STUDY REPORT

Full title
A non-interventional, open observational non-inferiority study in two cluster-assigned cohorts of children aged 14 months into the safety of NeisVac-C® vaccines manufactured at two different production sites and given simultaneously with measles-mumps-rubella vaccine, assessed by web-based intensive monitoring

Short title
Web-based intensive safety monitoring of NeisVac-C® vaccination in toddlers

Study running name
Peuterprik

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The Clinical Study Report report is structured according to required elements described in EU Guideline on good pharmacovigilance practices (GVP), Module VIII - Post-authorization safety studies (Rev1) (Ch VIII.B.6.3.2) [ref 1].

Chapter

Annexes in separate files

1. Title

Full title

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Short title

Web-based intensive safety monitoring of NeisVac-C® vaccination in toddlers

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2. Abstract

2.1. Summary

A non-interventional, open observational non-inferiority study was conducted in two cluster-assigned cohorts of children aged 14 months to investigate reactogenicity of NeisVac-C® vaccines manufactured at two different production sites, the "old" production site in Beltsville, MD, USA and the "new" production site in Orth/Donau, Austria (to be referred to as "old NeisVac-C®" vs "new NeisVac-C®") and given simultaneously with measles-mumps-rubella vaccine, by web-based intensive monitoring using questionnaires covering days 0-4, 5-14 and 15-28 post vaccination.
In the routine setting of the Netherlands Immunization Program, 2727 children were evaluable for the primary endpoint. Old NeisVac-C® was given to 1729 and new NeisVac-C® to 998 children. Enrollment was from May 2014 to April 2016.
Primary endpoint: we found similar incidences of rectally measured fever ≥38.0°C during days 0 to 4 after vaccination with new NeisVac-C® versus old NeisVac-C® (8.2% and 8.3% respectively), and similar relative risk (unadjusted 0.99, adjusted 0.96) with an upper margin of 95% CI below 1.50. Non-inferiority of the new vaccine to cause more fever than the old vaccine was demonstrated.
Secondary endpoints: NeisVac-C® related injection site reactions and systemic reactions were found in both groups at similar proportions of children from days 0 to 4, and days 5 to 28.
It was concluded from the study results that for both endpoints new NeisVac-C® is non-inferior to old NeisVac-C®. Both have similar reactogenicity as assessed by rectally measured fever ≥38.0°C, injection site reactions, and systemic reactions.

2.2. Structured abstract

1. Title
A non-interventional, open observational non-inferiority study in two cluster- assigned cohorts of children aged 14 months into the safety of NeisVac-C® vaccines manufactured at two different production sites and given simultaneously with measles-mumps-rubella vaccine, assessed by web-based intensive monitoring
Running study name in Dutch: Peuterprik
Abstract date 1 August 2016

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2. Keywords
NeisVac-C® - reactogenicity – toddlers - post authorization safety study – ENCePP study

3. Rationale and background

To compare the safety of the NeisVac-C® vaccine bulk material produced at Pfizer Inc (formerly Baxter Ltd) in Orth/Donau (new) with the material produced in Beltsville (old) in toddlers aged 14 months, administered simultaneously with measles-mumps-rubella (MMR) vaccine. The study was done at the request of the MHRA as primary reviewer of the dossier applying for approval of a new production site. MHRA required a post-authorization safety study (PASS) to obtain additional clinical safety data comparing vaccines from the old and the new production facility. It was specifically asked to monitor fever (rectally measured, 38 Celsius or above) during the first 4 days after vaccinations with old or new vaccine. Moreover it was proposed that the study would last at least one year to cover possible seasonal influence.
The Netherlands was selected for this study being a country where MenC vaccine is not given simultaneously with other non-replicating vaccines but only with live attenuated MMR vaccine. Therefore, fever during days 0-4 may be ascribed to only one product, i.e. MenC vaccine, while fever due to MMR vaccine is expected to occur later (between 5 to 12 days after vaccination).

4. Research question and objectives

The objective of this study was to show that the vaccines produced at the new production site in Orth/Donau, Austria would not be more reactogenic than the vaccines produced at the old production site in Beltsville, MD, USA. Fever defined as a rectally measured temperature ≥38.0°C during days 0 to 4 after vaccination was used as the most important marker for reactogenicity.
The primary endpoint is the proportions of subjects with fever reactions within 4 days after administration.
The secondary endpoints are proportions of subjects with
- solicited other systemic and local reactions within 4 days after administration
- non-solicited other systemic and local reactions within 4 days after administration
- non-solicited other systemic and local reactions from 5 to 28 days after administration
For these endpoints possible differences between old and new vaccines were assessed.

5. Study design

Non-interventional, open observational non-inferiority study with two cluster- assigned cohorts using three questionnaires to obtain data covering days 0-4, 5-14 and 15-28 after vaccination.

6. Setting

The study was conducted as an intensive web-based monitoring of children vaccinated according to the regular National Immunization Program. All children in The Netherlands receive childhood vaccinations at baby clinics. Study regions were defined where Child Health Care organizations agreed to facilitate the study. The baby clinics were supplied with either 'old' or 'new' NeisVac-C® vaccines according to a distribution plan that aimed at a 50-50% distribution of each. Thus, one clinic was supposed to vaccinate children with only 'old' or only 'new' vaccine. The study started in the province of Noord-Brabant (5 organizations, 135 clinics), and because of lower than expected enrollment, extended to two more provinces (Zeeland with one organization, and Limburg (with 3 organizations in the southern part). Baby clinic staff did not enroll children but facilitated that parents of the children had reliable data on the vaccinations to complete the web-based questionnaires if they decided on their own to enroll their child in the study.
Thus, two study groups of similar size were to be formed according to the MenC vaccination that was given at the baby clinics, i.e. NeisVac-C® from either the new production site in Orth/Donau or old production site in Beltsville. All children were given MMR vaccine (M-M-R-VaxPro®) simultaneously.

7. Subjects and study size

At least 2430 healthy toddlers aged 13-18 months old, eligible to receive MenC and MMR vaccinations according to the NIP, were required to study the primary endpoint parameter with sufficient power.
Inclusion criteria were age 13-18 months old (boundaries included) and eligibility to receive MenC and MMR vaccinations according to the NIP.
Exclusion criteria were children receiving either MenC or MMR vaccine and not both at the same time, and children with parents that are not able or willing to understand or complete the questionnaires.

Enrollment for the study started May 2014, and ended per April 2016. In April 2015 the recruitment area was enlarged because enrollment rates were lower than expected. There were 42,704 parents addressed to participate, and 3002 reported for enrollment. Of these, 2727 fulfilled criteria for in- and exclusion, and had valid data for questionnaire 1. Old NeisVac-C® was given to 1729 children, and new NeisVac-C® to 998 children. Despite these unequal proportions of children that received old or new NeisVac-C® (63 vs 37%), a revised power calculation indicated that sufficient numbers of children were enrolled to evaluate the primary endpoint, after which enrollment was stopped.

8. Variables and data sources

Primary: reactogenicity defined as % of children with rectal temperature 38.0°C and above during the first 4 days after vaccination.
Secondary: other solicited and unsolicited complaints and symptoms at the injection site as well as systemic reactogenicity.
These parameters were collected using three questionnaires, to be completed by the parents. Questionnaire 1 covered days 0-4 after vaccinations, questionnaire 2 covered days 5-14, and questionnaire 3 covered days 15-28. Parents had up to 4 days after each period to complete the questionnaire in order to obtain best remembered and most accurate data.

The primary endpoint of the study, fever cases observed within 4 days after vaccination was analyzed using logistic regression with vaccination groups ("old" / "new" NeisVac-C®) and potential confounders as explanatory factors, applying a log link in order to obtain relative risk estimates at the end. Relative risk and its 95% CI of occurrence of fever cases with the "new" and "old" NeisVac-C® was calculated from the regression model assessing a potential increase of fever reactions with the "new" NeisVac-C®. If the upper limit of the 95% CI was below 1.5 then the "new" NeisVac-C® was considered to be non-inferior to the old NeisVac-C® as far as fever reaction is concerned.
The secondary endpoints were analyzed similarly and descriptively without the non-inferiority considerations.

9. Results

Both groups were comparable for age, weight and height, house hold size, daycare attendance and medical history. The study ran for over one year to account for seasonal variations.
The main result was that the incidence of fever (38.0°C or above) during pooled days 0-4 was similar for both groups: 8.2% for children that received new NeisVac-C®, and 8.3% for children that received old NeisVac-C®. Also for the other endpoints no differences were found. Incidence of reactions at the injection sites of NeisVac-C® occurred in 3.4% in old NeisVac-C® and 3.3% in new NeisVac-C® recipients, and systemic reactions in 32.2% and 35.2% respectively. For the primary endpoint of fever the relative risk for the new vaccine to cause a higher incidence of fever than the old NeisVac-C® was 0.99 in the unadjusted analysis, and 0.96 for the adjusted analysis, with upper limits of the 95% CI of 1.28 and 1.24 respectively, remaining below the predefined non-inferiority margin of 1.50.
Also for the secondary endpoints similar incidences were found: reactions at NeisVac-C® injection sites during days 5 to 28 in 0.3% and 0.2% for old and new NeisVac-C®, and systemic reactions in 47.6% and 48.1%, respectively. In 13 children convulsions were reported, 11 of them had febrile convulsions. All occurred within 14 days after vaccinations, with similar numbers in the old and new vaccine groups.
During the study, 11 subjects experienced a Serious Adverse Event. The events did not indicate a higher risk associated with the new NeisVac-C® compared to the old NeisVac-C®. The nature of these events was within the expected pattern of earlier reported symptoms or disease, including coincidental infections as a plausible cause..

