A randomised, open label study, exploring the differences in immunogenicity and reactogenicity of infants after immunisation with either an acellular (aP) or whole cell pertussis (wP) vaccine

Pertussis Acellular **W**hole cell **A**dvanced **RE**search - **AWARE Study**

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Chief Investigator (CI)	Dr. Dominic Kelly Centre for Clinical Vaccinology & Tropical Medicine (CCVTM), Churchill Hospital, Oxford OX3 7LE, United Kingdom

	0044 (0)1865 611400 Email: dominic.kelly@paediatrics.ox.ac.uk
Primary research site	Centre for Clinical Vaccinology & Tropical Medicine (CCVTM), Churchill Hospital, Oxford OX3 7LE, United Kingdom
Sponsor	University of Oxford Research Governance, Ethics & Assurance Team 1st Floor, Boundary Brook House, Churchill Drive, Headington, Oxford OX3 7GB, United Kingdom
Co-investigators	Prof Ronald de Groot Head of IMI PERISCOPE Consortium Radboud University Medical Center (RUMC) Geert Grooteplein-Zuid 10 6525 GA Nijmegen The Netherlands Ronald.deGroot@radboudumc.nl Prof Beate Kampmann Lead for Vaccine Induced Immunity (WP3 of PERISCOPE consortium) Professor of Paediatric Infection & Immunity Director, The Vaccine Centre London School of Hygiene and Tropical Medicine Theme Leader Vaccines & Immunity MRC Unit The Gambia @ LSHTM E: beate.kampmann@lshtm.ac.uk bkampmann@mrc.gm T: +44 20 7 612 7925 A: Faculty of Infectious & Tropical Diseases| Room 367| Keppel Street London WC1E 7HT Prof J.J.M. van Dongen, MD, PhD Lead for Biomarker Discovery Platform (WP5 of PERISCOPE consortium) Professor of Medical Immunology

	Dept. of Immunohematology and Blood Transfusion (IHB) Leiden University Medical Center (LUMC) The Netherlands J.J.M.van_Dongen@lumc.nl Prof Matthew D Snape Associate Professor in Paediatics and Vaccinology Oxford Vaccine Group, University of Oxford, Centre for Clinical Vaccinology & Tropical Medicine (CCVTM), Churchill Hospital, Oxford OX3 7LE, United Kingdom matthew.snape@paediatrics.ox.ac.uk
Lead Trial Statistician	Dr. Xinxue Liu Lead Trial Statistician Oxford Vaccine Group University of Oxford Centre for Clinical Vaccinology & Tropical Medicine (CCVTM) Churchill Hospital, Oxford OX3 7LE, United Kingdom xinxue.liu@paediatrics.ox.ac.uk
Monitor for the UK	Research Governance, Ethics & Assurance Team

PROTOCOL SIGNATURE SHEET UK

Investigator Agreement

“I have read this protocol and agree to abide by all provisions set forth therein. I agree to comply with the International Conference on Harmonization Tripartite Guideline on Good Clinical Practice.”

Date:

Dr. Dominic Kelly

Chief Investigator

Oxford Vaccine Group

University of Oxford

Date:

Dr. Xinxue Liu

Lead Trial Statistician

University of Oxford

Conflicts of interest

No conflicts of interest to declare

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host institutions, regulatory authorities, members/partners of the PERISCOPE consortium and members of the Research Ethics Committee

