the consistent limb group vs. the alternating limb group

19. the anti-tetanus toxoid GMC response variable (persistence) at 12 months of age
    a. the consistent limb group vs. the alternating limb group

20. the anti-tetanus toxoid GMC response variable at 13 months of age
    a. the consistent limb group vs. the alternating limb group

21. the anti-tetanus toxoid GMC response variable at 24 months of age
    a. the consistent limb group vs. the alternating limb group

The binary variables will be analysed using logistic regression. The model will contain the terms dose group (4 levels), and alternating limb group (2 levels). There will also be a term for centre. The results of a comparison between two levels of a factor will be reported as an odds ratio and as a risk difference, with 95% confidence intervals.

For the analysis of variables relating only to 0 dose control group the term for dose group will be omitted.

The following binary outcomes will be analysed:

1. percentage of infants with MenC rSBA $\geq$ 1:8 and $\geq$ 1:128 assessed 6 days after the 12 month Hib-MenC booster
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group

b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group

c. Single dose MenC-TT Group vs 0 dose control Group

d. Two dose MenC Group vs. 0 dose control Group

e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

2. percentage of infants with MenC rSBA $\geq$ 1:8 and $\geq$ 1:128 assessed 28 days after the 12 month Hib-MenC booster

   a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group

   b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group

   c. Single dose MenC-TT Group vs 0 dose control Group

   d. Two dose MenC Group vs. 0 dose control Group

   e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

3. percentage of infants with MenC rSBA $\geq$ 1:8 and $\geq$1:128 assessed at 12 months of age

   a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group

   b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group

   c. Single dose MenC-TT Group vs 0 dose control Group

   d. Two dose MenC Group vs. 0 dose control Group

   e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

4. percentage of infants with MenC rSBA $\geq$ 1:8 and $\geq$1:128 at 5 months of age

   a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group

   b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group

   c. Single dose MenC-TT Group vs 0 dose control Group

   d. Two dose MenC Group vs. 0 dose control Group

   e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

5. percentage of infants with MenC rSBA $\geq$ 1:8 and $\geq$1:128 at 24 months of age

   a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group

b. Single dose MenC-CRM$_{197}$ Group vs. 0 dose control Group

c. Single dose MenC-TT Group vs 0 dose control Group

d. Two dose MenC Group vs. 0 dose control Group

e. Single dose MenC-CRM$_{197}$ Group vs Single dose MenC-TT Group

6. percentage of infants with MenC rSBA GMT $\geq$ 1000 assessed 6 days after the 12 month Hib-MenC booster

   a. Single dose MenC-CRM$_{197}$ Group vs. Two dose MenC Group

   b. Single dose MenC-CRM$_{197}$ Group vs. 0 dose control Group

   c. Single dose MenC-TT Group vs 0 dose control Group

   d. Two dose MenC Group vs. 0 dose control Group

   e. Single dose MenC-CRM$_{197}$ Group vs Single dose MenC-TT Group

7. percentage of infants with MenC rSBA GMT $\geq$ 1000 assessed 28 days after the 12 month Hib-MenC booster

   a. Single dose MenC-CRM$_{197}$ Group vs. Two dose MenC Group

   b. Single dose MenC-CRM$_{197}$ Group vs. 0 dose control Group

   c. Single dose MenC-TT Group vs 0 dose control Group

   d. Two dose MenC Group vs. 0 dose control Group

   e. Single dose MenC-CRM$_{197}$ Group vs Single dose MenC-TT Group

8. percentage of infants with *S. pneumoniae* IgG $\geq$ 0.35 μg/ml for each of the 13 serotypes at 5 months of age

   a. the consistent limb group vs. the alternating limb group

9. percentage of infants with *S. pneumoniae* IgG $\geq$ 0.35 μg/ml for each of the 13 serotypes at 12 months of age

   a. the consistent limb group vs. the alternating limb group

10. percentage of infants with *S. pneumoniae* IgG ≥ 0.35 μg/ml for each of the 13 serotypes at 13 months of age after the PCV-13 booster dose
    a. the consistent limb group vs. the alternating limb group

11. percentage of infants with *S. pneumoniae* IgG ≥ 0.35 μg/ml for each of the 13 serotypes at 24 months of age after the PCV-13 booster dose
    a. the consistent limb group vs. the alternating limb group

12. percentage of infants with anti-PRP IgG ≥ 0.15 μg/ml and ≥ 1.0 μg/ml at 5 months of age
    a. the consistent limb group vs. the alternating limb group

13. percentage of infants with anti- PRP IgG ≥ 0.15 μg/ml and ≥ 1.0 μg/ml at 12 months of age
    a. the consistent limb group vs. the alternating limb group

14. percentage of infants with anti-PRP IgG ≥ 0.15 μg/ml and ≥ 1.0 μg/ml at 13 months of age, after the Hib-MenC booster dose
    a. the consistent limb group vs. the alternating limb group

15. percentage of infants with anti-PRP IgG ≥ 0.15 μg/ml and ≥ 1.0 μg/ml at 24 months of age
    a. the consistent limb group vs. the alternating limb group

16. percentage of infants with anti- tetanus toxoid >0.1 IU/ml at 5 months of age
    a. the consistent limb group vs. the alternating limb group

17. percentage of infants with anti- tetanus toxoid >0.1 IU/ml at 12 months of age
    a. the consistent limb group vs. the alternating limb group

18. percentage of infants with anti- tetanus toxoid >0.1 lU/ml at 13 months of age
    a. the consistent limb group vs. the alternating limb group

19. percentage of infants with anti- tetanus toxoid >0.1 lU/ml at 24 months of age
    a. the consistent limb group vs. the alternating limb group

C) Analysis of safety

The data summaries will report the total number and percentage of infants experiencing each type of adverse event, and the total number and percentage of infants experiencing at least one adverse event of any type, for each dose group, and the total number of infants in each dose group, for local and general adverse events during the 4-day follow-up period after each MenC (including Hib-MenC), DTaP-IPV-Hib and PCV13 vaccination. The classification of severity of local adverse events and fever will follow the Brighton collaboration guidelines [35 - 38].

Where the adverse event is further classified with a grade (1, 2, 3) related to severity, the summary reports will include the numbers in each class.

Statistical analysis

The binary variables will be analysed using logistic regression. The model will contain the terms dose group (4 levels) and alternating limb group (2 levels). A term for centre will be included in the model. The results of a comparison between two levels of a factor will be reported as an odds ratio and as a risk difference, with 95% confidence intervals.

The following variables will be analysed:

Local adverse events

1. percentage of infants with each type of adverse event, and at least one local adverse event after each dose of MenC vaccine (at 3 and 4 months)
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT Group

2. percentage of infants with each type of adverse event, and at least one local adverse event after each dose of DTaP-IPV-Hib (at 2, 3 and 4 months) and PCV13 (at 2 and 4 months)
    a. the consistent limb group vs. the alternating limb group

3. percentage of infants with each type of adverse event, and at least one local adverse event after the 12 month booster Hib-MenC and PCV13 vaccination
    a. Two dose MenC Group vs. 0 dose control Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    d. Single dose MenC-TT Group vs 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group
    f. the consistent limb group vs. the alternating limb group

Systemic adverse events

1. percentage of infants with each type of adverse event, and at least one systemic adverse event after MenC (3 and 4 months), DTaP-IPV-Hib (2, 3 and 4 months) and PCV13 (2 and 4 months) vaccination
    a. Two dose MenC Group vs. 0 dose control Group

b. Single dose MenC-CRM$_{197}$ Group vs. 0 dose control Group

c. Single dose MenC-CRM$_{197}$ Group vs. Two dose MenC Group

d. Single dose MenC-TT Group vs 0 dose control Group

e. Single dose MenC-CRM$_{197}$ Group vs Single dose MenC-TT Group

f. the consistent limb group vs. the alternating limb group

2. percentage of infants with each type of adverse event, and at least systemic one adverse event after the 12 month booster Hib-MenC and PCV13 vaccination

    a. Two dose MenC Group vs. 0 dose control Group

    b. Single dose MenC-CRM$_{197}$ Group vs. 0 dose control Group

    c. Single dose MenC-CRM$_{197}$ Group vs. Two dose MenC Group

    d. Single dose MenC-TT Group vs 0 dose control Group

    e. Single dose MenC-CRM$_{197}$ Group vs Single dose MenC-TT Group

    f. The consistent limb group vs. the alternating limb group

9.2 The Number of Participants

This is a non-inferiority trial assessing whether there is any difference in the antibody level between infants who receive no priming doses of MenC and those who receive 1 priming dose or 2 priming doses of MenC-CRM$_{197}$.

The total sample size for the study is 498 participants randomised on a 10:10:7:4 basis to the 4 study groups. This will result in 160 participants in the Two Dose MenC group, 160 in the Single Dose MenC-CRM$_{197}$ group, 114 in the Single Dose MenC-TT group, and 64 in 0 dose MenC (Control) group. Based on a mean (SD) log SBA of 3.47 (0.9) at 1 month after booster for 2 dose MenC[12], 160 participants per group are required for the comparison between the Single Dose MenC-CRM$_{197}$ and the Two dose MenC groups to detect 10% non-inferiority with a 2.5% level of

significance (1-sided), 90% power and allowing for a 12.5% drop-out rate. An additional 114 participants will be required for the Single Dose MenC-TT group to allow a comparison with the Single Dose MenC-CRM_{197} primed group, allowing for a 12.5% drop-out rate.

No sample size calculation for the alternate limb component of the trial is included as the data generated here will be pilot data.

Non-inferiority margi	Estimated difference	Power	Sample size required per group	Total sample size required
5%	-0.18	90%	526	1052
		80%	393	786
7.5%	-0.26	90%	253	506
		80%	190	380
10%	**-0.35**	**90%**	**140***	**280**
		80%	105	210

** Requires 160 participants per group when allowing for a 'dropout' rate of 12.5%*

A previous study has shown the mean (SD) logSBA=2.85 (0.48) at 1 month following a 12 month dose of MenC vaccine with no prior MenC immmunisation[43]. Making the reasonable assumption that the SBA titres are relatively stable in the one month period after the vaccination, these statistics were used to calculate a sample size for a comparison between the one dose group and 0 dose control group at 6 days after the 12 month vaccination.

To detect a difference of 0.285 (10% of 2.85) between groups of equal size of 56 with a significance level of 0.05 (2-sided), would provide a power of 88%. Allowing for 12.5% drop outs, the initial size of 0 dose control group should be 64 infants. This therefore enables the 6 day blood test to be performed on only a subset of participants (64) in each of the other groups.

Assumptions

Sample size calculation has been extrapolated from the following MenC rSBA data taken from the infant MenC study[39], at various time points:

12 months

Log10 rSBA : mean= 0.9441395 Std. Dev = 0.8876458

Log10 IgG : mean= 0.02791 Std. Dev = 0.4380407

6 days-post 12 months booster

Log10 rSBA : mean= 3.672566 Std. Dev = 0.2518598

Log10 IgG : mean= 1.211286 Std. Dev.= 0.1606558

8 days-post 12 months booster

Log10 rSBA : mean= 4.264592 Std. Dev = 0.584236

Log10 IgG : mean= 1.72307 Std. Dev.= 0.3447821

30 days-post 12 months booster

Log10 rSBA: mean= 3.784377 Std. Dev = 0.4211916

Log10 IgG: mean= 1.446253 Std. Dev.= 0.3564013

9.3 Hypothesis Test

Non-inferiority of Single Dose MenC-CRM_{197} schedule over Two Dose MenC schedule would be demonstrated if the null hypothesis bellow is rejected.

Null Hypothesis H0:

The mean of ($\log_{10}$) of MenC rSBA values of the active current vaccine schedule (Two Dose) group μ_{exp}, exceeds the mean of ($\log_{10}$) of MenC rSBA values of the experimental vaccine schedule (Single Dose) group μ_{ac} by at least a margin "M" (M >0).

$$\mathbf{H_0}: \mu_{exp} - \mu_{ac} \leq -M$$

Alternative Hypothesis H_a: The mean of ($\log_{10}$) of MenC rSBA values of the active current vaccine schedule (Two Dose) may indeed have higher mean of ($\log_{10}$) of MenC rSBA values compared to mean of ($\log_{10}$) of MenC rSBA of the experimental vaccine schedule (Single Dose) group, but the difference is not more than "M" (M >0).

$$\mathbf{Ha}: \quad \mu_{exp} - \mu_{ac} > -M$$

- μ_{exp} refers to the population mean of $\log_{10}$ of MenC rSBA values for the experimental vaccine schedule (Single dose) group
- μ_{ac} refers to the population mean of $\log_{10}$ of MenC rSBA values of the active current vaccine schedule (Two Dose) group.

For the above comparison, M, the non-inferiority margin, is chosen to be 0.35, this value corresponds to a 10% of the value 3.47 $\log_{10}$ (MenC rSBAGMT) at 1 month after booster (Reference 14, Borrow et al)

μ_{exp} and μ_{ac} can be estimated as $\log_{10}$ (MenC rSBAGMT) in (Single Dose) and (Two Dose) groups respectively (which are equivalent to the arithmetic mean of $\log_{10}$ MenCrSBA in (Single Dose) group and the arithmetic mean of $\log_{10}$ MenCrSBA in (Two Dose) group).

The nominal significance level for this one-sided test is considered α=0.025 and power is 90%.

If the null hypothesis is rejected we can then conclude non-inferiority of the experimental Single Dose priming vaccine schedule compared to Two Dose priming vaccine schedule.

9.4 Criteria for the Termination of the Trial.

The end of trial is the date of the completion of all study assays. This is a Phase IV trial so it is not expected that the clinical trial would terminated prematurely because of adverse effects on the participants. It is possible, however, that an increase in the incidence of serogroup C meningococcal disease could change the risk/ benefit profile of participants (particularly those in the 0 dose MenC (Control) group). No such increase in MenC disease is anticipated, however if this were to occur the appropriateness or otherwise of continuing the study will be considered by the data monitoring committee and trial steering committee.

9.5 Procedure for Accounting for Missing, Unused, and Spurious Data.

Missing data will not be accounted for.

9.6 Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Any significant deviation from the original statistical plan will be discussed with the study statistician and, as appropriate, reflected in any publications arising out of this study.

9.7 Inclusion in Analysis

The immunogenicity analysis will be performed on both an intention to treat (ITT) population (all participants completing their designated primary and booster stage immunisation courses, and providing blood samples at the timepoint being analysed) and on a completers protocol (CP) population (all participants in the intention to treat analysis completing the study without any significant protocol deviations).

10 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

11 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

Regular monitoring will be performed according to ICH GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. A risk based monitoring plan will be developed and used by the monitors to verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, relevant standard operating procedures, GCP and all applicable regulatory requirements.

A trial steering committee will be formed that will include, but not be limited to, the chief investigator, a statistician, a quality assurance manager and project manager.

12 ETHICS

This study will recruit infants who are not old enough to give consent. Informed consent will be obtained from the parent/legal guardian of each participant.

12.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

12.2 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

12.3 Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to the Research Ethics Committee (REC) and a favourable opinion will be sought in both countries. Approval from the MHRA and from the relevant Research and Development departments in the Primary Care Trusts where the study will be conducted will be obtained prior to starting the study in the UK. In Malta approval from the MA and from the Chairman of Paediatrics and the Hospital's Superintendent at Mater Dei Hospital will be obtained prior to starting the study. The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

12.4 Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participant number on the CRF and any electronic database. All documents will be stored securely and only accessible by trial staff and authorised personnel. The study will comply with the Data Protection Act which requires data to be anonymised as soon as it is practical to do so. Given the possibility of enrolling twins into this study the study diaries will be labelled with the participant's first name to aid recording of data relevant to the appropriate child.

12.5 Other Ethical Considerations

Participants might sustain pain at the site of venepuncture. Pain will be reduced by applying a local anaesthetic cream which will temporarily desensitise the skin over the venepuncture site. Up to two attempts may be made to obtain a blood sample at each visit, if the parent gives verbal consent at the time of the procedure for a second attempt. If no blood is obtained, a second visit may be made only if the parent agrees to this.

Pain associated with immunisation will be reduced by allowing the infant to breastfeed or by using distraction methods.

If a participant was found to have antibody levels below the threshold of response for any of the vaccines received in this study on the 24 month blood sample (or after the 13 month blood sample if the participant did not return for the final study visit), then study staff would administer a booster dose of the relevant vaccine at a later visit to be organised with the participant's family. This booster may be administered by the participant's GP if necessary (for example if the participant has moved out of the area) and if this agreed with the participant's family and GP.

13 DATA HANDLING AND RECORD KEEPING

All study data will be entered into a sharepoint based database system.

The participants will be identified by a study specific participant number and/or initial in any database. The name and any other identifying detail will NOT be included in any study data electronic file.

14 FINANCING AND INSURANCE

Part financing has been obtained from the BRC feasibility and sustainability fund. Insurance will be provided by the University of Oxford in the UK.

15 PUBLICATION POLICY

Publications arising from this paper will be coordinated by the chief investigator.

16 STORAGE AND HANDLING OF SAMPLES

Samples will not be labelled with information that directly identifies the subjects but will be coded with the participant number/initials for the subject. After blood centrifugation and serum separation, samples will be stored at -20°C until analysis can be performed. Storage and handling

of biological samples will be according to the site specific Laboratory Standard Operating Procedures (SOPs).

B cell responses will be determined in participants enrolled at Oxford and, potentially, other study sites according to local arrangements. At Oxford this analysis will be conducted on fresh samples. For samples obtained elsewhere the analysis will be conducted either at Oxford on frozen samples transferred from the other study sites or analysed locally on fresh or frozen samples. Where applicable, antigen specific B cell responses to vaccines received in the study will be analysed in the Oxford Vaccine Centre laboratory at the Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford by ELISPOT and flow cytometric assays depending on the available volumes of blood.

DNA will be extracted from a sample of blood according to standard protocol. The cellular plug resting after centrifugation of the sample of blood may be kept in -20° C for later DNA extraction. These blood clots and the extracted DNA will be stored in the Biobank of the Oxford Vaccine Centre laboratory at the Centre of Clinical Vaccinology and Tropical Medicine, University of Oxford or at local study sites for later analysis for genetic polymorphisms. Genetic testing will be performed on these samples before January 2015.

17 STUDY SUBJECTS

17.1 Disposition of subjects

17.1.1 MenC Groups

In this multicentre study performed in the UK and Malta 509 subjects aged between 6.9 – 10.6 weeks were recruited and randomised into 4 groups to receive one dose of the MenC-CRM197 vaccine at 3 months of age (Group 1); two doses of the MenC-CRM197 vaccine at 3 and 4 months of age (Group 2), no MenC vaccine priming doses (Group 3: control group) or one dose of the MenC-TT vaccine (Group 4). There were 449 subjects recruited in 4 centres in the UK: 404 in Oxford, 30 in Bristol, 6 in Southampton and 9 in London and 60 subjects enrolled in one centre in Malta. The baseline demographic characteristics of each study dose group are summarised in tables 1 - 2. The CONSORT diagram for the Intention to Treat (ITT) Population is shown in Figure 1. Tables 3.1 and 3.2 summarise the demographic characteristics of the ITT population and the completers population (CP).

The trial completion rate was reported, together with the time and number of withdrawals and the reason for withdrawal, for each study dose group.

Table 1: Number of subjects, age and gender by group

Table 1. Number of babies, mean age (weeks), standard deviation, and range at visit 1 for group by sex.

	GROUP 1	**GROUP 2**	**GROUP 3**	**GROUP 4**	**Total**
	N mean (sd) range	N mean (sd) range	N mean (sd) range	N mean (sd) range	N mean (sd) range
males	90 8.5 (0.9) 6.6-11.1	77 8.6 (0.8) 7.0-11.9	28 8.9 (1.3) 6.6-14.6	68 8.5 (0.9) 6.9-10.6	263 8.6 (0.9) 6.6-14.6
females	75 8.5 (0.9) 6.3-12.0	84 8.6 (0.9) 6.6-12.4	38 8.5 (0.8) 7.1-11.0	49 8.5 (0.8) 6.9-10.6	246 8.5 (0.9) 6.3-12.4
total	165 8.5 (0.9) 6.3-12.0	161 8.6 (0.9) 6.6-12.4	66 8.7 (1.1) 6.6-14.6	117 8.5 (0.8) 6.9-10.6	509 8.5 (0.8) 6.9-10.6

1 baby in GROUP 1 age=12.4 1 baby in GROUP 3 age=14.6

Table 2: Ethnicity of subjects by group

ETHNIC ORIGIN	GROUP 1	GROUP 2	GROUP 3	GROUP 4	ALL GROUPS	Percentage
African/Afrocaribbean	0	1	0	3	4	90.177
Asian	3	2	0	1	6	1.179
Caucasian	152	145	58	104	459	0.786
other	10	13	8	9	40	7.859
total	165	161	66	117	509	100

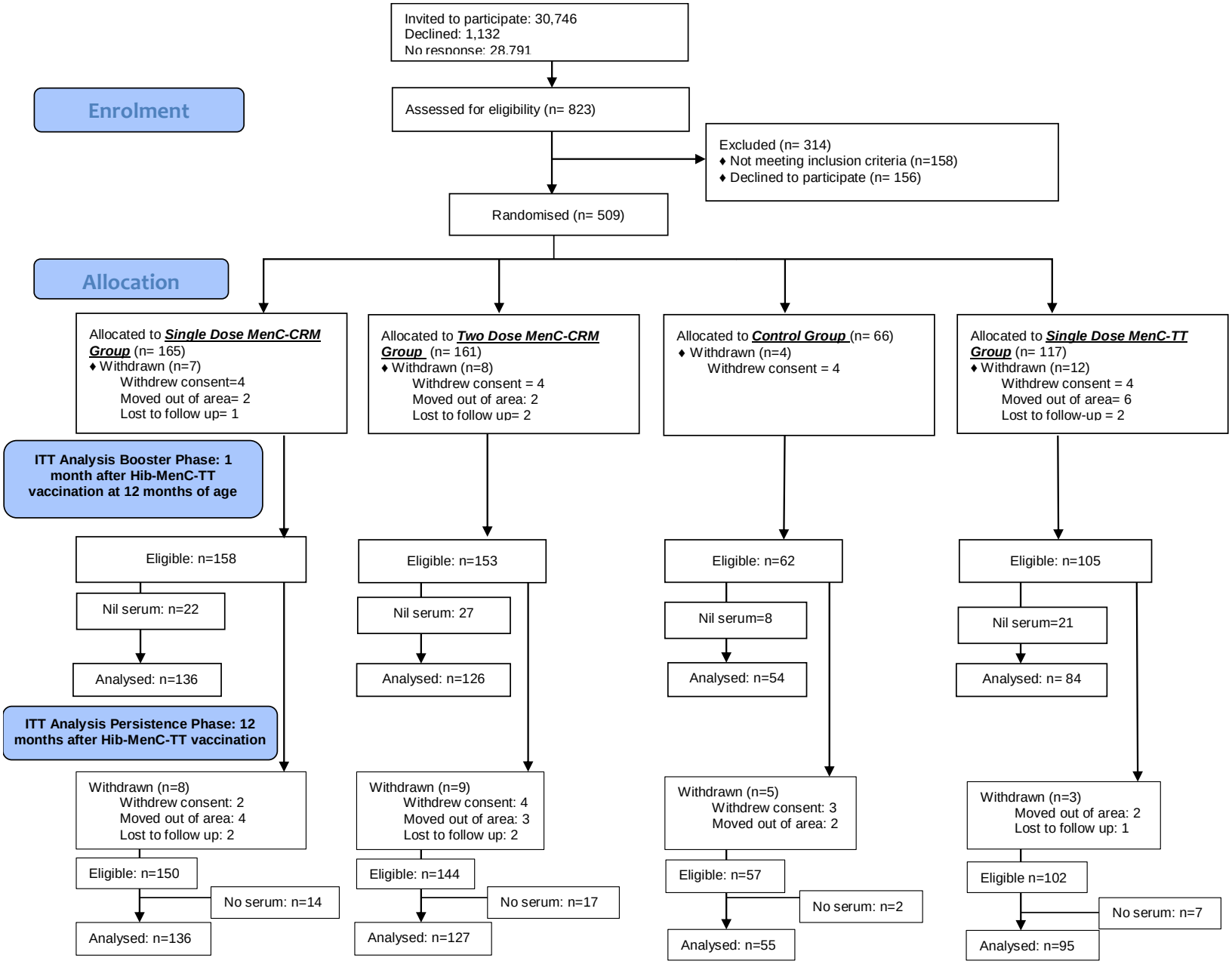


Figure 1: Consort Diagram: MenC Groups

Table 3.1: Demographics (Number and age) by Group: ITT population

Table 3. Number of babies, mean age (weeks), standard deviation, and range at each visit by group (ITT population).					
	GROUP 1	**GROUP 2**	**GROUP 3**	**GROUP 4**	**total**
	N mean (sd) range	N mean (sd) range	N mean (sd) range	N mean (sd) range	N mean (sd) range
Visit 2	165 13.0 (1.1) 10.3-18.4	159 13.1 (1.1) 10.7-17.4	65 13.2 (1.1) 11.1-18.6	117 13.0 (0.9) 11.0-16.6	506 13.0 (1.1) 10.3-18.6
Visit 3	165 17.6 (1.3) 14.4-23.3	158 17.6 (1.3) 15.0-22.4	65 17.7 (1.4) 15.3-25.4	114 17.6 (1.2) 15.0-20.9	502 17.6 (1.3) 14.4-25.4
Visit 4	165 22.3 (1.5) 19.0-28.3	157 22.3 (1.5) 19.0-27.6	63 22.5 (1.5) 19.4-29.6	112 22.4 (1.4) 19.1-26.7	497 22.3 (1.5) 19.0-26.6
Visit 5	161 54.3 (1.4) 52.3-58.1	154 54.3 (1.4) 51.9-59.0	62 54.2 (1.2) 52.4-57.9	106 54.2 (1.4) 51.6-58.7	483 54.3 (1.4) 51.6-59.0
Visit 6	61 55.3 (1.4) 53.3-58.9	61 55.3 (1.5) 53.3-59.0	61 55.0 (1.2) 53.3-58.7	63 55.3 (1.5) 53.0-59.7	246 55.2 (1.4) 53.0-59.7
Visit 7	158 59.1 (1.6) 56.6-63.0	153 59.3 (1.9) 56.6-70.4	62 59.1 (1.4) 56.7-63.4	105 59.0 (1.6) 56.0-63.6	478 59.1 (1.7) 56.0-70.4
Visit 8	149 104.8 (2.6) 100.4-116.1	144 104.7 (2.3) 96.1-116.4	57 104.3 (1.8) 100.7-108.9	102 105.0 (2.5) 100.4-118.7	452 104.8 (2.4) 96.1-118.7

Table 3.2: Demographics (Number and age) by MenC Group: CP population

Table 4. Number of babies, mean age (weeks), standard deviation, and range at each visit by group (CP population).					
	GROUP 1	**GROUP 2**	**GROUP 3**	**GROUP 4**	**total**
	N mean (sd) range	N mean (sd) range	N mean (sd) range	N mean (sd) range	N mean (sd) range
Visit 2	161 12.9 (1.0) 10.3-15.7	154 13.1 (1.0) 10.7-16.7	63 13.0 (0.8) 11.1-15.4	116 13.0 (0.9) 11.0-16.6	494 13.0 (1.0) 10.3-16.7
Visit 3	159 17.5 (1.2) 14.4-21.0	151 17.6 (1.2) 15.0-21.7	63 17.6 (1.0) 15.3-20.9	109 17.5 (1.1) 15.0-20.9	482 17.5 (1.1) 14.4-21.7
Visit 4	154 22.1 (1.5) 19.0-26.7	146 22.1 (1.3) 19.0-26.4	59 22.2 (1.2) 19.4-25.6	103 22.2 (1.2) 19.1-25.6	462 22.2 (1.3) 19.0-26.7
Visit 5	154 54.3 (1.4) 52.3-58.0	144 54.2 (1.2) 51.9-57.6	60 54.2 (1.2) 52.4-57.9	100 54.2 (1.3) 52.0-57.6	458 54.3 (1.3) 51.9-58.0
Visit 6	59 55.3 (1.4) 53.3-58.9	58 55.3 (1.4) 53.3-58.4	59 55.0 (1.2) 53.3-58.7	60 55.3 (1.3) 53.0-58.4	236 55.2 (1.3) 53.0-58.9
Visit 7	144 59.0 (1.6) 56.6-63.0	133 59.0 (1.4) 56.6-63.1	57 58.9 (1.4) 56.7-63.4	93 58.9 (1.5) 56.3-62.7	427 59.0 (1.5) 56.3-63.4
Visit 8	132 104.6 (2.2) 100.9-111.9	124 104.7 (1.8) 100.7-109.3	52 104.3 (1.8) 100.7-108.9	89 104.8 (2.1) 100.4-109.6	397 104.6 (2.0) 100.4-111.9

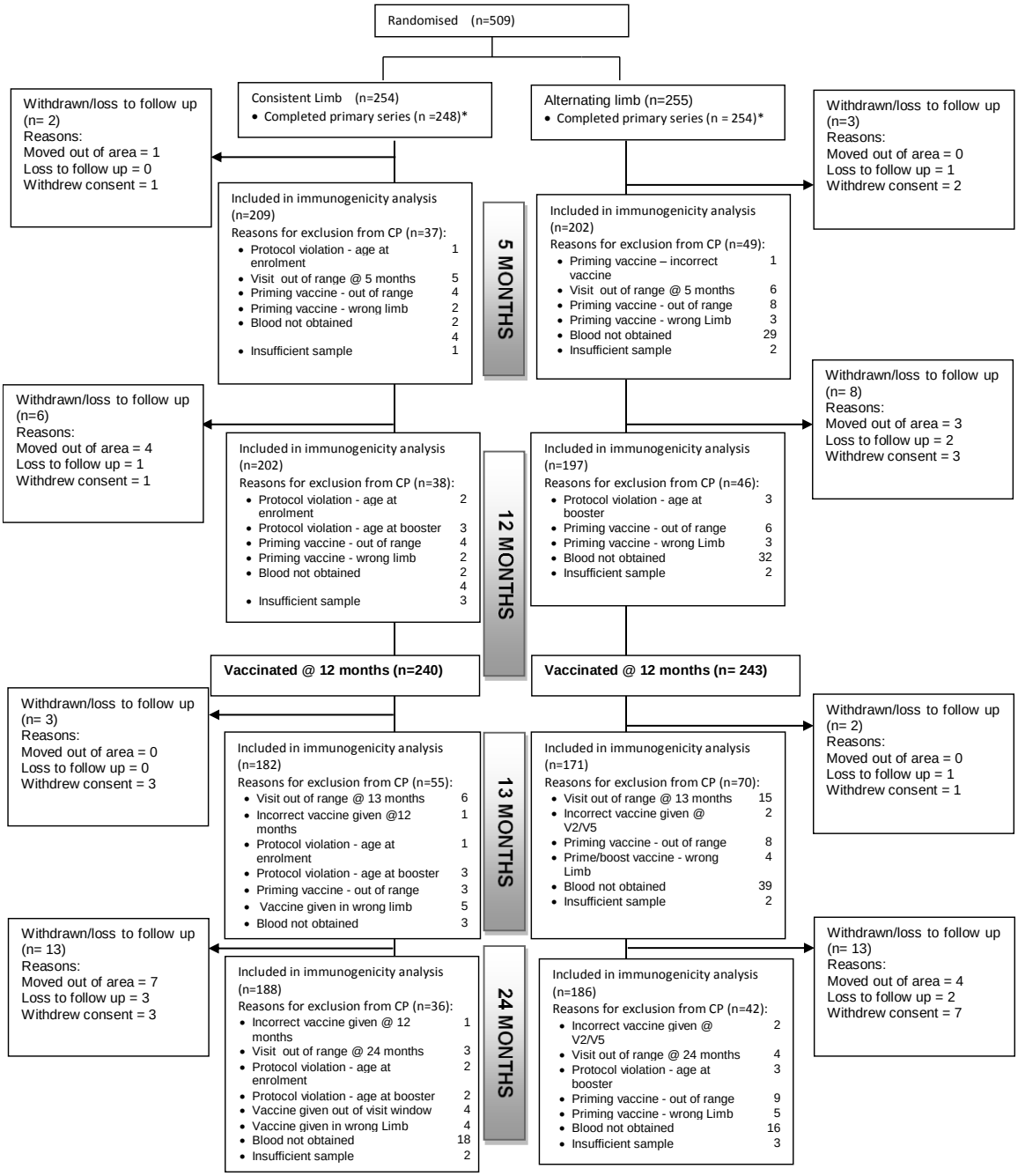
17.1.2 Consistent/Alternating Subgroups

The demographics of the subjects analysed in the consistent/alternating subgroups are shown in table 4.

Table 4. Demographics by Subgroup

	Consistent Limb	**Alternating Limb**
Total number of children	254	255
Median age (range)	59 days (51-102)	59 days (51-102)
Sex		
Female	122 (48%)	124 (49%)
Male	132 (52%)	131 (51%)
Ethnicity		
White Caucasian	228 (90%)	230 (90%)
Asian	3 (1%)	3 (1%)
African/Afrocaribean	0	4 (2%)
Other	22 (9%)	18 (7%)

Figure 2: Consort Diagram: Consistent/Alternating Limb subgroups



17.1.3 B-cell analysis

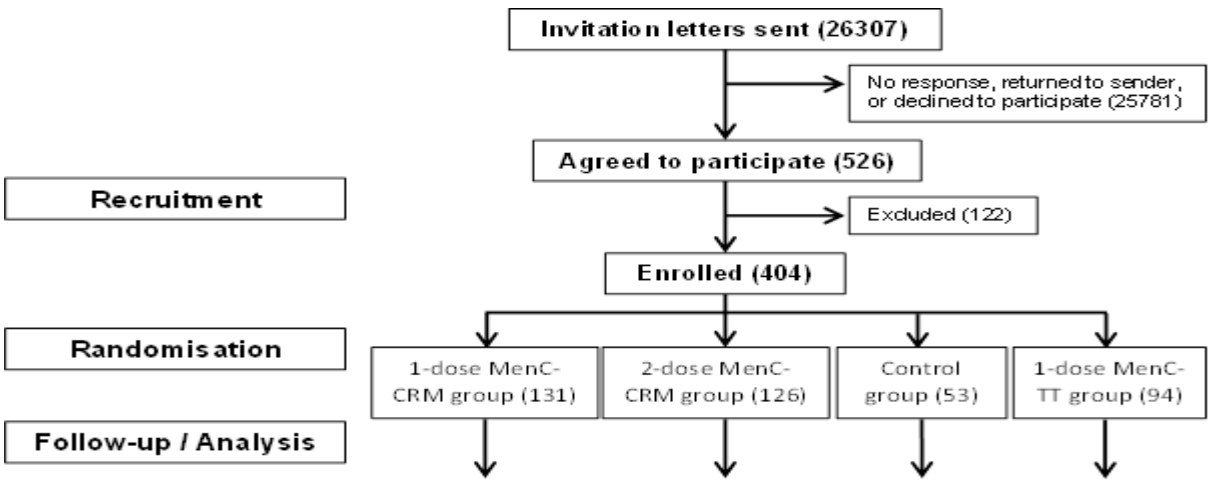
The subset of participants on whom memory B-cells were measured were selected pragmatically from those recruited at the Oxford research site, from whom a sufficient blood volume could be drawn (>4 mL), and where the sample could be processed within 6 hours of collection.