10. Discussion

The study was conducted to assess non-inferiority of new NeisVac-C® compared to old NeisVac-C® with regard to fever (rectally measured 38.0°C or above) occurring from 0 to 4 days after vaccination as a primary endpoint. The study demonstrated that new NeisVac-C® had a similar profile of reactogenicity as old NeisVac-C®, with similar rates of fever during the observation period: 8.2 vs 8.3% respectively, and similar relative risk values (0.99 and 0.96 for unadjusted and adjusted RR analysis) to cause fever.
During 5 to 28 days the pooled incidence of fever was higher than during days 0 to 4. These differences may be associated with the longer period of observation, but also because the MMR vaccine given simultaneously with NeisVac-C® is known to cause fever in 10-20% of recipients during this period.
The observed incidence of fever during days 0 to 4 of 8.2-8.3% is somewhat higher than the incidence found before in a similar study in Netherlands children (approximately 6%). A reason for this difference cannot be concluded.
The study was conducted in children that were given the vaccines as a part of the routine schedule in the National Immunization Program, in approximately 7% of the eligible population. The study population is considered representative for the general eligible population.

It was concluded that the NeisVac-C produced at the new production facility in Orth/Donau, Austria is non-inferior to the NeisVac-C produced at the old production facility in Beltsville, MD, USA.

11. Marketing authorization holder

NeisVac-C® vaccine: Netherlands Marketing Authorization Number RVG 26343, ATC code J07AH07, a product of Pfizer Inc.
Marketing authorization holder of NeisVac-C® vaccine is Pfizer Netherlands: Rivium Westlaan 142, 2909 LD Capelle aan den IJssel, The Netherlands.

12. Names and affiliations of principal investigators

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- Joep Scholl MSc, statistical analyses
- Annemarie van Gorp PharmD, data management

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Pfizer Inc is a Delaware Corporation with its principal place of business at 235 East 42nd Street, New York, New York 10017, United States of America.

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The study was performed in cooperation with the following partners:
1. National Institute of Public Health and Environment (RIVM), in particular with DVP. The DVP unit is responsible for purchase, supply and distribution of vaccines for the National Immunization Program.
2. Child health care organizations in the Netherlands provinces of Noord-Brabant, Zeeland and Limburg. These organizations execute the National Immunization Program, as a part of which the study was performed.

The partners had no 'investigator status'.
The study was done in The Netherlands only.

5. Milestones

The timelines of the study are described in Table 1.

Table 1. Timelines of the study

Year month	activity / step
2014 JAN	Medical Ethical Review Committee waiver granted
2014 MAR	study registration in the EU PAS Register
2014 APR	delivery of the appropriate batches in the study area
2014 APR	start sending weekly recruitment letters to parents of eligible children
2014 MAY	first inclusions (start of data collection)
2014 JUN	first fully completed questionnaires
2015 APR	regional extension of recruitment
2015 MAY	study progress report
2016 FEB	revised power analysis for unequal assignment of products under study
2016 FEB	decision to stop recruitment
2016 FEB	last letters sent
2016 APR	last inclusions
2016 MAY	last completed questionnaires (end of data collection)
2016 JUL	final draft study report
2016 SEP	final study report

6. Rationale and background

As described in the Study Protocol (Annex 1) the study was initiated for the reasons below.
NeisVac-C® is a meningococcal group C polysaccharide conjugate vaccine (tetanus toxoid protein conjugate). The vaccine stimulates the immune system to produce antibodies against group C meningococcal bacteria, thereby preventing the development of meningitis/meningococcal disease. It is indicated for active immunization of children aged 2 months and older, adolescents and adults, for the prevention of invasive disease caused by *Neisseria meningitidis* serogroup C.
NeisVac-C® is a product of Pfizer Inc, and until 01MAY2015 owned by Baxter Ltd. Due to a transfer of the manufacturing site of the drug substance from former Baxter's facility at Beltsville, Maryland, USA to former Baxter's facility at Orth/Donau, Austria, Baxter has applied for a Type II variation application (UK/H/0435/001/II/048/G) to allow release of Orth/Donau batches based on comparability to clinically qualified Beltsville batches to the United Kingdom's Medicines and Healthcare Products Regulatory Agency (MHRA) as the authority of the Reference Member State (RMS). MHRA required a post-authorization safety study (PASS) study [ref 2] to obtain additional clinical safety data comparing vaccines from the old and the new production facility. Baxter was also requested by the MHRA to submit a Risk Management Plan (RMP) in which it should be considered how the safety and efficacy of the vaccine manufactured at the new Orth/Donau site would be actively monitored in comparison with the Beltsville vaccine. In particular it was asked to consider the extent to which there would be co-marketing of the vaccines and to specifically address batch traceability, the limitations of passive surveillance to evaluate relative reactogenicity and to formulate proposals to either overcome this or to conduct active surveillance to demonstrate comparable reactogenicity. It was specifically asked to monitor fever (rectally measured, 38 Celsius or above) during the first 4 days after vaccinations with old or new vaccine. Moreover it was proposed that the study would last at least one year to cover possible seasonal influence. The PASS study was listed as EU RMP category 3.
From May 2015 Pfizer Inc is owner of NeisVac-C® and has adopted all obligations related to this product including the full commitment to the present study.
As a National Pharmacovigilance Centre of The Netherlands, Lareb required full independence to design and organize the study, followed by also independent data analysis and reporting. The study was funded by Baxter/Pfizer without restrictions, and Baxter/Pfizer consented with the full independence and transparency demanded by Lareb, including performing the study under an ENCePP seal [ref 3].
In The Netherlands Immunization Program NeisVac-C® is given to children of an age of about 14 months simultaneously with MMR vaccine. MMR is a live, attenuated virus vaccine, with a latency window of about 5 days for side effects that are associated with the replication of vaccine viruses. Any febrile reaction occurring during the first 4 days after administration of both vaccines, is more likely to be associated with NeisVac-C® than with MMR vaccine. Because of this co-administration, The Netherlands was considered a suitable country where reactogenicity of NeisVac-C® could be evaluated, compared to countries where NeisVac-C® is co-administered with non-live vaccines (as is the case with most other European countries).
We therefore have investigated systemic and local adverse events following NeisVac-C® and MMR administered simultaneously, with emphasis on the first 4 days after administration, and with subsequent follow-up during the remaining month.

7. Research question and objectives

As described in the Study Protocol (Chapter 2; Annex 1), the primary objective of this study in children aged 14 months was:

- to compare the proportions of vaccinees with fever (rectally measured temperature of ≥38.0ºC) within 4 days after injections of the Pfizer NeisVac-C® vaccine of which the bulk material was produced in Orth/Donau (new) or NeisVac-C® vaccine of which the bulk material was produced in Beltsville (old), and simultaneous MMR vaccine for both groups of NeisVac-C® recipients.

Secondary objective of this study was to investigate

- the safety profile with other parameters (solicited and non-solicited) within 4 days after simultaneous injections of either MenC, and MMR vaccines.
- the safety profile of these vaccines with non-solicited parameters from 5 to 28 days after injection.

8. Amendments and updates to the protocol

The study protocol had four versions:
Version 1.1, 13 January 2014
Version generally agreed upon by Baxter Ltd
Version 1.2, 15 January 2014
Version submitted to Medical Ethical Review Committee
Version 1.3, Amendment 1, 29 July 2014
Amendment 1 includes the following changes

1. Inclusion of an amendment section in the protocol
2. Inclusion of reference to ENCePP seal [ref 3]
3. Additions relating to processing questionnaire data from parents who measured their child's temperature using non-rectal thermometry

Version 1.4, Amendment 2, 21 July 2015 (Annex 1)
Amendment 2 includes the following changes

1. Changes related to changed ownership of study product NeisVac-C® from Baxter Ltd to Pfizer Inc
2. Changes related to extension of recruitment area of study subjects.
3. Changes related to update of recruitment material
4. Changes related to longer duration of study
5. Minor changes

Justifications for all amendments are described in Protocol version 1.4 (Annex 1).

9. Research methods

9.1. Study design

This is a non-interventional, open observational non-inferiority study with two cluster-assigned cohorts. The two groups differed with regard to the NeisVac-C® vaccine that has been given, old or new NeisVac-C®: the "old" lots produced in Beltsville, or "new" lots from Orth/Donau.
The assignment was done at the level of the baby clinics where the vaccines were to be administered (both to study subjects and non-study subjects). All children at each baby clinic were to receive the same vaccines regardless of their participation in the study. The vaccines given in this study were the vaccines that were used in the routine Netherlands Vaccination Program, and were not specifically labeled as 'study medication'. In addition, for this reason the baby clinics had no status as 'study sites', and their personnel were not regarded as 'investigators' or 'study teams'.

Lareb (and not RIVM as stated in the protocol) has generated a distribution list based on the numbers of children that received MenC vaccine at each child health center in the previous year. The distribution list was composed manually and aimed at an approximate 50-50% distribution for old and new NeisVac-C® vaccines within each region and within large towns, to ensure that local differences in confounding factors (e.g. concurrent infections, habits and attitudes towards vaccinations) were balanced out. Old or new NeisVac-C® vaccines were identified by their batch numbers.
After enrollment parents received 3 web-based questionnaires with questions about any ADRs that occurred after vaccination.
The 1st questionnaire was sent at day 5 and covered days 1, 2, 3 and 4.
The 2nd questionnaire was sent after two weeks and covered days 5 to 14.
The 3rd questionnaire was sent after four weeks and covered days 15 to 28.
The duration of the study for each participant is approximately one month from vaccination.