1 Table of Contents

2 SYNOPSIS

Trial Title	A randomised, open label study exploring the differences in immunogenicity and reactogenicity in infants after immunisation with either an acellular (aP) or whole cell pertussis (wP) vaccine	
Short Title	**Pertussis A**cellular **W**hole cell **A**dvanced **RE**search - **AWARE Study**	
Internal Ref. no. UK	OVG2016/02	
Clinical Phase	Phase III	
Trial Design	Open label, randomised controlled trial recruiting infants born to mothers who have received an aP vaccine during pregnancy. Infants will be randomised to receive a primary schedule of aP or wP vaccine.	
Trial Participants	Infants (aged 8-10 weeks) who have not yet received their first set of vaccinations. Infant mother's immunisation status will be recorded in the trial	
Planned Sample Size	A total of 114 infants will be recruited:and randomised 1:1 to receive either an aP or wP in primary immunisation schedule.	
Planned Study Period	4 years and 10 months (September 2019 – June 2024)	
	Objectives	Endpoints
Primary	To investigate Pertussis Toxin (PT) specific antibody responses, as a marker of memory, following an aP booster given at 12 months of age	PT-specific antibody GMC at 13 months in aP versus wP groups

	to infants primed with aP versus wP vaccines	
Secondary	1. To assess the effect of baseline PT, FHA, PRN-specific IgG antibody responses on the PT, FHA and PRN antibody responses in infants primed with aP versus wP vaccines	1.Percentage reduction in PT, FHA, PRN antibody GMC at 5, 12, and 13 months of age for each 2-fold higher baseline antibody concentration in the aP versus wP groups.
	2. To compare pertussis antigen-specific memory B-cell frequencies at 5, 12, and 13 months of age in infants primed with aP versus wP vaccines	2. Pertussis antigen-specific memory B-cell geometric mean frequencies at 5, 12, and 13 months of age measured by ELISpot in the aP versus wP groups.
	3. To compare pertussis antigen specific T cell responses at 5 months of age, in infants primed with aP versus wP vaccines	3.Pertussis antigen-specific T cell responses at 5 months following antigen-specific restimulation
	4. To compare PT-specific antibody responses in infants primed with aP versus wP vaccine	4. PT-specific antibody GMC at 2, 5 and 12months of age in the aP versus wP groups
	5. To compare other pertussis specific antibody responses including FHA, PRN- and FIM specific antibodies in infants primed with aP versus wP	5.FHA, PRN and FIM specific antibody GMCs prior to immunisation and at 2, 5, 12 and 13 months of age

	6. To compare serological responses to the non-pertussis vaccines (Hib, diphtheria, tetanus, pneumococcus) in infants primed with aP versus wP vaccines	6.Hib, diphtheria, tetanus, and pneumococcal-specific antibody responses at 2, 5, 12 and 13 months in the aP versus wP groups
	7. Assessment of pertussis specific functional antibodies prior to and after immunisation with aP versus wP vaccines	7.Assays of pertussis specific functional antibodies may include: adherence inhibition; bacterial agglutination; bactericidal activity; bacterial opsonization and phagocytosis undertaken on serum or plasma samples taken at 2 (prior to immunisation), 5, 12 and 13 months of age
	8. To determine the induction and persistence of mucosal antibodies and/or cytokines in mucosal lining fluid before vaccination (2 months) and after vaccination (at 5 and 12 months)	8. The soluble factors in the eluate from nasosorption will be analysed by Luminex multiplex immunoassay
	9. To assess vaccine reactogenicity in those primed with aP versus wP vaccines	9. Proportion of individuals with local and systemic symptoms (solicited and unsolicited) after vaccine doses at 2, 4 and 12 months in infants primed with aP versus wP vaccine.

Exploratory	1. Assessment of pertussis specific T-cell responses to aP versus wP vaccination	1.Exploratory T-cell assays (including, but not limited to measurement of intra-cellular and supernatant cytokines from ex-vivo stimulated PBMCs) to assess T-cell responses at a variety of time-points around dose 1 (baseline), 5, 12 and 13 months.
	2. Assessment of genetic and epigenetic determinants of the immune response to aP or wP immunisation	2. Measurement of genetic variation (e.g. SNPs in candidate genes, B-cell repertoire in bulk and individual B-cells) and epigenetic variation (e.g. methylation and histone modification) and correlation with vaccine immunogenicity and reactogenicity
	3. To assess vaccine reactogenicity in those primed with aP versus wP vaccines using novel methods	3. Continuous body temperature measurements in the first 24 hours after the administration of the study vaccines
	4. To explore parental motivations and experiences participating in the trial.	4. Parent motivation and experience questionnaire sent between the 5th and 6th visit