From the 404 children recruited in Oxford, memory B-cell results were available from at least one time-point for 355 children, of whom 164 were female. The median age at first vaccination was 59 days (range 51-102).

90% (320/355) of children were of Caucasian ethnicity; 5 children of Asian ethnicity; and 4 children of African or Afro-Carribean ethnicity. 26 children were identified as "other" ethnicity including mixed ethnic background.

Figure 3 describes the number of children included in the B-cell analysis.

Figure 3: Number of children enrolled and included in the final analysis for MenC-specific memory B-cells



Visit 4 (5 months of age)				
Included	**71**	**68**	**28**	**49**
Wrong vaccine administered (included in intention-to-treat analysis)	0	0	0	1 (MenC-CRM)
Exclusions:				
Blood sample not available, insufficient or unable to process	58	52	22	40
Withdrawn due to adverse event	0	1	0	0
Withdrawal of consent	0	2	2	1
Moved/Lost to follow-up	0	0	0	4
Failed positive control	0	3	0	0
ELISpot plate contamination	2	0	1	0
Visit 5 (12 months of age)				
Included	**63**	**45**	**27**	**39**
Exclusions:				
Blood sample not available, insufficient or unable to process	63	74	22	42
Previous withdrawal	0	3	2	5
Withdrawal of consent	1	0	1	2
Moved/Lost to follow-up	3	2	0	4
ELISpot plate contamination	0	0	1	0
Failed positive control	1	2	0	2
Visit 6 (12 months + 6 days)				
Randomised for blood sample	52	52	53	55
Included	**22**	**21**	**19**	**16**
Exclusions:				
Blood sample not available, insufficient or unable to process	25	26	30	27
Previous withdrawal	4	5	3	11
Withdrawal of consent	1	0	0	0
ELISpot plate contamination	0	0	0	1
Failed positive control	0	0	1	0
Visit 7 (13 months of age)				
Included	**58**	**51**	**20**	**34**
Wrong vaccine administered (included in intention-to-treat analysis)	0	0	0	1 (MenC-CRM)
Exclusions:				
Blood sample not available, insufficient or unable to process	64	68	30	47
Previous withdrawal	5	5	3	11
Withdrawal of consent	2	0	0	1
Moved/Lost to follow-up	0	2	0	0
ELISpot plate contamination	2	0	0	1
Visit 8 (24 months of age)				
Included	**70**	**73**	**30**	**53**
Exclusions:				
Blood sample not available, insufficient or unable to process	44	36	14	25
Previous withdrawal	7	7	3	12
Withdrawal of consent	3	4	3	0
Moved/Lost to follow-up	6	4	2	3
ELISpot plate contamination	0	1	1	0
Failed positive control	1	1	0	1

17.2 Trial Completion Rate

The trial completion rate as well as the reasons for withdrawal at each specific time point in the study is shown in table 5.

Table 5: Trial completion rate, time and reason for withdrawal by MenC group

Group	Enrolled	Withdrawn (completed visit)														Total	Reasons					
		V1	Reason	V2	Reason	V3	Reason	V4	Reason	V5	Reason	V6	Reason	V7	Reason		SAE	CW	MOA	LF	Completed Trial	%
Group 1	165	0		0		0		4	MOA:2, LF: 1, CW: 1	2	CW:2	1	CW	8	CW:2, LF:2; MOA:4	15	0	6	6	3	150	90.91
Group 2	161	2	CW	1	SAE	1	CW	3	MOA:2, LF: 1	1	LF	0		9	CW:4, LF:2, MOA:3	17	1	7	5	4	144	89.44
Group 3	66	1	CW	0		2	CW	1	CW	0		0		5	CW:3, MOA:2	9	0	7	2	0	57	86.36
Group 4	117	0		3	CW:1; MOA:2	2	MOA: 1; LF: 1	6	MOA:3, CW: 2, LF:1	0		1	CW	3	MOA: 2, LF1	15	0	4	8	3	102	87.18
Total	509	3		4		5		14		3		2		25		56	1	24	21	10	453	89.00

CW: Consent withdrawal; SAE: Serious Adverse Event; MOA: Moved out of area; LF: Lost to follow up

17.3 Protocol deviations

17.3.1 MenC Groups

The reasons for and the number of protocol deviations at each specific study visit are shown in table 6.1

Table 6.1: Protocol deviations By MenC group

Summary	Visit	Group 1	Group 2	Group 3	Group 4	Total
Protocol violation	**V1**		1	1		2
	V2				1	1
	V7		1			1
	Total					**4**
Out of timelines	V2	4	4	1	1	10
	V3	2	2	0	3	7
	V4	5	4	2	4	15
	V5	1	3	0	1	5
	V7	7	9	3	6	25
	V8	4	3	0	1	8
	Total*	18	21	4	12	**55**

*Those with delayed V4 only were included in subsequent immunogenicity analysis

Protocol violations: 2 subjects at V1 were aged >84 days when enrolled, at V2 one subject received Menjugate instead of NeisVac-C; and one subject was part vaccinated with Hib-MenC vaccine twice at V5 thus excluded from immunogenicity analysis at V7.

17.3.2 Consistent/Alternating subgroup analysis

The number of subjects excluded from the consistent/alternating limb analysis at each study visit is shown in table 6.2.

Table 6.2: Protocol deviations: Consistent/Alternating subgroup analysis

	5 months		**12 months**		**13 months**		**24 months**	
	CL	AL	CL	AL	CL	AL	CL	AL
Incorrect age at enrolment	1		2		1		2	
Incorrect age at booster			3	3	3		2	3
Incorrect vaccine given		1			1	2	1	2
Vaccine given out of visit window	4	8	4	6	3	8	4	9
Vaccine given in wrong limb	2	3	2	3	5	4	4	5
Visit out of range	5	6			6	15	3	4
Total exclusions due to protocol violations at each visit	**12**	**18**	**11**	**12**	**19**	**29**	**16**	**23**

17.3.3 B-cell Analysis

The number of protocol deviations and exclusions are as shown in figure 3.

18 ENDPOINTS

18.1 Primary End point assays

18.1.1 Meningococcal serogroup C serum bactericidal antibody assay

Meningococcal serogroup C antibody was measured using a serum bactericidal antibody (SBA) assay as described by Maslanka et al., 1997.[44] The SBA target strain was C11 (C:16:P1.7-1,1) and the complement source was baby rabbit (r) sera (Pel-Freeze Incorporated, Rodgerson, AZ). rSBA titres were expressed as the reciprocal of the final serum dilution giving ≥50% killing at 60 minutes. For analysis, rSBA titres <4 were assigned a value of 2. An rSBA cut off of ≥1:8 was taken as indicative of protection.[45] A threshold of ≥1:128 was also included as a more conservative protective titre.[46]

18.2 Secondary End point assays

18.2.1 Serotype specific Streptococcus pneumoniae IgG assays

Anti-pneumococcal serotype-specific IgG concentrations for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F using a multiplexed bead assay (Quantitative detection of serum IgG antibodies to 13 *Streptococcus pneumoniae* capsular polysaccharides using a multiplex fluorescent bead assay) as described by Lal G et al 2005 was performed. [47]

An antibody concentration threshold of ≥0.35 µg/ml as recommended by the World Health Organization (WHO) for an ELISA was used as the correlate of protection for all pneumococcal serotypes. The lower limit of quantification (LLOQ) for each pneumococcal serotype-specific IgG serum concentration was 0.10 µg/ml, and results below this level were reported as 0.05 µg/ml.

18.2.2 Anti-polyribosyl-ribitol phosphate (PRP) IgG and anti-tetanus toxoid multiplex assays

Hib anti-PRP and anti-tetanus toxoid IgG concentrations using a multiplexed fluorescent bead assay (Triplex fluorescent bead assay for the quantitative detection of serum IgG antibodies to *Haemophilus influenzae* type b capsular polysaccharide, diphtheria toxoid and Tetanus toxoid) as described by Pickering et al 2002.[48]

Anti-PRP concentrations of ≥ 0.15 µg/ml and ≥ 1.0 µg/ml were taken as correlates of short and long-term protection against Hib, respectively. The lower limit of quantification (LLOQ) for anti-PRP IgG GMC was 0.046 µg/ml and any results below this level were reported as 0.023 µg /ml. The correlate of protection for TT was ≥ 0.1 IU/ml. The LLOQ for anti-TT IgG GMC was 2.00 (IUx1000)/ml, and any results below this level were reported as 1.00 (IUx1000)/ml.

18.2.3 Men C memory B-cell assays

MenC specific memory B-cell numbers were measured by Enzyme-Linked Immunospot (ELISpot) in the peripheral of study participants at 5 months, 12 months, 6 days following the 12 month booster dose and 13 months of age. Specific memory B-cells against diphtheria and tetanus were measured and used as controls.

Separation of Peripheral Blood Mononuclear Cells (PBMCs) by density gradient centrifugation

Heparinised whole blood samples (2-4.5 ml) were processed within 6 hours of collection. PBMCs were isolated by diluting blood in R0 “complete” medium and layering over a density gradient medium (Lymphoprep™; Alere, UK) as previously described by Kelly *et al*, and Blanchard *et al*.[49,50]

In vitro stimulation of PBMCs for differentiation of memory B-cells into ASCs

$2x10^5$ cells/well of isolated PBMCs were seeded into 96-well, cell culture treated plates and stimulated with 100 µl/well of a mixture of 1:5000 *Staphylococcus aureus* Cowan 1 strain (SAC), 1.7 µg/ml CpG and 83.33 ng/ml pokeweed mitogen. Plates were incubated at 37ºC, 5% CO_2 and 95% humidity for 5-6 days. Harvested cells were washed in phosphate buffered saline with ethylenediaminetetraacetic acid (EDTA) di-sodium and 0.5% newborn bovine serum and re-suspended in R10 medium to a concentration of $2x10^6$ cells/ml.

Preparation of antigen coated ELISpot plates

96-well multiscreen-IP filter plates with polyvinylidene membranes were coated with the following antigens: 10 µg/ml goat anti-human Ig; 10 µg/ml diphtheria toxoid; 5 µg/ml tetanus toxoid; or 5 µg/ml MenC polysaccharide mixed with 5 µg/ml methylated human serum albumin. Anti-human Ig, tetanus and diphtheria toxoids were included as positive controls and phosphate buffered saline as background control.

ELISpot assay for detection of IgG-ASCs

As previously described,[49,50] $2x10^5$ cells/well cultured PBMCs were added to ELISpot plates and incubated with 50 µl/well (1:5000) of goat anti-human γ-chain-specific alkaline phosphatase conjugate. Spots were developed using 5-bromo-4-chloro-3-indolyl phosphate in nitroblue tetrazolium dissolved in aqueous dimethylformamide prepared from a kit.

Harvested cells were washed in buffer, counted and re-suspend in R10 medium to give a final concentration of $2x10^6$ cells/ml. 100 µL of the cell suspension was added to each of the antigen-specific wells ($2x10^5$ cells/well) and 100 µl of the Ig control dilutions (1:100 and 1:1000) to the Ig-coated wells. The plates were incubated at 37ºC/5%CO2/95% humidity overnight. After approximately 16-20 hours the cells and supernatants were discarded and the plates were repeatedly

washed with PBS and detergent (0.25% Tween), and then soaked in phosphate buffered saline (PBS) for 5 minutes. The PBS was then flicked out and 50 µL of filtered IgG-alkaline phosphatase conjugate diluted 1:5000 in R10 was added to each well and incubated for 4 hours at room temperature.

The plates were then washed repeatedly in PBS-T0.25% and sterile H_2O and left to soak in water. Bio-Rad #170-6432 kit development buffer [Tri(hyroxylmethyl)aminomethane] was diluted 1:25 in sterile H_2O and 50 µl each of solution A [5,5'-diphenyl-3,3'-bis(4-nitrophenyl)-2,2'-(3,3'dimethoxybiphenyl-4,4'-ylene)ditetrazolium dichloride] and solution B [N,N-dimethyl formamide] was added to 5 ml of diluted buffer. 50 µl of the filtered substrate mix was added to each well. Spots were allowed to develop without becoming too dark. The reactions were stopped with sterile H_2O and when all the wells were stopped, the plates were again washed in sterile H_2O and dried in a drying oven overnight.

Automated enumeration of IgG-ASC spots

ELISpot plates were scanned and counted using AID ELISpot reader version 5.0 and verified by visual inspection. Identical settings were used for all plates and antigens by a blinded operator. If <3 well replicates were available and variation was more than 15%, the sample was excluded from analysis for that antigen. For samples taken from children aged 12 months or older, if the total IgG spots/million PBMCs was <1000, the sample was excluded from analysis. For samples taken from children aged less than 12 months, if no IgG response was measured (zero results for both dilutions of IgG), the sample was excluded from analysis.

19 STATISTICAL ANALYSIS

19.1 MenC Group analysis

The analysis of the outcome variables was based on the intention–to-treat (ITT) population. An analysis on the Completers Population (CP) was also performed to complement the ITT population analysis. Subjects were included in the ITT population if they had at least one dose and at least one post-baseline assessment, and in the completers population if they received all doses of vaccine and had all planned assessments. Models used in the analyses contained the terms dose group (4 levels), and a term for centre. The term for dose group was omitted for the analysis of variables relating only to the 0 dose control group.

MenC Geometric Mean Titres (and their binomial exact 95% Confidence Intervals [CI]) were calculated from computational analysis of the $\log_{10}$ transformed MenC rSBA titres and subsequent antilog of the mean and the 95% CI. SBA titres <4 were given an arbitrary value of 2 for the analysis. A one way analysis of variance (ANOVA) of the $\log_{10}$ transformed SBA titres was performed for each of the blood sampling visit. Non-inferiority between the single dose and two dose MenC-CRM197 groups was concluded if the lower limit of the 95%CI of the difference between the mean log10SBA values was ≥-0.35 (equivalent to a non-inferiority margin of >-10%).

Binary variables were analysed using logistic regression. The results of a comparison between two levels of a factor were reported as odds ratios with 95% confidence intervals.

The software STATA 13 and StatXact 9 were used for the immunogenicity analyses.

19.2 Consistent/Alternating Limb subgroup analysis

There were no sample size calculations for the alternating limb against the consistent limb comparison as this was a secondary objective of the parent study. No statistical comparison of baseline characteristics was conducted.

In line with the pre-specified analysis plan, unadjusted comparisons between limb groups for log_{10}-transformed antibody concentration data were conducted using independent samples t-tests and results presented as geometric mean concentrations (GMCs) with 95% confidence intervals. Adjusted analyses were performed using linear regression adjusting for centre and randomised vaccine group from the main study. Binary immunogenicity outcomes using cut off points pertaining to standard immunogenicity thresholds were compared between groups using Fishers exact test and adjusted comparisons were conducted using logistic regression.

Analysis of immunogenicity was based on a pre-specified completers protocol (CP) population (all participants completing the study without any significant protocol deviations). Significant protocol deviation was predefined as visits outside of visit windows specified in the protocol, enrolment despite not meeting the specified inclusion and or exclusion criteria and incorrect administration of vaccines. Reactogenicity was analysed based on the Reactogenicity population (RP), which comprised all participants excluding those who received any study vaccine in the wrong limb or who received an incorrect vaccine at any time point.

The immunogenicity objective of the study was assessed by comparing S. *pneumonia*e IgG GMCs and percentage of infants with serum concentration of *S. pneumonia*e specific IgG ≥0.35 µg/ml for all 13 PCV13 serotypes; anti-PRP IgG GMCs and percentage of infants with serum concentration of anti-PRP IgG ≥0.15 µg/ml and ≥1.0 µg/ml; anti-TT IgG GMCs and percentage of infants with anti-TT >0.1 IU/ml. P values less than 0.05 were considered statistically significant.

For TT immunogenicity, a post hoc analysis was performed at 12 months after excluding participants who received a single dose of Men C-TT at 3 months of age. Anti-TT IgG GMCs were compared between both groups, using independent samples t-test.

19.3 B-cell Analysis

The sample size calculation for the full study was based on the primary objective to determine non-inferiority of antibody response following booster vaccination in group 1 (1 dose MenC-CRM priming) compared to group 2 (2 dose MenC-CRM197 priming). The subset of participants on whom memory B-cells were measured were selected pragmatically from those recruited by the Oxford research site, from whom a sufficient blood volume could be drawn (> 4 ml) and where the sample could be collected and returned to the lab in time for processing (< 6 hours). All comparisons of B-cell responses between the study groups were exploratory. Primary analyses were based on all available blood samples (intention-to-treat analysis [ITT]). All calculations were carried out using Microsoft Excel and GraphPad PRISM version 4.

As $2x10^5$ cells were added to each well, the lowest level of detection for memory B-cells is 0.625 cells/million PBMCs. Therefore negative results were assigned a value of half the lowest level of detection (0.31 cells/million PBMCs) for the purposes of calculating geometric mean concentrations (GMCs).

Differences between groups:

A one-way analysis of variance (ANOVA) was conducted of the $\log_{10}$ transformed data for the 4-level factor group, with each visit analysed separately, to test for differences amongst the groups in the number of memory B-cells detected. The residual variance was checked for normality, and the variances within groups were tested for heterogeneity (Bartlett's test). If the data appeared to be non-normal, non-parametric Kruskal Wallis analysis was carried out. The number of patients, mean and standard error for each group were reported, unless the data distribution was non-normal, when the mean, median and interquartile range were reported.

If the F-test for groups from the one-way ANOVA was significant, 3 degrees of freedom were partitioned to test for differences amongst groups 1, 2 and 4 (2 degrees of freedom) and between

the mean of groups 1, 2 and 4 and the control group (group 3) (1 degree of freedom). If the F-test for the mean of groups 1, 2 and 4 compared with the control was significant, a second partition was investigated, where the 3 degrees of freedom were partitioned in 3 comparisons, each group compared with the control. If the F-test for differences between groups 1, 2 and 4 was significant, comparisons of pairs of groups were investigated. The comparisons were reported with 95% CIs. As these analyses involved multiple comparisons, the Bonferroni adjusted p-values were reported. Similar analyses were conducted for non-normal data using Kruskal-Wallis analysis and chi-squared statistics were reported.

The data analysed are the ITT population.

Memory B-cell frequencies:

The proportion of MenC-specific memory B-cells out of the total pool of IgG positive memory B-cells was calculated at each time-point for all the primed children (pooled samples from 1-dose MenC-CRM, 2-dose MenC-CRM and 1-dose MenC-TT groups) as well as for un-primed children in the control group.

Differences over time:

To analyse the number of antigen specific memory B-cells (using $\log_{10}$ transformed data) linear mixed models were used to estimate the mean difference between two time points for each group. The terms in the models included the group, visit and the interaction between group and visit from which the change between two visits could be estimated for each group. P-values were reported for the comparison of each group mean difference with 0.

The data analysed are the ITT population.

20 RESULTS

20.1 Primary Objective

The primary objective of the study was met: the MenC rSBA GMTs induced one month after Hib-MenC-TT vaccination at 12 months of age in participants who were primed with one dose of MenC-CRM197 at 3 months of age was non-inferior to the MenC rSBA GMTs in vaccinees who had been primed with two doses of the same vaccine at 3 and 4 months of age. Non-inferiority was demonstrated from the analysis performed on both the ITT and CP populations (Tables 7-10).

In addition participants primed with a single MenC-CRM/TT dose had significantly higher GMTs than those primed with 2 MenC-CRM197 doses (Tables 8 and 10). Priming with one dose of MenC-TT at 3 months of age and boosting with the-Hib-MenC-TT vaccine at the age of 12 months resulted in significantly higher MenC rSBA GMTs when compared to those primed with one MenC-CRM197 dose. Vaccinees who had not been primed in infancy had significantly lower MenC rSBA GMTs compared to those who were primed with one or two MenC conjugate vaccine in infancy (Tables 8 and 10).

Comparisons performed:

1. MenC rSBA GMT response variable assessed 28 days after the Hib-MenC 12 month booster dose
    a. **Single dose MenC-CRM_{197} Group vs. Two dose MenC Group**
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT Group

Table 7: MenC rSBA GMT 28 days after Hib-MenC-TT 12 month dose (ITT population)

V7 ITT	ITT pop (N)	Serum (n)	%	Log_{10}SBA	95% CI		GMT	95% CI	
					LCL	UCL		LCL	UCL
Group 1 (Single dose MenC-CRM)	158	136	86.07595	2.819943	2.697263	2.942623	660.6067	498.0386	876.2398
Group 2 (Two dose MenC-CRM)	153	126	82.35294	2.470357	2.343	2.6	295.3636	220.2926	398.1072
Group 3 (Control)	62	54	87.09677	2.084911	1.891	2.279	121.5937	77.80366	190.1078
Group 4 (Single dose MenC-TT)	105	84	80	3.443926	3.288	3.6	2779.24	1940.886	3981.072

Table 8: Group differences in MenC log_{10} rSBA 28 days after Hib-MenC-TT 12 month dose (ITT population)

One way ANOVA of log_{10}SBA V7 ITT	Difference	95% CI		F	p value
		LCL	UCL		
Group 1 vs 2	0.3495855	0.1730472	0.5261238	15.16	**0.0001**
Group 1 vs 3	0.7350313	0.5053882	0.9646744	39.6	**<0.00001**
Group 1 vs 4	-0.6239837	-0.8221114	-0.425856	38.34	**<0.00001**
Group 2 vs 3	0.3854458	0.153	0.618	10.65	**0.0012**
Group 2 vs 4	-0.9735692	-1.174676	-0.772462	90.58	**<0.00001**
Group 4 vs 3	1.359015	1.109988	1.608042	115.11	**<0.00001**

No difference was noted when correcting for centre (p=0.9616).

Primary objective was met: Difference in log_{10}SBA: Group 1 – Group 2 = 0.349586 (95% LCL: **0.1730472**): Non-inferior if LCL ≥-0.35

Table 9: MenC rSBA GMT 28 days after Hib-MenC-TT 12 month dose (Completers population)

V7 CP	CP population	Serum (n)	%	Log_{10}SBA	95% CI		GMT	95% CI	
					LCL	UCL		LCL	UCL
Group 1 (Single dose MenC-CRM)	144	125	86.80556	2.841723	2.716	2.967	694.5812	519.996	926.8298
Group 2 (Two dose MenC-CRM)	133	111	83.45865	2.484175	2.351	2.618	304.9123	224.3882	414.954
Group 3 (Control)	57	49	85.96491	2.070349	1.87	2.271	117.5842	74.13102	186.638
Group 4 (Single dose MenC-TT)	93	76	81.72043	3.44204	3.281	3.603	2767.197	1909.853	4008.667

Primary objective was met: Difference in log_{10}SBA: Group 1 – Group 2 = 0.357548 (95% LCL: **0.174267**): Non-inferior if LCL ≥-0.35

Table 10: Group differences in MenC log_{10} rSBA 28 days after Hib-MenC-TT 12 month dose (Completers population)

One way ANOVA of log_{10}SBA V7 CP	Difference	95% CI		F	p value
		LCL	UCL		
Group 1 vs 2	0.357548	0.174267	0.5408284	14.72	**0.0001**
Group 1 vs 3	0.771374	0.5345101	1.008238	41.02	**<0.00001**
Group 1 vs 4	-0.60032	-0.8047326	-0.3959018	33.36	**<0.00001**
Group 2 vs 3	0.413826	0.1727926	0.65486	11.4	**0.0008**
Group 2 vs 4	0.957865	-1.167098	-0.7486321	81.06	**<0.00001**
Group 4 vs 3	1.371691	1.114221	1.629162	109.78	**<0.00001**

No difference was noted when correcting for centre (p= 0.9676).

20.2 Secondary Objectives

20.2.1 MenC rSBA titres 6 days following Hib-MenC-TT vaccination at 12 months of age

Six days following Hib-MenC-TT vaccination at 12 months of age the MenC rSBA GMTs were not different in those who had been primed with one/two MenC-CRM197 doses in infancy (Tables 11-13). However, MenC rSBA GMTs were significantly higher in those primed with one MenC-TT dose compared to all other schedules. Those who were never primed in infancy had significantly lower MenC rSBA titres than those primed with any MenC conjugate vaccine schedule.

Comparisons performed:

2. MenC rSBA GMT response variable assessed 6 days after the Hib-MenC 12 month booster dose
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 11: MenC rSBA GMT 6 days after Hib-MenC-TT 12 month dose (ITT population)

V6 ITT	ITT pop (N)	Serum (n)	%	Log_{10}SBA	95% CI		GMT	95% CI	
					LCL	UCL		LCL	UCL
Group 1 (Single dose MenC-CRM)	62	49	79.03226	3.053304	2.847	3.26	1130.587	703.0723	1819.701
Group 2 (Two dose MenC-CRM)	61	50	81.96721	2.992238	2.788	3.197	982.2861	613.762	1573.983
Group 3 (Control)	61	52	85.2459	2.344561	2.144	2.545	221.0859	139.3157	350.7519
Group 4 (Single dose MenC-TT)	63	52	82.53968	3.589204	3.389	3.79	3883.327	2449.063	6165.95

Table 12: Group differences in MenC log_{10} rSBA 6 days after Hib-MenC-TT 12 month dose (ITT population)

V6 ITT	Difference	95% CI		F	p value
One way ANOVA of log_{10}SBA		LCL	UCL		
Group 1 vs 2	0.061066	0.229653	0.351786	0.17	0.6792
Group 1 vs 3	0.708744	0.420805	0.996683	23.56	**<0.00001**
Group 1 vs 4	-0.5359	-0.82384	-0.24796	13.47	**0.0003**
Group 2 vs 3	0.647678	0.361225	0.93413	19.88	**<0.00001**
Group 2 vs 4	-0.59696	-0.88342	-0.31051	16.89	**0.0001**
Group 4 vs 3	1.244642	0.961013	1.528274	74.88	**<0.00001**

No difference was noted when correcting for centre (p= 0.6351).

Table 13: MenC rSBA GMT 6 days after Hib-MenC-TT 12 month dose (Completers population)

V6 CP	CP pop (N)	Serum (n)	%	Log_{10}SBA	95% CI		GMT	95% CI	
					LCL	UCL		LCL	UCL
Group 1 (Single dose MenC-CRM)	60	48	80	3.047929	2.837	3.258	1116.681	687.0684	1811.34
Group 2 (Two dose MenC-CRM)	58	49	84.48276	2.985726	2.777	3.194	967.6672	598.4116	1563.148
Group 3 (Control)	59	50	84.74576	2.31191	2.106	2.518	205.0737	127.6439	329.6097
Group 4 (Single dose MenC-TT)	60	49	81.66667	3.569356	3.361	3.778	3709.847	2296.149	5997.911

Table 14: Group differences in MenC log10 rSBA 6 days after Hib-MenC-TT 12 month dose (Completers population)

One way ANOVA of log10SBA V6 CP	Difference	95% CI		F	p value
		LCL	UCL		
Group 1 vs 2	0.622026	-0.2339	0.358302	0.17	0.6791
Group 1 vs 3	0.736018	0.441388	1.030649	24.28	**<0.00001**
Group 1 vs 4	-0.52143	-0.81753	-0.22533	12.06	**0.0006**
Group 2 vs 3	0.673816	0.380723	0.966909	20.56	**<0.00001**
Group 2 vs 4	-0.58363	-0.878	-0.289	15.27	**0.0001**
Group 4 vs 3	1.257445	0.964352	1.550538	71.61	**<0.00001**

No difference was noted when correcting for centre (p= 0.601)

20.2.2 MenC rSBA GMTs one year after Hib-MenC-TT vaccination at 12 months of age

Twelve months following Hib-MenC-TT vaccination, no significant differences in the MenC rSBA GMTs were observed between those primed with one/two MenC-CRM197 doses and those who had not been primed in infancy (Tables 15-18). Participants who were primed with one MenC-CRM197/TT dose had higher persistent GMTs than those primed with 2 MenC-CRM197 doses. MenC-TT priming resulted in higher persistent MenC rSBA GMTs when compared to all other schedules.

Comparisons performed:

3. MenC rSBA GMT response variable assessed 12 months after the Hib-MenC 12 month booster dose
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 15: MenC rSBA GMT 12 months after Hib-MenC-TT 12 month dose (ITT population)

V8 ITT	ITT pop (N)	Serum (n)	No serum	%	Log_{10}SBA	95% CI		GMT	95% CI	
						LCL	UCL		LCL	UCL
Group 1 (Single dose MenC-CRM)	152	136	16	89.47368	0.790204	0.658695	0.921713	6.168843	4.557163	8.350508
Group 2 (Two dose MenC-CRM)	147	127	20	86.39456	0.611541	0.476	0.747	4.088286	2.992265	5.584702
Group 3 (Control)	57	55	2	96.49123	0.700579	0.495	0.906	5.018557	3.126079	8.053784
Group 4 (Single dose MenC-TT)	102	95	7	93.13725	2.091366	1.935	2.248	123.4144	86.09938	177.0109

Table 16: Group differences in MenC log10 rSBA 24 months after Hib-MenC-TT 12 month dose (ITT population)

One way ANOVA of log_{10}SBA	Difference	95% CI		F	p value
		LCL	UCL		
Group 1 vs 2	0.1786625	-0.0093914	0.366716	3.49	0.0625
Group 1 vs 3	0.0896248	-0.1538991	0.333149	0.52	0.4698
Group 1 vs 4	-1.301163	-1.504937	-1.09739	157.56	**<0.00001**
Group 2 vs 3	-0.0890376	-0.3350338	0.156959	0.51	0.4772
Group 2 vs 4	-1.479825	-1.686548	-1.2731	198.02	**<0.00001**
Group 4 vs 3	1.390787	1.132574	1.649	112.11	**<0.00001**

No difference was noted when correcting for centre (p= 0.6605).

Table 17: MenC rSBA GMT 12 months after Hib-MenC-TT 12 month dose (Completers population)

V8 CP	CP population	Serum (n)	%	Log_{10}SBA	95% CI		GMT	95% CI	
					LCL	UCL		LCL	UCL
Group 1 (Single dose MenC-CRM)	134	120	89.55224	0.79773	0.66	0.935	6.276673	4.570882	8.609938
Group 2 (Two dose MenC-CRM)	124	108	87.09677	0.5714	0.426	0.716	3.727344	2.666859	5.19996
Group 3 (Control)	52	50	96.15385	0.722472	0.509	0.935	5.278032	3.228494	8.609938
Group 4 (Single dose MenC-TT)	89	82	92.13483	2.059486	1.893	2.226	114.6796	78.16278	168.2674

Table 18: Group differences in MenC log10 rSBA 12 months after Hib-MenC-TT 12 month dose (Completers population)

V8 CP	Difference	95% CI		F	p value
One way ANOVA of log10SBA		LCL	UCL		
Group 1 vs 2	0.22633	0.0265605	0.4260995	4.96	**0.0265**
Group 1 vs 3	0.0752575	-0.178262	0.328778	0.34	0.5597
Group 1 vs 4	-1.261756	-1.477552	-1.045961	132.23	**<0.00001**
Group 2 vs 3	-0.151073	-0.4087022	0.1065572	1.33	0.2496
Group 2 vs 4	-1.488086	-1.708695	-1.267478	175.98	**<0.00001**
Group 4 vs 3	1.337014	1.066768	1.60726	94.67	**<0.00001**

No difference was noted when correcting for centre (p= 0.2507).

20.2.3 MenC rSBA persistence at 12 months of age (following infant priming)

Prior Hib-MenC-TT vaccination at 12 months of age the MenC rSBA GMTs were significantly higher in participants who had been primed with two MenC-CRM197 doses compared to those who received just one MenC-CRM dose at 3 months of age; however a significant difference was not observed when compared to those primed with one MenC-TT dose (Tables 19-21). Priming with one MenC-TT dose induced significantly higher MenC rSBA GMTs than a single MenC-CRM197 dose priming.

Comparisons performed:

4. MenC rSBA GMT response variable (persistence) assessed at 12 months of age
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 19: MenC rSBA GMT persistence at 12 months of age (ITT population)

V5 ITT	ITT pop (N)	Serum (n)	%	Log_{10}SBA	95% CI		GMT	95% CI	
					LCL	UCL		LCL	UCL
Group 1 (Single dose MenC-CRM)	161	147	91.30435	0.645064	0.521	0.769	4.416358	3.318945	5.874894
Group 2 (Two dose MenC-CRM)	154	128	83.11688	0.919553	0.786	1.053	8.309073	6.10942	11.29796
Group 3 (Control)	62	54	87.09677	0.30103	0.30103	0.30103	2	2	2
Group 4 (Single dose MenC-TT)	106	93	87.73585	0.967828	0.811	1.124	9.285977	6.471426	13.30454

Table 20: Group differences in MenC log10 rSBA persistence at 12 months of age (ITT population)

One way ANOVA of log_{10}SBA V5 ITT	Difference	95% CI		F	p value
		LCL	UCL		
Group 1 vs 2	-0.2744883	-0.4568609	0.092116	8.76	**0.0033**
Group 1 vs 4	-0.3227633	-0.5226402	-0.1228865	10.08	**0.0016**
Group 2 vs 4	-0.0482751	-0.25382	0.15727	0.21	0.6445

A significant difference was noted when correcting for centre (p= 0.0113). However, adjustment for centre resulted in insignificant differences in the results.

Table 21: MenC rSBA GMT persistence at 12 months of age (Completers population)

V5 CP	CP pop (N)	Serum (n)	No serum	%	Log10SBA	95% CI		GMT	95% CI	
						LCL	UCL		LCL	UCL
Group 1 (Single dose MenC-CRM)	154	140	14	90.90909	0.621412	0.495	0.748	4.182268	3.126079	5.597576
Group 2 (Two dose MenC-CRM)	144	121	23	84.02778	0.905578	0.77	1.042	8.045959	5.888437	11.01539
Group 3 (Control)	60	53	7	88.33333	0.30103	0.30103	0.30103	2	2	2
Group 4 (Single dose MenC-TT)	100	89	11	89	0.943678	0.785	1.102	8.783716	6.095369	12.64736

Table 22: Group differences in MenC log10 rSBA persistence at 12 months of age (Completers population)

One way ANOVA of log_{10}SBA V5 CP	Difference	95% CI		F	p value
		LCL	UCL		
Group 1 vs 2	-0.28417	-0.46988	-0.0984544	9.06	0.0028
Group 1 vs 4	-0.32227	-0.5251	-0.1194355	9.77	0.0019
Group 2 vs 4	-0.038101	-0.24703	0.1708279	0.13	0.7201

No difference was noted when correcting for centre (p= 0.0783).