9.2. Setting

The study was embedded in the routine vaccination schedule, in which MenC vaccine (NeisVac-C®) and MMR vaccine (M-M-R-VaxPro®) are routinely given simultaneously to children at the age of 14 months (between 12 and 18 months). To organize the logistics of assigned distribution the study was performed in defined regions determined by a positive cooperation with the Child Health Care (CHC) organization in that region that was responsible for delivering child health care including routine childhood immunizations in their region. The study area included 9 regional CHC organizations in three provinces:
- Province Noord-Brabant: GGD HvB, Thebe, Zuidzorg, TZ West Brabant and Careyn, with 135 clinics (numbers of children per clinic range from 20 to 830 per year).
- Province Zeeland: GGD Zeeland, with 30 clinics (numbers of children per clinic range from 14 to 394 per year).
- Province Limburg: Orbis, Meander, Envida, with 24 clinics (numbers of children per clinic range from 41 to 441 per year).
Names of these organizations have changed during the study due to reorganizations and mergers. The designations of subjects according to their region is done using the name of the organizations at the start of the cooperation of the CHC organization.
Together these 9 organizations had 189 child health centers/clinics.

Shipments to these clinics were done by RIVM/DVP Zuid, a regional office of the National Institute of Public Health and the Environment. One of their responsibilities is vaccine delivery and accountability of the National Immunization Program. Deliveries to each baby clinic was done in the usual frequency of supply (every 2-3 months) according to the distribution list. In some regions, large clinics also stored vaccines for other (often smaller) clinics in the neighborhood. RIVM/DVP was free to choose if code 1 or 2 indicated old or new vaccine, without informing Lareb nor the baby clinic staff. Neither baby clinic staff nor the parents were informed whether

the clinic provided old or new NeisVac-C® vaccine.
There was no unblinding procedure.

The study started May 2014 in the province of Noord-Brabant, in the regions of 5 of the 6 CHC organizations who agreed to facilitate the study. It was chosen to vaccinate children with the vaccines that were available at the time of their visit to the baby clinic, and not to wait until the clinic had been supplied with the assigned vaccine to avoid a delayed start. Thus, in each clinic some children may have received the old vaccine where the clinic had been assigned to use new. It was expected that the imprecision caused by a running start would occur only during the starting phase, and would not affect the over-all 50-50% distribution for old or new vaccines.
The study was expected to last for a whole year, to cover all seasons, and multiple vaccine batches as supplied by Pfizer Inc.
Because of lower than expected recruitment rates, the study was extended in April 2015 to 2 other provinces: Province Zeeland with the child health care organization serving the entire province, and Province Limburg with 3 child health care organizations in the south of the province.
Enrollment was closed April 1st 2016, and last incoming 3rd questionnaires were received by May 6th 2016.

9.3. Subjects

Participants were recruited from regionally defined populations of healthy children who were eligible to receive regular vaccinations with MenC and MMR vaccines according to the Netherlands Immunization Program.
In the target region designated vaccination centers/baby clinics were supplied with NeisVac-C® vaccines with either “old” lots produced in Beltsville or “new” lots from Orth/Donau.

The time schedule for eligible participants is described in Table 2.

Table 2. Schedule per eligible participant

time	actor	Activity
- 4 wks	RIVM/DVP	sending letter to parents in region with predefined baby clinics. The letter is with headings of RIVM, and sent by RIVM.
-4 wks to-1 day	parents	pre-registration at Lareb LIM site, with contact details and planned date of vaccination
Day 1	baby clinic	- give MenC and MMR vaccinations - remind parents of the study described in the letter that parents received earlier. - record vaccinations on the study letter from the parents, and recording as usual for adequate registration by RIVM/DVP.
Day 4	parents	latest possibility to register for study, confirmation of date of vaccination
Day 5	Lareb	send questionnaire 1, reminder at day 7, valid time of return: day 8
Day 14	Lareb	send questionnaire 2, reminder at day 16, valid time of return: day 18
Day 28	Lareb	send questionnaire 3, reminder at day 32, valid time of return: day 36
Day 36	Lareb	closure

Numbering of days is defined in the protocol (Annex 1).

Approximately one month before the scheduled vaccination (usually at age 14 months) the parents/caretakers of children in the target region, received a letter and flyer with information about the study (see also Protocol, Annex 1) and the possibility to already sign up for the study, both online and through a paper form. First letters were sent 26 June 2014. Last letters were sent 29 February 2016. The total number of letters was 42,704, indicating the total recruitment population.
The letters were sent weekly by RIVM/DVP, and data for selection (age, postal codes linked to study region) were derived from the population register, which is also the basis for the National Immunization Program.

At the baby clinic, following NeisVac-C® and MMR vaccination, parents were asked by baby clinic staff to participate, and the information required for participation was written down on the study flyer (specification of injection sites and batch number per vaccine). Based on information in the letter and subsequent flyer parents could decide to participate and register at the study web-page. Parental consent was considered a valid equivalent of a signature if they voluntarily ticked a box stating that they have understood all information and confirmed their participation. Unique subjects were identified by the email address of the reporting parent (in case of twins a second email address would be required). Subjects were not registered on a named base. Only date of birth, sex, and address of CHC were asked.

Prior to vaccination, the child health physician or nurse reviewed the health status of the child to exclude any contra-indication for MenC and MMR vaccinations according to the guidelines of the National Immunization Program. These vaccinations were given to all eligible children, regardless of later (non)participation.
Health personnel at the baby clinic recorded the vaccinations (name clinic, name and batch number of vaccine, injection sites for both vaccines (left, right, arm, leg), date of vaccination) in the child health dossier, as well as on the vaccination cards, which were sent to the RIVM/DVP Zuid vaccination registry, as prescribed in the NIP.

After vaccination, parents were reminded to refer to the letter and flyer that they received previously, and that they had the option to sign-up for the study with the use of a reporting code. If they intended to participate, baby clinic staff would record the vaccines given that day, with their batch numbers, and injection sites. The flyer also contained a diary table, to be used by the parents as an aide-memoire, to record any health event that was to be completed in the electronic questionnaires. The flyers were kept by the parents and are not used as source data material.

Parents of children who had registered to participate received a web-based questionnaire at day 5 after the vaccines were administered with questions about any ADRs that occurred within 4 days of vaccination. A second questionnaire was sent after two weeks, and a third after 4 weeks.
Parents were sent reminders if the completed questionnaire was not received by Lareb. Questionnaires received after the valid time of return were not included in the Per Protocol analysis.

The following criteria for in- and exclusion were used:
Inclusion criteria
- children 14 months old (age range ≥12 to ≤18 months; boundaries included) who received the NeisVac-C® and MMR vaccination in one of the participating clinics according to the NIP

Exclusion criteria
- children receiving either MenC or MMR vaccine and not both at the same time
- children that received simultaneously any other vaccine than MenC and MMR
- children that were vaccinated at baby clinics that do not participate in the study
- children whose parents/caretakers registered for the study later than day 4 after vaccination.
- children with parents that were not able or willing to understand/complete the questionnaires in Dutch.

The participant could withdraw from the study at any moment without stating a reason. If a questionnaire was not completed after a reminder had been sent, the patient was considered lost to follow up and did not receive any further questionnaires. Reasons for drop-out were not recorded.

9.4. Variables

Primary study parameter: proportions of children with fever (MedDRA LLT codes based on rectally measured body temperature of ≥38.0°C within 4 days after vaccination with NeisVac-C® and MMR. Temperature values obtained by axillary or ear measurements were converted to rectal values (see Annexes 1 and 3).
Secondary study parameter(s): proportions of children with solicited other systemic and local reactions within 4 days after vaccination with NeisVac-C® and MMR.

All data that was asked to be completed in the questionnaires (outcomes, exposures, predictors, potential confounders, and effect modifiers) are further described in the Protocol sections 5.2 and 6.3 (Annex 1).
Details of solicited events were requested in preformatted questionnaire modules. Parents could make additional remarks in open text fields. If these open text field included symptoms or complaints that were compatible with solicited events, these were added manually. The remainder was regarded as 'other'.

9.5. Data sources and measurement

Database
At Lareb, the LIM Platform (Lareb Intensive Monitoring) is an application used for several LIM studies (Microsoft SQL server)[ref 4], and now applied for the first time for a vaccine study. Each study subject has a unique identifier coding for a study as well as personal identifiers. For the present study additional modules were designed, programmed and coupled to the LIM platform by an external party contracted by Lareb. All study data are kept on the central Lareb server. Backups are made daily.

Incoming data
For the reporting parent, limited instructions to complete data are given on the study flyer. Also explanations/help buttons are available on the entry module web page. Via a web-based entry module all information obtained during the study was directly recorded in the dedicated part of the LIM Platform. Data for the present study had a specific study code.
There were no other sources of information, and there were no paper documents that were regarded as source materials.
The email address of the reporting parent serves as the unique subject identifier. For a twin child, another email address was required.
Incoming data are checked for completeness through a forced-entry facility at the web-entry module. This module uses validation rules at the level of data entry, checking for completeness and logic (for instance: date of birth must precede date of vaccination), and precoded choices (e.g. list of Child Health Center; see under additional tables).
Reporter language is Dutch.

Additional tables
Three tables were added to the data set.
The first table described the Child Health Clinic (street address, village or town, and organization). This table was linked to the entry module. So the reporting parent could easily choose the CHC where the child had received the vaccinations.
The second table listed the batch numbers of NeisVac-C® that were in use at the CHC, and their origin (old or new production site). This table was not accessible for reporting parents nor health care workers at the CHC.
Batch numbers were manually entered by the reporting parent and compared to the batch numbers in this table. In case of non-matching batch numbers Lareb personnel tried to determine the correct batch numbers manually. In the majority of cases, there were easily solvable errors in typing, such as used small print instead of capitals, letter O instead of zero, letter L instead of 1, spaces and hyphens. In few instances, the reported batch numbers had to be compared with the batch numbers that were known to be used by the reported CHC (such data were obtained from RIVM).