3 ABBREVIATIONS

AE	Adverse Event
aP	Acellular Pertussis (vaccine)
APR	Annual Progress Report
AR	Adverse Reaction
AWARE	Pertussis acellular and whole cell advance research study
BAI	Bacterial adherence inhibition assay
Bp	*Bordetella pertussis*
CCMO	*Centrale Commissie Mensgebonden Onderzoek (MREC NL)*
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CDC	Centres for Disease Control and Prevention
CI	Chief Investigator
Cib	Centre for Infectious Disease Control (The Netherlands)
CHCD	Child Health Computer Department
CRF	Case Report Form
eCRF	(electronic) Case Report Form
CSM	Centre for Statistics in Medicine, University of Oxford
CYTOF	Cytometry by time of flight
DNA	Deoxyribonucleic Acid
DOB	Date of Birth
DSUR	Development Safety Update Report

DSMC	Data Safety Monitoring Committee
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ELISpot	Enzyme-Linked Immunosorbent Spot
EU	European Union
FHA	Filamentous haemaggluttinin
FIM	Fimbriae
FVFV	First Visit First Volunteer
GCP	Good Clinical Practice
GMC	Geometric mean concentration
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
GP	General Practitioner
GSK	GlaxoSmithKline
HHE	Hypotonic Hyporesponsive episodes
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IGH	Immunoglobulin heavy chain
IGK/IGL	Immunoglobulin κ and λ light chains

ISF	Investigator Site File
ITT	Intention-to-treat
IU	International units
JCVI	Joint Committee on Vaccination and Immunisation
LVLV	Last Visit Last Volunteer
MHRA	Medicines and Healthcare products Regulatory Agency
MIA	Multiplex immunoassay
MLF	Mucosal lining fluid
NL	The Netherlands
NHS	National Health Service
NIPs	National immunisation programs
NRES	National Research Ethics Service
OPA	Opsonophagocytosis assay
OVC	Oxford Vaccine Centre
OVG	Oxford Vaccine Group
OVGL	Oxford Vaccine Group Laboratory
OXTREC	Oxford Tropical Research Ethics Committee
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet

Prn	Pertactin
PT	Pertussis toxin
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RGEA	Research Governance, Ethics and Assurance Team
RIVM	National Institute for Public Health and the Environment
RNA	Ribonucleic Acid
RNA-seq	RNA-sequencing
SAE	Serious Adverse Event
SAM	Synthetic Absorptive Matrices
SAR	Serious Adverse Reaction
SBA	Serum bactericidal activity assay
SNPs	Single-nucleotide polymorphism
SmPC	Summary of product characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
DTaP dtap	Diphtheria, Tetanus and aP (vaccines for primary immunization) Diphtheria, Tetanus and aP (vaccines for adolescent and adult immunization)
TMF	Trial Master File

TOPS	The Over Volunteering Prevention System (http://www.tops.org.uk)
UK	United Kingdom
USA	United States of America
WHO	World Health Organization
WMA	World Medical Association
WMO	Medical research involving human subjects act
wP	Whole cell pertussis (vaccine)
WP	Work package

4 BACKGROUND AND RATIONALE

Introduction

Pertussis disease is an acute respiratory infection caused by the Gram-negative bacterium *Bordetella pertussis (Bp).* This is a highly contagious infection and the disease manifestations can depend on the individual immune response, age, pre-exposure to disease and immunisation status. The disease is spread by airborne transmission and the reservoir of the *Bp* is exclusively human [2]. Pertussis disease has several forms of presentation: from the classical presentation, known commonly as “whooping cough”; through severe forms in young infants with cyanosis and apnoea; to mild disease with coryzal symptoms or persistent cough. Infected individuals can also be asymptomatic. The incubation period is between 7 to 10 days and non- immunised infants and new-borns are more likely to develop severe disease[1, 3].