20.2.4 MenC rSBA GMT at 5 months of age following different infant MenC conjugate vaccine schedules

One month after primary vaccination infants who had been primed with two MenC-CRM 197 doses had significantly higher MenC rSBA GMTs than those primed with one MenC-CRM/TT vaccine dose (Tables 23-26). However priming with a single MenC-TT dose resulted in significantly higher GMTs when compared to single MenC-CRM dose priming.

Comparisons performed:

5. MenC rSBA GMT response variable assessed at 5 months of age
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 23: MenC rSBA GMT at 5 months of age (ITT population)

V4 ITT	ITT pop (N)	Serum (n)	%	Log10SBA	95% CI		GMT	95% CI	
					LCL	UCL		LCL	UCL
Group 1 (Single dose MenC-CRM)	165	144	87.27273	1.728832	1.633	1.825	53.55894	42.95364268	66.83439
Group 2 (Two dose MenC-CRM)	157	137	87.26115	2.792767	2.694	2.891	620.536	494.310687	778.0366
Group 3 (Control)	63	56	88.88889	0.3386567	0.263249	0.414068	2.181005	1.833365269	2.594588
Group 4 (Single dose MenC-TT)	112	99	88.39286	2.228838	2.113	2.345	169.3706	129.7179271	221.3095

Table 24: Group differences in MenC log10 rSBA at 5 months of age (ITT population)

V4 ITT	Difference	95% CI		F	p value
		LCL	UCL		
One way ANOVA of log10SBA					
Group 1 vs 2	-1.064	-1.202	-0.926	230.43	<0.00001
Group 1 vs 4	-0.500	-0.651	-0.350	47.15	<0.00001
Group 2 vs 4	0.564	0.412	0.716	42.53	<0.00001

No difference was noted when correcting for centre (p= 0.1798).

Table 25: MenC rSBA GMT at 5 months of age (Completers population)

V4 CP	CP pop (N)	Serum (n)	%	Log10SBA	95% CI		GMT	95% CI	
					LCL	UCL		LCL	UCL
Group 1 (Single dose MenC-CRM)	154	136	88.31169	1.726496	1.628	1.825	53.27163	42.46196	66.83439
Group 2 (Two dose MenC-CRM)	146	131	89.72603	2.7874	2.687	2.888	612.9146	486.4072	772.6806
Group 3 (Control)	59	53	89.83051	0.340789	0.261007	0.42057	2.191738	1.823925	2.633724
Group 4 (Single dose MenC-TT)	103	91	88.34951	2.216375	2.095	2.337	164.5792	124.4515	217.2701

Table 26: Group differences in MenC log10 rSBA at 5 months of age (Completers population)

CP population V4	Difference	95% CI		F	p value
One way ANOVA of log10SBA		LCL	UCL		
Group 1 vs 2	-1.061	-1.202	-0.920	218.47	<0.00001
Group 1 vs 4	-0.490	-0.646	-0.334	38.06	<0.00001
Group 2 vs 4	0.571	0.414	0.728	50.93	<0.00001

No difference was noted when correcting for centre (p= 0.1741).

MenC memory B cell Results

Memory B-cell results were available from at least one time-point for 355 children. 857 blood samples were available for analysis of memory B-cell responses. Details of sample inclusions and exclusions are outlined in Figure 3. Eleven samples (spread over 3 visits) were affected by a temporary incubator CO_2 regulator failure. These plates were placed into sealable plastic boxes with CO_2 sachets when the failure was discovered, and results included in the final analysis. One participant randomised to the 1-dose MenC-TT group received vaccinations according to the 1-dose MenC-CRM group. Results for this participant were analysed in the 1-dose MenC-TT group (intention-to-treat analysis).

At each visit there were large numbers of 0 cell counts in at least one group, and in all groups at visit 4 and visit 8. The data were not normally distributed and were not analysed by parametric methods.

The number of participants included in analysis for each group, at each visit is given in Table 27, along with the median and interquartile range for each based on the $\log_{10}$ transformed data of the number of MenC-specific memory B cells.

	1-dose MenC-CRM	**2-dose MenC-CRM**	**Control**	**1-dose MenC-TT**
	N median (IQR)	N median (IQR)	N median (IQR)	N median (IQR)
Visit 4	71 -0.51 (-0.51, 0.27)	68 0.05 (-0.51, 0.70)	28 -0.51 (-0.51, -0.20)	49 0.10 (-0.51, 0.54)
Visit 5	63 0.55 (0.27, 1.06)	45 0.40 (-0.20, 0.75)	27 -0.08 (-0.51, 0.40)	39 0.48 (-0.20, 0.88)
Visit 6	22 1.09 (1.00, 1.33)	21 0.82 (0.49, 1.18)	19 -0.20 (-0.51, 0.30)	16 1.10 (0.80, 1.45)
Visit 7	58 0.85 (0.40, 1.18)	51 0.75 (0.40, 0.94)	20 0.97 (0.45, 1.37)	34 1.33 (0.94, 1.71)
Visit 8	70 0.48 (-0.20, 0.84)	73 0.27 (-0.51, 1.04)	30 0.76 (0.27, 1.14)	53 0.64 (0.27, 0.98)

Table 27. Number of participants, median and interquartile range for the 4 study groups at each visit for $\log_{10}$ transformed number of MenC-specific memory B cells.

No statistically significant difference was seen between children who received 1- or 2-dose MenC-CRM_{197} priming at any time-point in the number of MenC-specific memory B-cells generated (Table 28-32). Control children had fewer MenC memory B-cells than all other groups after the primary vaccines and until 1 month after the booster. There were no statistically significant differences in the number of MenC memory B-cells generated one month after the Hib-MenC-TT booster by un-primed children (control group) and children who had received either 1 or 2 doses of MenC-CRM_{197} primary vaccines. By 13 months of age, children in the 1-dose TT group had generated more MenC-specific memory B-cells than children in either the 1-dose or 2-dose MenC-CRM groups. The greatest degree of waning of MenC memory B-cell numbers post-booster occurred in the 1-dose MenC-TT primed group, and the least occurred in the un-primed group (Table 33). By 24 months of age all study groups had similar number of MenC memory B-cells, with only a small but statistically significant difference in between the 2-dose MenC-CRM_{197} primed children and the 1-dose MenC-TT primed group (Table 28-32).

20.2.5 MenC specific memory B cells at 5 months of age (following infant priming)

Comparisons performed:

6. the numbers of MenC specific memory B cells in the blood at 5 months of age

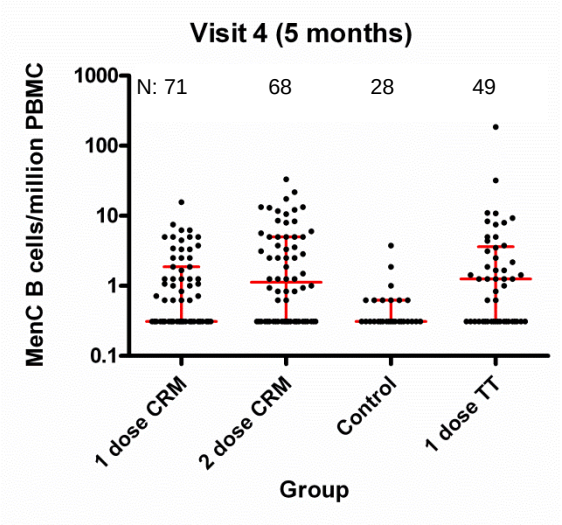


Figure 4: Number of MenC-specific memory B-cells (log_{10} scale) detected in the peripheral blood of individual participants after immunisation with different schedules of MenC conjugate vaccines at 5 months of age.

	Kruskal-Wallis statistic	*p-value*
5 months of age (1 month after primary vaccines)		
*Between groups 1-dose CRM, 2-dose CRM, and 1-dose TT and control	$X^2_3 = 17.0$	**p = 0.0007**
*Between groups 1-dose CRM, 2-dose CRM, and 1-dose TT	$X^2_2 = 6.0$	p = 0.051
Groups 1-dose CRM, 2-dose CRM, and 1-dose TT vs control	$X^2_1 = 11.2$	**p = 0.0008**
1-dose CRM vs control	$X^2_1 = 5.1$	**p = 0.02**
2-dose CRM vs control	$X^2_1 = 12.9$	**p = 0.0003**
1-dose TT vs control	$X^2_1 = 11.0$	**p = 0.0009**

***Groups are grouped together since there was no statistically significant differences between them (i.e. between Group 1 vs 2 vs 4 and Group 2 vs 4. Differences between pairs of groups were only compared if differences at the all group level comparison were significant.**

Table 28: Comparison between groups of the log_{10} transformed number of MenC-specific memory B-cells detected in the peripheral blood at 5 months of age.

20.2.6 MenC specific memory B cells at 12 months of age

Comparisons performed:

7. the numbers of MenC specific memory B cells in the blood at 12 months of age

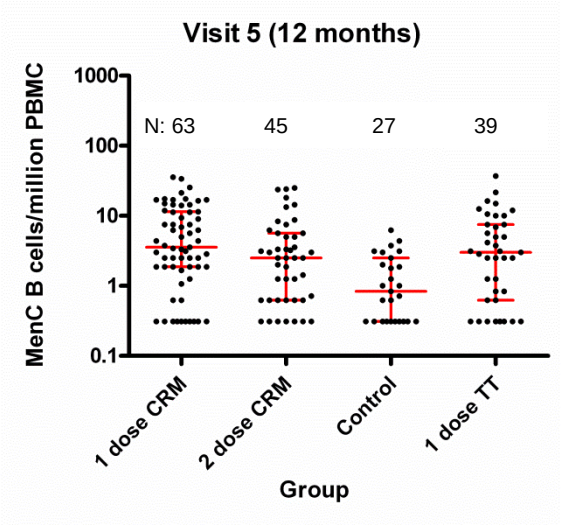


Figure 5: Number of MenC-specific memory B-cells (log_{10} scale) detected in the peripheral blood of individual participants after immunisation with different schedules of MenC conjugate vaccines at 12 months of age.

	Kruskal-Wallis statistic	*p-value*
12 months of age (pre-booster)		
Between groups 1-dose CRM, 2-dose CRM, and 1-dose TT and control	$X^2_3 = 16.8$	**p = 0.0008**
Between groups 1-dose CRM, 2-dose CRM, and 1-dose TT	$X^2_2 = 2.9$	p = 0.23
Groups 1-dose CRM, 2-dose CRM, and 1-dose TT vs control	$X^2_1 = 14.0$	**p = 0.0002**
1-dose CRM vs control	$X^2_1 = 15.9$	**p = 0.0001**
2-dose CRM vs control	$X^2_1 = 7.1$	**p = 0.008**
1-dose TT vs control	$X^2_1 = 8.2$	**p = 0.004**

Table 29: Comparison between groups of the $\log_{10}$ transformed number of MenC-specific memory B-cells detected in the peripheral blood at 12 months of age.

20.2.7 MenC specific memory B cells 6 days after Hib-MenC-TT vaccination at 12 months of age

Comparisons performed:

8. the numbers of MenC specific memory B cells in the blood at 6 days after the Hib-MenC 12 month booster dose

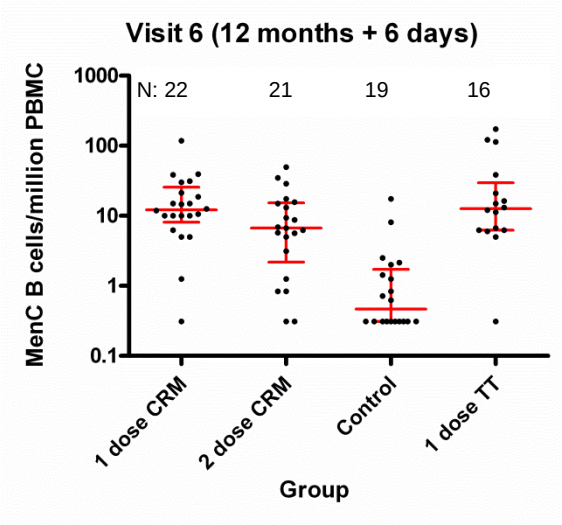


Figure 6: Number of MenC-specific memory B-cells (log_{10} scale) detected in the peripheral blood of individual participants after immunisation with different schedules of MenC conjugate vaccines 6 days after the 12-month booster.

	Kruskal-Wallis statistic	*p-value*
12 months + 6 days (6 days after booster vaccination)		
Between groups 1-dose CRM, 2-dose CRM, and 1-dose TT and control	$X^2_3 = 27.8$	**p = 0.0001**
Between groups 1-dose CRM, 2-dose CRM, and 1-dose TT	$X^2_2 = 4.3$	p = 0.12
Groups 1-dose CRM, 2-dose CRM, and 1-dose TT vs control	$X^2_1 = 24.5$	**p = 0.0001**
1-dose CRM vs control	$X^2_1 = 20.5$	**p = 0.0001**
2-dose CRM vs control	$X^2_1 = 12.6$	**p = 0.0004**
1-dose TT vs control	$X^2_1 = 16.8$	**p = 0.0001**

Table 30: Comparison between groups of the $\log_{10}$ transformed number of MenC-specific memory B-cells detected in the peripheral blood 6 days after the 12-month booster.

20.2.8 MenC specific memory B cells 28 days after Hib-MenC-TT vaccination at 12 months of age

Comparisons performed:

9. The numbers of specific memory B cells in the blood at 28 days after the Hib-MenC 12 month booster dose

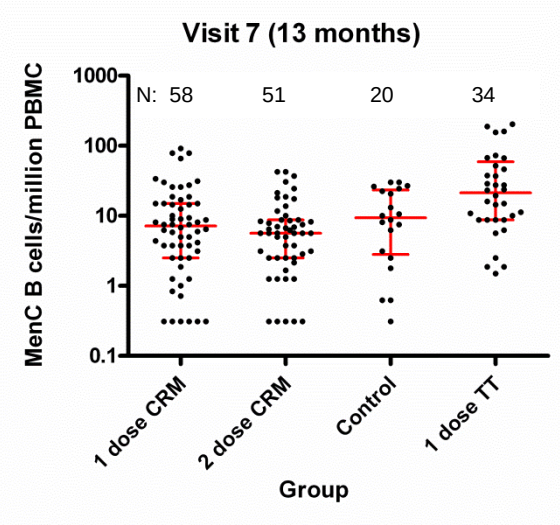


Figure 7: Number of MenC-specific memory B-cells (log_{10} scale) detected in the peripheral blood of individual participants after immunisation with different schedules of MenC conjugate vaccines at 13 months of age.

	Kruskal-Wallis statistic	*p-value*
13 months of age (1 month after booster vaccination)		
Between groups 1-dose CRM, 2-dose CRM, and 1-dose TT and control	$X^2_3 = 22.4$	**p = 0.0001**
Between groups 1-dose CRM, 2-dose CRM, and 1-dose TT	$X^2_2 = 21.9$	**p = 0.0001**
Groups 1-dose CRM, 2-dose CRM, and 1-dose TT vs control	$X^2_1 = 0.1$	p = 0.83
1-dose CRM vs control	$X^2_1 = 0.5$	p = 0.46
2-dose CRM vs control	$X^2_1 = 2.2$	p = 0.14
1-dose TT vs control	$X^2_1 = 6.0$	**p = 0.01**
1-dose CRM vs 2-dose CRM	$X^2_1 = 0.7$	p = 0.40
1-dose CRM vs 1-dose TT	$X^2_1 = 14.0$	**p = 0.0002**
2-dose CRM vs 1-dose TT	$X^2_1 = 20.6$	**p = 0.0001**

Table 31: Comparison between groups of the $\log_{10}$ transformed number of MenC-specific memory B-cells detected in the peripheral blood at 13 months of age.

20.2.9 MenC specific memory B cells 12 months after Hib-MenC-TT vaccination at 12 months of age

Comparisons performed:

10. The numbers of specific memory B cells in the blood at 11-12 months after the Hib-MenC booster dose

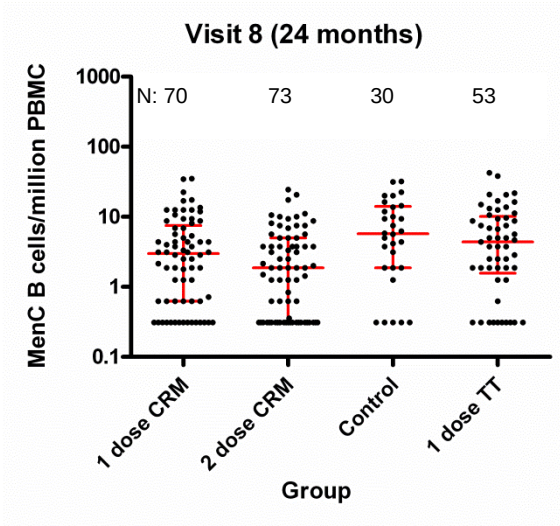


Figure 8: Number of MenC-specific memory B-cells (log_{10} scale) detected in the peripheral blood of individual participants after immunisation with different schedules of MenC conjugate vaccines at 24 months of age.

	Kruskal-Wallis statistic	*p-value*
13 months of age (1 month after booster vaccination)		
Between groups 1-dose CRM, 2-dose CRM, and 1-dose TT and control	$X^2_3 = 14.4$	**p = 0.0024**
Between groups 1-dose CRM, 2-dose CRM, and 1-dose TT	$X^2_2 = 8.5$	**p = 0.014**
Groups 1-dose CRM, 2-dose CRM, and 1-dose TT vs control	$X^2_1 = 6.0$	**p = 0.014**
1-dose CRM vs control	$X^2_1 = 4.5$	**p = 0.0024**
2-dose CRM vs control	$X^2_1 = 10.4$	**p = 0.0012**
1-dose TT vs control	$X^2_1 = 0.8$	p = 0.37
1-dose CRM vs 2-dose CRM	$X^2_1 = 2.8$	p = 0.10
1-dose CRM vs 1-dose TT	$X^2_1 = 2.0$	p = 0.16
2-dose CRM vs 1-dose TT	$X^2_1 = 8.2$	**p = 0.004**

Table 32: Comparison between groups of the $\log_{10}$ transformed number of MenC-specific memory B-cells detected in the peripheral blood at 24 months of age.

Group	**Mean difference**	**Standard error**	**Estimated 95% CI around difference**	**p-value**
1-dose CRM	-0.407	0.102	-0.61 to -0.21	**<0.0001**
2-dose CRM	-0.501	0.105	-0.71 to -0.30	**<0.0001**
Control	-0.193	0.165	-0.52 to 0.13	0.24
1-dose TT	-0.789	0.126	-1.04 to -0.54	**<0.0001**

Table 33: Differences in $\log_{10}$ transformed number of MenC-specific memory B-cells detected in the peripheral blood between 24-month and 13-month samples for each study group

20.2.10 Additional exploratory analyses on memory B cell results

A statistically significant rise was seen in the number of MenC-specific memory B-cells between the pre- and post-booster blood samples for each study group with a greater rise seen in the control group and the 1-dose MenC-TT group compared to the 1- and 2-dose MenC-CRM groups (table 34).

Group	Mean difference	Standard error	Estimated 95% CI around difference	p-value
1-dose CRM	0.229	0.099	0.04 to 0.42	**0.021**
2-dose CRM	0.337	0.112	0.12 to 0.56	**0.003**
Control	0.876	0.160	0.56 to 1.19	**<0.0001**
1-dose TT	0.937	0.128	0.68 to 1.19	**<0.0001**

Table 34: Differences in $\log_{10}$ transformed number of MenC-specific memory B-cells detected in the peripheral blood between 13-month and 12-month samples for each study group.

Frequencies of MenC-specific memory B-cells

The median proportion of MenC-specific memory B-cells in the control group was 0% until 1 month after the Hib-MenC-TT booster (Table 35). By 13 months of age 0.10% (median) of control children's IgG positive memory B-cells were MenC-specific, similar to the median percentage of MenC-specific memory B-

cells in previously primed children (0.09%). These median frequencies had waned to 0.04% and 0.01% in un-primed and primed children respectively by 24 months of age.

		5 months of age	12 months of age	6 days after 12-month booster	13 months of age	24 months of age
Primed children	Median	0.03%	0.05%	0.14%	0.09%	0.01%
	IQR	0% – 0.10%	0.01% – 0.11%	0.06% – 0.30%	0.04% - 0.26%	0% - 0.04%
Un-primed children (Control group)	Median	0.0%	0.0%	0.0%	0.10%	0.04
	IQR	0% – 0.02%	0% – 0.03%	0% – 0.03%	0.04% - 0.20%	0.01% - 0.07%

IQR: Interquartile range

Table 35: Proportion of MenC-specific memory B-cells out of the total pool of IgG positive memory B-cells detected in the peripheral blood at each time-point.

Differentiating primed and un-primed children

Blood samples drawn 6 days after the Hib-MenC-TT booster were used to compare primed children (groups 1, 2, and 4) and un-primed (control) children (Figure 9). A threshold of 2.5 MenC memory B-cells/million PBMCs was picked by visually inspecting the data and drawing a line through the point that would

best separate results from the primed compared to the un-primed children, excluding outliers. Use of this "threshold" as a test to detect primed children gives a sensitivity of 0.86 and specificity of 0.89.

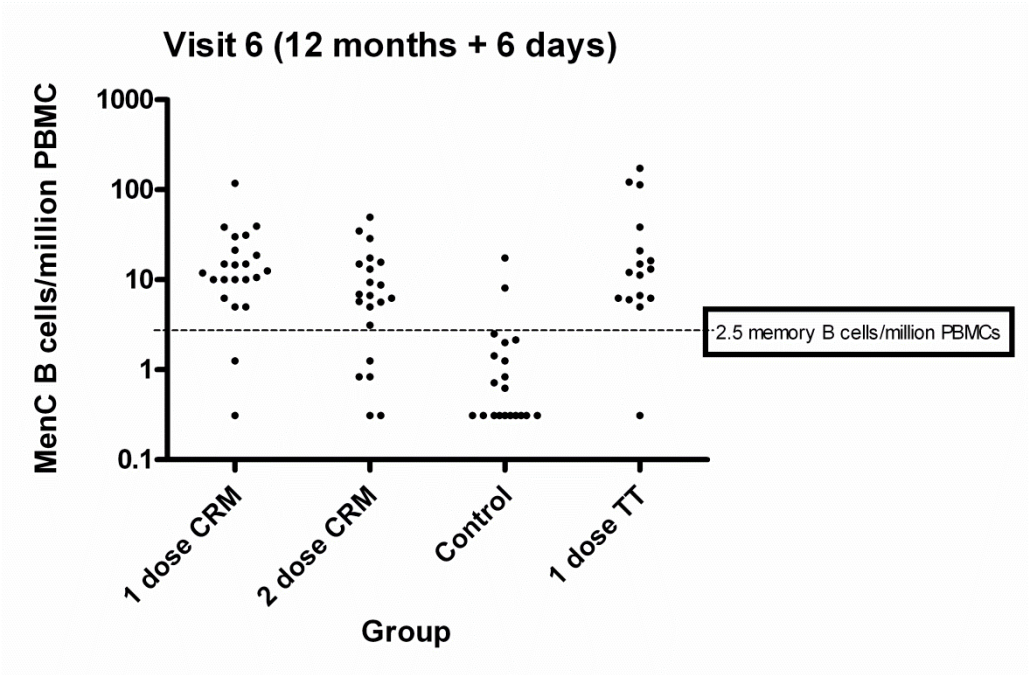
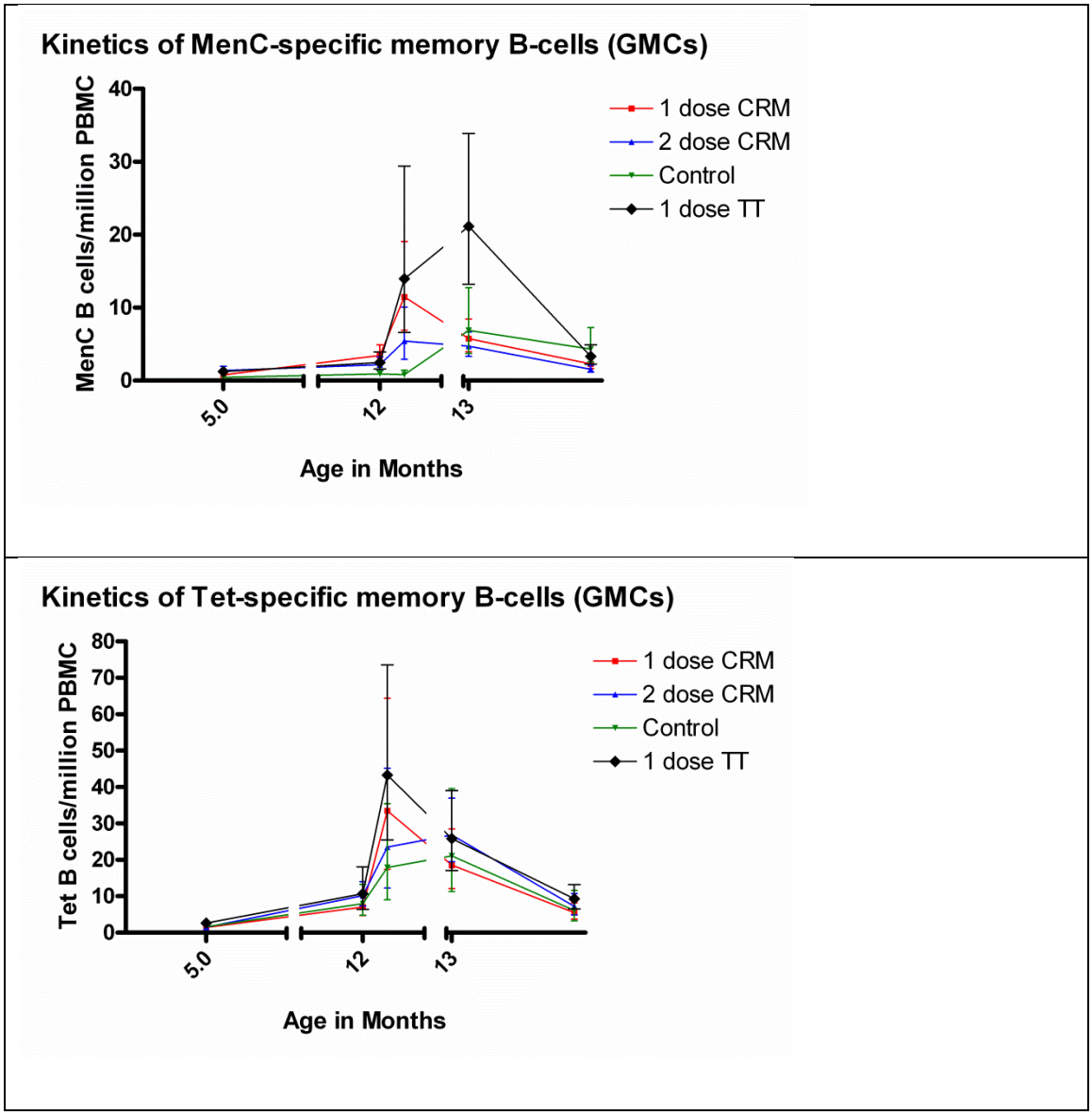


Figure 9: Number of MenC-specific memory B-cells detected in the peripheral blood of infants 6 days after a Hib-MenC-TT booster at 12 months of age, according to different primary immunisation schedules.

Kinetics of memory B-cell generation

The kinetics of MenC memory B-cell generation over time for each group is shown in Figure 10 based on geometric mean concentrations calculated for the number of MenC-specific memory B-cells detected at each time-point for each group. The kinetics of memory B-cell production for the control antigens, diphtheria (Dip) and tetanus (Tet) are also included. The patterns for each group appear to be antigen-specific.



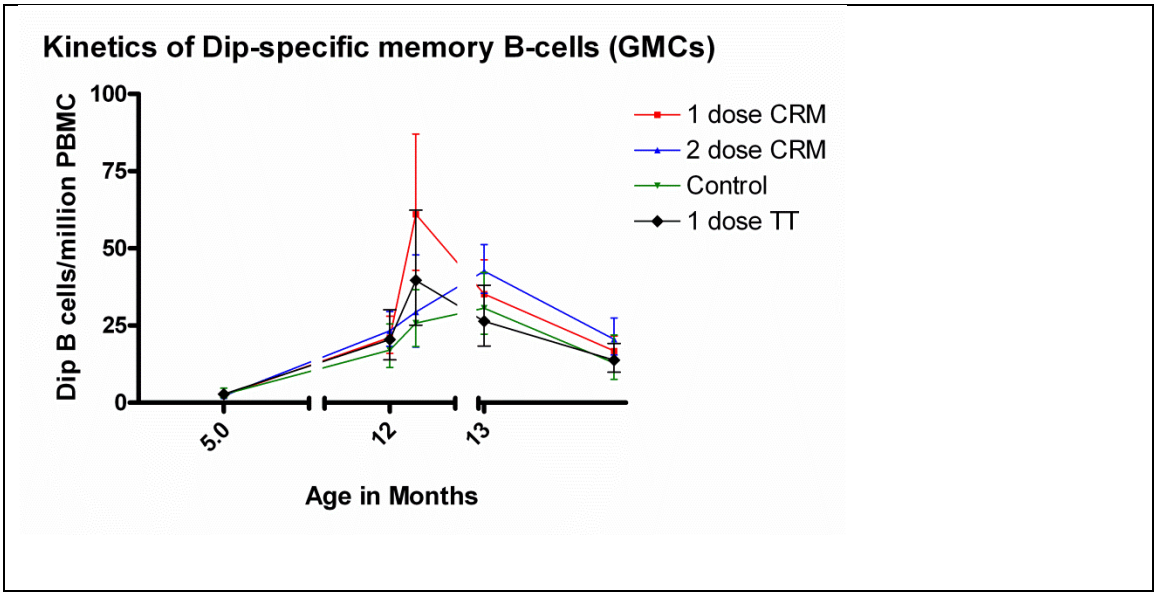


Figure 10: Kinetics of the number of antigen-specific memory B-cells detected in the peripheral blood of infants after immunisation with different schedules of MenC conjugate vaccines, at each time-point following primary and booster vaccines, based on geometric mean concentrations for each study group at each visit.

A statistically significant rise was seen in the number of memory B-cells between 5 months (1 month after primary vaccines) and 12 months of age (prior to booster vaccines) across all antigens tested (Table 36). To exclude variability in the assay over time, MenC-specific memory B-cell responses for all children were plotted according to calendar month of blood sampling for the 5-month and 12-month samples. No change in the pattern of results was seen over time (Figure 11).

		MenC	**Diphtheria**	**Tetanus**
1-dose CRM group	Mean difference	0.671	0.958	0.697
	95% CI	0.50, 0.84	0.78, 1.13	0.50, 0.90
	P-value	**<0.0001**	**<0.0001**	**<0.0001**
2-dose CRM group	Mean difference	0.245	1.032	0.819
	95% CI	0.05, 0.44	0.83, 1.23	0.60, 1.04
	P-value	**0.014**	**<0.0001**	**<0.0001**
Control	Mean difference	0.323	0.791	0.664
	95% CI	0.05, 0.59	0.51, 1.07	0.36, 0.97
	P-value	**0.019**	**<0.0001**	**<0.0001**
1-dose TT group	Mean difference	-0.789	0.883	0.616
	95% CI	-1.04, -0.54	0.66, 1.11	0.37, 0.87
	P-value	**<0.0001**	**<0.0001**	**<0.0001**

Table 36: Differences in the log_{10} transformed number of antigen-specific memory B-cells detected in the peripheral blood between 5 months and 12 months for each study group.

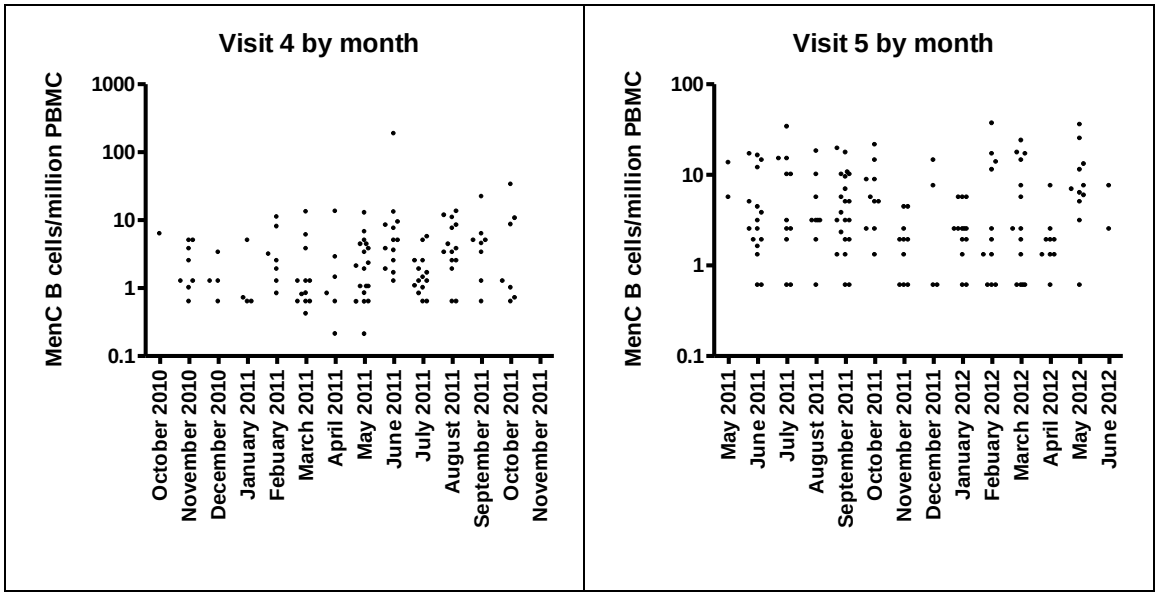


Figure 11: MenC-specific memory B-cells detected in the peripheral blood at 5 months (visit 4) and 12 months (visit 5) of age by calendar month for all participants.