The third table included the batch numbers that were accepted before and after correction, and was used for analysis.

Processing of incoming data
On a daily basis, incoming data were monitored for quality and consistency by dedicated and instructed Lareb personnel. Only administrative data in the 1st questionnaire were corrected if needed which is explained in a separate comment field (e.g. in case parents initially state that their son was a female, but later ask Lareb to correct this). Checks of batch numbers are described above. Subsequent questionnaire data are never changed, but coded/corrected in separate field and explained in a comment field. Both solicited events and complaints recorded in free text field were manually coded. Coding into MedDRA terminology was performed using an internal list to use appropriate and consistent coding where possible. Reported temperature values were manually translated into MedDRA coding as described in the Statistical Analysis Plan (Annex 3).

Study related data tables
Using a study code data tables in csv-format were extracted from the LIM database. A scheme of relations between these data tables is shown in Annex 2. These tables form the basis for the data and their analysis described in this report.

9.6. Bias
See Statistical Analysis Plan (Annex 3).

9.7. Study size
The study was done to investigate if recipients of the new NeisVac-C® vaccine would not have a higher incidence of fever within 4 days after vaccination than recipients of the old NeisVac-C® vaccine. The statistical formulation of the study hypothesis to address the primary endpoint (related to the number of children with fever reactions (defined as rectally measured temperatures 38.0º Celsius or higher) within 4 days after vaccination with NeisVac-C®, and the resulting sample size calculations are described in protocol sections 9.1 and 9.2 (Annex 1). The primary endpoint is based on the pooled daily incidence of fever from days 0 to 4 after vaccination, which was expected to occur in about 6% of the children (95% CI 4.44-7.71, when 52/863 cases). Expected frequency was based on a study performed in The Netherlands earlier [ref 5].
For a non-inferiority approach the following assumptions and choices were made:
- null hypothesis: group A receiving the new product, has a higher prevalence of fever than children in group B. The risk ratio between group A and B is 1.5 or more.
- alternative hypothesis (one-sided): the risk ratio between group B and group A is less than 1.5.
- study groups equally sized (approximately)
- type 1 error alpha: rejection null hypothesis when it is actually true, is set at 0.025, one-sided
- type 2 error beta: rejection null hypothesis, is set at 0.80
- clinically acceptable or meaningful difference between endpoints in group A and group B: risk ratio of 1.5 indicating a higher risk of fever in group A (corresponding with a fever prevalence of 9% in group A).
- sample size is calculated to obtain numbers needed for the Per Protocol analysis set.

We needed to study 1215 children in each group to be able to reject the null hypothesis that the fever rates between in group A are higher than in group B, with probability (power) of at least 0.8. The type I error probability associated with this test of this null hypothesis is set at 0.05 (two-sided, 0.025% one-sided). If there is truly no difference in fever prevalence between the two groups, then 2430 children are required to be 80% sure that the upper limit of a one-sided 97.5% confidence interval (or equivalently a 95% two-sided confidence interval) of the risk ratio will exclude a difference in favor of the reference group of more than 3%.
In order to evaluate 1215 children per group we needed to recruit higher numbers of children. With 1215 per group we needed 2430 fully evaluable and complete datasets. We assumed that 90% of data from an estimated 2700 respondents that had completed all questionnaires were evaluable. We assumed that after starting the questionnaires 75% of responders would also complete the last questionnaire, thus giving an estimation of 3600 responding parents that start and agree to participation. Experience of RIVM indicated that about 40% of parents participated to a similar questionnaire proposal. However, to be sure to include the desired number of respondents we assumed a lower percentage: 15-20%. Thus we assumed that 18,000 to 24,000 parents needed to be addressed for taking part in the investigation. In addition, to compensate for other interferences or loss, we intended to address 25,000 parents.
At a process evaluation it was found that instead of an expected participation rate of 15-20% only 6% of children actually enrolled in the study despite multiple efforts to stimulate taking part. In January 2015 it was therefore decided to extend the study both in time and regions, in order to obtain the required numbers of subjects. Geographically, the basis of recruitment of 25,000 children annually was increased with 7300 from additional regions. The extension was effected from April 2015. The study timeline was limited by the expiration date of the last product manufactured at Beltsville, ie MAR2017.
In the first phase of the study we monitored total enrollment rates without distinguishing the enrolled children as 'old' or 'new' NeisVac-C® recipients.
In June 2015 we performed a progress analysis showing that during days 0-4 the average pooled incidence of fever was higher than expected, around 8% instead of the anticipated 6%. At that time, the percentages of fever in the 'old' and 'new' NeisVac-C® group were deliberately not studied.
From fall 2015 we further looked into this distribution, and noticed that the sizes of the two groups were not approximately equal as intended, but about 63-37% distributed. We decided to continue enrollment.
In February 2016 we performed new power calculations (Annex 4) to see if we would have recruited sufficient numbers of subjects to address the primary endpoint, taking into account the higher than anticipated rate of fever (8.0%) and the unequal group sizes (1.7:1). With these new assumptions, 841 cases and 1430 controls would be required to reject a difference. On the basis of this

revised assessment, we concluded that it would be justified to terminate enrollment. We submitted this proposal to Pfizer Inc, who agreed with our intention to stop enrollment. At that time 42,704 invitation letters had been sent.

9.8. Data transformation

See Statistical Analysis Plan (Annex 3).

9.9. Statistical methods

See Statistical Analysis Plan (Annex 3).

9.10. Quality control

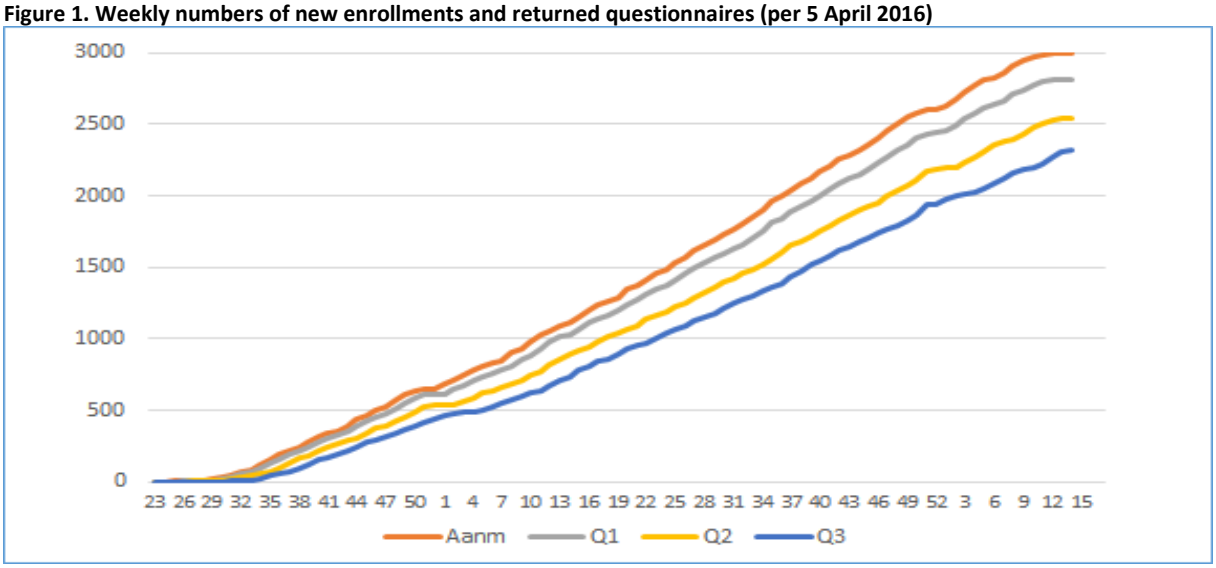
All incoming data were manually checked case by case for possible errors and inconsistencies, e.g. when potentially unrealistic dates or values were reported. At the level of the entry module there were no logic tests to calculate if the date of birth could be realistic. When inconsistencies or otherwise doubtful entries were encountered, the reporting parents were asked by email for possible corrections. In case of changes, these were recorded in a separate comment field. There was a low threshold for consulting colleagues in case of doubt for MedDRA coding, or other critical aspects of data processing.
See also Statistical Analysis Plan (Annex 3).