Pertussis vaccines have been one of the cornerstones of national immunisation programs (NIPs) since their introduction in the 1940s-1950s. From that period, the widespread use of whole cell pertussis (wPs) vaccines in NIPs has resulted in a huge reduction of pertussis-related deaths and disease, especially in young infants [1, 4, 5]. The reactogenicity of the wP vaccine, with symptoms like high fever, irritability and hypotonic-hyporesponsive episodes contributed to a low acceptance of the vaccine in the 1970s in the UK with a significant negative impact in the vaccination coverage rates[5, 6]. This situation stimulated the development of a less reactogenic vaccine, the acellular pertussis vaccine (aP), currently widely used in industrialised countries. At the time of introduction, the aP was expected to have an efficacy similar to wP vaccines based on the antibody responses observed in clinical trials. However, more recently increases in the incidence of pertussis infection, including fatal cases in young infants, have been observed in many regions using aP vaccines[7]. Whilst several hypotheses have been considered to explain the increased incidence, it is increasingly clear that aP does not provide a similar duration of protection to that seen with wP[8-11]. This difference in long-term effectiveness is likely to be due to differences in the type of immune response after immunisation with wP and aP that are still not completely understood in humans.

In view of the problems with long-term effectiveness seen with routine use of aP vaccines the World Health Organization (WHO) continues to recommend wP vaccine in the NIPs for infants for countries that have not already introduced aP vaccine[12]. A clearer understanding of how these two vaccines work is fundamental when considering adjustments or rethinking vaccination strategies and such knowledge will help the future development of new vaccines.

Epidemiology and disease burden

Since the start of the pertussis vaccination era, the incidence of pertussis decreased dramatically in the countries covered by the vaccine. *Amirthalingam G et al* reported a significant decrease in the number of notified cases in England and Wales since the introduction in the routine schedule of the wPs vaccine (DTwP) in 1957[5].

Despite effective vaccines the WHO estimates that there were still 16 million cases of pertussis in 2008 with 95% of these in developing countries, making it one of the leading causes of vaccine-preventable deaths[13]. However, the disease continues to be underreported. According to the WHO in 2015 only 139,786 cases were reported globally[14]. The global mortality due to pertussis was estimated in 195,465 cases by *Black et al [15]*. The highest morbidity and mortality due to pertussis occurs in low-income countries, where vaccine coverage is often still lower when compared with high income countries[14]. Although, according to the WHO it is estimated that global pertussis vaccination coverage was 86% in 2015 (with only 7 countries with vaccination coverage bellow 50%) [14].

In many areas of the world where aP vaccines are used routinely, there has been a rise in the incidence of disease, with significant numbers of cases occurring in Europe, Australia and the US in the last decade[16]. In the UK, disease seems to occur in 3-4 year cycles, with activity peaking each year in the Northern Hemisphere normally on the 3rd quarter[17]. In the Netherlands the cycles are described to be shorter and occur every 2-3 years[18].

In Europe, two of the countries affected by increases in pertussis cases were the UK and the Netherlands. In the UK the pertussis outbreak started in the 2nd half of 2011, and in 2012 a significant increase was reported (2011: 1256 cases, 2012: 11986 cases)[19]. The Netherlands also had a significant increase of the number the cases from 2011 (5447 cases) to 2012 (12853 cases). But not all of the European countries suffered the same epidemics[19]. In Finland, the number of cases has been stable throughout the years, with small variations. In the period previously described, cases reported were 555 in 2011 and 541 in 2012[19]. In 2015, the incidence of pertussis was very low in Finland (3.02/100.000) and the number of patients less than one year of age was only 11.

Different reasons have been hypothesised as possible causes of the described outbreaks including: a more rapid waning of immunity provided by the widespread use of aP vaccines in infancy; higher awareness of pertussis with increased disease reporting; increased circulation of 93Bp [20] and resurgence of new strains.