20.2.10 Pneumococcal IgG GMCs for each of the 13 serotypes at 5 months, 12 months, 13 months and 24 months of age

Pneumococcal IgG GMCs for each of the 13 serotypes at 5, 12, 13 and 24 months were similar for participants in both groups with numerical differences not reaching statistical significance.

Comparisons performed:

11. The *S. pneumoniae* IgG GMC response variable for each of the thirteen serotypes separately at 5 months of age
    a. the consistent limb group vs. the alternating limb group

12. the *S. pneumoniae* IgG GMC response variable for each of the thirteen serotypes separately, (persistence) at 12 months of age
    a. the consistent limb group vs. the alternating limb group

13. the *S. pneumoniae* IgG GMC response variable for each of the thirteen serotypes separately at 13 months of age after the PCV-13 booster dose
    a. the consistent limb group vs. the alternating limb group

14. the *S. pneumoniae* IgG GMC response variable for each of the 13 serotypes separately at 24 months of age.
    a. the consistent limb group vs. the alternating limb group

TABLE 37 PNEUMOCOCCAL SEROTYPE SPECIFIC GEOMETRIC MEAN RATIOS BY VISIT (ITT POPULATION)

		Consistent Limbs		*Alternating Limbs*		*Unadjusted*		*Adjusted*	
Serotype	*Visit*	*N*	*Geometric Mean (CI)*	*N*	*Geometric Mean (CI)*	*GMR (CI)*	*p value*	*GMR (CI)**	*p value**
1	V4	221	1.33 (1.14 ,1.55)	220	1.42 (1.20 ,1.69)	0.94 (0.74 ,1.18)	0.5686	0.92 (0.73 ,1.16)	0.4899
1	V5	213	0.58 (0.51 ,0.65)	209	0.59 (0.53 ,0.67)	0.97 (0.82 ,1.14)	0.7136	0.97 (0.82 ,1.14)	0.6992
1	V7	200	9.16 (7.99 ,10.49)	200	10.18 (8.78 ,11.81)	0.90 (0.74 ,1.10)	0.2986	0.90 (0.73 ,1.10)	0.3002
1	V8	204	1.02 (0.92 ,1.14)	209	1.12 (1.00 ,1.26)	0.91 (0.78 ,1.07)	0.2452	0.91 (0.77 ,1.06)	0.2144
3	V4	220	7.37 (6.59 ,8.24)	219	6.44 (5.74 ,7.23)	1.14 (0.97 ,1.34)	0.0990	1.14 (0.97 ,1.34)	0.1061
3	V5	213	1.45 (1.27 ,1.66)	209	1.36 (1.18 ,1.56)	1.07 (0.88 ,1.29)	0.5179	1.07 (0.88 ,1.30)	0.5072
3	V7	200	13.03 (11.47 ,14.80)	200	15.34 (13.40 ,17.56)	0.85 (0.71 ,1.02)	0.0846	0.86 (0.71 ,1.03)	0.1053
3	V8	204	2.36 (1.98 ,2.81)	209	2.32 (1.99 ,2.71)	1.02 (0.80 ,1.28)	0.8990	1.02 (0.81 ,1.29)	0.8853
4	V4	221	1.71 (1.48 ,1.97)	220	1.73 (1.46 ,2.05)	0.99 (0.79 ,1.23)	0.9039	0.98 (0.79 ,1.23)	0.8784
4	V5	213	0.48 (0.42 ,0.53)	209	0.45 (0.39 ,0.51)	1.07 (0.90 ,1.26)	0.4515	1.07 (0.91 ,1.27)	0.4213
4	V7	201	10.52 (9.31 ,11.89)	200	10.53 (9.16 ,12.10)	1.00 (0.83 ,1.20)	0.9929	1.01 (0.84 ,1.21)	0.9190
4	V8	204	0.93 (0.83 ,1.04)	209	0.93 (0.83 ,1.04)	1.00 (0.85 ,1.18)	0.9728	1.00 (0.85 ,1.18)	0.9970
5	V4	220	2.18 (1.85 ,2.57)	219	1.99 (1.69 ,2.34)	1.09 (0.87 ,1.38)	0.4395	1.09 (0.87 ,1.37)	0.4602
5	V5	213	0.60 (0.54 ,0.68)	209	0.58 (0.51 ,0.65)	1.04 (0.88 ,1.23)	0.6140	1.03 (0.88 ,1.22)	0.6851
5	V7	200	10.62 (9.30 ,12.12)	200	10.69 (9.31 ,12.28)	0.99 (0.82 ,1.20)	0.9390	1.00 (0.82 ,1.21)	0.9619
5	V8	204	1.42 (1.25 ,1.63)	209	1.59 (1.41 ,1.80)	0.89 (0.75 ,1.07)	0.2210	0.89 (0.75 ,1.07)	0.2143

		Consistent Limbs		Alternating Limbs		Unadjusted		Adjusted	
Serotype	*Visit*	*N*	*Geometric Mean (CI)*	*N*	*Geometric Mean (CI)*	*GMR (CI)*	*p value*	*GMR (CI)**	*p value**
6A	V4	221	2.29 (1.91 ,2.75)	220	1.98 (1.62 ,2.40)	1.16 (0.89 ,1.51)	0.2774	1.15 (0.88 ,1.50)	0.3184
6A	V5	213	1.01 (0.87 ,1.17)	209	0.97 (0.83 ,1.14)	1.04 (0.83 ,1.29)	0.7461	1.05 (0.84 ,1.30)	0.6891
6A	V7	200	31.99 (27.76 ,36.86)	200	31.63 (26.93 ,37.14)	1.01 (0.82 ,1.25)	0.9169	1.02 (0.82 ,1.26)	0.8494
6A	V8	204	3.09 (2.68 ,3.57)	209	2.88 (2.52 ,3.28)	1.07 (0.88 ,1.31)	0.4681	1.08 (0.89 ,1.31)	0.4557
6B	V4	221	0.20 (0.17 ,0.24)	220	0.22 (0.18 ,0.26)	0.91 (0.71 ,1.18)	0.4883	0.91 (0.70 ,1.17)	0.4590
6B	V5	213	0.38 (0.32 ,0.44)	209	0.36 (0.31 ,0.43)	1.04 (0.83 ,1.29)	0.7442	1.06 (0.85 ,1.31)	0.6210
6B	V7	201	16.17 (13.55 ,19.30)	200	15.10 (12.67 ,17.99)	1.07 (0.84 ,1.37)	0.5871	1.08 (0.84 ,1.38)	0.5480
6B	V8	204	1.79 (1.54 ,2.08)	209	1.80 (1.55 ,2.08)	1.00 (0.81 ,1.23)	0.9695	1.00 (0.81 ,1.23)	0.9935
7F	V4	221	3.90 (3.42 ,4.46)	220	3.52 (3.08 ,4.03)	1.11 (0.92 ,1.34)	0.2866	1.11 (0.92 ,1.33)	0.2879
7F	V5	213	1.31 (1.20 ,1.43)	209	1.28 (1.15 ,1.41)	1.03 (0.90 ,1.17)	0.7053	1.02 (0.89 ,1.17)	0.7472
7F	V7	200	11.10 (9.92 ,12.42)	200	10.47 (9.21 ,11.91)	1.06 (0.89 ,1.26)	0.5001	1.06 (0.89 ,1.26)	0.4979
7F	V8	204	2.24 (2.06 ,2.43)	209	2.57 (2.33 ,2.84)	0.87 (0.76 ,0.99)	0.0340	0.87 (0.76 ,0.99)	0.0341
9V	V4	221	1.70 (1.44 ,2.00)	220	1.72 (1.45 ,2.04)	0.98 (0.78 ,1.25)	0.8959	0.97 (0.76 ,1.22)	0.7793
9V	V5	213	0.49 (0.43 ,0.55)	209	0.46 (0.41 ,0.51)	1.07 (0.90 ,1.27)	0.4599	1.06 (0.90 ,1.26)	0.4922
9V	V7	201	11.05 (9.80 ,12.45)	200	10.78 (9.58 ,12.13)	1.02 (0.87 ,1.21)	0.7746	1.02 (0.86 ,1.21)	0.8212
9V	V8	204	1.17 (1.04 ,1.33)	209	1.23 (1.08 ,1.38)	0.96 (0.81 ,1.14)	0.6251	0.96 (0.81 ,1.14)	0.6216
14	V4	221	1.90 (1.57 ,2.30)	219	2.41 (2.01 ,2.90)	0.79 (0.60 ,1.03)	0.0765	0.78 (0.60 ,1.02)	0.0700
14	V5	213	1.02 (0.88 ,1.18)	209	0.96 (0.81 ,1.14)	1.06 (0.84 ,1.32)	0.6369	1.06 (0.85 ,1.32)	0.6253
14	V7	201	10.80 (9.24 ,12.61)	200	10.24 (8.61 ,12.18)	1.05 (0.84 ,1.33)	0.6534	1.06 (0.83 ,1.34)	0.6514

		Consistent Limbs		Alternating Limbs		Unadjusted		Adjusted	
Serotype	*Visit*	*N*	*Geometric Mean (CI)*	*N*	*Geometric Mean (CI)*	*GMR (CI)*	*p value*	*GMR (CI)**	*p value**
14	V8	204	1.59 (1.39 ,1.82)	209	1.54 (1.33 ,1.78)	1.03 (0.85 ,1.26)	0.7518	1.03 (0.84 ,1.25)	0.7975
18C	V4	221	2.75 (2.32 ,3.27)	219	2.52 (2.13 ,2.99)	1.09 (0.86 ,1.39)	0.4824	1.08 (0.85 ,1.38)	0.5229
18C	V5	213	0.59 (0.52 ,0.65)	209	0.56 (0.50 ,0.62)	1.05 (0.91 ,1.22)	0.5018	1.06 (0.91 ,1.23)	0.4501
18C	V7	200	10.87 (9.63 ,12.26)	200	10.69 (9.46 ,12.09)	1.02 (0.86 ,1.21)	0.8548	1.02 (0.86 ,1.21)	0.8107
18C	V8	204	1.01 (0.89 ,1.15)	209	0.99 (0.88 ,1.11)	1.02 (0.87 ,1.21)	0.7734	1.03 (0.87 ,1.22)	0.7299
19A	V4	221	1.64 (1.40 ,1.93)	220	1.72 (1.45 ,2.03)	0.96 (0.76 ,1.21)	0.7100	0.95 (0.75 ,1.20)	0.6561
19A	V5	212	0.61 (0.52 ,0.71)	208	0.50 (0.43 ,0.59)	1.21 (0.96 ,1.52)	0.1082	1.21 (0.96 ,1.52)	0.1047
19A	V7	201	11.18 (9.49 ,13.18)	200	11.83 (9.97 ,14.05)	0.95 (0.75 ,1.20)	0.6391	0.94 (0.74 ,1.19)	0.6231
19A	V8	204	1.46 (1.23 ,1.73)	209	1.37 (1.16 ,1.62)	1.06 (0.84 ,1.35)	0.6141	1.06 (0.83 ,1.34)	0.6446
19F	V4	221	6.58 (5.68 ,7.62)	220	6.66 (5.71 ,7.77)	0.99 (0.80 ,1.22)	0.9069	0.99 (0.80 ,1.22)	0.8955
19F	V5	213	1.09 (0.95 ,1.25)	209	1.07 (0.92 ,1.25)	1.02 (0.83 ,1.25)	0.8684	1.02 (0.83 ,1.25)	0.8388
19F	V7	200	19.58 (17.18 ,22.31)	200	22.58 (19.66 ,25.94)	0.87 (0.72 ,1.05)	0.1409	0.87 (0.72 ,1.05)	0.1499
19F	V8	204	1.96 (1.69 ,2.27)	209	2.04 (1.76 ,2.37)	0.96 (0.78 ,1.18)	0.6854	0.95 (0.77 ,1.17)	0.6224
23F	V4	221	0.83 (0.69 ,1.01)	220	0.85 (0.71 ,1.03)	0.98 (0.75 ,1.28)	0.8662	0.97 (0.74 ,1.27)	0.8209
23F	V5	212	0.44 (0.37 ,0.52)	209	0.40 (0.33 ,0.48)	1.09 (0.84 ,1.41)	0.5145	1.09 (0.84 ,1.41)	0.5189
23F	V7	200	14.59 (12.42 ,17.13)	200	15.20 (13.06 ,17.69)	0.96 (0.77 ,1.20)	0.7123	0.97 (0.78 ,1.20)	0.7549
23F	V8	204	2.75 (2.25 ,3.37)	209	2.62 (2.18 ,3.14)	1.05 (0.80 ,1.38)	0.7206	1.06 (0.81 ,1.39)	0.6759

**Adjusted for centre and dose group*

TABLE 38: PNEUMOCOCCAL SEROTYPE SPECIFIC GEOMETRIC MEAN RATIOS BY VISIT (CP POPULATION)

		Consistent Limbs		*Alternating Limbs*		*Unadjusted*		*Adjusted*	
Serotype	*Visit*	*N*	*Geometric Mean (CI)*	*N*	*Geometric Mean (CI)*	*GMR (CI)*	*p value*	*GMR (CI)**	*p value**
1	V4	209	1.30 (1.12 ,1.52)	202	1.37 (1.15 ,1.64)	0.95 (0.75 ,1.20)	0.6530	0.93 (0.74 ,1.18)	0.5505
1	V5	202	0.57 (0.51 ,0.64)	197	0.58 (0.52 ,0.65)	0.98 (0.83 ,1.16)	0.8333	0.98 (0.83 ,1.16)	0.8341
1	V7	181	9.43 (8.17 ,10.89)	171	10.65 (9.08 ,12.49)	0.89 (0.72 ,1.10)	0.2656	0.89 (0.71 ,1.10)	0.2684
1	V8	188	1.03 (0.93 ,1.15)	186	1.10 (0.97 ,1.23)	0.94 (0.80 ,1.10)	0.4513	0.94 (0.80 ,1.10)	0.4503
14	V4	209	1.89 (1.56 ,2.30)	201	2.47 (2.04 ,2.98)	0.77 (0.58 ,1.01)	0.0568	0.76 (0.58 ,1.00)	0.0529
14	V5	202	1.00 (0.85 ,1.16)	197	0.94 (0.79 ,1.12)	1.06 (0.84 ,1.33)	0.6385	1.06 (0.84 ,1.33)	0.6359
14	V7	182	10.78 (9.15 ,12.69)	171	10.03 (8.27 ,12.17)	1.07 (0.83 ,1.38)	0.5773	1.07 (0.83 ,1.38)	0.5925
14	V8	188	1.54 (1.34 ,1.77)	186	1.50 (1.29 ,1.74)	1.03 (0.84 ,1.26)	0.7930	1.00 (0.81 ,1.23)	0.9970
18C	V4	209	2.77 (2.31 ,3.30)	201	2.52 (2.10 ,3.01)	1.10 (0.85 ,1.41)	0.4664	1.09 (0.84 ,1.40)	0.5193
18C	V5	202	0.57 (0.51 ,0.64)	197	0.55 (0.49 ,0.61)	1.04 (0.89 ,1.22)	0.6200	1.04 (0.89 ,1.22)	0.5802
18C	V7	181	10.99 (9.68 ,12.46)	171	10.74 (9.41 ,12.27)	1.02 (0.85 ,1.23)	0.8100	1.02 (0.85 ,1.23)	0.8002
18C	V8	188	0.98 (0.87 ,1.12)	186	0.96 (0.85 ,1.08)	1.03 (0.86 ,1.22)	0.7621	1.03 (0.86 ,1.23)	0.7513
19A	V4	209	1.63 (1.38 ,1.93)	202	1.72 (1.44 ,2.05)	0.95 (0.75 ,1.21)	0.6771	0.94 (0.74 ,1.20)	0.6285
19A	V5	201	0.60 (0.51 ,0.72)	196	0.49 (0.42 ,0.58)	1.22 (0.96 ,1.54)	0.0972	1.22 (0.96 ,1.54)	0.0989
19A	V7	182	11.60 (9.80 ,13.74)	171	11.94 (9.88 ,14.44)	0.97 (0.75 ,1.25)	0.8222	0.97 (0.76 ,1.26)	0.8419
19A	V8	188	1.48 (1.24 ,1.76)	186	1.33 (1.11 ,1.59)	1.11 (0.87 ,1.42)	0.4040	1.12 (0.88 ,1.44)	0.3564
19F	V4	209	6.58 (5.65 ,7.67)	202	6.73 (5.72 ,7.92)	0.98 (0.78 ,1.22)	0.8474	0.97 (0.78 ,1.22)	0.8214
19F	V5	202	1.10 (0.95 ,1.26)	197	1.05 (0.90 ,1.23)	1.04 (0.84 ,1.28)	0.7147	1.04 (0.85 ,1.29)	0.6801
19F	V7	181	19.54 (17.01 ,22.45)	171	23.35 (20.10 ,27.12)	0.84 (0.68 ,1.03)	0.0866	0.84 (0.68 ,1.03)	0.0864
19F	V8	188	1.98 (1.70 ,2.31)	186	1.99 (1.71 ,2.32)	0.99 (0.80 ,1.23)	0.9583	0.99 (0.80 ,1.23)	0.9100
23F	V4	209	0.81 (0.67 ,0.98)	202	0.84 (0.69 ,1.02)	0.97 (0.73 ,1.27)	0.8024	0.96 (0.72 ,1.27)	0.7564
23F	V5	201	0.43 (0.36 ,0.52)	197	0.40 (0.32 ,0.49)	1.09 (0.83 ,1.43)	0.5321	1.09 (0.83 ,1.43)	0.5491

		Consistent Limbs		Alternating Limbs		Unadjusted		Adjusted	
Serotype	*Visit*	*N*	*Geometric Mean (CI)*	*N*	*Geometric Mean (CI)*	*GMR (CI)*	*p value*	*GMR (CI)**	*p value**
23F	V7	181	14.33 (12.07 ,17.02)	171	15.41 (13.01 ,18.24)	0.93 (0.73 ,1.18)	0.5542	0.93 (0.73 ,1.18)	0.5524
23F	V8	188	2.70 (2.19 ,3.32)	186	2.61 (2.16 ,3.17)	1.03 (0.78 ,1.37)	0.8230	1.06 (0.80 ,1.40)	0.7000
3	V4	208	7.40 (6.59 ,8.30)	201	6.38 (5.65 ,7.21)	1.16 (0.98 ,1.37)	0.0828	1.16 (0.98 ,1.37)	0.0864
3	V5	202	1.45 (1.26 ,1.67)	197	1.29 (1.12 ,1.48)	1.13 (0.93 ,1.37)	0.2320	1.14 (0.93 ,1.38)	0.2025
3	V7	181	13.43 (11.75 ,15.35)	171	14.87 (12.82 ,17.25)	0.90 (0.74 ,1.10)	0.3142	0.93 (0.76 ,1.13)	0.4500
3	V8	188	2.37 (1.97 ,2.86)	186	2.16 (1.86 ,2.52)	1.09 (0.86 ,1.39)	0.4569	1.11 (0.88 ,1.42)	0.3781
4	V4	209	1.70 (1.47 ,1.96)	202	1.70 (1.42 ,2.03)	1.00 (0.79 ,1.26)	0.9938	0.99 (0.79 ,1.25)	0.9523
4	V5	202	0.48 (0.42 ,0.53)	197	0.44 (0.39 ,0.50)	1.08 (0.91 ,1.28)	0.4022	1.08 (0.91 ,1.28)	0.3990
4	V7	182	10.73 (9.47 ,12.16)	171	11.10 (9.56 ,12.89)	0.97 (0.80 ,1.17)	0.7301	0.97 (0.80 ,1.18)	0.7902
4	V8	188	0.93 (0.82 ,1.04)	186	0.90 (0.80 ,1.01)	1.03 (0.87 ,1.21)	0.7358	1.03 (0.87 ,1.21)	0.7362
5	V4	208	2.16 (1.83 ,2.56)	201	1.97 (1.66 ,2.34)	1.10 (0.86 ,1.39)	0.4471	1.09 (0.86 ,1.38)	0.4888
5	V5	202	0.60 (0.53 ,0.67)	197	0.57 (0.51 ,0.65)	1.05 (0.88 ,1.24)	0.5998	1.04 (0.87 ,1.23)	0.6858
5	V7	181	10.81 (9.43 ,12.40)	171	11.06 (9.48 ,12.89)	0.98 (0.80 ,1.20)	0.8310	0.98 (0.80 ,1.21)	0.8531
5	V8	188	1.39 (1.21 ,1.58)	186	1.56 (1.38 ,1.77)	0.89 (0.74 ,1.07)	0.2007	0.89 (0.74 ,1.06)	0.1926
6A	V4	209	2.26 (1.87 ,2.73)	202	1.95 (1.58 ,2.40)	1.16 (0.88 ,1.54)	0.2927	1.14 (0.86 ,1.51)	0.3635
6A	V5	202	1.02 (0.87 ,1.19)	197	0.96 (0.81 ,1.13)	1.07 (0.85 ,1.33)	0.5787	1.08 (0.86 ,1.35)	0.4957
6A	V7	181	32.01 (27.50 ,37.24)	171	31.80 (26.64 ,37.95)	1.01 (0.80 ,1.27)	0.9559	1.01 (0.80 ,1.28)	0.9253
6A	V8	188	3.03 (2.60 ,3.53)	186	2.81 (2.44 ,3.23)	1.08 (0.88 ,1.32)	0.4727	1.08 (0.88 ,1.33)	0.4830
6B	V4	209	0.19 (0.16 ,0.23)	202	0.21 (0.18 ,0.26)	0.88 (0.68 ,1.15)	0.3477	0.88 (0.67 ,1.14)	0.3278
6B	V5	202	0.37 (0.32 ,0.43)	197	0.36 (0.30 ,0.42)	1.04 (0.83 ,1.31)	0.7193	1.07 (0.86 ,1.34)	0.5497
6B	V7	182	16.39 (13.55 ,19.81)	171	15.54 (12.79 ,18.89)	1.05 (0.80 ,1.38)	0.7027	1.06 (0.81 ,1.39)	0.6695
6B	V8	188	1.74 (1.50 ,2.03)	186	1.72 (1.47 ,2.01)	1.01 (0.81 ,1.26)	0.9050	1.02 (0.82 ,1.27)	0.8596

		Consistent Limbs		Alternating Limbs		Unadjusted		Adjusted	
Serotype	*Visit*	*N*	*Geometric Mean (CI)*	*N*	*Geometric Mean (CI)*	*GMR (CI)*	*p value*	*GMR (CI)**	*p value**
7F	V4	209	3.92 (3.43 ,4.48)	202	3.50 (3.03 ,4.04)	1.12 (0.92 ,1.36)	0.2546	1.11 (0.92 ,1.35)	0.2780
7F	V5	202	1.31 (1.19 ,1.43)	197	1.28 (1.15 ,1.42)	1.02 (0.89 ,1.18)	0.7354	1.02 (0.89 ,1.17)	0.8082
7F	V7	181	11.26 (10.00 ,12.69)	171	10.68 (9.30 ,12.26)	1.05 (0.88 ,1.26)	0.5668	1.05 (0.87 ,1.26)	0.6031
7F	V8	188	2.22 (2.05 ,2.40)	186	2.54 (2.28 ,2.83)	0.87 (0.76 ,1.00)	0.0482	0.88 (0.76 ,1.00)	0.0528
9V	V4	209	1.67 (1.41 ,1.98)	202	1.68 (1.40 ,2.01)	1.00 (0.78 ,1.27)	0.9734	0.97 (0.76 ,1.25)	0.8254
9V	V5	202	0.49 (0.43 ,0.56)	197	0.45 (0.40 ,0.51)	1.09 (0.91 ,1.30)	0.3601	1.08 (0.90 ,1.29)	0.4097
9V	V7	182	11.33 (9.98 ,12.85)	171	11.16 (9.79 ,12.72)	1.01 (0.85 ,1.22)	0.8725	1.00 (0.83 ,1.20)	0.9811
9V	V8	188	1.15 (1.02 ,1.30)	186	1.19 (1.05 ,1.35)	0.97 (0.81 ,1.15)	0.6977	0.97 (0.81 ,1.15)	0.7134
Hib	V4	207	406.56 (307.02 ,538.37)	202	609.89 (453.34 ,820.51)	0.67 (0.44 ,1.00)	0.0510	0.64 (0.43 ,0.95)	0.0268
Hib	V8	159	2562.54 (2060.53 ,3186.86)	159	2783.03 (2235.01 ,3465.42)	0.92 (0.68 ,1.25)	0.5984	0.95 (0.69 ,1.29)	0.7271
Tet	V4	207	538.45 (485.21 ,597.54)	202	557.20 (493.00 ,629.77)	0.97 (0.82 ,1.13)	0.6747	0.96 (0.82 ,1.12)	0.5956
Tet	V8	159	423.75 (347.28 ,517.06)	159	607.64 (501.82 ,735.78)	0.70 (0.53 ,0.92)	0.0104	0.69 (0.52 ,0.91)	0.0093

**Adjusted for centre and dose group*

20.2.11 Hib anti-PRP IgG GMCs for each of the 13 serotypes at 5 months, 12 months, 13 months and 24 months of age

At 5 months (one month following completion of the primary immunisation series), anti-PRP (Hib) IgG GMCs were significantly higher for participants in the alternating limb compared to those in the consistent limb group (Table 39). Waning of antibodies was observed for both groups between completion of the primary series and boosting, with anti-PRP IgG GMCs remaining significantly higher in the alternating limb group. An anamnestic response was seen following boosting with Hib-MenC-TT at twelve months for participants in both groups. After boosting at 12 months, anti-PRP IgG GMCs did not differ significantly (Table 39).

Comparisons performed:

15. the anti-PRP IgG GMC response variable at 5 months of age
    a. the consistent limb group vs. the alternating limb group

16. the anti-PRP IgG GMC response variable (persistence) at 12 months of age
    a. the consistent limb group vs. the alternating limb group

17. the anti-PRP IgG GMC response variable at 13 months of age, after the Hib-MenC booster dose
    a. the consistent limb group vs. the alternating limb group

18. the anti-PRP IgG GMC response variable at 24 months of age
    a. the consistent limb group vs. the alternating limb group

TABLE 39 ANTI-PRP IGG GMCS BY VISIT (ITT & CP POPULATION)

	Consistent Limbs		*Alternating Limbs*		*Unadjusted*		*Adjusted*	
Visit	*N*	*Geometric Mean (CI)*	*N*	*Geometric Mean (CI)*	*GMR (CI)*	*p value*	*GMR (CI)**	*p value**
CP Population								
V4	207	0.41 (0.31 ,0.54)	202	0.61 (0.45 ,0.82)	0.67 (0.44 ,1.00)	0.0510	0.64 (0.43 ,0.95)	0.0268
V5	202	0.35 (0.28 ,0.43)	197	0.50 (0.40 ,0.62)	0.70 (0.51 ,0.95)	0.0238	0.68 (0.50 ,0.92)	0.0136
V7	182	20.58 (16.29 ,26.01)	171	26.57 (21.61 ,32.67)	0.77 (0.57 ,1.06)	0.1074	0.77 (0.56 ,1.05)	0.0969
V8	187	2.45 (2.01 ,2.98)	186	2.65 (2.16 ,3.25)	0.92 (0.69 ,1.23)	0.5814	0.95 (0.71 ,1.26)	0.7054
ITT Population								
V4	219	0.44 (0.33 ,0.58)	220	0.66 (0.50 ,0.88)	0.66 (0.44 ,0.98)	0.0389	0.64 (0.43 ,0.94)	0.0229
V5	213	0.35 (0.28 ,0.44)	209	0.50 (0.40 ,0.62)	0.71 (0.52 ,0.95)	0.0235	0.69 (0.51 ,0.92)	0.0135
V7	201	19.85 (15.73 ,25.06)	200	26.01 (21.40 ,31.61)	0.76 (0.56 ,1.03)	0.0806	0.76 (0.56 ,1.03)	0.0784
V8	203	2.41 (1.99 ,2.93)	209	2.79 (2.31 ,3.38)	0.86 (0.66 ,1.13)	0.2893	0.86 (0.66 ,1.13)	0.2915

**Adjusted for centre and MenC dose group*

20.2.12 Anti-tetanus toxoid GMCs for each of the 13 serotypes at 5 months, 12 months, 13 months and 24 months of age

Anti-TT IgG GMCs were similar for both groups at 5 and 12 months but were significantly higher in the alternating limb group compared to the consistent limb group at 13 months and 24 months (Table 40)

Comparisons performed:

19. the anti-tetanus toxoid GMC response variable at 5 months of age ,
    a. the consistent limb group vs. the alternating limb group

20. the anti-tetanus toxoid GMC response variable (persistence) at 12 months of age
    a. the consistent limb group vs. the alternating limb group

21. the anti-tetanus toxoid GMC response variable at 13 months of age
    a. the consistent limb group vs. the alternating limb group

22. the anti-tetanus toxoid GMC response variable at 24 months of age

    a. the consistent limb group vs. the alternating limb group

TABLE 40. ANTI-TETANUS TOXOID GMCS BY VISIT (ITT & CP POPULATION)

	Consistent Limbs		*Alternating Limbs*		*Unadjusted*		*Adjusted*	
Visit	*N*	*Geometric Mean (CI)*	*N*	*Geometric Mean (CI)*	*GMR (CI)*	*p value*	*GMR (CI)**	*p value**
CP Population								
V4	207	0.54 (0.49 ,0.60)	202	0.56 (0.49 ,0.63)	0.97 (0.82 ,1.13)	0.6747	0.96 (0.82 ,1.12)	0.5956
V5	201	0.18 (0.16 ,0.21)	197	0.21 (0.18 ,0.24)	0.86 (0.71 ,1.05)	0.1376	0.84 (0.70 ,1.02)	0.0828
V7	182	1.63 (1.40 ,1.90)	171	2.30 (1.97 ,2.68)	0.71 (0.57 ,0.88)	0.0020	0.69 (0.56 ,0.86)	0.0008
V8	187	0.44 (0.37 ,0.52)	186	0.61 (0.51 ,0.73)	0.71 (0.56 ,0.92)	0.0093	0.71 (0.55 ,0.91)	0.0074
CP Population (with MenC-TT group removed)								
V4	158	0.53 (0.47 ,0.59)	160	0.51 (0.45 ,0.59)	1.03 (0.86 ,1.23)	0.7490	1.02 (0.85 ,1.22)	0.8113
V5	150	0.17 (0.15 ,0.20)	160	0.19 (0.17 ,0.23)	0.87 (0.70 ,1.08)	0.2053	0.87 (0.70 ,1.08)	0.2136
V7	140	1.55 (1.30 ,1.84)	140	2.11 (1.78 ,2.50)	0.74 (0.58 ,0.94)	0.0131	0.73 (0.57 ,0.93)	0.0101
V8	141	0.42 (0.34 ,0.51)	148	0.56 (0.46 ,0.68)	0.74 (0.56 ,0.99)	0.0410	0.75 (0.57 ,1.00)	0.0524
ITT Population								
V4	219	0.55 (0.50 ,0.61)	220	0.57 (0.51 ,0.64)	0.96 (0.82 ,1.12)	0.6027	0.95 (0.82 ,1.11)	0.5355
V5	212	0.18 (0.16 ,0.21)	209	0.21 (0.18 ,0.24)	0.86 (0.71 ,1.04)	0.1211	0.84 (0.70 ,1.02)	0.0713
V7	201	1.67 (1.44 ,1.93)	200	2.23 (1.93 ,2.57)	0.75 (0.61 ,0.92)	0.0054	0.74 (0.60 ,0.91)	0.0040
V8	203	0.45 (0.38 ,0.54)	209	0.62 (0.53 ,0.74)	0.73 (0.57 ,0.92)	0.0093	0.72 (0.57 ,0.92)	0.0082

**Adjusted for centre and MenC dose group*

20.2.13 Percentage of infants with MenCrSBA ≥ 1:8 and ≥ 1:128 six days following Hib-MenC-TT vaccination at 12 months of age

Six days after Hib-MenC-TT vaccination there was no significant difference in the percentage of participants with MenC rSBA≥1:8 and ≥1:128 between those who were primed with a single

MenC-CRM/TT dose compared to those primed with two MenC-CRM197 doses (Tables 41-44). Furthermore no difference was observed between the percentage of participants having MenC seroprotective titres following one dose of either of the conjugates used in the study. Priming with one MenC-CRM197/TT or two doses of MenC-CRM197 resulted in a higher percentage of vaccinees with MenC rSBA ≥1:8 or ≥128 when compared to no MenC conjugate vaccine priming in infancy.