10. Results

10.1. Participants

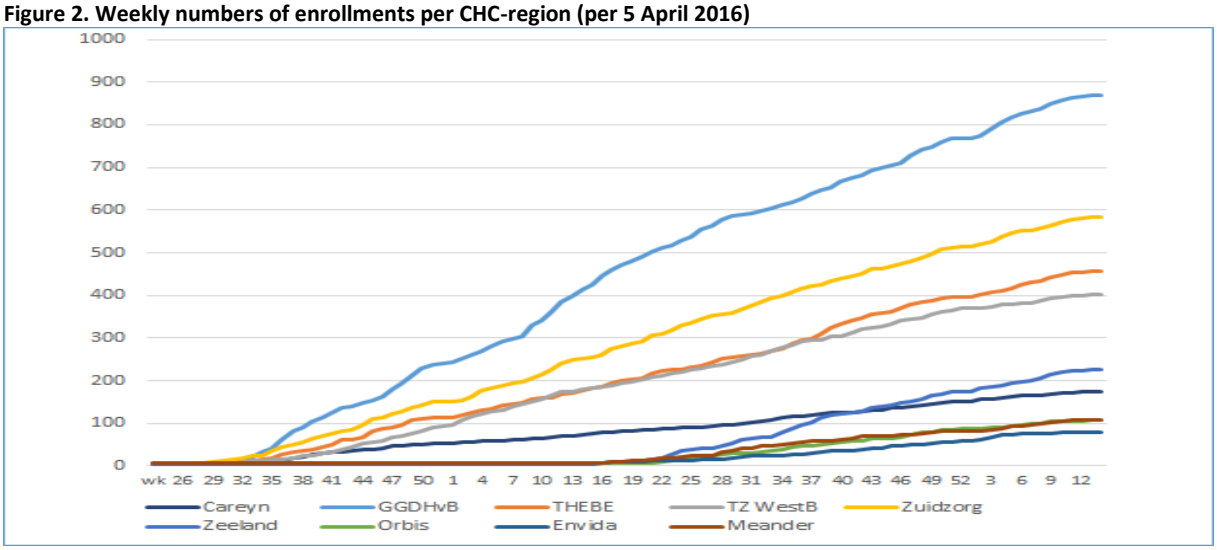
First invitation letters were sent April 2014 to children living in the study regions (5) in Province Noord-Brabant, from 20 March 2015 also to children in study regions in Province Limburg (3) and Province Zeeland (1). Last letters were sent 29 February 2016. Altogether, 42,704 letters were sent.
The first enrollment in Province Noord-Brabant was in week 24, 2014, in week 16, 2015 in Province Zeeland, and week 17, 2015 in Province Limburg. The numbers of weeks in which children enrolled was 88 in Province Noord-Brabant, and 51 in Provinces Limburg and Zeeland.
Enrollment started in May 2014 with low numbers (summer holidays, new study) and gradually rose to 20-50 per week (shown in Figure 1). From March 2015, after extension to the new study regions the numbers of enrollment increased slightly to 35-55 per week. The enrollment pattern was steady.
With a start of enrollment in May 2014, and end of enrollment by March 2016, quarters 3 and 4 contributed in 2014 and 2015, quarters 1 in 2015 and 2016, and quarter 2 only in 2015.

Figure 1. Weekly numbers of new enrollments and returned questionnaires (per 5 April 2016)



Explanation figure 1: x-axis: weeks in 2014, 2015 and 2016; y-axis: cumulative numbers of subjects; abbreviations: Aanm: new enrollments; Q1: received questionnaire 1; Q2: received questionnaire 2; Q3: received questionnaire 3.

Figure 2. Weekly numbers of enrollments per CHC-region (per 5 April 2016)

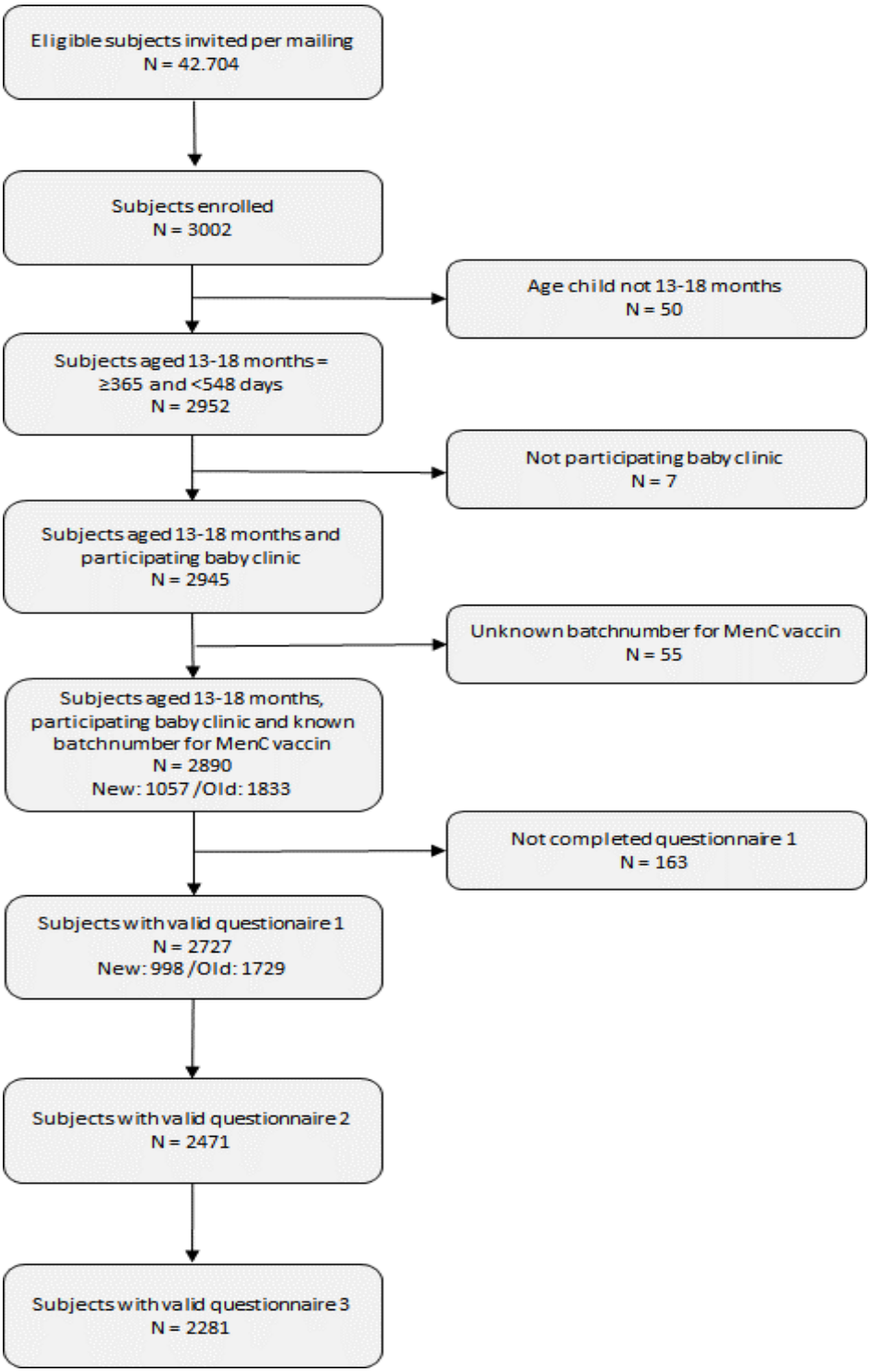


Explanation figure 2: x-axis: weeks in 2014, 2015 and 2016; y-axis: cumulative numbers of subjects per CHC-region.

Over the full study period on average 3002 (7.1%) of eligible children enrolled, with variations in time and CHC region (shown in Figure 2).

Of these 3002 subjects, 2727 had valid data for evaluation of the primary endpoint, fulfilling in- and exclusion criteria (specified in Protocol sections 4.2 and 4.3; Annex 1), and other criteria for data quality. Figure 3 shows the flow chart of subject disposition.

Figure 3. Flow chart of subject disposition.



The subject disposition included the steps described below:

- children between 13 and 18 months of age: from few children (n=50) out of 3002, implausible birth dates were received (e.g. more plausible as a birth date from a parent, or a birth year one year earlier than expected). These birth dates led to exclusion for the PP analysis. Ages were calculated to days. Children with ages between 365 and 548 days were included (n=2952).
- children should have their MenC and MMR vaccinations at the same time. This was a forced entry in the web-based questionnaire. The data do thus not contain children that have different vaccination dates for MenC and MMR vaccines.
- children that have received any vaccine other than MenC and MMR vaccine at the completion date should be excluded. If parents ticked YES for the question if more vaccines were given at that date, further enrollment was no longer possible. In addition, the baby clinic where the children were vaccinated never administer other vaccines for children at this age.
- children should have received their vaccinations at baby clinics that were pre-listed on the web-based form. In a few cases, a non-listed clinic was entered by parents. In 7 cases the parent reported vaccinations at an unknown clinic address which led to exclusion, leaving 2945 participants.
- children whose parents tried to register for the study later than day 4 after vaccination were automatically excluded as the web-based questionnaire rejected the enlistment.

- in 55 children an unknown batch number of MenC vaccine was reported which appeared unverifiable. This lead to exclusion, leaving 2890 subjects.
- children with parents that were not able/willing to understand/complete the questionnaires. There were some implausible answers that might have been related to not understanding the question, e.g. completing the parents birth date instead of the child's birth date; completing the year of birth a year earlier than the correct year; when asked for medical history, completing data that applied to the recent vaccination; completing adverse events in a free text field that had been earlier asked with a structured questionnaire for solicited events. In these instances, it was assessed case by case whether all data from such subject should be kept. We discarded 163 subjects for missing or inconsistent 1st questionnaires, leaving 2727 subjects for analysis of data from the 1st questionnaire.
- 2471 of the 2727 gave valid data for the 2nd questionnaire, and 2281 of the 2727 gave valid data for the 3rd questionnaire.

To address the endpoints some data from questionnaires 2 and 3 are pooled for analysis and description. The base number of subjects for pooled analysis from questionnaires 2 and 3 together is 2471.

Enrollment rates per CHC-region were calculated by the numbers of enrolled children in the numerator, and the numbers of doses of MenC vaccine used in each region in the year before (2013 for Province Noord-Brabant, and 2014 for Provinces Limburg and Zeeland as a fixed reference denominator. Table 3 shows that the highest percentual participation rates of these 2727 subjects (corrected for the different regional duration of recruitment) was in the regions of TZ WB (8.8%), Thebe (8.5%) and Orbis (8.6%), and lowest in the region of GGDHvB (4.3%). However, the latter region was by far the largest with 10,813 resident children (35% of total annual recruitment population), and in absolute numbers most of the children came from this region (792 of 2727).