A genomic analysis of the 2012 UK outbreak strains showed that many of the isolated Bp strains were genetically distinct, but due to high similarities between the strains they were considered closely related[8]. These strains were also considered similar to the ones that normally circulate during low disease incidence. Of concern, making this problem an even bigger challenge is the fact that in several countries an increase has been observed in the circulation of *Bordetella pertussis* strains that do not express one or more vaccine antigens[21].

There is accumulating evidence that the true burden of disease in industrialised countries is much larger than previously assumed, and there is a resurgence of disease, particularly in (vaccinated) populations such as older children, adults and the elderly, which are not typically considered at risk[22]. In this population, a considerable proportion of this burden is related with the lost in time for work or other activities. A prolonged duration of symptoms is reported when compared with other upper respiratory infectious illness, with an associated disruption of sleep and daily activities. This was associated with a decrease in quality of life in the symptomatic population but also in undiagnosed household contacts [23].

In infants, increased mortality and morbidity are one of the main consequences. During the period between 2001 and 2011, 48 deaths in children under the age of 12 months were reported in England, 85% of them in infants that weren't fully immunised[24]. In 2012, in the peak of the outbreak 14 deaths were reported in England and Wales, all in children under 3 months of age[17]. In the same outbreak of 2012, 3 deaths in unvaccinated neonates between 0-2 months of age were reported in the Netherlands. In Finland, more booster doses have been used in the adolescent and adult population and the last higher outbreak was 2004 with 1631 cases.

Pertussis Vaccines

Two types of pertussis vaccines are available worldwide: whole cell pertussis vaccines and the acellular vaccine. The whole cell vaccines are suspensions of formalin inactivated *B pertussis* adsorbed with aluminium[25]. The acellular pertussis vaccines consist of highly-purified proteins from *B pertussis* including toxins (pertussis toxin (PT)) and adhesins (filamentous haemagglutinin (FHA), Pertactin (Prn), Fimbriae (FIM) types 2 and 3) adsorbed to aluminium[7, 26]. Individual aP vaccines differ in the number and type of antigens, but also in the bacterial clone, adjuvants and preservatives used during manufacturing[7]. Multicomponent vaccines (≥ 3 Bp antigens) are recommended for use in the NIPs since they are more efficacious in preventing mild to severe pertussis disease when compared with other aP vaccines[26].

After safety and effectiveness trials showing low reactogenity and a comparable immune response, based on PT antibodies, the aP vaccine was introduced particularly in high income countries during the 80's and 90's decades[1, 12].

WHO continues to recommend the wP in infants, but it is not recommended in adolescents and adults, due to a possible interference with other vaccines or vaccine antigens in combined vaccines[12]. Currently in high-income countries, aP vaccines are part of the routine NIPs, with a schedule that varies between countries. In Europe different immunisation schedules are used[27]. A minimum of two doses are required for protection according to the WHO [13] and the antibody responses in the 3+1 schedule or the 2+1 schedule seems similar in terms of antibody concentration and response rates[27]. The 2+1 schedule (as employed in Finland and elsewhere in Nordic countries) has the advantages of fewer injections, and provides early protection in the most vulnerable age group, while the use of a booster at 12 months may provide a sustained elevation of vaccine specific antibodies compared to a 3+0 schedule (as employed in the UK). The booster will allow induction of measurable antibody responses in 100% of children and result in higher antibody levels than after the priming doses[27].

Immune mechanisms

Epidemiological, clinical and preclinical studies have shown that immunity in humans wanes rapidly after immunisation with pertussis vaccines, especially with aP[9]. This suggests that the improvements in the reactogenicity profile of aP, as compared to wP, may be accompanied by differences in the elicited immune response. The estimation of the duration of immunity after any of the vaccines or even natural infection has several limitations, since there is no clear correlate of protection and substantial differences between vaccines and immunisation schedules need to be addressed[28]. A review from *Wendelboe et al* showed that immunity against Bp after vaccination can last between 4-12 years in children, with no significant differences between the aP and the wP vaccines[29]. A different perspective in duration of immunity was presented by *Sheridan et al* in a retrospective study in an Australian cohort. The study showed that an infant fully primed with wP would have a lower associated risk of developing pertussis disease when compared with infants primed with aP, particularly if this vaccine was used in the first administration[30].