Comparisons performed:

23. percentage of infants with MenC rSBA ≥ 1:8 and ≥ 1:128 assessed 6 days after the 12 month Hib-MenC booster
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 41. Percentage of infants with MenC rSBA ≥1:8 and ≥1:128 6 days after Hib-MenC-TT vaccination at 12 months of age (ITT population)

V6	ITT pop (N)	Serum (n)	%	≥1:8 (n)	≥1:8 (%)	LCL	UCL	≥1:128 (n)	≥1:128 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	62	49	79.03	49	100	92.75	100*	48	97.96	89.15	99.95
Group 2 (Two dose MenC-CRM)	61	50	81.97	50	100	92.89	100*	47	94	83.45	98.75
Group 3 (Control)	61	52	85.25	42	80.77	67.47	90.37324	41	78.85	65.30	88.94
Group 4 (Single dose MenC-TT)	63	52	82.54	52	100	93.15	100*	52	100	93.15	100*
Total	247	203									

*one sided 97.5% CI

Table 42: Differences in Proportions of infants with MenC rSBA ≥1:8 and ≥1:128 6 days after Hib-MenC-TT vaccination at 12 months of age (ITT population)

V6 ITT Population	**Difference in proportions** (95% CI), ***2-sided p value***	
Group Differences	**1:8**	**1:128**
GROUP1 VS GROUP 2	0(-7.48 , 7.48), 1.0	3.96 (-5.46 , 14.84), 0.38
GROUP1 VS GROUP 3	19.23 (9.36 , 32.77), **0.001**	19.11 (6.98 , 32.77),**0.003**
GROUP1 VS GROUP 4	0 (-7.25 , -6.98), 1.0	-2.04 (-10.85 , 5.02), 0.31
GROUP2 VS GROUP 3	19.23 (9.29 , 32.53), **0.001**	15.15 (1.64 , 29.65), **0.03**
GROUP2 VS GROUP 4	0 (7.15 , 6.85), 1.0	-6.00 (-16.55 , 1.17), 0.07
GROUP3 VS GROUP 4	-19.23 (-32.54 , -9.38), **0.0008**	-21.15 (-34.70 , -10.98), **0.0004**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

Table 43. Percentage of infants with MenC rSBA ≥1:8 and ≥1:128 6 days after Hib-MenC-TT vaccination at 12 months of age (Completers population)

V6 Completers Population	CP pop (N)	Serum (n)	%	≥1:8 (n)	≥1:8 (%)	LCL	UCL	≥1:128 (n)	≥1:128 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	60	48	80	48	100	92.60	100*	47	97.92	88.93	99.95
Group 2 (Two dose MenC-CRM)	58	49	84.48	49	100	92.75	100*	46	93.88	83.13	98.72
Group 3 (Control)	59	50	84.75	40	80	66.28	89.97	39	78	64.04	88.47
Group 4 (Single dose MenC-TT)	60	49	81.67	49	100	92.75	100*	49	100	92.75	100*
Total	237	196									

*one sided 97.5% CI

Table 44: Differences in Proportions of infants with MenC rSBA ≥1:8 and ≥1:128 6 days after Hib-MenC-TT vaccination at 12 months of age (Completers population)

V6 CP	Difference in proportions (95% CI), ***2-sided p value***	
Group Difference	**1:8**	**1:128**
GROUP1 VS GROUP 2	0 (-7.61 , 7.37), 1.0	4.04 (-5.64 , 15.29), 0.38
GROUP1 VS GROUP 3	20.00 (9.68 , 33.72), **0.0009**	19.92 (7.41 , 34.29),**0.002**
GROUP1 VS GROUP 4	0 (-7.61 , 7.37), 1.0	-2.08 (-11.07 , 5.29), 0.34
GROUP2 VS GROUP 3	20.00 (9.73 , 33.72), **0.0009**	15.88 (1.81 , 31.12), **0.024**
GROUP2 VS GROUP 4	0 (-7.42 , 7.34), 1.0	-6.12 (-16.87 , 1.39), 0.09
GROUP3 VS GROUP 4	-20.00 (-33.72 , 9.73), **0.0009**	-22.00 (--35.96 , -11.41), **0.0004**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

20.2.14 Percentage of infants with MenCrSBA ≥ 1:8 and ≥ 1:128 one month following Hib-MenC-TT vaccination at 12 months of age

One month after Hib-MenC-TT vaccination there was no difference in the percentage of subjects with MenC rSBA ≥1:8 following single dose MenC-CRM197/MenC-TT priming compared to two MenC-CRM197 dose priming (Tables 45-48). The percentage of vaccinees with MenC rSBA ≥1:128 was higher following single dose MenC-CRM/TT priming compared to two MenC-CRM197 infant doses or no priming. However, no significant difference was observed between those who were unprimed compared to those primed with 2 MenC-CRM197 doses in infancy. This is in contrast to the percentage of vaccinees with MenC rSBA≥1:8 or ≥1:128 of those primed with one dose of MenC-CRM/TT vaccine which was significantly higher compared to those who were not primed in infancy. The percentage of vaccinees with MenC rSBA ≥1:128 was significantly higher with one MenC-TT dose compared to one MenC-CRM197 dose priming but no significant difference was seen when comparing the percentage of subjects with MenC rSBA≥1:8.

Comparisons performed:

24. percentage of infants with MenC rSBA ≥ 1:8 and ≥ 1:128 assessed 28 days after the 12 month Hib-MenC booster

    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group

    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group

    c. Single dose MenC-TT Group vs 0 dose control Group

    d. Two dose MenC Group vs. 0 dose control Group

    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 45. Percentage of infants with MenC rSBA ≥1:8 and ≥1:128 one month after Hib-MenC-TT vaccination at 12 months of age (ITT population)

V7 ITT	**ITT pop (N)**	**Serum (n)**	**No serum**	**%**	**≥1:8 (n)**	**≥1:8 (%)**	**LCL**	**UCL**	**≥1:128 (n)**	**≥1:128 (%)**	**LCL**	**UCL**
Group 1 (Single dose MenC-CRM)	158	136	22	86.08	132	97.06	92.64	99.19	125	91.91	85.99	95.89
Group 2 (Two dose MenC-CRM)	153	126	27	82.35	122	96.82	92.07	99.13	102	80.95	73.00	87.40
Group 3 (Control)	62	54	8	87.10	45	83.33	70.71	92.08	37	68.52	54.45	80.48
Group 4 (Single dose MenC-TT)	105	84	21	80	84	100	95.70	100*	83	98.81	93.54	99.97
Total	478	400	78									

*one sided 97.5% CI

Table 46: Differences in Proportions of infants with MenC rSBA ≥1:8 and ≥1:128 one month after Hib-MenC-TT vaccination at 12 months of age (ITT Population)

V7 ITT population	Difference in proportions (95% CI), ***2-sided p value***	
Group Differences	**1:8**	**1:128**
GROUP1 VS GROUP 2	0.23 (-4.60 , 5.30), 0.99	10.96 (2.02 , 19.64), **0.009**
GROUP1 VS GROUP 3	13.72 (3.08 , 26.01), **0.002**	23.39 (11.27 , 32.33), **0.00004**
GROUP1 VS GROUP 4	-2.94 (-7.48 , 1.32), 0.12	-6.90 (-12.94 , -0.98), **0.029**
GROUP2 VS GROUP 3	13.49 (3.07 , 25.81), **0.0005**	12.43 (-1.81 , 27.45), 0.089
GROUP2 VS GROUP 4	-3.18 (-8.01 , 1.32), 0.10	-17.86 (-25.98 , -9.87), **0.00013**
GROUP3 VS GROUP 4	-16.67 (-29.29 , -7.92), **0.0001**	-30.29 (-43.75 , -18.99), <**0.0001**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

Table 47. Percentage of infants with MenC rSBA ≥1:8 and ≥1:128 one month after Hib-MenC-TT vaccination at 12 months of age (Completers population)

V7 CP	**CP population**	**Serum (n)**	**No serum**	**%**	**≥1:8 (n)**	**≥1:8 (%)**	**LCL**	**UCL**	**≥1:128 (n)**	**≥1:128 (%)**	**LCL**	**UCL**
Group 1 (Single dose MenC-CRM)	144	125	19	86.81	121	96.8	92.01	99.12	116	92.8	86.77	96.66
Group 2 (Two dose MenC-CRM)	133	111	22	83.46	109	98.20	93.64	99.78	91	81.98	73.55	88.63
Group 3 (Control)	57	49	8	85.96	41	83.67	70.34	92.68	33	67.35	52.45	80.05
Group 4 (Single dose MenC-TT)	93	76	17	81.72	76	100	95.26	100*	75	98.68	92.89	99.97
Total	427	361	66									

* one sided 97.5% CI

Table 48: Differences in Proportions of infants with MenC rSBA ≥1:8 and ≥1:128 one month after Hib-MenC-TT vaccination at 12 months of age (Completers Population)

V7 CP	**Difference in proportions** (95% CI), 2-sided p value	
Group Differences	**1:8**	**1:128**
GROUP1 VS GROUP 2	-1.40 (-6.49 , 3.48), 0.58	10.82 (2.48 , 19.85), **0.012**
GROUP1 VS GROUP 3	13.13 (2.24 , 26.05), **0.004**	25.45 (12.76 , 40.06), **<0.0001**
GROUP1 VS GROUP 4	-3.20 (-8.19 , 1.52), 0.12	-5.88 (-12.05 , 0.47), 0.063
GROUP2 VS GROUP 3	14.52 (4.63 , 27.32), **0.001**	14.64 (0.52 , 30.13), **0.041**
GROUP2 VS GROUP 4	-1.80 (-6.47 , 2.84), 0.34	-16.70 (-25.13 , -9.14), **0.0004**
GROUP3 VS GROUP 4	-16.33 (-29.66 , -7.32), **0.0003**	-31.34 (-45.53 , -19.30), **<0.00001**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

20.2.15 Percentage of infants with MenCrSBA ≥ 1:8 and ≥ 1:128 at 12 months of age

Before Hib-MenC-TT vaccination at 12 months of age the percentage of subjects with MenC rSBA≥1:8 was significantly higher following two priming doses of MenC-CRM197 compared to a single dose of MenC-CRM in infancy (Tables 49-52). No difference in the percentage of vaccinees with MenC rSBA ≥1:8 was observed when comparing two MenC-CRM197 priming with a single priming dose of MenC-TT. The percentage of those primed with one dose of MenC-CRM197 with MenC rSBA≥1:128 was not significantly different when compared to two dose MenC-CRM197 priming, however the percentage of subjects with MenC rSBA≥1:8 and ≥1:128 was significantly lower when compared to those primed with MenC-TT. Furthermore, more vaccinees primed with MenC-TT had SBA ≥1:128 compared to those primed with two MenC-CRM197 doses.

Comparisons performed:

25. percentage of infants with MenC rSBA ≥ 1:8 and ≥1:128 assessed at 12 months of age
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 49. Percentage of infants with MenC rSBA ≥1:8 and ≥1:128 just before Hib-MenC-TT vaccination at 12 months of age (ITT population)

V5 ITT population	ITT pop (N)	Serum (n)	No serum	%	≥1:8 (n)	≥1:8 (%)	LCL	UCL	≥1:128 (n)	≥1:128 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	161	147	14	91.30	38	25.85	18.99	33.71	10	6.80	3.31	12.15
Group 2 (Two dose MenC-CRM)	154	128	26	83.12	53	41.41	32.77	50.45	16	12.50	7.32	19.50
Group 3 (Control)	62	54	8	87.10	0	0	0	0	0	0	0	0
Group 4 (Single dose MenC-TT)	106	93	13	87.74	37	39.78	29.78	50.46	21	22.58	14.55	32.42
Total	483	422	61	87.37								

Table 50: Differences in Proportions of infants with MenC rSBA ≥1:8 and ≥1:128 just before Hib-MenC-TT vaccination at 12 months of age (ITT Population)

V5 ITT	**Difference in proportions** (95% CI), ***2-sided p value***	
Group Differences	**1:8**	**1:128**
GROUP1 VS GROUP 2	-15.56 (-26.61 , -4.27), **0.006**	-5.70 (-13.30 , 1.37), 0.124
GROUP1 VS GROUP 3	25.85 (18.48 , 33.79), **<0.00001**	6.80 (2.62 , 12.23), **0.045**
GROUP1 VS GROUP 4	-13.94 (-26.34 , -1.68), **0.023**	-15.78 (-25.95 , 6.21), **0.0002**
GROUP2 VS GROUP 3	41.41 (32.70 , 50.55), **<0.00001**	12.50 (5.82 , 19.57), **0.005**
GROUP2 VS GROUP 4	1.62 (-11.64 , 14.64), 0.82	-10.08 (-20.88 , 0.15), **0.047**
GROUP3 VS GROUP 4	-39.78 (-50.46 , -29.76), **<0.00001**	-22.58 (-32.42 , -14.29), **0.0003**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

Table 51. Percentage of infants with MenC rSBA ≥1:8 and ≥1:128 just before Hib-MenC-TT vaccination at 12 months of age (Completers population)

V5 CP	**CP pop (N)**	**Serum (n)**	**No serum**	**%**	**≥1:8 (n)**	**≥1:8 (%)**	**LCL**	**UCL**	**≥1:128 (n)**	**≥1:128 (%)**	**LCL**	**UCL**
Group 1 (Single dose MenC-CRM)	154	140	14	90.91	33	23.57	16.81	31.48	9	6.43	2.98	11.85
Group 2 (Two dose MenC-CRM)	144	121	23	84.03	49	40.50	31.67	49.80	14	11.57	6.47	18.65
Group 3 (Control)	60	53	7	88.33	0	0	0	0	0	0	0	0
Group 4 (Single dose MenC-TT)	100	89	11	89.00	33	37.08	27.07	47.97	20	22.47	14.30	32.55
Total	458	403	55	87.99								

Table 52: Differences in Proportions of infants with MenC rSBA ≥1:8 and ≥1:128 just before Hib-MenC-TT vaccination at 12 months of age (Completers Population)

V5 CP	**Difference in proportions** (95% CI), ***2-sided p value***	
Group Differences	**1:8**	**1:128**
GROUP1 VS GROUP 2	-16.92 (-28.13 , -5.48),**0.003**	-5.14 (-12.83 , 1.89), 0.176
GROUP1 VS GROUP 3	23.57 (16.22 , 31.48), <**0.00001**	6.43 (-0.22 , 12.03), 0.055
GROUP1 VS GROUP 4	-13.51 (-25.92 , -1.18), **0.028**	-16.04 (-26.43 , -6.26), **0.0002**
GROUP2 VS GROUP 3	40.50 (31.60 , 49.80), <**0.00001**	11.57 (4.78 , 18.66), **0.008**
GROUP2 VS GROUP 4	3.42 (-10.07 , 16.63), 0.63	-10.90 (-21.88 , -0.45), **0.036**
GROUP3 VS GROUP 4	-37.08 (-47.97 , 27.05), <**0.0001**	-22.47 (-32.55 , -14.03), **0.0003**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

20.2.16 Percentage of infants with MenCrSBA ≥ 1:8 and ≥ 1:128 at 5 months of age

Following vaccination with 2 doses of MenC-CRM197 at 3 and 4 months of age, a higher percentage of vaccinees had MenC rSBA ≥1:8 and ≥1:128 when compared to those immunised with one dose of MenC-CRM or MenC-TT at 3 months of age (Tables 53-56). A significantly higher percentage of those primed with one dose of MenC-TT had MenC rSBA≥1:8 and ≥1:128 compared to those primed with a single MenC-CRM197 dose.

Comparisons performed:

26. percentage of infants with MenC rSBA ≥ 1:8 and ≥1:128 at 5 months of age
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 53. Percentage of infants with MenC rSBA ≥1:8 and ≥1:128 at 5 months of age after MenC infant priming (ITT population)

V4 ITT population	ITT pop (N)	No serum	Serum (n)	%	≥1:8 (n)	≥1:8 (%)	LCL	UCL	≥1:128 (n)	≥1:128 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	165	21	144	87.27	121	84.03	77.00	89.60	70	48.61	40.20	57.08
Group 2 (Two dose MenC-CRM)	157	20	137	87.26	137	100	0.97	100*	136	99.27	96.00	99.98
Group 3 (Control)	63	7	56	88.89	1	1.79	0.05	9.55	1	1.79	0.05	9.55
Group 4 (Single dose MenC-TT)	112	13	99	88.39	93	93.94	87.27	97.74	79	79.80	70.54	87.20
Total	497	61	436	87.73								

(*) one-sided, 97.50% confidence interval

Table 54: Differences in Proportions of infants with MenC rSBA ≥1:8 and ≥1:128 at 5 months of age after MenC priming (ITT Population)

V4 ITT	**Difference in proportions** (95% CI), ***2-sided p value***	
Group Differences	**1:8**	**1:128**
GROUP1 VS GROUP 2	-15.97 (-23.00 , -10.40), <**0.00001**	-50.660 (-59.12 , -42.19), <**0.00001**
GROUP1 VS GROUP 3	82.24 (72.96 , 88.44), <**0.00001**	46.82 (36.64 , 55.80),< **0.00001**
GROUP1 VS GROUP 4	-9.91 (-17.83 , -1.74), **0.018**	-31.19 (-42.23 , -19.26), <**0.00001**
GROUP2 VS GROUP 3	98.21 (90.45 , 99.95), <**0.00001**	97.48 (90.21 , 99.65), <**0.00001**
GROUP2 VS GROUP 4	6.06 (2.12 , 12.73), **0.004**	19.47 (11.44 , 27.51), <**0.00001**
GROUP3 VS GROUP 4	-92.15 (-96.65 , -83.30), <**0.00001**	-78.01 (-85.82 , -67.20), <**0.00001**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

Table 55. Percentage of infants with MenC rSBA ≥1:8 and ≥1:128 at 5 months of age after MenC priming (Completers population)

V4 CP	CP pop (N)	No serum	Serum (n)	%	≥1:8 (n)	≥1:8 (%)	LCL	UCL	≥1:128 (n)	≥1:128 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	154	18	136	88.31	115	84.56	77.37	90.18	67	49.26	40.59	57.97
Group 2 (Two dose MenC-CRM)	146	15	131	89.73	131	100	97.22	100*	130	99.24	95.82	99.98
Group 3 (Control)	59	6	53	89.83	1	1.89	0.048	10.07	1	1.89	0.048	10.07
Group 4 (Single dose MenC-TT)	103	12	91	88.35	85	93.41	86.20	97.54	72	79.12	69.33	86.94
Total	462	51	411	88.96								

(*) one-sided, 97.50% confidence interval

Table 56: Differences in Proportions of infants with MenC rSBA ≥1:8 and ≥1:128 at 5 months of age after MenC priming (Completers Population)

V4 CP	**Difference in proportions** (95% CI), ***2-sided p value***	
Group Differences	**1:8**	**1:128**
GROUP1 VS GROUP 2	-15.44 (-22.63 , -9.82), <**0.00001**	-49.97 (-58.71 , -41.25), <**0.00001**
GROUP1 VS GROUP 3	82.67 (73.02 , 89.16), <**0.00001**	47.38 (36.20 , 56.64), <**0.00001**
GROUP1 VS GROUP 4	-8.85 (-17.05 , -0.29), **0.043**	-29.86 (-41.31 , -17.34), <**0.00001**
GROUP2 VS GROUP 3	98.11 (89.93 , 99.95), <**0.0001**	97.35 (89.68 , 99.64), <**0.00001**
GROUP2 VS GROUP 4	6.59 (2.36 , 13.80), **0.004**	20.12 (12.14 , 29.68), <**0.00001**
GROUP3 VS GROUP 4	-91.52 (-96.37 , -81.95), <**0.00001**	-77.23 (-85.41 , -66.46), <**0.00001**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

20.2.17 Percentage of infants with MenC rSBA ≥ 1:8 and ≥ 1:128 at 24 months of age

Twelve months after Hib-MenC-TT vaccination no significant difference in the percentage of subjects with MenC rSBA ≥1:8 and ≥1:128 was observed following two MenC-CRM197 priming doses compared to a single dose of MenC-CRM197 and to infants who were not primed with a MenC vaccine (Tables 57-60). The percentage of subjects with MenC rSBA ≥1:8 and ≥1:128 of those primed with one MenC-TT vaccine dose in infancy was significantly higher than those who were unprimed or primed with one/two MenC-CRM197 doses.

Comparisons performed:

27. percentage of infants with MenC rSBA ≥ 1:8 and ≥1:128 at 24 months of age
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 57. Percentage of infants with MenC rSBA ≥1:8 and ≥1:128, 12 months after Hib-MenC-TT vaccination at 12 months of age (ITT population)

V8 ITT population	ITT pop (N)	Serum (n)	No serum	%	≥1:8 (n)	≥1:8 (%)	LCL	UCL	≥1:128 (n)	≥1:128 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	152	136	16	89.47	42	30.88	23.25	39.37	15	11.03	6.31	17.54
Group 2 (Two dose MenC-CRM)	147	127	20	86.39	25	19.69	13.16	27.67	7	5.51	2.24	11.03
Group 3 (Control)	57	55	2	96.49	15	27.27	16.14	40.96	6	10.91	4.11	22.25
Group 4 (Single dose MenC-TT)	102	95	7	93.14	78	82.11	72.90	89.22	66	69.47	59.18	78.51
Total	458	413	45		160				94			

Table 58. Percentage of infants with MenC rSBA ≥1:8 and ≥1:128, 12 months after Hib-MenC-TT vaccination at 12 months of age (Completers population)

V8 CP	CP population	Serum (n)	No serum	%	≥1:8 (n)	≥1:8 (%)	LCL	UCL	≥1:128 (n)	≥1:128 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	134	120	14	89.55	38	31.67	23.48	40.78	14	11.67	6.53	18.80
Group 2 (Two dose MenC-CRM)	124	108	16	87.10	20	18.52	11.69	27.14	5	4.63	1.52	10.47
Group 3 (Control)	52	50	2	96.15	14	28.00	16.23	42.49	6	12.00	4.53	24.31
Group 4 (Single dose MenC-TT)	89	82	7	92.13	66	80.49	70.26	88.42	55	67.07	55.81	77.06
Total	399	360	39									

Table 59: Differences in Proportions of infants with MenC rSBA ≥1:8, 12 months after Hib-MenC-TT vaccination at 12 months of age (ITT and Completers Populations)

V8	**Difference in proportions** (95% CI), ***2-sided p value***	
Group Differences	**ITT**	**CP**
GROUP1 VS GROUP 2	11.20(0.80, 21.60) 0.05	13.15 (2.06, 24.24) 0.03
GROUP1 VS GROUP 3	3.61(-10.49 , 17.70) 0.73	3.67 (-11.31, 18.64) 0.72
GROUP1 VS GROUP 4	-51.22 (-62.16 ,- 40.28) <**0.00001**	-48.82 (-60.77, -36.87)<0.00001
GROUP2 VS GROUP 3	-7.59 (-21.24, 6.06) 0.33	-9.48 (-23.92, 4.96) 0.21
GROUP2 VS GROUP 4	-62.42 (-72.78 , -52.06) <**0.00001**	-61.97 (-72.25, -50.69) <0.00001
GROUP3 VS GROUP 4	-54.83 (-68.90 , -40.76), <**0.00001**	-52.49 (-67.60, -37.37) <0.00001
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

Table 60: Differences in Proportions of infants with MenC rSBA ≥1:128, 12 months after Hib-MenC-TT vaccination at 12 months of age (ITT and Completers Populations)

V8	**Difference in proportions** (95% CI), ***2-sided p value***	
Group Differences	**ITT**	**CP**
GROUP1 VS GROUP 2	5.52 (-1.08, 12.11) 0.12	7.04 (0.06, 14.02) 0.06
GROUP1 VS GROUP 3	0.12 (-0.96, 9.90) 1.0	-0.33 (-11.02, 10.35) 1.0
GROUP1 VS GROUP 4	-58.44 (-69.10, -47.79) <**0.00001**	-55.41 (-67.09, -43.73) <**0.00001**
GROUP2 VS GROUP 3	-5.40 (-14.54, 3.75) 0.22	-7.37 (-17.21, 2.47) 0.10
GROUP2 VS GROUP 4	-63.96(-74.04, -53.89) <**0.00001**	-62.44 (-73.36, -41.49) <**0.00001**
GROUP3 VS GROUP 4	-58.56 (-70.96, -46.17) <**0.00001**	-55.07 (-68.66, -41.49) <**0.00001**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

20.2.18 Percentage of infants with MenC rSBA≥1:1000, 6 days after Hib-MenC-TT vaccination at 12 months of age

The percentage of vaccinees with MenC rSBA>1:1000 was not significantly different when comparing subjects primed with a single MenC-CRM197 dose to those primed with two dose of MenC-CRM197 but was significantly less compared to a single MenC-TT dose priming (Tables 61-63). Following two MenC-CRM197 dose or one MenC-CRM197/MenC-TT priming resulted in a significantly higher percentage of subjects with MenC rSBA≥1:1000 compared to those who were not primed in infancy. Priming with MenC-TT vaccine in infancy resulted in a significant higher percentage of vaccinees with MenC rSBA≥1:1000 compared to subjects who were primed with 2 doses of MenC-CRM197.

Comparisons performed:

28. percentage of infants with MenC rSBA ≥ 1:1000 assessed 6 days after the 12 month Hib-MenC booster
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 61: **Percentage of infants with MenC rSBA≥1:1000, 6 days after Hib-MenC-TT vaccination at 12 months of age (ITT population)**

V6 ITT	ITT pop (N)	Serum (n)	%	SBA >1:1000 (n)	SBA > 1:1000 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	62	49	79.03	29	59.18	44.21	73.00
Group 2 (Two dose MenC-CRM)	61	50	81.97	29	58.00	43.21	71.81
Group 3 (Control)	61	52	85.25	15	28.85	17.13	43.08
Group 4 (Single dose MenC-TT)	63	52	82.54	48	92.31	81.46	97.86
Total	247	203					

Table 62: Percentage of infants with SBA≥1:1000, 6 days after Hib-MenC-TT vaccination at 12 months of age (Completers population)

V6 CP	CP pop (N)	Serum (n)	%	SBA >1:1000 (n)	SBA >1:1000 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	60	48	80	28	58.33	43.21	72.39
Group 2 (Two dose MenC-CRM)	58	49	84.48	28	57.14	42.21	71.18
Group 3 (Control)	59	50	84.75	13	26.00	14.63	40.34
Group 4 (Single dose MenC-TT)	60	49	81.67	45	91.84	80.40	97.73
Total	237	196					

Table 63: Differences in Proportions of infants with MenC rSBA ≥1:1000, 6 days after Hib-MenC-TT vaccination at 12 months of age (ITT and Completers Populations)

Group Differences	Difference in proportions (95% CI), ***2-sided p value***	
	ITT	**CP**
GROUP1 VS GROUP 2	1.18 (-18.42 , 20.83), 0.97	1.19 (-18.69 , 21.13), 0.97
GROUP1 VS GROUP 3	30.34 (9.31 , 48.11), **0.0026**	32.33 (10.65 ,50.11),**0.0013**
GROUP1 VS GROUP 4	-33.12 (-49.28 , -14.94), **0.001**	-33.50 (-49.60 , -15.26), **0.0001**
GROUP2 VS GROUP 3	29.15 (8.90 , 47.11), **0.0033**	31.14 (10.33 , 48.94), **0.0017**
GROUP2 VS GROUP 4	-34.31 (-49.93 , -16.23), **0.0001**	-34.69 (-51.13 , -16.58), **0.0001**
GROUP3 VS GROUP 4	-63.46 (-76.67 , -47.04), <**0.00001**	-65.84 (-79.01 , -49.21), <**0.00001**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

20.2.19 Percentage of infants with MenC rSBA≥1:1000, 28 days after Hib-MenC-TT vaccination at 12 months of age

One month after HIb-MenC-TT vaccination a significantly higher percentage of subjects who had been primed with a single MenC-CRM/TT dose had MenC rSBA≥1:1000 compared to those primed with two MenC-CRM197 doses (Tables 64-66). There was no difference in the percentage of subjects with MenC rSBA≥1:1000 when comparing those primed with two MenC-CRM197 doses and the control group. Subjects who had been primed with MenC-TT had higher MenC rSBA≥1:1000 compared to those primed with one dose of MenC-CRM197.

Comparisons performed:

29. percentage of infants with MenC rSBA ≥ 1:1000 assessed 28 days after the 12 month Hib-MenC booster
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-TT Group vs 0 dose control Group
    d. Two dose MenC Group vs. 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group

Table 64: Percentage of infants with SBA≥1:1000, 28 days after Hib-MenC-TT vaccination at 12 months of age (ITT population)

V7 ITT	ITT pop (N)	Serum (n)	No serum	%	SBA >1:1000 (n)	SBA >1:1000 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	158	136	22	86.08	69	50.74	42.03	59.41
Group 2 (Two dose MenC-CRM)	153	126	27	82.35	41	32.54	24.46	41.46
Group 3 (Control)	62	54	8	87.10	10	18.52	9.25	31.43
Group 4 (Single dose MenC-TT)	105	84	21	80.00	71	84.52	74.99	91.49
Total	478	400	78					

Table 65: Percentage of infants with SBA≥1:1000, 28 days after Hib-MenC-TT vaccination at 12 months of age (Completers population)

V7 CP	CP population	Serum (n)	No serum	%	SBA >1:1000 (n)	SBA >1:1000 (%)	LCL	UCL
Group 1 (Single dose MenC-CRM)	144	125	19	86.81	66	52.80	43.67	61.79
Group 2 (Two dose MenC-CRM)	133	111	22	83.46	35	31.53	23.04	41.04
Group 3 (Control)	57	49	8	85.96	8	19.51	8.82	34.87
Group 4 (Single dose MenC-TT)	93	76	17	81.72	64	84.21	74.04	91.57
Total	427	361	66					

Table 66: Differences in Proportions of infants with MenC rSBA ≥1:1000, 28 days after Hib-MenC-TT vaccination at 12 months of age (ITT and Completers Populations)

Group Differences	Difference in proportions (95% CI), ***2-sided p value***	
	ITT	**CP**
GROUP1 VS GROUP 2	18.20 (6.17 , 29.83), **0.0029**	21.27 (8.67 , 33.3525), **0.001**
GROUP1 VS GROUP 3	32.22 (17.43 , 44.71), <**0.00001**	36.47 (21.23 , 49.07), **0.00001**
GROUP1 VS GROUP 4	-33.79 (-44.76 , -21.28), <**0.00001**	-31.41 (-42.93 , -18.30), **0.00001**
GROUP2 VS GROUP 3	14.02 (-0.49 , 26.57), 0.058	15.21 (3.08 , 28.17), **0.046**
GROUP2 VS GROUP 4	-51.98 (-62.57 , -39.78), <**0.00001**	-52.68 (-63.85 , -39.74), <**0.00001**
GROUP3 VS GROUP 4	-66.01 (-77.64 , -51.13), <**0.00001**	-67.88 (-79.75 , -52.72), <**0.00001**
Data are difference in proportions and their corresponding exact 95% CI (Confidence interval) and corresponding p values: GROUP1 VS GROUP 2: Proportions GROUP 1- PROPORTION GROUP 2		

20.2.20 Percentage of infants with S. pneumoniae IgG ≥ 0.35 µg/ml for each of the 13 serotypes at 5 months, 12 months, 13 months and 24 months of age

The percentage of infants in both limb groups achieving S.pneumoniae IgG seroprotective levels was similar at all time points studied.

Comparisons performed:

30. percentage of infants with *S. pneumoniae* IgG ≥ 0.35 µg/ml for each of the 13 serotypes at 5 months of age
    b. the consistent limb group vs. the alternating limb group

31. percentage of infants with *S. pneumoniae* IgG ≥ 0.35 µg/ml for each of the 13 serotypes at 12 months of age
    b. the consistent limb group vs. the alternating limb group

32. percentage of infants with *S. pneumoniae* IgG ≥ 0.35 µg/ml for each of the 13 serotypes at 13 months of age after the PCV-13 booster dose
    b. the consistent limb group vs. the alternating limb group

33. percentage of infants with *S. pneumoniae* IgG ≥ 0.35 µg/ml for each of the 13 serotypes at 24 months of age after the PCV-13 booster dose
    a. the consistent limb group vs. the alternating limb group

Adjusted odds ratios from a logistic regression model are presented in the last 4 columns of Table 67, in addition to a Fisher's Exact Test p value for comparison. For timepoints and serotypes where there were no participants with values below 0.35, or only one participant, a logistic model could not be run.