Table 3. Contributions per CHC-region, participation rates

Organization Name	# clinics	#tot	% of tot	#old	#new	elig pop/yr	recr weeks	%recr
Careyn	5	153	5.6	76	77	1610	88	5.6
GGD HvB	66	792	29.0	500	292	10813	88	4.3
THEBE	23	405	14.9	269	136	2811	88	8.5
Thuiszorg WB	16	382	14.0	200	182	2570	88	8.8
Zuidzorg	25	553	20.3	351	202	5273	88	6.2
Envida	9	60	2.2	47	13	1399	51	4.4
Meander	9	91	3.3	55	36	1906	51	4.9
Orbis	6	90	3.3	68	22	1061	51	8.6
GGD Zeeland	30	201	7.4	163	38	2986	51	6.9
Total	189	2727	100	1729	998	30429		6.5

Explanation table 3: # clinics: number of baby clinics; # tot: total number of children enrolled per CHC-organization; % of tot: percentage of children per CHC-region; #old: number of children that received old NeisVac-C®; #new: number of children that received new NeisVac-C®; elig pop/yr: denominator data with numbers of children that received MenC vaccine in the previous year (as a reference number); recr weeks: total weeks of active recruitment per organization; % recr: over-all recruitment percentage per organization.

Assignment and distribution of old and new NeisVac-C®

At the start of inclusions all clinics used old MenC vaccines. Only after the start of enrollment the clinics were supplied with the old or new MenC vaccines according to the assignment list. Old MenC vaccine was assigned to 95 (50.3%) baby clinics, and new vaccine to 94 (49.7%) baby clinics, each serving wide ranges of numbers of children.

During the study period 14 old and 8 new batches were distributed in the study region (as listed in Annex 3). However, 4 batches of old NeisVac-C® and 3 batches of new NeisVac-C® were not used in study participants.

At baby clinics that were assigned to administer old MenC vaccine 1425 doses were given, of which 1412 (99.1%) children indeed received old MenC vaccine, and 13 received new MenC vaccine. At baby clinics that were assigned to administer new MenC vaccine, 1302 doses were given, of which 985 (75.7%) were new vaccine and 317 (24.3%) were old vaccine.

Table 4 shows which old and new batches of NeisVac-C® were used in each organization. Of the new NeisVac-C® 5 batches were used, of which NeisVac-C® batch VNS1N09A was given to 500 of 998 children, and thus contributed to half of the data on the new NeisVac-C®. Of the old NeisVac-C® 10 batches were used, of which 557 of 1729 children received NeisVac-C® batch VNS1N06C as the largest contributor.
Batch numbers of MMR vaccines were not verified, and not further analysed and reported.

Table 4. Distribution per CHC-region of batch numbers of NeisVac-C®

	Organizations									
NeisVac-C® batches	**Careyn**	**Envida**	**GGD HvB**	**GGD Zeeland**	**Meander**	**Orbis**	**THEBE**	**Thuiszorg WB**	**Zuidzorg**	**Total per batch**
New NeisVac-C®										
VNS1M10A	11		69				21	26	29	156
VNS1M10B	24		104				44	60	46	278
VNS1N09A	36	9	112	33	25	15	65	91	114	500
VNS1P11B						6				6
VNS1Q04A	6	4	7	5	11	1	6	5	13	58
New Total	77	13	292	38	36	22	136	182	202	998
Old NeisVac-C®										
VNS1L07D			1				1			2
VNS1M07B			3				3			6
VNS1M09C			11	1			8	2	2	24
VNS1M15A	11		75				18	18	26	148
VNS1N01B				2					5	7
VNS1N05A	12	12	89	15	12	3	65	48	108	364
VNS1N06C	22	15	164	55	15	23	86	67	110	557
VNS1N06F	15	11	75	41	8	13	59	16	50	288
VNS1N07E	13	8	67	35	16	19	28	46	44	276
VNS1N08A	3	1	15	14	4	10	1	3	6	57
Old Total	76	47	500	163	55	68	269	200	351	1729
New and Old Total	**153**	**60**	**792**	**201**	**91**	**90**	**405**	**382**	**553**	**2727**

Summarizing, altogether 2727 children of 3002 enrolled that fulfilled criteria for in- and exclusion were available for Per Protocol analysis. These children had been vaccinated at 189 baby clinics, that had been supplied with old or new NeisVac-C® vaccines. Old NeisVac-C® vaccine was given to 1729 (63,4%) and 998 (36,6%) received new NeisVac-C® vaccines, as identified by their batch numbers.

10.2. Descriptive data

The demographic characteristics of the two study groups are given in Table 5. Both study groups are similar with regard to age, weight and height, household size, daycare attendance, medical history.

Table 5. Demographic characteristics of the per-protocol population and comparability of groups 'old' vs 'new'

	Old vaccine (N=1729)	New vaccine (N=998)	p-value
Sex: male (n/%)	877 (50.7)	524 (52.5)	0.39#
Missing (n/%)	0	0	
Age (months)			
Mean (SD)	14.44 (0.64)	14.40 (0.57)	0.62$
Median (range)	14.23 (12.10 – 17.57)	14.26 (12.36 – 17.11)	
Missing (n/%)	0	0	
Length (cm)			
Mean (SD)	78.4 (3.0)	78.4 (2.9)	0.94$
Median (range)	78.0 (67.5 – 89.0)	78.5 (67.0 – 88.0)	
Missing (n/%)	20 (1.2)	5 (0.5)	
Weight (kg)			
Mean (SD)	10.2 (1.1)	10.2 (1.1)	0.47$
Median (range)	10.0 (6.4 – 14.3)	10.0 (6.1 – 13.6)	
Missing (n/%)	18 (1.0)	4 (0.4)	
Household size (n/%)			0.055*
1 child	921 (53.3)	597 (59.8)	
2 children	617 (35.7)	315 (31.6)	
3 children	133 (7.7)	64 (6.4)	
4 children	28 (1.6)	12 (1.2)	
5 children	7 (0.4)	3 (0.3)	
6 children	1 (0.1)	1 (0.1)	
7 children	0 (0)	0 (0)	
8 children	1 (0.1)	0 (0)	
Missing (n/%)	21 (1.2)	6 (0.6)	
Daycare: yes (n/%)	1041 (60.2)	603 (60.4)	0.94#
Missing (n/%)	21 (1.2)	5 (0.5)	
Medical history: yes (n/%)	502 (29.0)	295 (29.6)	0.81#
Missing (n/%)	0	0	
Season (n/%)			< 0.001#
Quarter 1	497(28.8)	253(25.4)	
Quarter 2	261(15.1)	175(17.5)	
Quarter 3	405(23.4)	342(34.3)	
Quarter 4	566(32.7)	228(22.8)	

* p-value for differences among all groups (Fisher's exact test)
# p-value for differences among all groups (Chi square test)
$ p-value for difference in mean values (Mann-Whitney U test)

Body weight and length
Among the reported values for body weight and length, we found 15 outliers values in 10 children (e.g. with apparently reported figures for weight and length around birth instead of actual figures) (Annex 5.2). These were further handled as missing values.

Household size
Household size may be an important determinant for children to acquire infections which cause fever: the more children the more infections. Children that received old vaccine tended to come from families with 2-3 children, while children that received new vaccine tended to come from families with 1-2 children. Overall however, the household sizes for both groups are not significantly different (p=0.055).

Season
As infectious agents circulate more in fall, winter and early spring, they may constitute another cause of fever. The seasons in which old or new vaccines were administered, proved different. Old vaccines were given more during quarters 1 and 4, while new vaccines were given more in quarters 2 and 3. The differences probably have logistic reasons, but are not fully understood.

10.3. Outcome data

The main outcome results for the primary and secondary endpoints are described in tables 6 and 7. Table 6 shows the incidence of the primary and secondary endpoints for both groups of subjects, based on LLT MedDRA codings.
As to the primary endpoint of the study, we found no difference in the percentages of children with fever during the first 4 days after old (8.3%) or new MenC vaccine (8.2%). Nor did we find differences between injection site reactions and systemic reactions other than fever.
Table 7 shows that percentages of children with injection site reactions and pooled systemic reactions (including fever) occurring during the 5-28 days after vaccinations (secondary endpoint) were also similar.

Table 6. Relative risk of fever, injection site reactions and systemic reactions for the new vaccine within 4 days after vaccination with MenC / MMR

	Incidence (n/%)		Relative risk (95% CI), unadjusted	Relative risk (95% CI), adjusted*
	Old vaccine (N=1729)	New Vaccine (N=998)		
Fever	144 (8.3)	82 (8.2)	0.99 (0.76 – 1.28)	0.96 (0.74 – 1.24)
Injection site reactions$	58 (3.4)	33 (3.3)	0.99 (0.64 – 1.49)	0.90 (0.58 – 1.37)
Systemic reactions#	556 (32.2)	351 (35.2)	1.09 (0.98 – 1.22)	1.07 (0.96 – 1.20)

$ Injection site reactions at the MenC injection site
# Fever is not included in systemic reactions
* Adjusted for length, weight, household size, daycare, medical history

Table 7. Relative risk of fever, injection site reactions and systemic reactions for the new vaccine within 5 - 28 days after vaccination with MenC / MMR

	Incidence (n/%)		Relative risk (95% CI), unadjusted	Relative risk (95% CI), adjusted*
	Old vaccine (N=1575)	New Vaccine (N=896)		
Injection site reactions$	5 (0.3)	2 (0.2)	N.A. **	N.A. **
Systemic reactions#	750 (47.6)	431 (48.1)	1.01 (0.93 – 1.10)	1.00 (0.92 – 1.09)

$ Injection site reactions at the MenC injection site
# Fever is included in systemic reactions
* Adjusted for length, weight, household size, daycare, medical history
** No reliable estimate could be calculated due to the low number of events

10.4. Main results

For the primary and secondary endpoints, we also determined relative risks. The assessment of relative risk was done both crude (unadjusted) and adjusted for potential confounders using log-binomial regression (see Annexes 1 and 3). For fever based on measured temperatures 38.0°C or higher, injection site reactions and systemic reactions other than fever during days 0 to 4 after vaccination we found no higher risk (both crude and adjusted) for the new NeisVac-C® vaccine to cause such reactions (Table 6). Upper limits of the 95% confidence intervals of the relative risks for fever between 0-4 days were 1.28 and 1.24 for the unadjusted and adjusted tests respectively, which is below the non-inferiority margin of 1.50 (See also Annexes 1 and 3). Similarly, we did not find such increased risk for the period 5 to 28 days after vaccination (Table 7). We concluded that the new NeisVac-C®vaccine is non-inferior to the old NeisVac-C® vaccine.