Immunity after a natural infection can wane after 4-20 years[29], but more optimistic results were described by Wearing et al that according to the developed mathematical model, natural immunity could last on average 30 years[28].

Different animal models have been developed to better understand disease pathogenesis and the host response, but the best model developed so far, due to the genetic similarity with humans but also in the disease presentation, was the baboon model.

Studies in the baboon model have demonstrated that aPs prevent severe disease but do not prevent asymptomatic infection, i.e. colonization, or transmission to naive animals, whereas infection and to some extent wPs can prevent colonization, transmission and disease [31]. Studies in mice are consistent with these findings and demonstrate that protective immunity is more effective and persistent when induced by infection or wP than by aP[11].The most effective immunisation with wP gave a similar skewing on the T-helper responses as observed when compared with natural infection, i.e. type Th1/Th17, although to a milder degree[10]. The less effective aP immunisation gave a skewing to Th2/Th17 type responses, instead, with the Th2 component being non-protective. In the aP vaccinated baboon model the type of skewing was a mixed Th1/Th2 response, but no Th17 response was found, while wP vaccinated and infected animals had a type Th1/Th17 responses Warfel, Zimmerman [31], Warfel and Merkel [32]

In human studies with children vaccinated with a aP vaccine, as expected, the immune responses were similar to the baboon model with induction of Th1 and Th2, but with no Th17 stimulation, showing a possible explanation for the differences in long-standing protection for disease and colonization conferred by the vaccines[33]. With a Th2 mediated immune response primarily associated to aP, after immunisation, protection against symptomatic disease can be achieved, but susceptibility to infection, carriage and transmission remains, allowing vaccinated individuals to act as a reservoir to *Bp* transmitting disease to vulnerable individuals[32, 34]. *Bordetella pertussis* specific B and T cell responses can persist for a longer period than serum antibody levels[35].

Previous studies show that memory B cells could have a protective role, even in the absence of specific antibodies in vaccinated children, a mechanism that should be better explored in order to fully understand the duration and associated physiology to the immune responses to the pertussis vaccine and natural infection[35]. The B cell memory responses can also be influenced by age, and a weak correlation between specific IgG pertussis antibodies and memory B cells was described [36, 37].

There is also evidence of a role for Th17 cells as previously described, although this has not yet been confirmed in humans[38] . The role of functional antibodies and T cell responses in protection against disease and/or carriage has been demonstrated already by different laboratories[34].

However, there are still important knowledge gaps, in particular relating to human immunity to Bp and whether observations in animal models translate to clinical practice.

Adverse events after pertussis immunisation – aP and wP

The nature of local and systemic reactions following aP and wP are similar to those recognised for other childhood vaccines. These include redness, swelling and pain in the limb where the vaccine was given (local reactions) and fever, irritability and crying (systemic reactions). The incidence of such reactions is higher after wP than after immunisation with aP[26].

More significant reactions such as febrile convulsions and hypotonic-hypo responsive episodes (HHE) are rare. Febrile convulsions are relatively common in the setting of infectious causes of fever but can more rarely follow fever caused by immunisation. The incidence of febrile convulsions after immunisation has been estimated at 1/200,000 and 1/16,000 immunisations for aP and wP vaccines respectively[39]. Hypotonic-hyporesponsive episodes (also called “floppy baby”) involve the sudden onset of limpness, reduced responsiveness and looking pale usually within 48 hours of receipt of immunisation. Although these can be experienced by parents as frightening, the children recover spontaneously and no sequelae were reported in children with no underlying neurological conditions [40, 41]. These episodes are rare and can occur after any childhood vaccine. A recent Cochrane review demonstrated that HHE can occur after the administration of aP or wP vaccines, although they are more likely to happen after wP[26]. The estimated incidence for HHE is of ~1/1,600-7,000 versus ~1/400-1,700 for aP versus wP vaccines[39]. Typically, HHE occur with the first immunisation dose and do not recur with subsequent doses. They are not a contra-indication to further doses of vaccine.