TABLE 67. COUNT AND PERCENTAGE OF PARTICIPANTS WITH *S. PNEUMONIAE* IGG >= 0.35 MCG/ML (ITT POPULATION)

sero	*Visit*	*Consistent Limbs*	*Alternating Limbs*	*p value (Fishers Exact Test)*	*OR**	*LCL**	*UCL*	*p value**
1	V4	198 (89.6%)	196 (89.1%)	0.8787	1.031	0.561	1.896	0.9218
1	V5	163 (76.5%)	160 (76.6%)	1.0000	0.996	0.631	1.571	0.9851
1	V7	199 (99.5%)	200 (100.0%)	1.0000				
1	V8	188 (92.2%)	191 (91.4%)	0.8586	1.111	0.544	2.267	0.7734
3	V4	220 (100.0%)	218 (99.5%)	0.4989				
3	V5	196 (92.0%)	193 (92.3%)	1.0000	0.977	0.475	2.011	0.9499
3	V7	199 (99.5%)	199 (99.5%)	1.0000	1.081	0.065	17.846	0.9566
3	V8	200 (98.0%)	205 (98.1%)	1.0000	1.009	0.246	4.135	0.9905
4	V4	209 (94.6%)	197 (89.5%)	0.0545	2.019	0.974	4.183	0.0587
4	V5	136 (63.8%)	126 (60.3%)	0.4830	1.173	0.789	1.745	0.4300
4	V7	201 (100.0%)	200 (100.0%)	.	.	.	.	.
4	V8	185 (90.7%)	189 (90.4%)	1.0000	1.024	0.524	1.999	0.9447
5	V4	205 (93.2%)	204 (93.2%)	1.0000	1.009	0.478	2.130	0.9821
5	V5	163 (76.5%)	148 (70.8%)	0.1868	1.331	0.857	2.065	0.2026
5	V7	200 (100.0%)	200 (100.0%)	.	.	.	.	.
5	V8	191 (93.6%)	200 (95.7%)	0.3871	0.644	0.261	1.590	0.3395
6A	V4	201 (91.0%)	193 (87.7%)	0.2845	1.422	0.768	2.631	0.2625
6A	V5	187 (87.8%)	177 (84.7%)	0.3973	1.314	0.751	2.300	0.3387
6A	V7	199 (99.5%)	199 (99.5%)	1.0000	1.020	0.061	17.012	0.9890
6A	V8	203 (99.5%)	208 (99.5%)	1.0000	1.118	0.067	18.564	0.9382
6B	V4	70 (31.7%)	78 (35.5%)	0.4209	0.837	0.561	1.248	0.3826
6B	V5	126 (59.2%)	113 (54.1%)	0.3261	1.302	0.877	1.933	0.1911
6B	V7	200 (99.5%)	199 (99.5%)	1.0000	0.904	0.055	14.802	0.9436
6B	V8	187 (91.7%)	193 (92.3%)	0.8571	0.942	0.457	1.939	0.8708
7F	V4	218 (98.6%)	218 (99.1%)	1.0000	0.697	0.115	4.238	0.6954
7F	V5	209 (98.1%)	206 (98.6%)	1.0000	0.853	0.174	4.182	0.8441
7F	V7	200 (100.0%)	199 (99.5%)	1.0000				
7F	V8	204 (100.0%)	209 (100.0%)	.	.	.	.	.
9V	V4	199 (90.0%)	192 (87.3%)	0.3718	1.269	0.697	2.310	0.4356
9V	V5	143 (67.1%)	127 (60.8%)	0.1880	1.320	0.880	1.980	0.1798
9V	V7	201 (100.0%)	200 (100.0%)	.	.	.	.	.

sero	*Visit*	*Consistent Limbs*	*Alternating Limbs*	*p value (Fishers Exact Test)*	*OR**	*LCL**	*UCL*	*p value**
9V	V8	194 (95.1%)	196 (93.8%)	0.6693	1.302	0.552	3.073	0.5467
14	V4	197 (89.1%)	197 (90.0%)	0.8764	0.914	0.493	1.697	0.7762
14	V5	179 (84.0%)	175 (83.7%)	1.0000	1.026	0.608	1.731	0.9239
14	V7	198 (98.5%)	195 (97.5%)	0.5028	1.586	0.371	6.785	0.5340
14	V8	193 (94.6%)	195 (93.3%)	0.6812	1.239	0.545	2.817	0.6096
18C	V4	207 (93.7%)	200 (91.3%)	0.3713	1.364	0.659	2.824	0.4024
18C	V5	160 (75.1%)	155 (74.2%)	0.8240	1.058	0.678	1.649	0.8046
18C	V7	200 (100.0%)	200 (100.0%)	.	.	.	.	.
18C	V8	183 (89.7%)	189 (90.4%)	0.8699	0.942	0.487	1.819	0.8581
19A	V4	203 (91.9%)	196 (89.1%)	0.3356	1.386	0.725	2.651	0.3241
19A	V5	148 (69.8%)	133 (63.9%)	0.2144	1.314	0.867	1.991	0.1976
19A	V7	201 (100.0%)	199 (99.5%)	0.4988				
19A	V8	186 (91.2%)	191 (91.4%)	1.0000	0.947	0.474	1.893	0.8771
19F	V4	218 (98.6%)	217 (98.6%)	1.0000	1.008	0.200	5.084	0.9920
19F	V5	189 (88.7%)	185 (88.5%)	1.0000	1.056	0.572	1.950	0.8609
19F	V7	200 (100.0%)	200 (100.0%)	.	.	.	.	.
19F	V8	198 (97.1%)	204 (97.6%)	0.7690	0.815	0.243	2.731	0.7399
23F	V4	163 (73.8%)	166 (75.5%)	0.7430	0.916	0.595	1.412	0.6915
23F	V5	113 (53.3%)	102 (48.8%)	0.3809	1.211	0.822	1.786	0.3329
23F	V7	199 (99.5%)	199 (99.5%)	1.0000	1.040	0.063	17.079	0.9781
23F	V8	194 (95.1%)	198 (94.7%)	1.0000	1.103	0.455	2.675	0.8288

**Adjusted for centre and MenC dose group*

TABLE 68. COUNT AND PERCENTAGE OF PARTICIPANTS WITH *S. PNEUMONIAE* IGG >= 0.35 MCG/ML (CP POPULATION)

sero	*Visit*	*Consistent Limbs*	*Alternating Limbs*	*p value (Fishers Exact Test)*	*OR**	*LCL**	*UCL*	*p value**
1	V4	188 (90.0%)	179 (88.6%)	0.7501	1.122	0.597	2.109	0.7213
1	V5	156 (77.2%)	151 (76.6%)	0.9059	1.042	0.650	1.671	0.8646
1	V7	180 (99.4%)	171 (100.0%)	1.0000				
1	V8	174 (92.6%)	169 (90.9%)	0.5789	1.304	0.617	2.754	0.4873
14	V4	187 (89.5%)	182 (90.5%)	0.7445	0.892	0.463	1.719	0.7325
14	V5	168 (83.2%)	164 (83.2%)	1.0000	0.993	0.585	1.685	0.9780
14	V7	179 (98.4%)	166 (97.1%)	0.4910	1.687	0.393	7.249	0.4820
14	V8	177 (94.1%)	174 (93.5%)	0.8330	1.028	0.431	2.451	0.9504
18C	V4	195 (93.3%)	183 (91.0%)	0.4629	1.303	0.622	2.728	0.4829
18C	V5	150 (74.3%)	143 (72.6%)	0.7346	1.084	0.690	1.702	0.7263
18C	V7	181 (100.0%)	171 (100.0%)	.	.	.	.	.
18C	V8	169 (89.9%)	168 (90.3%)	1.0000	0.988	0.493	1.978	0.9719
19A	V4	191 (91.4%)	181 (89.6%)	0.6146	1.225	0.627	2.394	0.5522
19A	V5	140 (69.7%)	126 (64.3%)	0.2861	1.269	0.826	1.949	0.2768
19A	V7	182 (100.0%)	170 (99.4%)	0.4844				
19A	V8	174 (92.6%)	170 (91.4%)	0.7075	1.177	0.551	2.517	0.6738
19F	V4	206 (98.6%)	199 (98.5%)	1.0000	1.028	0.203	5.194	0.9737
19F	V5	179 (88.6%)	174 (88.3%)	1.0000	1.075	0.573	2.016	0.8217
19F	V7	181 (100.0%)	171 (100.0%)	.	.	.	.	.
19F	V8	183 (97.3%)	181 (97.3%)	1.0000	1.079	0.304	3.835	0.9063
23F	V4	152 (72.7%)	150 (74.3%)	0.7387	0.932	0.599	1.451	0.7561
23F	V5	106 (52.7%)	94 (47.7%)	0.3669	1.235	0.828	1.842	0.3015
23F	V7	180 (99.4%)	170 (99.4%)	1.0000	1.171	0.071	19.305	0.9122
23F	V8	179 (95.2%)	176 (94.6%)	0.8184	1.167	0.460	2.960	0.7456
3	V4	208 (100.0%)	200 (99.5%)	0.4914				
3	V5	186 (92.1%)	181 (91.9%)	1.0000	1.072	0.514	2.235	0.8523
3	V7	180 (99.4%)	170 (99.4%)	1.0000	1.232	0.074	20.496	0.8844
3	V8	184 (97.9%)	182 (97.8%)	1.0000	1.098	0.267	4.516	0.8970
4	V4	199 (95.2%)	179 (88.6%)	0.0175	2.504	1.155	5.428	0.0201
4	V5	130 (64.4%)	119 (60.4%)	0.4694	1.193	0.792	1.796	0.3986
4	V7	182 (100.0%)	171 (100.0%)	.	.	.	.	.

sero	*Visit*	*Consistent Limbs*	*Alternating Limbs*	*p value (Fishers Exact Test)*	*OR**	*LCL**	*UCL*	*p value**
4	V8	171 (91.0%)	167 (89.8%)	0.7289	1.155	0.574	2.326	0.6856
5	V4	195 (93.8%)	186 (92.5%)	0.6974	1.199	0.551	2.606	0.6476
5	V5	155 (76.7%)	139 (70.6%)	0.1736	1.360	0.865	2.138	0.1824
5	V7	181 (100.0%)	171 (100.0%)	.	.	.	.	.
5	V8	176 (93.6%)	177 (95.2%)	0.6544	0.740	0.296	1.853	0.5208
6A	V4	189 (90.4%)	177 (87.6%)	0.4301	1.336	0.713	2.503	0.3664
6A	V5	180 (89.1%)	166 (84.3%)	0.1844	1.569	0.870	2.830	0.1342
6A	V7	180 (99.4%)	170 (99.4%)	1.0000	1.026	0.061	17.252	0.9860
6A	V8	187 (99.5%)	185 (99.5%)	1.0000	1.286	0.077	21.470	0.8611
6B	V4	62 (29.7%)	71 (35.1%)	0.2474	0.769	0.506	1.169	0.2192
6B	V5	120 (59.4%)	104 (52.8%)	0.1910	1.425	0.947	2.145	0.0894
6B	V7	181 (99.5%)	170 (99.4%)	1.0000	0.945	0.057	15.553	0.9683
6B	V8	173 (92.0%)	170 (91.4%)	0.8533	1.130	0.535	2.388	0.7495
7F	V4	207 (99.0%)	200 (99.0%)	1.0000	1.077	0.149	7.779	0.9415
7F	V5	198 (98.0%)	195 (99.0%)	0.6853	0.448	0.071	2.821	0.3924
7F	V7	181 (100.0%)	170 (99.4%)	0.4858				
7F	V8	188 (100.0%)	186 (100.0%)	.	.	.	.	.
9V	V4	188 (90.0%)	175 (86.6%)	0.3570	1.313	0.711	2.425	0.3845
9V	V5	137 (67.8%)	118 (59.9%)	0.1178	1.398	0.922	2.120	0.1148
9V	V7	182 (100.0%)	171 (100.0%)	.	.	.	.	.
9V	V8	179 (95.2%)	175 (94.1%)	0.6534	1.271	0.507	3.188	0.6085

**Adjusted for centre and MenC dose group*

20.2.21 Percentage of infants with Hib anti-PRP IgG ≥ 0.15 µg/ml and ≥ 1.0 µg/ml and anti-tetanus toxoid IgG ≥ 0.1 IU/ml at 5 months, 12 months, 13 months and 24 months of age

The percentage of infants in both limb groups achieving seroprotective Hib anti-PRP IgG levels was similar at all time points studied.

Comparisons performed:

34. percentage of infants with anti-PRP IgG ≥ 0.15 μg/ml and ≥ 1.0 μg/ml at 5 months of age
    b. the consistent limb group vs. the alternating limb group
35. percentage of infants with anti- PRP IgG ≥ 0.15 μg/ml and ≥ 1.0 μg/ml at 12 months of age
    b. the consistent limb group vs. the alternating limb group
36. percentage of infants with anti-PRP IgG ≥ 0.15 μg/ml and ≥ 1.0 μg/ml at 13 months of age, after the Hib-MenC booster dose
    a. the consistent limb group vs. the alternating limb group
37. percentage of infants with anti-PRP IgG ≥ 0.15 μg/ml and ≥ 1.0 μg/ml at 24 months of age
    a. the consistent limb group vs. the alternating limb group
38. percentage of infants with anti- tetanus toxoid >0.1 IU/ml at 5 months of age
    b. the consistent limb group vs. the alternating limb group
39. percentage of infants with anti- tetanus toxoid >0.1 IU/ml at 12 months of age
    b. the consistent limb group vs. the alternating limb group
40. percentage of infants with anti- tetanus toxoid >0.1 lU/ml at 13 months of age
    a. the consistent limb group vs. the alternating limb group
41. percentage of infants with anti- tetanus toxoid >0.1 lU/ml at 24 months of age
    a. the consistent limb group vs. the alternating limb group

TABLE 69. PERCENTAGE OF PARTICIPANTS WITH ANTI-PRP IGG AND ANTI-TETANUS TOXOID CONCENTRATION ≥0.1 IU/L AND ≥0.15 IU/L (ITT & CP POPULATION)

			ITT Populations			*Completers Population*		
	Visit	*Table*	*Consistent Limbs N=219*	*Alternating Limbs N=220*	*p value (Fishers Exact Test)*	*Consistent Limbs N=207*	*Alternating Limbs N=202*	*p value (Fishers Exact Test)*
Anti-PRP IgG	V4	N (%) >=1.0 IU/L	86 (39.3%)	102 (46.4%)	0.1482	78 (37.7%)	90 (44.6%)	0.1612
	V4	N (%) >=0.15 IU/L	145 (66.2%)	160 (72.7%)	0.1477	135 (65.2%)	145 (71.8%)	0.1672
	V5	N (%) >=1.0 IU/L	59 (27.7%)	67 (32.1%)	0.3401	56 (27.7%)	62 (31.5%)	0.4433
	V5	N (%) >=0.15 IU/L	148 (69.5%)	160 (76.6%)	0.1246	140 (69.3%)	151 (76.6%)	0.1147
	V7	N (%) >=1.0 IU/L	188 (93.5%)	196 (98.0%)	0.0444	172 (94.5%)	168 (98.2%)	0.0884
	V7	N (%) >=0.15 IU/L	197 (98.0%)	200 (100.0%)	0.1231	180 (98.9%)	171 (100.0%)	0.4991
	V8	N (%) >=1.0 IU/L	150 (73.5%)	164 (78.5%)	0.2510	138 (73.4%)	142 (76.3%)	0.5520
	V8	N (%) >=0.15 IU/L	197 (96.6%)	204 (97.6%)	0.5713	183 (97.3%)	181 (97.3%)	1.0000
Anti-tetanus toxoid	V4	N (%) >=0.1 IU/L	215 (98.2%)	217 (98.6%)	0.7240	203 (98.1%)	199 (98.5%)	1.0000

			ITT Populations			*Completers Population*		
	Visit	*Table*	*Consistent Limbs N=219*	*Alternating Limbs N=220*	*p value (Fishers Exact Test)*	*Consistent Limbs N=207*	*Alternating Limbs N=202*	*p value (Fishers Exact Test)*
	V5	N (%) >=0.1 IU/L	157 (74.1%)	157 (75.1%)	0.8236	148 (73.6%)	148 (75.1%)	0.8185
	V7	N (%) >=0.1 IU/L	200 (99.5%)	199 (99.5%)	1.0000	181 (99.5%)	171 (100.0%)	1.0000
	V8	N (%) >=0.1 IU/L	177 (86.8%)	192 (91.9%)	0.1109	162 (86.2%)	171 (91.9%)	0.0972

20 ANALYSIS OF SAFETY

The data summaries report the total number and percentage of infants experiencing each type of adverse event, and the total number and percentage of infants experiencing at least one adverse event of any type, for each dose group, and the total number of infants in each dose group, for local and general adverse events during the 4-day follow-up period after each MenC (including Hib-MenC), DTaP-IPV-Hib and PCV13 vaccination. The classification of severity of local adverse events and fever followed the Brighton collaboration guidelines [35 - 38].

Where the adverse event is further classified with a grade (1, 2, 3) related to severity, the summary reports include the numbers in each class.

21.1 Local Adverse events

21.1.1 Primary Vaccination Phase

21.1.1.1 MenC Group analysis

The binary variables were analysed using logistic regression. The model contained the terms dose group (4 levels) and alternating limb group (2 levels). A term for centre was included in the model. The results of a comparison between two levels of a factor were reported as an odds ratio, with 95% confidence intervals. Analysis of safety was carried out using SAS version 9.3.

The following variables were analysed:

4. percentage of infants with each type of adverse event, and at least one local adverse event after each dose of MenC vaccine (at 3 and 4 months)
    a. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    b. Single Dose MenC-CRM_{197} Group vs Single Dose MenC-TT Group

Table 70: Number and percentage of participants experiencing injection site pain after each dose of a MenC vaccine (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
2	Absent	141 (85%)	123 (78%)		97 (84%)
	Mild	16 (10%)	24 (15%)		15 (13%)
	Moderate	7 (4%)	10 (6%)		2 (2%)
	Severe	1 (1%)	1 (1%)		1 (1%)
	Present	24 (15%)	35 (22%)		18 (16%)
3	Absent		129 (83%)		
	Mild		20 (13%)		
	Moderate		5 (3%)		
	Severe		2 (1%)		
	Present				

Table 71: Odds ratios adjusted for centre and limb group for the differences in MenC injection site pain between groups at 3 months of age (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
2	Single CRM vs Two-dose	0.8326	0.5814	1.1923	0.3174
	Single TT vs Single CRM	1.0524	0.5382	2.0581	0.8813
	Single TT vs Two-dose	0.8763	0.5928	1.2953	0.5077

Table 72: Number and percentage of participants with injection site erythema after each dose of a MenC vaccine (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
2	Absent	104 (65%)	82 (55%)		70 (63%)
	Mild	42 (26%)	47 (32%)		24 (21%)
	Moderate	14 (9%)	13 (9%)		15 (13%)
	Severe	1 (1%)	6 (4%)		3 (3%)
	Present	57 (35%)	66 (45%)		42 (38%)
3	Absent		75 (51%)		
	Mild		48 (32%)		
	Moderate		17 (11%)		
	Severe		8 (5%)		
	Present		73 (49%)		

Table 73: Odds ratios adjusted for centre and limb group for the differences in injection site erythema between groups at 3 months of age

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
2	Single CRM vs Two-dose	0.8421	0.6382	1.1111	0.2243
	Single TT vs Single CRM	1.1149	0.6695	1.8566	0.6759
	Single TT vs Two-dose	0.9388	0.6934	1.2712	0.6832

Table 74: Number and percentage of participants with swelling at the injection site after each dose of a MenC vaccine (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
2	Absent	145 (88%)	130 (84%)		97 (86%)
	Mild	13 (8%)	17 (11%)		11 (10%)
	Moderate	5 (3%)	5 (3%)		5 (4%)
	Severe	1 (1%)	2 (1%)		0 (0%)
	Present	19 (12%)	24 (16%)		16 (14%)
3	Absent		127 (84%)		
	Mild		19 (13%)		
	Moderate		6 (4%)		
	Severe		0 (0%)		
	Present		25 (16%)		

Table 75: Odds ratios adjusted for centre and limb group for the differences in swelling at the injection site between groups at 3 months of age (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
2	Single CRM vs Two-dose	0.8235	0.5547	1.2225	0.3354
	Single TT vs Single CRM	1.2715	0.6197	2.6089	0.5124
	Single TT vs Two-dose	1.0471	0.6901	1.5887	0.8287

Table 76: Number and percentage of participants with induration at the injection site after each dose of a MenC vaccine (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
2	Absent	132 (81%)	116 (75%)		92 (80%)
	Mild	19 (12%)	33 (21%)		14 (12%)
	Moderate	8 (5%)	5 (3%)		8 (7%)
	Severe	4 (2%)	1 (1%)		1 (1%)
	Present	31 (19%)	39 (25%)		23 (20%)
3	Absent		115 (74%)		
	Mild		31 (20%)		
	Moderate		8 (5%)		
	Severe		1 (1%)		
	Present		40 (26%)		

Table 77: Odds ratios adjusted for centre and limb group for the differences in induration at the injection site between groups at 3 months of age (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
2	Single CRM vs Two-dose	0.8654	0.6228	1.2024	0.3890
	Single TT vs Single CRM	1.0664	0.5804	1.9591	0.8360
	Single TT vs Two-dose	0.9228	0.6458	1.3187	0.6592

21.1.1.2 Consistent/Alternating Subgroup Analysis

Comparisons performed:

1. percentage of infants with each type of adverse event, and at least one local adverse event after each dose of DTaP-IPV-Hib (at 2, 3 and 4 months) and PCV13 (at 2 and 4 months)
    a. the consistent limb group vs. the alternating limb group

Table 78. Number and percenatage of participants with Maximum Local Reaction: Induration, erythema, swelling– alternating Limbs comparison – DTaP-IPV-Hib (ITT & Reactogenicity Populations)

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
V1	Hardness	Absent	195 (77%)	208 (82%)	0.1685	0.1795	194 (77%)	206 (82%)	0.1848	0.1866
		Mild < 2.5cm	58 (23%)	45 (18%)	.	.	58 (23%)	45 (18%)	.	.
		Mod-Sev >2.5cm	0 (0%)	1 (0%)	.	.	0 (0%)	1 (0%)	.	.
	Redness	Absent	141 (56%)	138 (54%)	0.6542	0.7513	140 (56%)	137 (54%)	0.6691	0.7882
		Mild < 2.5cm	108 (43%)	114 (45%)	.	.	108 (43%)	113 (45%)	.	.
		Mod-Sev >2.5cm	4 (2%)	2 (1%)	.	.	4 (2%)	2 (1%)	.	.
	Swelling	Absent	212 (84%)	213 (84%)	0.9026	0.9035	211 (84%)	212 (84%)	0.8805	0.8224
		Mild < 2.5cm	35 (14%)	36 (14%)	.	.	35 (14%)	35 (14%)	.	.
		Mod-Sev >2.5cm	6 (2%)	4 (2%)	.	.	6 (2%)	4 (2%)	.	.
V2	Hardness	Absent	166 (66%)	180 (71%)	0.2494	0.2524	165 (66%)	178 (71%)	0.2095	0.2103
		Mild < 2.5cm	85 (34%)	73 (29%)	.	.	85 (34%)	71 (28%)	.	.
		Mod-Sev >2.5cm	0 (0%)	1 (0%)	.	.	0 (0%)	1 (0%)	.	.
	Redness	Absent	118 (47%)	127 (50%)	0.4767	0.5017	117 (47%)	125 (50%)	0.4741	0.4740
		Mild < 2.5cm	133 (53%)	126 (50%)	.	.	133 (53%)	124 (50%)	.	.
		Mod-Sev >2.5cm	0 (0%)	1 (0%)	.	.	0 (0%)	1 (0%)	.	.
	Swelling	Absent	190 (76%)	197 (78%)	0.6903	0.6210	189 (76%)	196 (78%)	0.6134	0.4569
		Mild < 2.5cm	57 (23%)	55 (22%)	.	.	57 (23%)	52 (21%)	.	.
		Mod-Sev >2.5cm	4 (2%)	2 (1%)	.	.	4 (2%)	2 (1%)	.	.
V3	Hardness	Absent	159 (65%)	147 (58%)	0.1673	0.1486	158 (65%)	144 (58%)	0.1510	0.1276
		Mild < 2.5cm	87 (35%)	104 (41%)	.	.	86 (35%)	103 (42%)	.	.
		Mod-Sev >2.5cm	0 (0%)	1 (0%)	.	.	0 (0%)	1 (0%)	.	.
	Redness	Absent	105 (43%)	105 (42%)	0.5210	0.8184	104 (43%)	103 (42%)	0.5192	0.8064
		Mild < 2.5cm	140 (57%)	143 (57%)	.	.	139 (57%)	141 (57%)	.	.
		Mod-Sev >2.5cm	1 (0%)	4 (2%)	.	.	1 (0%)	4 (2%)	.	.
	Swelling	Absent	173 (70%)	183 (73%)	0.6610	0.5708	172 (70%)	180 (73%)	0.7106	0.6077
		Mild < 2.5cm	72 (29%)	67 (27%)	.	.	71 (29%)	66 (27%)	.	.
		Mod-Sev >2.5cm	1 (0%)	2 (1%)	.	.	1 (0%)	2 (1%)	.	.

Table 79: 1 Number and percentage of participants with Maximum Local pain – alternating Limbs comparison – DTaP-IPV-Hib (ITT & Reactogenicity Populations)

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
V1	Pain	Absent	173 (68%)	180 (71%)	0.8423	0.5427	172 (68%)	179 (71%)	0.8086	0.4977
		Mild	49 (19%)	42 (17%)	.	.	49 (19%)	41 (16%)	.	.
		Moderate	19 (8%)	21 (8%)	.	.	19 (8%)	21 (8%)	.	.
		Severe	12 (5%)	11 (4%)	.	.	12 (5%)	11 (4%)	.	.
V2	Pain	Absent	200 (80%)	202 (80%)	0.9238	0.9658	200 (80%)	199 (80%)	0.9305	0.9113
		Mild	36 (14%)	35 (14%)	.	.	35 (14%)	34 (14%)	.	.
		Moderate	11 (4%)	14 (6%)	.	.	11 (4%)	14 (6%)	.	.
		Severe	4 (2%)	3 (1%)	.	.	4 (2%)	3 (1%)	.	.
V3	Pain	Absent	187 (76%)	188 (75%)	0.7784	0.7147	185 (76%)	186 (75%)	0.7791	0.8328
		Mild	44 (18%)	46 (18%)	.	.	44 (18%)	44 (18%)	.	.
		Moderate	13 (5%)	13 (5%)	.	.	13 (5%)	13 (5%)	.	.
		Severe	2 (1%)	5 (2%)	.	.	2 (1%)	5 (2%)	.	.

Table 80. Number and percenatage of participants with Maximum Local Reaction : Induration, erythema, swelling– alternating Limbs comparison – PCV13 (ITT & Reactogenicity Populations)

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
V1	Hardness	Absent	197 (78%)	212 (83%)	.	0.1104	196 (78%)	210 (83%)	.	0.1151
		Mild < 2.5cm	56 (22%)	42 (17%)	.	.	56 (22%)	42 (17%)	.	.
		Mod-Sev >2.5cm	0 (0%)	0 (0%)	.	.	0 (0%)	0 (0%)	.	.
	Redness	Absent	156 (62%)	149 (59%)	0.2926	0.4905	155 (62%)	148 (59%)	0.3051	0.5243
		Mild < 2.5cm	95 (38%)	105 (41%)	.	.	95 (38%)	104 (41%)	.	.
		Mod-Sev >2.5cm	2 (1%)	0 (0%)	.	.	2 (1%)	0 (0%)	.	.
	Swelling	Absent	217 (86%)	216 (85%)	0.2831	0.8156	216 (86%)	215 (85%)	0.3082	0.8993
		Mild < 2.5cm	30 (12%)	36 (14%)	.	.	30 (12%)	35 (14%)	.	.
		Mod-Sev >2.5cm	6 (2%)	2 (1%)	.	.	6 (2%)	2 (1%)	.	.
V3	Hardness	Absent	169 (69%)	175 (69%)	.	0.8572	168 (69%)	172 (69%)	.	0.9040
		Mild < 2.5cm	77 (31%)	77 (31%)	.	.	76 (31%)	76 (31%)	.	.
		Mod-Sev >2.5cm	0 (0%)	0 (0%)	.	.	0 (0%)	0 (0%)	.	.
	Redness	Absent	117 (48%)	119 (47%)	0.6212	0.9397	116 (48%)	117 (47%)	0.6198	0.9356
		Mild < 2.5cm	129 (52%)	131 (52%)	.	.	128 (52%)	129 (52%)	.	.
		Mod-Sev >2.5cm	0 (0%)	2 (1%)	.	.	0 (0%)	2 (1%)	.	.
	Swelling	Absent	190 (77%)	198 (79%)	.	0.7194	189 (77%)	195 (79%)	.	0.7539
		Mild < 2.5cm	56 (23%)	54 (21%)	.	.	55 (23%)	53 (21%)	.	.
		Mod-Sev >2.5cm	0 (0%)	0 (0%)	.	.	0 (0%)	0 (0%)	.	.

Table 81: 2 Number and percentage of participants with Maximum Local pain – alternating Limbs comparison – PCV13 (ITT & Reactogenicity Populations)

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
V1	Pain	Absent	177 (70%)	185 (73%)	0.9055	0.4739	176 (70%)	184 (73%)	0.8819	0.4302
		Mild	46 (18%)	41 (16%)	.	.	46 (18%)	40 (16%)	.	.
		Moderate	18 (7%)	17 (7%)	.	.	18 (7%)	17 (7%)	.	.
		Severe	12 (5%)	11 (4%)	.	.	12 (5%)	11 (4%)	.	.
V3	Pain	Absent	190 (77%)	198 (79%)	0.7692	0.7194	188 (77%)	196 (79%)	0.7288	0.5952
		Mild	41 (17%)	40 (16%)	.	.	41 (17%)	38 (15%)	.	.
		Moderate	13 (5%)	10 (4%)	.	.	13 (5%)	10 (4%)	.	.
		Severe	2 (1%)	4 (2%)	.	.	2 (1%)	4 (2%)	.	.

21.1.2 Booster vaccination phase

21.1.2.1 MenC Groups

2. percentage of infants with each type of adverse event, and at least one local adverse event after the 12 month booster Hib-MenC and PCV13 vaccination
    a. Two dose MenC Group vs. 0 dose control Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    d. Single dose MenC-TT Group vs 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group
    f. the consistent limb group vs. the alternating limb group

Table 82: Number and percentage of participants experiencing injection site pain after the Hib-MenC-TT vaccine (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
5	Absent	123 (78%)	114 (75%)	50 (81%)	75 (71%)
	Mild	23 (15%)	33 (22%)	9 (15%)	26 (25%)
	Moderate	11 (7%)	5 (3%)	3 (5%)	2 (2%)
	Severe	1 (1%)	0 (0%)	0 (0%)	2 (2%)
	Present	35 (22%)	38 (25%)	12 (19%)	30 29%)

Table 83: Odds ratios adjusted for centre and limb group for the differences in Hib-MenC-TT injection site pain between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
5	Ctl vs Two-dose	0.7799	0.4709	1.2916	0.3341
	Ctl vs Single TT	0.5962	0.2776	1.2802	0.1847
	Ctl vs Single CRM	0.8529	0.4082	1.7823	0.6723
	Single CRM vs Two-dose	0.9143	0.6423	1.3016	0.6191
	Single TT vs Single CRM	1.4306	0.8098	2.5274	0.2174
	Single TT vs Two-dose	1.3081	0.8938	1.9143	0.1669

Table 84: Number and percentage of participants with injection site redness after the Hib-MenC-TT vaccine (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
5	Absent	77 (51%)	60 (44%)	32 (53%)	48 (50%)
	Mild	43 (28%)	46 (34%)	12 (20%)	25 (26%)
	Moderate	23 (15%)	22 (16%)	13 (22%)	17 (18%)
	Severe	8 (5%)	8 (6%)	3 (5%)	6 (6%)
	Present	74 (49%)	76 (56%)	28 (47%)	48 (50%)

Table 85: Odds ratios adjusted for centre and limb group for the differences in Hib-MenC-TT injection site redness between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
5	Ctl vs Two-dose	0.8734	0.5684	1.3422	0.5370
	Ctl vs Single TT	0.9001	0.4574	1.7709	0.7604
	Ctl vs Single CRM	0.9436	0.5040	1.7667	0.8561
	Single CRM vs Two-dose	0.9256	0.6754	1.2686	0.6308
	Single TT vs Single CRM	1.0484	0.6131	1.7926	0.8629
	Single TT vs Two-dose	0.9704	0.6750	1.3952	0.8712

Table 86: Number and percentage of participants with injection site swelling after the Hib-MenC-TT vaccine (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
5	Absent	122 (80%)	114 (77%)	46 (78%)	79 (77%)
	Mild	21 (14%)	22 (15%)	7 (12%)	13 (13%)
	Moderate	7 (5%)	9 (6%)	4 (7%)	7 (7%)
	Severe	3 (2%)	3 (2%)	2 (3%)	3 (3%)
	Present	31 (20%)	34 (23%)	13 (22%)	23 (23%)

Table 87: Odds ratios adjusted for centre and limb group for the differences in Hib-MenC-TT injection site swelling between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
5	Ctl vs Two-dose	1.0126	0.6110	1.6782	0.9614
	Ctl vs Single TT	0.9762	0.4450	2.1416	0.9521
	Ctl vs Single CRM	1.1257	0.5341	2.3724	0.7556
	Single CRM vs Two-dose	0.8995	0.6195	1.3060	0.5777
	Single TT vs Single CRM	1.1531	0.6193	2.1470	0.6533
	Single TT vs Two-dose	1.0372	0.6865	1.5670	0.8622

Table 88: Number and percentage of participants with injection site induration after the Hib- MenC-TT vaccine (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
5	Absent	106 (71%)	109 (72%)	45 (74%)	69 (69%)
	Mild	29 (19%)	28 (19%)	11 (18%)	21 (21%)
	Moderate	12 (8%)	11 (7%)	4 (7%)	5 (5%)
	Severe	3 (2%)	3 (2%)	1 (2%)	5 (5%)
	Present	44 (29%)	42 (28%)	16 (26%)	31 (31%)

Table 89: Odds ratios adjusted for centre and limb group for the differences in Hib-MenC-TT injection site induration between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
5	Ctl vs Two-dose	0.8874	0.5553	1.4182	0.6176
	Ctl vs Single TT	0.7942	0.3835	1.6447	0.5350
	Ctl vs Single CRM	0.8584	0.4321	1.7055	0.6630
	Single CRM vs Two-dose	1.0338	0.7364	1.4512	0.8478
	Single TT vs Single CRM	1.0809	0.6135	1.9044	0.7879
	Single TT vs Two-dose	1.1174	0.7640	1.6341	0.5672

Table 90: Number and percentage of participants with injection site pain after the PCV13 vaccine at 12 months of age (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
V5	Absent	117 (74%)	108 (71%)	50 (81%)	68 (65%)
	Mild	25 (16%)	31 (20%)	8 (13%)	30 (29%)
	Moderate	16 (10%)	12 (8%)	2 (3%)	5 (5%)
	Severe	1 (1%)	1 (1%)	2 (3%)	2 (2%)
	Present	42 (26%)	44 (29%)	12 (19%)	37 (35%)

Table 91: Odds ratios adjusted for centre and limb group for the differences in PCV13 injection site pain at 12 months of age between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
5	Ctl vs Two-dose	0.8874	0.5553	1.4182	0.6176
	Ctl vs Single TT	0.7942	0.3835	1.6447	0.5350
	Ctl vs Single CRM	0.8584	0.4321	1.7055	0.6630
	Single CRM vs Two-dose	1.0338	0.7364	1.4512	0.8478
	Single TT vs Single CRM	1.0809	0.6135	1.9044	0.7879
	Single TT vs Two-dose	1.1174	0.7640	1.6341	0.5672

Table 92: Number and percentage of participants with injection erythema after the PCV13 vaccine at 12 months of age (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
V5	Absent	73 (53%)	60 (46%)	29 (50%)	46 (48%)
	Mild	35 (26%)	40 (31%)	12 (21%)	30 (32%)
	Moderate	22 (16%)	21 (16%)	9 (16%)	12 (13%)
	Severe	7 (5%)	9 (7%)	8 (14%)	7 (7%)
	Present	64 (47%)	70 (54%)	29 (50%)	49 (52%)

Table 93: Odds ratios adjusted for centre and limb group for the differences in PCV13 injection site redness at 12 months of age between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
5	Ctl vs Two-dose	0.8874	0.5553	1.4182	0.6176
	Ctl vs Single TT	0.7942	0.3835	1.6447	0.5350
	Ctl vs Single CRM	0.8584	0.4321	1.7055	0.6630
	Single CRM vs Two-dose	1.0338	0.7364	1.4512	0.8478
	Single TT vs Single CRM	1.0809	0.6135	1.9044	0.7879
	Single TT vs Two-dose	1.1174	0.7640	1.6341	0.5672

Table 94: Number and percentage of participants with injection swelling after the PCV13 vaccine at 12 months of age (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
V5	Absent	116 (78%)	111 (79%)	46 (81%)	76 (78%)
	Mild	20 (13%)	16 (11%)	6 (11%)	14 (14%)
	Moderate	10 (7%)	8 (6%)	5 (9%)	6 (6%)
	Severe	3 (2%)	6 (4%)	0 (0%)	2 (2%)
	Present	33 (22%)	30 (21%)	11 (19%)	22 (22%)

Table 95: Odds ratios adjusted for centre and limb group for the differences in PCV13 injection site swelling at 12 months of age between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
5	Ctl vs Two-dose	0.8874	0.5553	1.4182	0.6176
	Ctl vs Single TT	0.7942	0.3835	1.6447	0.5350
	Ctl vs Single CRM	0.8584	0.4321	1.7055	0.6630
	Single CRM vs Two-dose	1.0338	0.7364	1.4512	0.8478
	Single TT vs Single CRM	1.0809	0.6135	1.9044	0.7879
	Single TT vs Two-dose	1.1174	0.7640	1.6341	0.5672

Table 96: Number and percentage of participants with injection induration after the PCV13 vaccine at 12 months of age (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
V5	Absent	106 (71%)	97 (67%)	38 (64%)	69 (71%)
	Mild	26 (17%)	29 (20%)	14 (24%)	19 (20%)
	Moderate	11 (7%)	13 (9%)	6 (10%)	8 (8%)
	Severe	6 (4%)	5 (3%)	1 (2%)	1 (1%)
	Present	43 (29%)	47 (33%)	21 (36%)	28 (29%)

Table 97: Odds ratios adjusted for centre and limb group for the differences in PCV13 injection site induration at 12 months of age between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
5	Ctl vs Two-dose	0.8874	0.5553	1.4182	0.6176
	Ctl vs Single TT	0.7942	0.3835	1.6447	0.5350
	Ctl vs Single CRM	0.8584	0.4321	1.7055	0.6630
	Single CRM vs Two-dose	1.0338	0.7364	1.4512	0.8478
	Single TT vs Single CRM	1.0809	0.6135	1.9044	0.7879
	Single TT vs Two-dose	1.1174	0.7640	1.6341	0.5672

21.1.2.2 Consistent/Alternating Limb Subgroup

Table 98: Maximum Local Reaction – Induration, Erythema and ~Swelling Alternating Limbs – Hib-MenC-TT at 12 months of age (ITT & Reactogenicity Population)

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
V5	Hardness	Absent	157 (66%)	172 (72%)	.	0.1567	155 (67%)	168 (72%)	.	0.1916
		Mild < 2.5cm	81 (34%)	67 (28%)	.	.	78 (33%)	65 (28%)	.	.
		Mod-Sev >2.5cm	0 (0%)	0 (0%)	.	.	0 (0%)	0 (0%)	.	.
	Redness	Absent	103 (43%)	114 (48%)	0.3415	0.3322	101 (43%)	112 (48%)	0.3266	0.3064
		Mild < 2.5cm	134 (56%)	122 (51%)	.	.	131 (56%)	118 (51%)	.	.
		Mod-Sev >2.5cm	1 (0%)	3 (1%)	.	.	1 (0%)	3 (1%)	.	.
	Swelling	Absent	188 (79%)	173 (72%)	0.2268	0.0926	184 (79%)	171 (73%)	0.3902	0.1574
		Mild < 2.5cm	49 (21%)	64 (27%)	.	.	48 (21%)	60 (26%)	.	.
		Mod-Sev >2.5cm	1 (0%)	2 (1%)	.	.	1 (0%)	2 (1%)	.	.