10.5. Other analyses

Table 8 shows that solicited and separately grouped systemic reactions have similar pooled incidences for days 0-4 and 5-28 for subjects vaccinated with either old or new MenC vaccine. Systemic reactions together were treated as a composite endpoint for regression analysis, and no differences were found.

Table 8. Incidence of systemic reactions per study group within 4 days and 5 – 28 days after vaccination with MenC / MMR

	Incidence within 4 days (n/%)		Incidence within 5 - 28 days (n/%)	
	Old vaccine (N=1729)	New Vaccine (N=998)	Old vaccine (N=1575)	New Vaccine (N=896)
Fever	144 (8.3)	82 (8.2)	385 (24.4)	211 (23.6)
Rash	74 (4.3)	56 (5.6)	249 (15.8)	166 (18.5)
Decreased appetite	184 (10.6)	123 (12.3)	264 (16.8)	150 (16.7)
Less sleeping	194 (11.2)	110 (11.0)	173 (11.0)	94 (10.5)
Somnolence	411 (23.8)	252 (25.3)	418 (26.5)	244 (27.2)
Vomiting	48 (2.8)	39 (3.9)	65 (4.1)	39 (4.4)
Diarrhoea	108 (6.3)	68 (6.8)	134 (8.5)	82 (9.2)
Seizures	1 (0.1)	3 (0.3)	3 (0.2)	3 (0.3)

Unspecified reports of fever (in Dutch reported as 'koorts' (without temperature measurement) or 'verhoging' (with measured temperatures >37.5°C and < 38.0°C); and MedDRA coded as 'fever' and 'body temperature increased') were not accepted for Per Protocol analysis, and are not presented in the tables. From days 0 to 4, this was reported in 22 subjects in the new NeisVac-C® group, and 49 in the old NeisVac-C® group, and for days 5-28 reported numbers were 25 for the new, and 63 for the old NeisVac-C® group.

Fever is not evaluated as a numeric continuous variable but via coding. Table 9 shows that high grade fever occurred in a few subjects, and in similar percentages of children in the old and new NeisVac-C® groups. The percentages of children with hyperpyrexia seem to differ, but the significance is uncertain because of the very small numbers.

Table 9. Incidences of pyrexia, hyperpyrexia and hyperthermia per study group within 4 days and 5 – 28 days after vaccination with MenC / MMR

	Incidence within 4 days (n/%)		Incidence within 5 – 28 days (n/%)	
	Old vaccine (N=1729)	New vaccine (N=998)	Old vaccine (N=1575)	New Vaccine (N=896)
Pyrexia	142 (98.6)	78 (95.1)	370[#] (95.9)	203 (96.2)
Hyperpyrexia	2 (1.4)	4 (4.9)	16[#] (4.2)	8 (3.8)
Hyperthermia[*]	0	0	0	0

Pyrexia, hyperpyrexia and hyperthermia are defined in the Statistical Analysis Plan (Annex 3).

[*] Including malignant hyperthermia

[#] Combined numbers of pyrexia and hyperpyrexia exceed the total number of events as specified in table 4 due to 1 case of combined reporting of both events

Influence of season

Table 10 shows that the percentages of febrile reactions during days 0-4 are all below 10%, but have some seasonal variation (lower in quarters 2 and 3, and higher in quarters 1 and 4). The overall percentage of fever in the period 5-28 days is much higher, from approximately 20 to 30%. The percentage during the second period may be higher not only because fever caused by MMR vaccine is expected to occur in the first part of that period, but also because the base line rate of fever is collected during a longer period.

Table 10. Incidence of fever per season per study group within 4 days and 5 – 28 days after vaccination with MenC / MMR

	Incidence within 4 days (n/%)		Incidence within 5 – 28 days (n/%)	
	Old vaccine (N=1729)	New vaccine (N=998)	Old vaccine (N=1575)	New Vaccine (N=896)
Quarter 1	44 (8.9)	23 (9.1)	127 (27.9)	48 (20.8)
Quarter 2	18 (6.9)	13 (7.4)	56 (23.7)	46 (29.1)
Quarter 3	26 (6.4)	23 (6.7)	88 (23.5)	60 (19.9)
Quarter 4	56 (9.9)	23 (10.1)	114 (22.4)	57 (27.8)

The percentages are calculated using the numbers of subjects with fever per quarter, while the denominator is the number of children that received old or new vaccine during that quarter.

Table 11 shows numbers of subjects with fever per individual batch of NeisVac-C® (grouped per old or new NeisVac-C®). Incidences are calculated using the denominator figures given in table 4. The incidence rates are similar between batches. Most of the higher rates are found with small numbers of observations.

Table 11. Incidences of fever per batch per study group within 4 days and 5 – 28 days after vaccination with MenC / MMR

		Incidence within 4 days (n/%)	Incidence within 5 – 28 days (n/%)
New vaccine	VNS1M10A	14 (9.0)	37 (26.4)
	VNS1M10B	26 (9.4)	59 (24.2)
	VNS1N09A	36 (7.2)	101 (22.4)
	VNS1P11B	1 (16.7)	3 (60.0)
	VNS1Q04A	5 (8.6)	11 (19.3)
Old vaccine	VNS1L07D	1 (50)	1 (50.0)
	VNS1M07B	2 (33.3)	2 (33.3)
	VNS1M09C	0 (0)	7 (35.0)
	VNS1M15A	7 (4.7)	36 (26.3)
	VNS1N01B	1 (14.3)	1 (16.7)
	VNS1N05A	37 (10.2)	86 (26.5)
	VNS1N06C	44 (7.9)	105 (20.9)
	VNS1N06F	22 (7.6)	74 (27.7)
	VNS1N07E	27 (9.8)	55 (21.6)
	VNS1N08A	3 (5.3)	18 (33.3)

10.6. Adverse events and adverse reactions

The study is exclusively aimed at investigating adverse events and the results are described above. An overview of any PT codes of complaints that have been reported in Questionnaires 1, 2 and 3 together (covering days 0 to 28) are listed per Old or New NeisVac-C® group in Annex 5.2. This list included also rarely occurring PT's.

In only 20 subjects no adverse event was coded (6 in the 'new' group, and 14 in the 'old' group). This indicates the low threshold of reporting any complaints in the study. We had no reports of acute allergic reactions, nor anaphylaxis.

In 11 subjects febrile convulsions occurred (5 in the 'new' group and 6 in the old group), and 2 subjects with convulsion (fever not coded)(one in each group). Three of the subjects with seizures had invalid reported latency times and are therefore not included in Table 8 that describes the secondary endpoint. These three were all from recipients of old NeisVac-C®. Their data were rejected because the convulsions were reported in the 2nd questionnaire covering days 5 to 14, but were reported to have occurred at 9 hours, 3 and 4 days, and should have been reported already in the 1st questionnaire. It appeared that the child (see Table 12, LIMPAT no 9461) who had the febrile convulsion at 9 hours actually had this febrile convulsion 9 hours after the start of the fever 8 days after vaccination.
If we would have included these three cases, the numbers for seizures in Table 8 would read: incidence within 4 days (n/%) old vaccine 3 (0.2), new vaccine 3 (0.3), incidence within 5-28 days old vaccine 4 (0,3), new vaccine 3 (0.3). Also with these three subjects included the incidences are low and similar for old and new vaccine recipients.

Criteria for Serious Adverse Events (SAE) that would occur during the study were defined by the protocol section 10.1 (Annex 1). Lareb does not use a list of events that automatically should be regarded as serious.
In 11 subjects the parents reported in Questionnaires 1 or 2 events that could be qualified as an SAE: 10 because of hospitalization and 1 because of involvement of ambulance personnel. Table 12 describes the main characteristics of these cases. Case summaries are described in Annex 5.3. Six cases were vaccinated with the old NeisVac-C® (Beltsville), and 5 were vaccinated with new NeisVac-C® (Orth). In most cases hospitalization was considered related to concomitant infection (e.g. viral gastro-enteritis) which is common at toddler age. None of the SAE cases were considered as a safety signal, as they were in the expected range of events usually reported after simultaneous MenC and MMR vaccinations [ref 6].