As for all vaccines, there is a very small chance of a severe allergic reaction to both aP and wP vaccines.

Prior to the 1970s pertussis immunisation was well accepted given clear evidence of effectiveness against disease. It was recognised that wP was a relatively reactogenic vaccine and a causal relationship with neurological complications was suggested in 1974[5, 6, 42]. Adverse publicity led to reduced vaccine coverage with the occurrence of subsequent disease epidemics during the 1970s with significant morbidity and mortality from pertussis disease. As a result the National Childhood Encephalopathy Study (NCES) was set-up in order to determine if wP was associated with an increased risk of neurological illness[42]. This study included 1000 cases of acute severe neurological illness and found that 3.5% of cases versus 1.7% of controls had received the wP vaccine within the previous 7 days. The study concluded that there was a 1/310,000 risk

(confidence interval 1/54,000 – 1/5,310,000) of a healthy child developing a neurological illness that persisted at a year. The limitations of this study have been highlighted as it did not mask the participant immunisation record from those classifying the neurological disease and there were no alternative sources of data to independently verify that all cases of neurological illness had been ascertained. The JCVI stated in 1981 that the risk associated with wP immunisation was low and outweighed by the benefits of the vaccine[5]. A follow up study NCES was published in 1993[42] and concluded that the low number of cases reduced the ability to determine if there was a causal effect between the vaccine and the events. They also concluded that only in rare cases could the vaccine be associated with a severe acute neurological illness and that even in those cases the role of the vaccine as an aetiological factor could not be determined and attribution of a cause should be considered speculative[42].

Subsequent advances in the genetic diagnosis of epilepsy have demonstrated that in children with encephalopathy previously attributed to vaccination, all of them were diagnosed with a specific epilepsy syndrome and 11 of the 14, had a SCN1A mutation identified[43]. This condition, called severe myoclonic epilepsy of infancy (SMEI) also known as Dravet syndrome, is a severe epileptic encephalopathy, normally diagnosed in the first year of life in previous healthy children [43, 44]. Receipt of the wP vaccine did not modify the course of the illness. This suggests that many cases where neurological disease has previously been attributed to pertussis vaccine instead are due to a genetically determined form of epileptic encephalopathy, which first becomes apparent with seizures following fever.

Several subsequent studies of large numbers of immunised children have provided further reassurance on the issue of pertussis related adverse events and supported the WHO position that countries who have not already introduced aP to National Immunisation Programs should continue to use wP vaccines. The study by Gale et al including data from the surveillance of 218,000 USA children (estimated 368,000 DTP immunisations) failed to show an increased risk of severe acute neurological illness, including encephalopathy or complicated seizures within the 7 days after vaccination[45]. A USA study investigating cohorts from 4 large health-care maintenance organisations, which included data on 679,942 children after 340,386 vaccinations with DTP and MMR vaccines. Whilst there was an increased risk of febrile seizures on the day after immunisation for both vaccines (which is not unexpected), there was no increase of afebrile seizures and no evidence of long-term consequences of such seizures [46]. A further USA study using data from health-care maintenance organisations with data on 2,197,000 children 0-6 years

of age from a 15-year period identified 442 cases of encephalopathy. There was no increased odds for cases having received DTP in the previous 7 days versus controls [47].