Table 99: MAXIMUM LOCAL REACTION – Pain ALTERNATING LIMBS – Hib-MenC-TT AT 12 MONTHS OF AGE (ITT & REACTOGENICITY POPULATION)

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
V5	Pain	Absent	186 (78%)	176 (74%)	0.2703	0.2495	184 (79%)	172 (74%)	0.2490	0.1905
		Mild	41 (17%)	50 (21%)	.	.	39 (17%)	48 (21%)	.	.
		Moderate	11 (5%)	10 (4%)	.	.	10 (4%)	10 (4%)	.	.
		Severe	0 (0%)	3 (1%)	.	.	0 (0%)	3 (1%)	.	.

Table 100: MAXIMUM LOCAL REACTION – INDURATION, ERYTHEMA AND ~SWELLING ALTERNATING LIMBS – PCV13 AT 12 MONTHS OF AGE (ITT & REACTOGENICITY POPULATION)

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
V5	Hardness	Absent	146 (61%)	164 (68%)	0.2732	0.1096	143 (61%)	159 (68%)	0.3559	0.1372
		Mild < 2.5cm	89 (37%)	73 (30%)	.	.	87 (37%)	72 (31%)	.	.
		Mod-Sev >2.5cm	3 (1%)	3 (1%)	.	.	3 (1%)	3 (1%)	.	.
	Redness	Absent	97 (41%)	111 (46%)	0.4987	0.2258	95 (41%)	109 (47%)	0.4402	0.2057
		Mild < 2.5cm	132 (55%)	121 (50%)	.	.	129 (55%)	117 (50%)	.	.
		Mod-Sev >2.5cm	9 (4%)	8 (3%)	.	.	9 (4%)	8 (3%)	.	.
	Swelling	Absent	174 (73%)	175 (73%)	0.5434	0.9622	170 (73%)	172 (74%)	0.5932	0.8946
		Mild < 2.5cm	59 (25%)	63 (26%)	.	.	58 (25%)	60 (26%)	.	.
		Mod-Sev >2.5cm	5 (2%)	2 (1%)	.	.	5 (2%)	2 (1%)	.	.

Table 101:3 MAXIMUM LOCAL REACTION – PAIN ALTERNATING LIMBS – PCV13 AT 12 MONTHS OF AGE (ITT & REACTOGENICITY POPULATION)

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
V5	Pain	Absent	174 (73%)	169 (70%)	0.3648	0.5132	171 (73%)	166 (71%)	0.3256	0.5547
		Mild	49 (21%)	45 (19%)	.	.	48 (21%)	43 (18%)	.	.
		Moderate	13 (5%)	22 (9%)	.	.	12 (5%)	21 (9%)	.	.
		Severe	2 (1%)	4 (2%)	.	.	2 (1%)	4 (2%)	.	.

21.2 Systemic adverse events

Comparisons performed

3. percentage of infants with each type of adverse event, and at least one systemic adverse event after MenC (3 and 4 months), DTaP-IPV-Hib (2, 3 and 4 months) and PCV13 (2 and 4 months) vaccination
    a. Two dose MenC Group vs. 0 dose control Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    d. Single dose MenC-TT Group vs 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group
    f. the consistent limb group vs. the alternating limb group

4. percentage of infants with each type of adverse event, and at least systemic one adverse event after the 12 month booster Hib-MenC and PCV13 vaccination
    a. Two dose MenC Group vs. 0 dose control Group
    b. Single dose MenC-CRM_{197} Group vs. 0 dose control Group
    c. Single dose MenC-CRM_{197} Group vs. Two dose MenC Group
    d. Single dose MenC-TT Group vs 0 dose control Group
    e. Single dose MenC-CRM_{197} Group vs Single dose MenC-TT Group
    f. The consistent limb group vs. the alternating limb group

21.2.1 MenC Groups

Table 102: Number and percentage of participants with fever after vaccination at 2, 3, 4 and 12 months of age (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
1	Normal	161 (98%)	157 (98%)	65 (98%)	112 (97%)
	Fever >=38 deg	3 (2%)	3 (2%)	1 (2%)	4 (3%)
	High Fever >=39 deg	0 (0%)	0 (0%)	0 (0%)	0 (0%)
2	Normal	160 (98%)	153 (97%)	62 (95%)	113 (97%)
	Fever >=38 deg	4 (2%)	5 (3%)	3 (5%)	2 (2%)
	High Fever >=39 deg	0 (0%)	0 (0%)	0 (0%)	1 (1%)
3	Normal	159 (96%)	150 (96%)	61 (97%)	110 (97%)
	Fever >=38 deg	5 (3%)	7 (4%)	2 (3%)	3 (3%)
	High Fever >=39 deg	1 (1%)	0 (0%)	0 (0%)	0 (0%)
5	Normal	143 (90%)	135 (89%)	55 (89%)	98 (93%)
	Fever >=38 deg	12 (8%)	15 (10%)	6 (10%)	4 (4%)
	High Fever >=39 deg	4 (3%)	2 (1%)	1 (2%)	3 (3%)

Table 103: Odds ratios adjusted for centre and limb group for the differences in fever≥38ºC at 2, 3, 4 and 12 months of age between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
1	Ctl vs Two-dose	1.3833	0.2900	6.5971	0.6839
	Ctl vs Single TT	2.4065	0.2601	22.2669	0.4392
	Ctl vs Single CRM	1.2140	0.1228	12.0033	0.8683
	Single CRM vs Two-dose	1.1395	0.3931	3.3031	0.8100
	Single TT vs Single CRM	0.5045	0.1094	2.3251	0.3801
	Single TT vs Two-dose	0.5748	0.2132	1.5495	0.2738
2	Ctl vs Two-dose	0.6628	0.2495	1.7606	0.4093
	Ctl vs Single TT	0.5492	0.1071	2.8173	0.4726
	Ctl vs Single CRM	0.5122	0.1110	2.3639	0.3912
	Single CRM vs Two-dose	1.2942	0.5374	3.1164	0.5652
	Single TT vs Single CRM	0.9325	0.2041	4.2609	0.9282
	Single TT vs Two-dose	1.2068	0.4579	3.1810	0.7038
3	Ctl vs Two-dose	1.0663	0.3416	3.3289	0.9120
	Ctl vs Single TT	0.8256	0.1320	5.1626	0.8377
	Ctl vs Single CRM	1.1292	0.2194	5.8112	0.8844
	Single CRM vs Two-dose	0.9443	0.4289	2.0787	0.8867
	Single TT vs Single CRM	1.3678	0.3318	5.6376	0.6647
	Single TT vs Two-dose	1.2915	0.4855	3.4357	0.6083
5	Ctl vs Two-dose	0.8081	0.4216	1.5491	0.5211
	Ctl vs Single TT	0.5373	0.1775	1.6264	0.2717
	Ctl vs Single CRM	0.8392	0.3256	2.1626	0.7166
	Single CRM vs Two-dose	0.9630	0.5878	1.5778	0.8810
	Single TT vs Single CRM	1.5617	0.6162	3.9578	0.3474
	Single TT vs Two-dose	1.5040	0.7941	2.8482	0.2104

Table 104: Number and percentage of participants with decreased appetite after vaccination at 2, 3, 4 and 12 months of age (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
1	Absent	113 (68%)	106 (66%)	49 (74%)	80 (69%)
	Mild	48 (29%)	47 (29%)	13 (20%)	26 (22%)
	Moderate	4 (2%)	7 (4%)	4 (6%)	10 (9%)
	Severe	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Present	52 (32%)	54 (34%)	17 (26%)	36 (31%)
2	Absent	123 (75%)	126 (79%)	54 (83%)	87 (75%)
	Mild	36 (22%)	25 (16%)	10 (15%)	24 (21%)
	Moderate	6 (4%)	7 (4%)	1 (2%)	5 (4%)
	Severe	0 (0%)	1 (1%)	0 (0%)	0 (0%)
	Present	42 (25%)	33 (21%)	11 (17%)	29 (25%)
3	Absent	130 (79%)	116 (74%)	53 (84%)	86 (76%)
	Mild	29 (18%)	33 (21%)	9 (14%)	19 (17%)
	Moderate	5 (3%)	8 (5%)	1 (2%)	7 (6%)
	Severe	0 (0%)	0 (0%)	0 (0%)	1 (1%)
	Present	34 (21%)	41 (26%)	10 (16%)	27 (24%)
5	Absent	106 (68%)	98 (65%)	44 (71%)	71 (68%)
	Mild	42 (27%)	40 (27%)	13 (21%)	25 (24%)
	Moderate	5 (3%)	10 (7%)	3 (5%)	8 (8%)
	Severe	3 (2%)	2 (1%)	2 (3%)	0 (0%)
	Present	50 (32%)	52 (35%)	18 (29%)	33 (32%)

Table 105: Odds ratios adjusted for centre and limb group for the differences in decreased appetite at 2, 3, 4 and 12 months of age between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
1	Ctl vs Two-dose	0.7824	0.5010	1.2218	0.2805
	Ctl vs Single TT	0.7667	0.3863	1.5216	0.4475
	Ctl vs Single CRM	0.7335	0.3833	1.4036	0.3492
	Single CRM vs Two-dose	1.0667	0.7799	1.4589	0.6862
	Single TT vs Single CRM	0.9566	0.5698	1.6062	0.8669
	Single TT vs Two-dose	1.0204	0.7192	1.4479	0.9098
2	Ctl vs Two-dose	0.7315	0.4360	1.2275	0.2365
	Ctl vs Single TT	0.6192	0.2843	1.3485	0.2274
	Ctl vs Single CRM	0.5920	0.2819	1.2433	0.1661
	Single CRM vs Two-dose	1.2357	0.8774	1.7403	0.2258
	Single TT vs Single CRM	0.9561	0.5508	1.6596	0.8731
	Single TT vs Two-dose	1.1814	0.8076	1.7283	0.3904
3	Ctl vs Two-dose	0.6786	0.3970	1.1600	0.1563
	Ctl vs Single TT	0.5777	0.2581	1.2933	0.1821
	Ctl vs Single CRM	0.7049	0.3242	1.5329	0.3776
	Single CRM vs Two-dose	0.9627	0.6718	1.3794	0.8357
	Single TT vs Single CRM	1.2201	0.6852	2.1727	0.4992
	Single TT vs Two-dose	1.1745	0.7951	1.7350	0.4189
5	Ctl vs Two-dose	0.8979	0.5770	1.3972	0.6330
	Ctl vs Single TT	0.9205	0.4610	1.8380	0.8144
	Ctl vs Single CRM	0.8896	0.4660	1.6984	0.7230
	Single CRM vs Two-dose	1.0093	0.7332	1.3893	0.9550
	Single TT vs Single CRM	0.9665	0.5633	1.6581	0.9014
	Single TT vs Two-dose	0.9754	0.6781	1.4031	0.8932

Table 106: Number and percentage of participants with drowsiness after vaccination at 2, 3, 4 and 12 months of age (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
1	Absent	66 (40%)	68 (43%)	31 (47%)	46 (40%)
	Mild	71 (43%)	67 (42%)	29 (44%)	46 (40%)
	Moderate	26 (16%)	20 (13%)	6 (9%)	21 (18%)
	Severe	2 (1%)	5 (3%)	0 (0%)	3 (3%)
	Present	99 (60%)	92 (58%)	35 (53%)	70 (60%)
2	Absent	91 (55%)	79 (50%)	37 (57%)	71 (61%)
	Mild	59 (36%)	52 (33%)	18 (28%)	32 (28%)
	Moderate	14 (8%)	26 (16%)	10 (15%)	13 (11%)
	Severe	1 (1%)	2 (1%)	0 (0%)	0 (0%)
	Present	74 (45%)	80 (50%)	28 (43%)	45 (39%)
3	Absent	100 (61%)	92 (59%)	40 (63%)	66 (58%)
	Mild	50 (30%)	49 (31%)	17 (27%)	34 (30%)
	Moderate	15 (9%)	14 (9%)	6 (10%)	11 (10%)
	Severe	0 (0%)	2 (1%)	0 (0%)	2 (2%)
	Present	65 (39%)	65 (41%)	23 (37%)	47 (42%)
5	Absent	95 (61%)	100 (66%)	41 (66%)	68 (65%)
	Mild	43 (27%)	35 (23%)	17 (27%)	18 (17%)
	Moderate	16 (10%)	12 (8%)	4 (6%)	14 (13%)
	Severe	3 (2%)	4 (3%)	0 (0%)	5 (5%)
	Present	62 (39%)	51 (34%)	21 (34%)	37 (35%)

Table 107: Odds ratios adjusted for centre and limb group for the differences in drowsiness at 2, 3, 4 and 12 months of age between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
1	Ctl vs Two-dose	0.8278	0.5577	1.2286	0.3482
	Ctl vs Single TT	0.7504	0.4048	1.3911	0.3619
	Ctl vs Single CRM	0.7505	0.4194	1.3428	0.3336
	Single CRM vs Two-dose	1.1030	0.8228	1.4785	0.5120
	Single TT vs Single CRM	1.0001	0.6124	1.6332	0.9998
	Single TT vs Two-dose	1.1031	0.7953	1.5299	0.5567
2	Ctl vs Two-dose	0.9362	0.6281	1.3954	0.7461
	Ctl vs Single TT	1.1707	0.6278	2.1832	0.6200
	Ctl vs Single CRM	0.9013	0.5024	1.6170	0.7276
	Single CRM vs Two-dose	1.0387	0.7768	1.3888	0.7979
	Single TT vs Single CRM	0.7699	0.4724	1.2548	0.2941
	Single TT vs Two-dose	0.7997	0.5759	1.1104	0.1820
3	Ctl vs Two-dose	0.8731	0.5777	1.3197	0.5198
	Ctl vs Single TT	0.8044	0.4250	1.5226	0.5038
	Ctl vs Single CRM	0.8902	0.4872	1.6265	0.7053
	Single CRM vs Two-dose	0.9808	0.7305	1.3170	0.8975
	Single TT vs Single CRM	1.1067	0.6786	1.8049	0.6846
	Single TT vs Two-dose	1.0855	0.7807	1.5092	0.6258
5	Ctl vs Two-dose	0.9355	0.6091	1.4369	0.7608
	Ctl vs Single TT	0.9528	0.4874	1.8626	0.8876
	Ctl vs Single CRM	0.7972	0.4271	1.4880	0.4766
	Single CRM vs Two-dose	1.1735	0.8631	1.5955	0.3075
	Single TT vs Single CRM	0.8367	0.4979	1.4062	0.5009
	Single TT vs Two-dose	0.9818	0.6902	1.3967	0.9188

Table 108: Number and percentage of participants with irritability after vaccination at 2, 3, 4 and 12 months of age (ITT population)

Visit	*category*	*Single dose MenC-CRM197*	*Two dose MenC*	*Control group*	*Single Dose MenC-TT*
1	Absent	63 (38%)	45 (28%)	23 (35%)	38 (33%)
	Mild	62 (38%)	70 (44%)	22 (33%)	43 (37%)
	Moderate	34 (21%)	37 (23%)	20 (30%)	31 (27%)
	Severe	6 (4%)	8 (5%)	1 (2%)	4 (3%)
	Present	102 (62%)	115 (72%)	43 (65%)	78 (67%)
2	Absent	63 (38%)	54 (34%)	28 (43%)	43 (37%)
	Mild	56 (34%)	53 (33%)	22 (34%)	35 (30%)
	Moderate	35 (21%)	44 (28%)	13 (20%)	33 (28%)
	Severe	11 (7%)	8 (5%)	2 (3%)	5 (4%)
	Present	102 (62%)	105 (66%)	37 (57%)	73 (63%)
3	Absent	70 (42%)	65 (41%)	25 (40%)	42 (37%)
	Mild	47 (28%)	49 (31%)	16 (25%)	39 (35%)
	Moderate	38 (23%)	30 (19%)	19 (30%)	25 (22%)
	Severe	10 (6%)	13 (8%)	3 (5%)	7 (6%)
	Present	95 (58%)	92 (59%)	38 (60%)	71 (63%)
5	Absent	74 (47%)	66 (43%)	24 (39%)	47 (45%)
	Mild	52 (33%)	52 (34%)	23 (37%)	32 (30%)
	Moderate	25 (16%)	27 (18%)	13 (21%)	19 (18%)
	Severe	8 (5%)	7 (5%)	2 (3%)	7 (7%)
	Present	85 (53%)	86 (57%)	38 (61%)	58 (55%)

Table 109: Odds ratios adjusted for centre and limb group for the differences in irritability at 2, 3, 4 and 12 months of age between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
1	Ctl vs Two-dose	0.9354	0.6182	1.4153	0.7518
	Ctl vs Single TT	0.9297	0.4872	1.7741	0.8250
	Ctl vs Single CRM	1.1433	0.6256	2.0894	0.6634
	Single CRM vs Two-dose	0.8181	0.6060	1.1046	0.1901
	Single TT vs Single CRM	1.2297	0.7427	2.0363	0.4216
	Single TT vs Two-dose	1.0061	0.7146	1.4165	0.9722
2	Ctl vs Two-dose	0.7991	0.5320	1.2003	0.2800
	Ctl vs Single TT	0.7763	0.4116	1.4641	0.4340
	Ctl vs Single CRM	0.7942	0.4370	1.4434	0.4497
	Single CRM vs Two-dose	1.0062	0.7450	1.3590	0.9678
	Single TT vs Single CRM	1.0231	0.6192	1.6904	0.9289
	Single TT vs Two-dose	1.0295	0.7354	1.4411	0.8656
3	Ctl vs Two-dose	1.0327	0.6847	1.5576	0.8779
	Ctl vs Single TT	0.9189	0.4841	1.7440	0.7958
	Ctl vs Single CRM	1.1388	0.6256	2.0731	0.6707
	Single CRM vs Two-dose	0.9069	0.6753	1.2178	0.5157
	Single TT vs Single CRM	1.2393	0.7545	2.0359	0.3968
	Single TT vs Two-dose	1.1239	0.8034	1.5722	0.4952
5	Ctl vs Two-dose	1.2254	0.8072	1.8602	0.3400
	Ctl vs Single TT	1.3013	0.6792	2.4929	0.4272
	Ctl vs Single CRM	1.4053	0.7648	2.5822	0.2730
	Single CRM vs Two-dose	0.8720	0.6466	1.1758	0.3691
	Single TT vs Single CRM	1.0799	0.6533	1.7852	0.7643
	Single TT vs Two-dose	0.9417	0.6703	1.3229	0.7290

21.2.2 Consistent/Alternating Limb Subgroups

Table 110: Odds ratios adjusted for centre and limb group for the differences in irritability at 2, 3, 4 and 12 months of age between groups (ITT population)

Visit	*Comparison*	*OR*	*LCL*	*UCL*	*P Value*
1	Ctl vs Two-dose	0.9354	0.6182	1.4153	0.7518
	Ctl vs Single TT	0.9297	0.4872	1.7741	0.8250
	Ctl vs Single CRM	1.1433	0.6256	2.0894	0.6634
	Single CRM vs Two-dose	0.8181	0.6060	1.1046	0.1901
	Single TT vs Single CRM	1.2297	0.7427	2.0363	0.4216
	Single TT vs Two-dose	1.0061	0.7146	1.4165	0.9722
2	Ctl vs Two-dose	0.7991	0.5320	1.2003	0.2800
	Ctl vs Single TT	0.7763	0.4116	1.4641	0.4340
	Ctl vs Single CRM	0.7942	0.4370	1.4434	0.4497
	Single CRM vs Two-dose	1.0062	0.7450	1.3590	0.9678
	Single TT vs Single CRM	1.0231	0.6192	1.6904	0.9289
	Single TT vs Two-dose	1.0295	0.7354	1.4411	0.8656
3	Ctl vs Two-dose	1.0327	0.6847	1.5576	0.8779
	Ctl vs Single TT	0.9189	0.4841	1.7440	0.7958
	Ctl vs Single CRM	1.1388	0.6256	2.0731	0.6707
	Single CRM vs Two-dose	0.9069	0.6753	1.2178	0.5157
	Single TT vs Single CRM	1.2393	0.7545	2.0359	0.3968
	Single TT vs Two-dose	1.1239	0.8034	1.5722	0.4952
5	Ctl vs Two-dose	1.2254	0.8072	1.8602	0.3400
	Ctl vs Single TT	1.3013	0.6792	2.4929	0.4272
	Ctl vs Single CRM	1.4053	0.7648	2.5822	0.2730
	Single CRM vs Two-dose	0.8720	0.6466	1.1758	0.3691
	Single TT vs Single CRM	1.0799	0.6533	1.7852	0.7643
	Single TT vs Two-dose	0.9417	0.6703	1.3229	0.7290

Table 111: MAXIMUM SEVERITY OF systemic REACTIONs (MILD, MODERATE, SEVERE) – change in appetite, drowsiness and irritability: ALTERNATING LIMB COMPARISON (chisquared tests) after vaccination at 2, 3, 4 and 12 months of age (ITT & Reacto population)

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
V1	Appetite	Absent	171 (68%)	177 (70%)	0.1801	0.6110	170 (67%)	176 (70%)	0.6125	0.5645
		Mild	65 (26%)	69 (27%)	.	.	65 (26%)	69 (27%)	.	.
		Moderate	17 (7%)	8 (3%)	.	.	17 (7%)	7 (3%)	.	.
		Severe	0 (0%)	0 (0%)	.	.	0 (0%)	0 (0%)	.	.
	Drowsiness	Absent	104 (41%)	107 (42%)	0.3447	0.8159	104 (41%)	107 (42%)	0.3519	0.7865
		Mild	104 (41%)	109 (43%)	.	.	103 (41%)	107 (42%)	.	.
		Moderate	42 (17%)	31 (12%)	.	.	42 (17%)	31 (12%)	.	.
		Severe	3 (1%)	7 (3%)	.	.	3 (1%)	7 (3%)	.	.
	Irr	Absent	78 (31%)	91 (36%)	0.2217	0.2327	77 (31%)	91 (36%)	0.2012	0.1859
		Mild	104 (41%)	93 (37%)	.	.	104 (41%)	92 (37%)	.	.
		Moderate	58 (23%)	64 (25%)	.	.	58 (23%)	63 (25%)	.	.
		Severe	13 (5%)	6 (2%)	.	.	13 (5%)	6 (2%)	.	.
V2	Appetite	Absent	198 (79%)	192 (76%)	0.6130	0.3775	197 (79%)	189 (76%)	0.6327	0.3938
		Mild	43 (17%)	52 (20%)	.	.	43 (17%)	51 (20%)	.	.
		Moderate	10 (4%)	9 (4%)	.	.	10 (4%)	9 (4%)	.	.
		Severe	0 (0%)	1 (0%)	.	.	0 (0%)	1 (0%)	.	.
	Drowsiness	Absent	144 (57%)	134 (53%)	0.7089	0.2973	144 (58%)	132 (53%)	0.6943	0.2805
		Mild	76 (30%)	85 (33%)	.	.	75 (30%)	84 (34%)	.	.
		Moderate	30 (12%)	33 (13%)	.	.	30 (12%)	32 (13%)	.	.
		Severe	1 (0%)	2 (1%)	.	.	1 (0%)	2 (1%)	.	.
	Irr	Absent	97 (39%)	91 (36%)	0.4101	0.5124	96 (38%)	91 (36%)	0.4361	0.6440
		Mild	87 (35%)	79 (31%)	.	.	87 (35%)	77 (31%)	.	.
		Moderate	57 (23%)	68 (27%)	.	.	57 (23%)	66 (26%)	.	.
		Severe	10 (4%)	16 (6%)	.	.	10 (4%)	16 (6%)	.	.
V3	Appetite	Absent	196 (80%)	189 (75%)	0.1358	0.1823	195 (80%)	185 (75%)	0.1137	0.1345
		Mild	43 (18%)	47 (19%)	.	.	42 (17%)	47 (19%)	.	.
		Moderate	6 (2%)	15 (6%)	.	.	6 (2%)	15 (6%)	.	.
		Severe	0 (0%)	1 (0%)	.	.	0 (0%)	1 (0%)	.	.

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
	Drowsiness	Absent	146 (59%)	152 (60%)	0.5732	0.8257	146 (60%)	148 (60%)	0.5367	0.9714
		Mild	71 (29%)	79 (31%)	.	.	69 (28%)	79 (32%)	.	.
		Moderate	27 (11%)	19 (8%)	.	.	27 (11%)	19 (8%)	.	.
		Severe	2 (1%)	2 (1%)	.	.	2 (1%)	2 (1%)	.	.
	Irr	Absent	100 (41%)	102 (40%)	0.6163	0.9684	99 (41%)	101 (41%)	0.6593	0.9726
		Mild	80 (33%)	71 (28%)	.	.	79 (32%)	70 (28%)	.	.
		Moderate	52 (21%)	60 (24%)	.	.	52 (21%)	58 (23%)	.	.
		Severe	14 (6%)	19 (8%)	.	.	14 (6%)	19 (8%)	.	.
V5	Appetite	Absent	165 (69%)	160 (67%)	0.9077	0.5329	163 (70%)	157 (67%)	0.9026	0.5053
		Mild	58 (24%)	62 (26%)	.	.	55 (24%)	59 (25%)	.	.
		Moderate	12 (5%)	14 (6%)	.	.	12 (5%)	14 (6%)	.	.
		Severe	3 (1%)	4 (2%)	.	.	3 (1%)	4 (2%)	.	.
	Drowsiness	Absent	141 (59%)	165 (69%)	0.0407	0.0257	138 (59%)	162 (70%)	0.0388	0.0203
		Mild	68 (29%)	45 (19%)	.	.	66 (28%)	43 (18%)	.	.
		Moderate	21 (9%)	25 (10%)	.	.	21 (9%)	24 (10%)	.	.
		Severe	8 (3%)	4 (2%)	.	.	8 (3%)	4 (2%)	.	.
	Irr	Absent	108 (45%)	103 (43%)	0.1928	0.5879	107 (46%)	101 (43%)	0.1765	0.5484
		Mild	69 (29%)	90 (38%)	.	.	67 (29%)	88 (38%)	.	.
		Moderate	47 (20%)	37 (15%)	.	.	47 (20%)	35 (15%)	.	.
		Severe	14 (6%)	10 (4%)	.	.	12 (5%)	10 (4%)	.	.

Table 112. Maximum Fever – alternating limb comparison AFTER VACCINATION AT 2, 3, 4 AND 12 MONTHS OF AGE (ITT & Reactogenicity populations)

			ITT Population				*Reactogenicity Population*			
Visit	*Symptom*	*Severity*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*	*Consistent Limbs*	*Alternating Limbs*	*P Value (Exact)*	*P Value (Any vs None)*
V1	Temp	Normal	244 (97%)	251 (99%)	0.1404	0.1242	243 (97%)	249 (99%)	.	0.1258
		Fever >=38 deg	8 (3%)	3 (1%)	.	.	8 (3%)	3 (1%)	.	.
		High Fever >=39 deg	0 (0%)	0 (0%)	.	.	0 (0%)	0 (0%)	.	.
V2	Temp	Normal	242 (96%)	246 (98%)	0.5089	0.4271	241 (96%)	242 (98%)	0.7875	0.4409
		Fever >=38 deg	8 (3%)	6 (2%)	.	.	8 (3%)	6 (2%)	.	.
		High Fever >=39 deg	1 (0%)	0 (0%)	.	.	1 (0%)	0 (0%)	.	.
V3	Temp	Normal	241 (98%)	239 (95%)	0.1122	0.0617	239 (98%)	235 (95%)	0.1124	0.0593
		Fever >=38 deg	5 (2%)	12 (5%)	.	.	5 (2%)	12 (5%)	.	.
		High Fever >=39 deg	0 (0%)	1 (0%)	.	.	0 (0%)	1 (0%)	.	.
V5	Temp	Normal	218 (92%)	213 (89%)	0.3732	0.2960	213 (91%)	207 (88%)	0.3865	0.2886
		Fever >=38 deg	17 (7%)	20 (8%)	.	.	17 (7%)	20 (9%)	.	.
		High Fever >=39 deg	3 (1%)	7 (3%)	.	.	3 (1%)	7 (3%)	.	.

21 DISCUSSION

21.1 MenC Groups

21.1.1 Immunogenicity

Priming with two MenC-CRM doses at 3 and 4 months of age does not offer any advantage over priming with a single MenC-CRM or MenC-TT dose at 3 months of age, since MenC antibodies wane below these thresholds for the majority of participants in all three groups, and at least 97% of children had MenC rSBA≥1:8 following a Hib-MenC-TT boost at 12 months of age, irrespective of the number of MenC doses used for infant priming. Our findings are similar to those reported in another study which showed that 98% of infants had MenC rSBA≥1:8 in response to a 12 month Hib-MenC-TT booster dose following one MenC infant priming dose,[54] however no comparison was made with the response to a two dose MenC infant priming schedule or a control group.

Intriguingly, priming with a single MenC-CRM dose induced higher post Hib-MenC-TT rSBA GMTs than two priming doses, suggesting that the administration of a greater amount of MenC antigen during priming reduces the subsequent immune response to the 12 month MenC conjugate vaccine booster dose. The underlying mechanism, which is not reflected in the frequencies of MenC-specific memory B-cells in peripheral blood detected at 5, 12 or 13 months, as shown in the B-cell memory section of this study, may still be related to differences in memory B-cell numbers if the pool is considered to be resident in lymphoid tissues and therefore inaccessible using peripheral blood sampling. Furthermore, this phenomenon might be the result of dose dependant carrier protein differences which are manifested when different MenC glycoconjugate vaccine formulations are used for priming and boosting. A similar effect has also been observed in children challenged with a MenC pure polysaccharide formulation following infant priming with one dose of MenC-TT which induced significantly higher post boost MenC

rSBA GMTs compared to a 2 dose MenC-TT infant priming.[14] The relatively reduced post-booster response seen with an increase in the number of MenC conjugate vaccine priming doses is not the same as the hyporesponsiveness that occurs in children repeatedly vaccinated with a pure polysaccharide MenC vaccine compared to others who are being vaccinated with the same MenC polysaccharide formulation for the first time.[55]The latter is thought to result from the terminal differentiation of B-cells into plasma cells without the formation of memory B-cells which is induced by repeated immunisation with a T-cell independent antigen that, as a net result, depletes the MenC specific B-cell pool.[8]

Two months after infant vaccination, one MenC-TT dose was significantly more immunogenic than one dose of MenC-CRM and following a Hib-MenC-TT boost at 12 months of age, the MenC rSBA GMTs were significantly higher in those primed with MenC-TT than all other study groups. Such immunogenicity differences are known to persist following a MenC boost in the second year of life, irrespective of whether MenC-TT or MenC-CRM are used for boosting.[53] Furthermore, at 2 years of age 82% of vaccinees primed with MenC-TT, whose MenC rSBA GMTs were significantly higher compared to the MenC rSBA GMTs measured in participants primed with a MenC-CRM schedule and in those who were not primed at all, still had MenC rSBA ≥1:8 in contrast to ≤30% of those primed with other MenC schedules. Despite evidence of immune memory after MenC disease and vaccination[56] the antibody response following MenC exposure is not rapid enough to prevent disease in those with a MenC rSBA titre <1:8,[20] demonstrating the importance of generating high post boost rSBA GMTs, leading to a higher proportion of children maintaining rSBA titres above the 1:8 threshold through early childhood.

Such results are assumed to indicate differences in immunogenicity of the vaccines that relate to the T-cell help induced by the different carrier proteins, though there are other

manufacturing differences between MenC-TT and MenC-CRM which make it difficult to formally draw this conclusion. Differences in the persistence of post-boost MenC bactericidal antibody are consistent with observations from other studies of the persistence of MenC bactericidal antibody following priming with different MenC glycoconjugates in infancy.[23,57] Our findings show that it would be more rational to prime infants with MenC-TT, rather than MenC-CRM, when boosting with Hib-MenC-TT.

Part of the study looked at the magnitude of the MenC rSBA GMTs six days after Hib-MenC-TT vaccination at 12 months of age. Participants who were previously primed with a MenC conjugate vaccine had significantly higher MenC rSBA GMTs (1130.59; 982.29; 3883.34 vs 221.09 for MenC-CRM197, two dose MenC-CRM197 and MenC-TT vs control groups, respectively with p=<0.00001 for all) and MenC rSBA titres ≥1:8 (100% of all those previously primed compared to 80.77% of those unprimed, p=0.001 for all) compared to those who were unprimed. This suggests that the magnitude of the MenC rSBA GMTs six days following a MenC glycoconjugate vaccine boost may be used as a test for the induction of immunological memory by MenC conjugate vaccines. The anamnestic response may however differ with the type of MenC conjugate vaccine formulation used for priming and boosting.

We also assessed the percentage of vaccinees with MenC rSBA titres ≥1:1000 following the 12 month Hib-MenC-TT vaccination to determine if this cut off can be used to distinguish those who were primed from those unprimed in infancy. Six days following Hib-MenC-TT vaccination significant differences were seen between subjects primed with the MenC conjugate vaccine schedules used in the study and those who were unprimed (p=≤0.003 for all). One month later a significant difference persisted between those unprimed and those primed with a single MenC-CRM197/TT vaccine (p=<0.00001 for both) but not when compared to those primed with two doses of MenC-CRM197 vaccines (p=0.058).