Table 12. Characteristics of subjects with Serious Adverse Events

LIMPAT no	Lareb no	DOB	sex	V date	NeisVac-C® batch	SAE reason	lat main LLT	coded LLT	overall caus MenC	overall caus MMR	other cause
4972	187065	11-09-13	M	04-12-14	VNS1M10B ORTH	OTH	10d	sleepiness, rash face, appetite decreased nos, febrile seizure, listlessness	unlikely	probable	
4936	188116	02-10-13	F	28-11-14	VNS1M10B ORTH	HO	3w	body temperature decreased, diarrhea, sleep problem, apathy	unlikely	possible	
5224	189072	11-10-13	M	23-01-15	VNS1M10B ORTH	HO	2d	epileptiform fits nos, drowsiness, vomiting, diarrhoea, decreased appetite, pyrexia, listlessness	possible	unlikely	
5404	193289	25-12-13	M	24-02-15	VNS1N09A ORTH	HO	16h	diarrhea, vomiting, appetite decreased nos, viral gastroenteritis, malaise, pyrexia	unlikely	unlikely	rota virus infection
5612	194868	27-01-14	M	25-03-15	VNS1N05A BELTSVILLE	HO	2d	somnolence, vomiting, appetite decreased nos, pyrexia,	unlikely	unlikely	viral gastro-enteritis
6137	200180	12-04-14	F	10-06-15	VNS1N05A BELTSVILLE	HO	2d	somnolence, diarrhea, pyrexia, malaise	unlikely	unlikely	viral gastro-enteritis
6748	203961	04-06-14	F	31-08-15	VNS1N09A ORTH	HO	32h	somnolence, diarrhea, appetite decreased nos, febrile convulsion, malaise, hyperpyrexia	possible	unlikely	infection (5th disease) became apparent the days after
6808	204402	01-07-14	F	09-09-15	VNS1N06C BELTSVILLE	HO	1d	somnolence, dyspnea, cough, appetite decreased nos, pyrexia, malaise, sleep disturbed	unlikely	unlikely	respiratory tract infection
8691	207976	10-09-14	M	10-11-15	VNS1N06C BELTSVILLE	HO	2d	vomiting, pyrexia, respiratory infection	unlikely	unlikely	infection?
9189	213397	26-10-14	M	12-01-16	VNS1N06F BELTSVILLE	HO	7d	malaise, pyrexia, vomiting, sleep decreased	unlikely	possible	infection?
9461	214858	01-11-14	F	22-02-16	VNS1N08A BELTSVILLE	HO	8d	febrile convulsion, malaise, hyperpyrexia	unlikely	possible	infection?

Explanation: Lareb no: number under which subjects data are stored in the regular pharmacovigilance database for spontaneous reporting known as Lareb2010; LIMPAT no: study identification number (is in database preceded by 000 000 0000; DOB: date of birth, V date: vaccination date; dates have format dd-mm-yy; NeisVac-C® batch: batch number of NeisVac-C®, and its production site (Orth for new vaccine; Beltsville for old vaccine); SAE reason: the criterion for which the event is/are regarded as a Serious Adverse event (HO indicated hospitalization, OTH indicates otherwise serious); lat main LLT: the latency time of the LLT that was the most important to consider the event as serious (time units: h=hours, d=days, w=weeks); coded LLT: all symptoms of complaints that were applicable were coded as a MedDRA Lower Level Term; overall caus MenC/MMR: the probability for either vaccine to have caused the SAE.

11. Discussion

11.1. Key results

The study was done to assess non-inferiority of new NeisVac-C® compared to old NeisVac-C® with regard to fever (rectally measured 38°C or above) occurring from 0 to 4 days after vaccination as a primary endpoint. The study concluded that the new NeisVac-C® has a similar profile of reactogenicity as the old NeisVac-C®. We found similar rates of fever (8.2% for new NeisVac-C® and 8.3% for old NeisVac-C®), which was higher than the initially assumed rate of 6% that was found in a previous study also done in The Netherlands in a comparable setting [ref 5]. Also for other parameters for reactogenicity (injection site reactions and systemic reactions other than fever) we found no indication that the new NeisVac-C® would cause these at a higher rate than the old NeisVac-C®. In addition, considering all reported complaints together, as well as all encountered Serious Adverse Events (described in Annex 5), we did not find unexpected reactions (neither the nature nor the rates of these reactions), which confirmed the typical safety profile of NeisVac-C® as determined in our national spontaneous reporting system of pharmacovigilance [ref 6].
Pooled incidence rates of fever were higher between 5-28 days after vaccination compared to 0-4 days after vaccination. For both periods of observation these rates were similar for old and new NeisVac-C® recipients. It is plausible that the higher rates for the 5-28 days period are not only caused by the longer observation period (being cumulative) but also are influenced by the fact that the concomitantly administered live attenuated MMR virus vaccine is known to cause fever and associated complaints from day 5 to 12 after vaccination.

11.2. Limitations

There were three unexpected findings that had potential for influencing the overall conclusions:

1. The number of children that received old NeisVac-C® was larger than the number of children that receive new NeisVac-C®. The anticipated proportions were 50-50%, while the actual proportions were 63-37%. However, the revised power calculations done prior to termination of enrollment, and prior to any analysis of endpoint, confirmed that the obtained numbers of enrollments of either group were large enough to allow the analysis of the primary endpoint with sufficient power.

2. The disproportional enrollment in favor of the old NeisVac-C® may have been caused by our decision to start enrollment and begin a gradual vaccine allocation from then on. Before the start of this allocation, all children were to receive the old NeisVac-C®, which may have caused the higher number of recipients of old NeisVac-C® than new NeisVac-C®.
As an alternative explanation, it may be hypothesized that that the old NeisVac-C® vaccine was more trusted than the new NeisVac-C® which may lead to selective enrollment and subsequent bias. However, the 'old or new status' of NeisVac-C® was not communicated. In practice, parents were not informed if their child would receive old or new vaccine, nor was it possible for the health care personnel administering the vaccines to know if they had old or new vaccine in stock, unless they understood the batch numbers as a key to this information. We consider that the disproportional enrollment did not influence our conclusions.

3. Differences were identified in baseline characteristics for household size and seasonality. The latter was significantly different between the groups.
- It was determined that children that received old NeisVac-C® had on average slightly more children in the household than children that received new NeisVac-C®, possibly making them more susceptible to acquire infections that may cause fever. Overall the differences in household sizes were not significant. Thus we are confident that we may conclude as above.
- Old and new NeisVac-C® were given over a period longer than one year to allow for seasonal variation as shown in Table 5. Table 10 shows that there may be seasonal fluctuation, but in all seasons the incidences of fever appear similar for old and new NeisVac-C®. Based on the outcomes of the unadjusted and adjusted analysis, we consider confounding by any of the covariates unlikely.

11.3. Interpretation

The study demonstrated that old and new NeisVac-C® have a similar profile of reactogenicity during observation periods of 0-4 days and 5-28 days after vaccination. The percentages of children with fever during days 0-4 after vaccination in the two groups were 8.2% and 8.3%, which is somewhat higher than the percentage found earlier in The Netherlands (approximately 6%) that formed the basis for our power calculations [ref 5].

11.4. Generalisability

This study has investigated children that followed the routine National Immunization Program of The Netherlands, in which the NeisVac-C® vaccine under study has been administered since 2002. The study was done in approximately 7% of the eligible population, which we may regard as representative for the general eligible population.

We found no indication that the new NeisVac-C® would be more reactogenic than the old NeisVac-C® in the presently studied children. In addition, we did not find any other safety signal. In fact, all reported complaints were compatible with earlier experience [ref 5,6].

12. References

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3. European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP). www.encepp.eu/index.shtml
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6. Lareb. Annual Pharmacovigilance reports Vaccines National Immunization Program, 2011-2014. (http://www.lareb.nl/Vaccins/Lareb-rapportages-en-publicaties) (in Dutch).

13. Other information

13.1 Study products

All vaccines used in this investigation are regularly licensed products, currently used in the NIP. Their good record of safety and effectiveness is well established. None of the vaccines had specific labeling as 'study medication'. The study products are described in Protocol Chapter 7 (Annex 1).

13.2 Medical-ethical aspects

The study protocol and related documents were submitted for review by a Medical-Ethical Review Board. On a preview the Board considered that according to Dutch law no formal assessment was required for the presented study, being a non-invasive questionnaire, in a non-interventional study with non-experimental medication. Their decision was communicated with a Waiver letter dated 23 January 2014.

13.3 Funding and influence

This study was funded by an unrestricted investigational grant by Baxter Ltd / Pfizer Inc. It was agreed that Lareb is free to publish any data obtained from the study. A pre-final version of this report was sent early July 2016 to Pfizer for information before the results would be public. Pfizer had useful editorial comments that did not affect the description of the results and their interpretation. Apart from this Study Report, results of the study will be published in a scientific journal.

ABBREVIATIONS

Term	Explanation
AE	Adverse events
CI	Confidence Interval
CHC	Child Health Clinic
DVP	Dienst Vaccinvoorziening en Preventieprogramma's, RIVM-unit responsible for regional vaccine distribution and registry
ENCePP	European Network of Centers for Pharmacoepidemiology and Pharmacovigilance
EUPAS	European Union Post-Authorisation Studies (Register)
LIM	Lareb Intensive Monitoring
LLT	lower level term MedDRA
MedDRA	Medical Dictionary for Regulatory Activities
MenC	NeisVac-C® vaccine to prevent infections by meningococci group C
MHRA	Medicines and Healthcare products Regulatory Agency
MMR	measles-mumps-rubella vaccine
NeisVac-C	meningococcal group C polysaccharide conjugate vaccine
NIP	Netherlands/National Immunization Program (in Dutch: Rijksvaccinatieprogramma or RVP)
PASS	Post-Authorization Safety Study
PP	Per Protocol
PT	preferred term MedDRA
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (National Institute of Public Health and the Environment)
RMP	Risk Management Plan
SAE	Serious adverse events