A MenC rSBA ≥1:1000 is a too high cut off to identify children who were primed from those who were not primed with a MenC vaccine in infancy, although this is affected by the type of carrier

protein used for priming and for MenC-CRM197, the number of doses used for priming. The findings also support blunting of the anamnestic response to a challenge when two MenC-CRM197 doses, rather than one, are used for infant priming.

The percentage of participants with a MenC rSBA titre ≥1:8 following immunisation with a single dose of Hib-MenC-TT at 12 months of age, without infant priming, reached up to 83%, a proportion that might be acceptable in countries where MenC disease is currently under control. The introduction of just a single dose of MenC vaccine at 12 months of age might not be appropriate in other countries where herd immunity has not been established through the initiation of the programme with a "catch up campaign" and subsequent adolescent boosting (used to maintain herd immunity). A routine 12 month only MenC immunisation programme, in the absence of such herd immunity would leave unvaccinated infants as well as vaccinated children, whose immunity has waned over time, at risk. The low titres of bactericidal antibodies in infancy and from 2 years of age onwards observed with just a single MenC vaccination at 12 months of age, suggests prevention of breakthrough cases in infants and pre-school aged children would be dependent on herd immunity induced by a catch-up vaccination campaign which could then be sustained through adolescent boosting. A single MenC toddler dose was successful in controlling MenC disease in The Netherlands[58] Australia[59] and in Canada[60] where infants were protected through herd protection induced by an initial catch up campaign targeting older children and adolescents. An alternative, as in the US, is to provide the first MenC dose in adolescence.[61] However, a MenC priming dose at 12 months of age might still be important for a robust anamnestic response following a MenC adolescent boost.[62] Indeed if herd immunity in the UK is maintained through a robust adolescent MenC booster programme, the 3 month infant MenC vaccine might conceivably be dropped from the MenC vaccination programme without any change in the current excellent population protection. Furthermore, the anticipated introduction of a routine MenB vaccination schedule in infancy, utilising a MenB vaccine which contains relatively well conserved

meningococcal sub-capsular proteins that may also be common amongst different meningococcal strains independent of the capsular polysaccharide type,[63] is predicted to protect against other serogroups, including some clones of MenC, in the first 12 months of life, potentially supporting the removal of the infant MenC doses.

21.1.2 Reactogenicity

No significant differences were observed in the frequency of local/systemic adverse events following a single dose MenC-CRM197 compared to MenC-TT vaccination at 3 months of age or when comparing a single dose MenC-CRM197/TT infant schedule to two doses of MenC-CRM197 given at 3 and 4 months of age. Similarly no significant differences were seen following Hib-MenC-TT vaccination at 12 months of age between those who were primed with the MenC-CRM197 vaccine and the MenC-TT vaccine and those who were primed with MenC-CRM197/TT compared to two doses of MenC-CRM197. In addition no significant differences were noted when comparing vaccinees primed with any of the priming schedules with those who were unprimed in infancy.

21.2 Consistent/Alternating Limb Subgroup

21.2.1 Immunogenicity

This study is the first randomised controlled trial to include an investigation of the potential effect of administering routine infant vaccinations in the same versus different limbs on vaccine immunogenicity. The findings of this component of the study suggest that alternating the limb used does not reduce, and might even improve, immunogenicity, at least for some antigens used in routine infant immunisation programmes.

The vaccination strategy that was best suited to address the effect of same versus different limb

use on immunogenicity in this study was that for PCV13 because the vaccine was always given in the same limb (the right leg) in the consistent limb group, and a different limb was used for each dose (left leg, right leg, or left arm) in the alternating limb group. With this approach, anti-pneumococcal IgG concentrations and the proportion of participants achieving the threshold of protection was similar in both groups. This finding is in contrast with that of a rabies vaccine study in which the proportion of participants responding with detectable neutralising antibody following an intradermal rabies vaccination schedule was higher in those immunised in the same arm (92%) than in those immunised in different limbs (68%) .[64] Although the reason for the dissimilar findings is not known, several factors might have contributed. In the rabies vaccine study, the vaccine was given in rapid succession with only a 7-day interval between each dose, whereas there were 2 months between the two primary doses of PCV13 in this study. With vaccine doses administered close together as in the rabies vaccine study, the germinal centres primed with the first dose will not have involuted before the second vaccine is administered, whereas the immune response is rather more mature with a 2-month gap between doses. Furthermore, the rabies vaccine was given intradermally, whereas all vaccines studied here were given intramuscularly, with likely differences in early interactions with resident immune cell populations. Additionally, the resulting immune response after vaccination with a viral protein antigen (such as rabies) is likely to differ from that seen with bacterial polysaccharide–protein conjugate vaccines like those assessed in this study.

In this study, Hib (anti-PRP) responses were higher in the AL group compared to the CL group after completion of a 3 dose infant priming schedule but not after boosting. Since immunisation in the arm in early infancy is not seen as acceptable due to the small muscle mass in the arm, for practical reasons, the only difference between the two groups for the delivery of the three-dose DTaP–IPV–Hib priming series was the limb used for the 2 month dose. Participants in the alternating limb group received the first dose in the left leg followed by the 3 and 4 month doses in the right leg, whereas those in the consistent limb group received all three doses in the right

leg. Thus the Hib data suggest that differences in the administration of the DTaP–IPV–Hib vaccine in a three-dose primary series can significantly change the immunogenicity of the schedule. The observed differences in Hib immunogenicity are most likely due to differences in the priming and stimulation of lymph nodes draining the site of vaccination. This is suggested by studies in animals that show a higher number of antibody-forming cells in draining than non-draining lymph nodes[65-67] perhaps driven by stimulation with antigen trapped and retained by the follicular dendritic cells for several months.[68-71] This phenomenon was not specifically tested in this study but warrants further investigation and should be taken into account in the design of clinical trials.

Anti-tetanus toxoid IgG concentration was higher in the alternating limb group than in the consistent limb group at 13 and 24 months (one and twelve months after boosting with Hib–MenC–TT respectively), but was similar for both groups after priming. To allow proper assessment of the MenC vaccines in line with the primary objective of the parent study, the same limb (left leg) was used for all MenC vaccines. Administration of Hib–MenC–TT in the left leg at 12 months therefore meant that for assessment of tetanus toxoid responses, the limb used was not consistent for the consistent limb group. Nevertheless, the observed difference in anti-tetanus toxoid IgG concentration after boosting could be because the limb allocation strategy used in the priming phase for the alternating limb group generated more immune memory and, consequently, a heightened response to the booster, than the strategy used for the consistent limb group. Conversely, it could be that a three-dose priming in the same leg in the consistent limb group might not favour generation of immunological memory.

21.2.2 **Reactogenicity**

No difference was observed in the proportion of participants in both groups that experienced any solicited local or systemic adverse event

21.3 B-cell analysis

21.3.1 Differences between groups based priming doses and vaccine types:

This is the first study to evaluate serogroup C meningococcal (MenC) memory B-cell responses following different priming schedules in infants with different conjugate vaccines, and has surprisingly found that priming does not produce a significant improvement in the memory response to a booster dose of vaccine 7 months later in comparison to un-primed controls, nor to the persistence of memory B-cells for 12 months after booster vaccination. Furthermore, no evidence was seen in this study for a relationship between the number of priming doses of MenC-CRM_{197}, and the generation of MenC-specific memory B-cells at any time-point following primary or booster immunisations in infants. These findings are in contrast to previously published studies which examined immunological memory after immunisation using antibody responsiveness as a surrogate and found that antibody responses following a booster were higher in those who had received fewer priming vaccine doses.[45,46] These previous studies suggested that limiting the number of "priming" doses of vaccine may favour memory formation, at the expense of initial antibody response.

In the current study, the number of MenC-specific memory B-cells detected by the ELISpot assay was shown to be related to the type of vaccine used for priming and boosting. Children primed with MenC-TT conjugate vaccine generated more memory B-cells following a Hib-MenC-TT booster than children previously primed with MenC-CRM_{197}, suggesting that the carrier protein used for priming and boosting may have an important role in determining the polysaccharide-specific response to conjugate vaccines. The differences between vaccines in induction of MenC-specific memory B-cells may explain the differential antibody response noted following primary and booster vaccines when children were primed with either a MenC-CRM_{197} vaccine or Hib-MenC-TT in the first year of life and given a Hib-MenC-TT booster as toddlers.[21,51] These observations

may be related to a greater number of MenC memory B-cells generated by Hib-MenC-TT and MenC-TT than by MenC-CRM_{197} primary immunisations.

21.3.2 Differences over time – number and frequencies of antigen specific memory B-cells:

In this study, a rise in antigen-specific memory B-cells was seen following primary immunisations until booster vaccination at 12 months of age for all antigens tested. This finding was consistent with an increase in the proportion of MenC-specific memory B-cells out of the total pool of IgG positive memory B-cells from 0.03% at 5 months to 0.05% at 12 months of age, suggesting that this is an antigen-specific increase, rather than a non-specific effect of immune maturation in infants. In the context of waning bactericidal antibody described in the first year of life following infant immunisations,[20,21] an increase in the number of MenC-specific memory B-cells between 5 and 12 months of age suggests that after primary MenC vaccines, memory B-cells continue to be generated for several months, but there is a decline in the proportion that differentiate into plasma cells. One explanation for this discordance may be that separate B-cell precursors are responsible for the primary antibody-producing and memory B-cell populations.[23]

21.3.3 Differentiating primed from un-primed children:

Primary and secondary GC reactions have been shown to be qualitatively very similar[52] although there are several quantitative differences, including the speed with which they develop. During a secondary response, IgG memory B-cells appear in the circulation more rapidly, and in adolescents receiving a booster dose of MenC conjugate vaccine memory B-cells were detected by day 6.[53] In the current study, detection of at greater than 2.5 MenC memory B-cells/million PBMCs in the peripheral blood, 6 days after a booster dose of Hib-MenC-TT was able to differentiate MenC-primed from un-primed children with a sensitivity and specificity of >85%. This could potentially

be used as a novel way to test the ability of MenC conjugate vaccines to induce immune memory in infants and young children with a single blood test.

22 CONCLUSIONS

22.1 MenC group

In countries where the incidence of invasive MenC disease in infancy has been controlled or practically eliminated following a routine MenC vaccination programme, two MenC infant priming doses may be reduced to a single priming dose without loss of immediate post-booster immunogenicity and without any effect on reactogenicity. In the absence of a herd immune effect induced by previous MenC vaccination catch up campaigns in countries where MenC infant disease is still prevalent, infant vaccination against MenC is the only way of providing protection in the first months of life with the most effective infant schedule being a single MenC-TT vaccination at 3 months of age followed by Hib-MenC-TT at 12 months of age. Vaccinating children against MenC disease for the first time at 12 months of age results in adequate seroprotection but, considering their susceptibility to MenC disease in infancy would not be adequate in countries where MenC transmission is still prevalent secondary to the lack of herd immunity from previous mass vaccination of at risk age groups.

Following a Hib-MenC-TT boost at 12 months of age, protection up till 24 months of age can only be sustained if children are primed with a MenC-TT, rather than a MenC-CRM197 vaccine, in infancy. Differences in the immunogenicity of MenC conjugate vaccines utilising different carrier proteins during priming and boosting will impact the need for further boosting beyond 2 years of age.

22.2 Consistent/Alternating limb subgroups

The administration of sequential doses of the PCV13 in different limbs does not result in reduced immunogenicity. There is some evidence that for some antigens (Hib), improved immunogenicity is seen when sequential vaccine doses are administered in different rather than same limb although the size of the difference is unlikely to be of clinical significance. Notwithstanding the limitations relating to the design of this component of the study, these observations provide confidence that immunisation programmes do not need to specify what limb should be used in schedules that contain several doses of a vaccine. Careful analysis of vaccine delivery, including the immunological effects and local or systemic reactions, is necessary to provide continued confidence in vaccine programmes and ensure that population protection is optimised.

22.3 B-cell analysis

MenC-specific memory B-cell production may be more dependent on the type of vaccine used for primary immunisation of infants, and perhaps the matching of carrier proteins, than the number of doses administered. Although the mechanistic differences between MenC-CRM197 and MenC-TT priming are unclear, it is possible that structural differences in these vaccines may underly differential interactions with B- and T-cell populations and thus different effects on various memory B-cell subsets. The differences between these vaccines in induction of MenC-specific memory B-cells may explain the differential antibody response noted following primary and post Hib-MenC-TT booster immunisations. The MenC-TT/Hib-MenC-TT combination of vaccines may offer a practical advantage for infant immunisation schedules in terms of long-term persistence of antibody. Irrespective of the priming vaccine received, MenC primed children can be separated from un-primed children with a sensitivity and specificity of >85% by the detection of at least 2.5 MenC memory B-cells/million PBMCs in the peripheral blood, 6 days after a Hib-MenC-TT booster vaccine.

23 REFERENCES

1. Bilukha OO, Rosenstein N, National Center for Infectious Diseases, Centers for Disease Control and Prevention (CDC). Prevention and control of meningococcal disease. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2005 May 27; 54(RR-7):1-21.

2. European Union Invasive Bacterial Infections Surveillance Network. 2003 Invasive *Neisseira meningitidis* in Europe-2002. Available from www.euibis.org/documents/2002_meningo.pdf.

3. Whalen CM, Hockin JC, Ryan A et al. The changing epidemiology of invasive meningococcal disease in Canada, 1985 through 1992: emergence of a virulent clone of Neisseria meningitidis. *JAMA.* 1995;273:390-394.

4. Pollard AJ, Levin M. Vaccines for prevention of meningococcal disease. Pediatr Infect Dis J. 2000; 19(4): 333-44.

5. Environmental Science and Research Limited. 2006. The Epidemiology of Meningococcal Disease in New Zealand in 2006. Wellington: Ministry of Health. Available from http://www.moh.govt.nz/moh.nsf/pagesmh/6647 /$File/epidemiology-of-meningococcal-disease-2006.pdf

6. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. N Engl J Med. 2001 May 3;344(18):1378-88.

7. Snape MD, Pollard AJ. Meningococcal polysaccharide-protein conjugate vaccines. Lancet Infect Dis. 2005; 5(1): 21-30.

8. Granoff DM, Pollard AJ. Reconsideration of the use of meningococcal polysaccharide vaccine. Pediatr Infect Dis J. 2007 Aug;26(8):716-22.

9. Schittek B, Rajewsky K. Maintenance of B-cell memory by long-lived cells generated from proliferating precursors. Nature. 1990 Aug 23;346(6286):749-51.

10. MacLennan IC, Gulbranson-Judge A, Toellner KM, Casamayor-Palleja M, Chan E, Sze DM, et al. The changing preference of T and B cells for partners as T-dependent antibody responses develop. Immunol Rev. 1997 Apr;156:53-66.

11. Pace D, Cuschieri P, Galea Debono A, Attard-Montalto S. Epidemiology of pathogenic Neisseria meningitidis serogroup B serosubtypes in Malta: implications for introducing PorA based vaccines. Vaccine. 2008 Nov 5;26(47):5952-6.

12. Muscat M, Spiteri G, Calleja N, Haider J, Gray SJ, Melillo JM, et al. Invasive meningococcal disease in Malta: an epidemiological overview, 1994-2007. J Med Microbiol. 2009 Nov;58(Pt 11):1492-8.

13. Miller E, Pollard AJ, Borrow R, et al. Safety and immunogenicity of Novartis meningococcal serogroup B vaccine after three doses administered in infancy. Paper presented at: ESPID 2008; Graz.

14. Borrow R, Goldblatt D, Finn A, et al. Immunogenicity of, and immunologic memory to, a reduced primary schedule of meningococcal C-tetanus toxoid conjugate vaccine in infants in the United Kingdom. Infect Immun. Oct 2003;71(10):5549-5555.

15. Southern J, Crowley-Luke A, Borrow R, Andrews N, Miller E. Immunogenicity of one, two or three doses of a meningococcal C conjugate vaccine conjugated to tetanus toxoid, given as a three-dose primary vaccination course in UK infants at 2, 3 and 4 months of age with acellular pertussis-containing DTP/Hib vaccine. Vaccine. Jan 12 2006;24(2):215-219.

16. Richmond P, Borrow R, Miller E, et al. Meningococcal serogroup C conjugate vaccine is immunogenic in infancy and primes for memory. J Infect Dis. Jun 1999;179(6):1569-1572.

17. Richmond P, Borrow R, Findlow J, et al. Evaluation of De-O-acetylated meningococcal C polysaccharide-tetanus toxoid conjugate vaccine in infancy: reactogenicity, immunogenicity, immunologic priming, and bactericidal activity against O-acetylated and De-O-acetylated serogroup C strains. Infect Immun. 2001;69:2378-2382.

18. Snape MD, Kelly DF, Lewis S, Banner C, Kibwana L, Moore CE, et al. Seroprotection against serogroup C meningococcal disease in adolescents in the United Kingdom: observational study. BMJ. 2008 Jun 28;336(7659):1487-91.

19. Miller E, Salisbury D, Ramsay ME. Planning, registration and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story. Vaccine 2001; 20 Suppl 1: S58-67.

20. Trotter C, Andrew N, Kaczmarski E, Miller E, Ramsay M. Effectiveness of meningococcal serogroup C conjugate vaccine 4 years after introduction. Lancet. 2004; 364 (9431): 365-367.

21. Maiden MC, Stuart JM. Carriage of serogroup C meningococci 1 year after meningococcal C conjugate polysaccharide vaccination. *Lancet.* May 25 2002;359(9320):1829-1831.

22. A Phase IV, Randomized Study to Evaluate the Immune Response of UK Infants Receiving DTaP/Hib/IPV, Meningococcal C Conjugate and Pneumococcal Conjugate Vaccines, Antibody Persistence and Responses to Booster Doses in the Second Year of Life (Sched2). http://clinicaltrials.gov/ct2/show/NCT00625677?term=sched2&rank=1. Accessed 15th November, 2008.

23. Borrow R, Andrews N, Findlow H, Waight P, Southern J, Crowley-Luke A, Stapley L, England A, Findlow J, Miller E. Kinetics of antibody persistence following administration of a combination meningococcal serogroup C and haemophilus

influenzae type b conjugate vaccine in healthy infants in the United Kingdom primed with a monovalent meningococcal serogroup C vaccine. Clin Vaccine Immunol 2010; 17: 154-9.

24. *Recommendations for the production and control of meningococcal group C conjugate vaccines*: World Health Organisation; 2004. 924.

25. Pollard AJ, Granoff DM. Reconsideration of the Use of Meningococcal Polysaccharide Vaccine. *Paed Infect Dis J.* 2007;26:716 - 722.

26. World Health Authority ECoBS. Proposed replacement of: TRS 926, Annex 3. Part C. Clinical evaluation of group C meningococcal conjugate vaccines. Accessed 28 September, 2007. http://www.who.int/biologicals/expert_committee/BS2065%20Mening%20+%20line%20number.pdf.

27. Ponzio NM, Chapman-Alexander JM, Thorbecke GJ. Transfer of memory cells into antigen-pretreated hosts. I. Functional detection of migration sites for antigen-specific B cells. Cell Immunol. 1977 Nov;34(1):79-92.

28. Peck FB Jr, Kohlstaedt KC. Pre-exposure rabies prophylaxis problems and procedures. Ind Med Surg. 1964 Jan;33:17-21.

29. Tan PL, Jacobson RM, Poland GA, Jacobsen SJ, Pankratz VS. Twin studies of immunogenicity--determining the genetic contribution to vaccine failure. Vaccine 2001; 19(17-19): 2434–9.

30. Newport MJ, Goetghebuer T, Weiss HA, Whittle H, Siegrist CA, Marchant A. Genetic regulation of immune responses to vaccines in early life. Genes Immun 2004;5:122–9.

31. Ovsyannikova IG, Pankratz VS, Vierkant RA, Jacobson RM, Poland GA. Human

leukocyte antigen haplotypes in the genetic control of immune response to measles mumps-rubella vaccine. J Infect Dis 2006; 19:655-63.

32. Yucesoy B, Sleijffers A, Kashon M, Garssen J, de Gruijl FR, Boland GJ, et al. IL-1beta gene polymorphisms influence hepatitis B vaccination. Vaccine 2002; 20: 3193-6.

33. Summary of product characteristics; Menjugate Kit. Novartis Vaccines and Diagnostics. Available at http://emc.medicines.org.uk

34. Summary of product characteristics; Pediacel, Sanofi Pasteur, MSD. Available at http://www.medicines.org.uk

35. Summary of product characteristics; NeisVac-C. Baxter Vaccines. Available at http://www.medicines.org.uk

36. Summary of product characteristics; Prevenar-13, Wyeth Lederle Vaccines. Available

37. at http://www.medicines.org.uk

38. Summary of product characteristics; Menitorix, GlaxoSmithKline. Available at http://www.medicines.org.uk

39. UK Department of Health. Immunisation against infectious diseases. Green book, 2006. Chapter 4: Immunisation procedures.

40. Michael Marcy S, Kohl KS, Dagan R, Nalin D, Blum M, Jones MC, et al. Brighton Collaboration Fever Working Group. Fever as an adverse event following immunization: case definition and guidelines of data collection, analysis, and presentation. Vaccine. 2004 Jan 26;22(5-6):551-6.

41. Kohl KS, Walop W, Gidudu J, Ball L, Halperin S, Hammer SJ, et al. Brighton Collaboration Local Reactions Working Group for Induration at or near Injection Site. Induration at or near injection site: case definition and guidelines for collection,

analysis, and presentation of immunization safety data. Vaccine. 2007 Aug 1;25(31):5839-57.

42. Kohl KS, Walop W, Gidudu J, Ball L, Halperin S, Hammer SJ, et al. Brighton Collaboration Local Reaction Working Group for Swelling at or near Injection Site. Swelling at or near injection site: case definition and guidelines for collection, analysis and presentation of immunization safety data. Vaccine. 2007 Aug 1;25(31):5858-74.

43. Gidudu J, Kohl KS, Halperin S, Hammer SJ, Heath PT, Hennig R, et al. Brighton Collaboration Local Reactions Working Group for a Local Reaction at or near Injection Site. A local reaction at or near injection site: case definition and guidelines for collection, analysis, and presentation of immunization safety data. Vaccine. 2008 Dec 9;26(52):6800-13.

44. Snape MD, MacLennan J, Lockhart S, et al. Demonstration of immunologic memory using serogroup C meningococcal glyco-conjugate vaccine. *Paed Infect Dis J.* 2009. Feb;28(2):92-7.

45. Maslanka SE, Gheesling LL, Libutti DE, Donaldson KB, Harakeh HS, Dykes JK, et al. Standardization and a multilaboratory comparison of Neisseria meningitidis serogroup A and C serum bactericidal assays. The Multilaboratory Study Group. Clin Diagn Lab Immunol. 1997 Mar;4(2):156-67.

46. Borrow R, Andrews N, Goldblatt D, Miller E. Serological basis for use of meningococcal serogroup C conjugate vaccines in the United Kingdom: reevaluation of correlates of protection. Infect Immun. 2001 Mar;69(3):1568-73.

47. Andrews N, Borrow R, Miller E. Validation of serological correlate of protection for meningococcal C conjugate vaccine by using efficacy estimates from postlicensure surveillance in England. Clin Diagn Lab Immunol. 2003 Sep;10(5):780-6.

48. Lal G, Balmer P, Stanford E, Martin S, Warrington R, Borrow R. Development and validation of a nonaplex assay for the simultaneous quantitation of antibodies to nine Streptococcus pneumoniae serotypes. J Immunol Methods. 2005 Jan;296(1-2):135-47.

49. Pickering JW, Martins TB, Greer RW, Schroder MC, Astill ME, Litwin CM, et al. A multiplexed fluorescent microsphere immunoassay for antibodies to pneumococcal capsular polysaccharides. Am J Clin Pathol. 2002 Apr;117(4):589-96.

50. Kelly DF, Snape MD, Perrett KP, Clutterbuck EA, Lewis S, Blanchard Rohner G, et al. Plasma and memory B-cell kinetics in infants following a primary schedule of CRM 197-conjugated serogroup C meningococcal polysaccharide vaccine. Immunology. 2009 May;127(1):134-43. Erratum in: Immunology. 2011 Apr;132(4):589..

51. Blanchard-Rohner G, Watt H, Kelly DF, Yu LM, Snape MD, Pollard AJ. Baseline polysaccharide-specific antibodies may not consistently inhibit booster antibody responses in infants to a serogroup C meningococcal protein-polysaccharide conjugate vaccine. Vaccine. 2012;30(28):4153-9.

52. Ramsay, M.E., et al., Herd immunity from meningococcal serogroup C conjugate vaccination in England: database analysis. BMJ, 2003. 326(7385): p. 365-6.

53. Richmond P., et al., Ability of 3 different meningococcal C conjugate vaccines to induce immunologic memory after a single dose in UK toddlers. J Infect Dis, 2001. 183(1): p. 160-3.

54. Diez-Domingo, J., et al., A randomized, multicenter, open-label clinical trial to assess the immunogenicity of a meningococcal C vaccine booster dose administered to children aged 14 to 18 months. Pediatr Infect Dis J, 2010. 29(2): p. 148-52.

55. Findlow H, Borrow R, Andrews N, Waight P, Sheasby E, Matheson M, et al. Immunogenicity of a single dose of meningococcal group C conjugate vaccine

given at 3 months of age to healthy infants in the United Kingdom. Pediatr Infect Dis J. 2012 Jun;31(6):616-22.

56. Leach A, Twumasi PA, Kumah S, Banya WS, Jaffar S, Forrest BD, et al. Induction of immunologic memory in Gambian children by vaccination in infancy with a group A plus group C meningococcal polysaccharide-protein conjugate vaccine. J Infect Dis. 1997 Jan;175(1):200-4.
57. Borrow R, Goldblatt D, Andrews N, Southern J, Ashton L, Deane S, et al. Antibody persistence and immunological memory at age 4 years after meningococcal group C conjugate vaccination in children in the United Kingdom. J Infect Dis. 2002 Nov 1;186(9):1353-7.
58. Khatami A, Snape MD, John T, Westcar S, Klinger C, Rollinson L, et al. Persistence of immunity following a booster dose of *Haemophilus influenzae* type B-Meningococcal serogroup C glycoconjugate vaccine: follow-up of a randomized controlled trial. Pediatr Infect Dis J. 2011 Mar;30(3):197-202.
59. Kaaijk P, van der Ende A, Berbers G, van den Dobbelsteen GP, Rots NY. Is a single dose of meningococcal serogroup C conjugate vaccine sufficient for protection? Experience from the Netherlands. BMC Infect Dis. 2012 Feb 8;12:35.
60. Simpkins D, Wood N, Jelfs J, McIntyre PB, Menzies R, Lawrence G, et al. Modern trends in mortality from meningococcal disease in Australia. Pediatr Infect Dis J. 2009 Dec;28(12):1119-20.
61. De Wals P, Deceuninck G, Lefebvre B, Boulianne N, De Serres G. Effectiveness of serogroup C meningococcal conjugate vaccine: a 7-year follow-up in Quebec, Canada. Pediatr Infect Dis J. 2011 Jul;30(7):566-9.

62. Committee on Infectious Diseases. Updated recommendations on the use of meningococcal vaccines. Pediatrics. 2014 Aug;134(2):400-3.

63. Stoof SP, van der Klis FR, van Rooijen DM, Knol MJ, Sanders EA, Berbers GA. Timing of an adolescent booster after single primary meningococcal serogroup C conjugate immunization at young age; an intervention study among Dutch teenagers. PLoS One. 2014 Jun 25;9(6):e100651.

64. Pollard AJ, Riordan A, Ramsay M. Group B meningococcal vaccine: recommendations for UK use. Lancet. 2014 Mar 29;383(9923):1103-4.

65. Peck FB, Kohlstaedt KC. Pre-exposure rabies prophylaxis problems and procedures. Ind Med Surg 1964; 33: 17–21.

66. Thorbecke GJ, Bell MK. The proliferative and anamnestic antibody response of rabbit lymphoid cells in vitro. II. Effect of passive antibody on immunologic memory in lymph nodes contralateral to the site of antigen injection. J Immunol 1973; 111: 1043–47.

67. Jacobson EB, Thorbecke GJ. The proliferative and anamnestic antibody response of rabbit lymphoid cells in vitro. I. Immunological memory in the lymph nodes draining and contralateral to the site of a primary antigen injection. J Exp Med 1969; 130: 287–97.

68. Donaldson SL, Kosco MH, Szakal AK, Tew JG. Localization of antibody-forming cells in draining lymphoid organs during long-term maintenance of the antibody response. J Leukoc Biol 1986; 40: 147–57.

69. Mandel TE, Phipps RP, Abbot AP, Tew JG. Long-term antigen retention by

dendritic cells in the popliteal lymph node of immunized mice. Immunology 1981; 43: 353–62.

70. Tew JG, Mandel T, Burgess A, Hicks JD. The antigen binding dendritic cell of the lymphoid follicles: evidence indicating its role in the maintenance and regulation of serum antibody levels. Adv Exp Med Biol 1979; 114: 407–10.

71. Tew JG, Mandel TE, Phipps RP, Szakal AK. Tissue localization and retention of antigen in relation to the immune response. Am J Anat 1984; 170: 407–20.

72. Tew JG, Phipps RP, Mandel TE. The maintenance and regulation of the humoral immune response: persisting antigen and the role of follicular antigen-binding dendritic cells as accessory cells. Immunol Rev 1980; 53: 175–201.

24 APPENDIX A: STUDY FLOW CHART

Details of vaccine administration for each treatment group			**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6***	**Visit 7**	**Visit 8**
						Blood	**Blood**	**Blood**	**Blood**	**Blood**
Two Dose MenC Group **(2 doses MenC-CRM$_{197}$)** **Group 2**	**a**	Right leg	DTaP-IPV-Hib PCV13	DTaP-IPV-Hib	DTaP-IPV-Hib PCV13		PCV13		MMR	
		Left leg		*MenC-**CRM$_{197}$***	*MenC-**CRM$_{197}$***		Hib-MenC			
	b	Right leg		DTaP-IPV-Hib	DTaP-IPV-Hib PCV13				MMR	
		Left leg/ arm	DTaP-IPV-Hib PCV13	*MenC-**CRM$_{197}$***	*MenC-**CRM$_{197}$***		Hib-MenC (leg), PCV13 (arm)			
Single Dose MenC-CRM$_{197}$ Group **(1 dose MenC-CRM$_{197}$)** **Group 1**	**a**	Right leg	DTaP-IPV-Hib PCV13	DTaP-IPV-Hib	DTaP-IPV-Hib PCV13		PCV13		MMR	
		Left leg/ arm		*MenC-**CRM$_{197}$***			Hib-MenC			
	b	Right leg		DTaP-IPV-Hib	DTaP-IPV-Hib PCV13				MMR	
		Left leg/ arm	DTaP-IPV-Hib PCV13	*MenC-**CRM$_{197}$***			Hib-MenC (leg) PCV13 (arm)			
Control Group **(0 dose MenC priming)** **Group 3**	**a**	Right leg	DTaP-IPV-Hib PCV13	DTaP-IPV-Hib	DTaP-IPV-Hib PCV13		PCV13		MMR	
		Left leg/ arm					Hib-MenC			
	b	Right leg		DTaP-IPV-Hib	DTaP-IPV-Hib PCV13				MMR	
		Left leg/ arm	DTaP-IPV-Hib PCV13				Hib-MenC (leg) PCV13 (arm)			
Single Dose MenC-TT Group **(1 dose MenC-TT)** **Group 4**	**a**	Right leg	DTaP-IPV-Hib PCV13	DTaP-IPV-Hib	DTaP-IPV-Hib PCV13		PCV13		MMR	
		Left leg/ arm		*MenC-TT*			Hib-MenC			
	b	Right leg		DTaP-IPV-Hib	DTaP-IPV-Hib PCV13				MMR	

		Left leg/ arm	DTaP-IPV-Hib PCV13	*MenC-TT*			Hib-MenC (leg) PCV13 (arm)			

* This would be for all participants in the Control Group (ie 64), and a matching number of participants in the Two Dose MenC, Single Dose MenC-CRM_{197} and the Single Dose MenC-CRM_{197} groups as determined by randomisation at enrolment. Timelines: Visit 1: age 6-12 weeks, Visit 2: age approximately 3 months, Visit 3: age approximately 4 months, Visit 4: age approximately 5 months, Visit 5: age approximately 12 months, Visit 6:6 days after visit 5, Visit 7: age approximately 13 months, Visit 8: age approximately 24 months

25 APPENDIX B: SCHEDULE OF PROCEDURES

Visit	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6*	Visit 7	Visit 8
Informed consent	X							
Check inclusion criteria	X							
Check exclusion criteria	X							
Check elimination criteria		X	X	X	X	X	X	X
Check contraindications		X	X		X		X	
Medical history	X							
Vaccination history	X							
Pre-vaccination body temperature	X	X	X		X		X	
Randomisation	X							
Blood sampling: for antibody and B memory cell determination (5ml at 5 months, 7.5ml for older ages)				X	X	X	X	X
Vaccination**	X	X	X		X		X	
Daily post-vaccination recording of solicited symptoms (Days 0–3) by subjects' parents/guardians	X	X	X		X			
Return of diary cards		X	X	X		X		
Diary card transcription		X	X	X		X		
Record any concomitant immunosuppressive medication/ vaccination	X	X	X	X	X	X	X	X
Reporting of Serious Adverse Events		X	X	X	X	X	X	X
Conclusion of study								X

* This would be for all participants in the Control Group (ie 64), and a matching number of participants in the Two Dose MenC, Single Dose MenC-CRM_{197} and the Single Dose MenC-CRM_{197} groups, as determined by randomisation at enrolment. Timelines: Visit 1: age 6-12 weeks, Visit 2: age approximately 3 months, Visit 3: age approximately 4 months, Visit 4: age approximately 5 months, Visit 5: age approximately 12 months, Visit 6:6 days after visit 5, Visit 7: age approximately 13 months, Visit 8: age approximately 24 months

** Vaccination as per study flow chart in Appendix A.

26 APPENDIX C: INTERVALS BETWEEN VISITS

Primary phase (initial visit at 6 to 12 weeks of age)

Interval	**Length of interval**
1 (Visit 1 to Visit 2)	28-42 days
2 (Visit 2 to Visit 3)	28-42 days
3 (Visit 3 to Visit 4)	28-42 days

Booster Phase (initial visit at 12 to 13 months of age)

5 (Visit 5 to Visit 6)	6 days
6 (Visit 5 to visit 7)	28 – 42 days
7 (Visit 5 to visit 8)	11-12 months