Study whole-cell pertussis vaccine – ComVac5® (Bharat Biotec)

The wP vaccine that is going to be used in this study is Comvac 5 ® (Bharat Biotec). This is a pentavalent vaccine containing DTwP-Hib-HepB antigens. This vaccine obtained a manufacturing license in India in 2009. Since that time until 2016 over 2 million doses have been supplied almost exclusively within India. Vaccine reactogenicity and immunogenicity has been assessed in a variety of clinical trials prior to and following the granting of a manufacturing licence (detailed in IB and IMPD). In 2007, a total of 180 infants received one of ComVac5 or Easyfive® (Panacaea Biotech), a comparator wP vaccine, in a 3-dose primary immunisation regime. Both reactogenicity and immunogenicity was similar between groups.

In 2011 a further clinical trial enrolled 330 subjects received one of ComVac5 or Easyfive® (Panacaea Biotech) and reactogenicity and immunogencity were assessed. Again there were no significant differences between the immunogenicity and reactogenicity of the vaccines. No Serious Adverse Reactions to the IMP were observed.

ComVac5 has also been used as the concomitant pentavalent vaccine in clinical trials of RotaVac® (Bharat Biotech) rotavirus vaccine [48, 49]. As part of these clinical trials safety data was systematically collected on the 6799 [48] and 1356 infants[49] enrolled and the summary data published. While the focus of these studies was the RotaVac® vaccine they are the largest body of published safety data on ComVac5 and are in keeping with the profile of other whole cell pertussis vaccines.

Maternal immunisation

In the UK, the Department of Health recommended aP immunisation of all the pregnant women from autumn of 2012, in the peak of the outbreak. The vaccine is still recommended in the UK for all pregnant women, between 16-32 weeks of gestational age[50].

This recommendation was also announced by the WHO since it is considered to be the best cost-effective additional preventing strategy for infection[7]. Previous studies using tetanus, diphtheria and aP-containing combination vaccines during pregnancy have shown to reduce pertussis-related infant mortality and morbidity [51]. It has also been shown that infants born to

mothers who were vaccinated in pregnancy with aP, have higher infant PT antibody concentrations in the first 2 months of life, prior to receiving their primary immunisations, when compared with infants born to non-vaccinated mothers[52, 53]. Despite this finding, and the reduction in mortality and morbidity as previously described, after the primary immunisations with a acellular pertussis vaccine a blunting of the infant responses to the acellular antigens were identified in different countries like the UK, Belgium and USA, although not always with statistical significance[52-54].

However, there is still a significant lack of comprehensive data on infant responses to aP and wP priming (the recommended vaccine in developing countries), to other vaccine components in the priming phase of the infant vaccination schedule and also what is the clinical meaning of the findings previously described[7]. Other information that is not completely understood is when is the ideal time to vaccinate mothers during gestation. Vaccinating mothers after the 20-21 weeks ultra-sound seems a very common strategy, and normally after 26 weeks. The rational for this recommendation was based on observational studies showing that if immunisation occurred in the third trimester it was associated with higher antibody titers at birth[55].

Another question still not completely clarified is the possibility that maternal immunisation could contribute to high antibody concentrations in the infant at birth, which could inhibit the infant antibody response to immunisation. A study from *Jones* et al, showed a higher antibody concentration at birth seemed to inhibit the infants antibody response for specific immunisations, like pneumococcus and tetanus[56]. The clinical relevance of this finding remains uncertain.

Furthermore, it is unclear how vaccination in pregnancy affects the long-term quality and quantity of pertussis immunity of infants.

Rationale for the study

This study will directly compare the immune responses of infants given aP and wP vaccines as part of a 2 dose-schedule of primary immunisation in order to better understand the immunological features that distinguish a vaccine that we have some evidence generates better long-term protection against pertussis infection and carriage (wP) than aP. A detailed investigation of the immune responses will be possible through the application of cutting-edge novel functional antibody and cellular assays using the technology platforms developed by the PERISCOPE consortium team of experts (see below). Specifically, we will compare wP and aP primary schedule of infant immunisation for: the development of pertussis antigen-specific B-cell memory (measured both as a secondary antibody response and by enumeration of memory B-cells); the

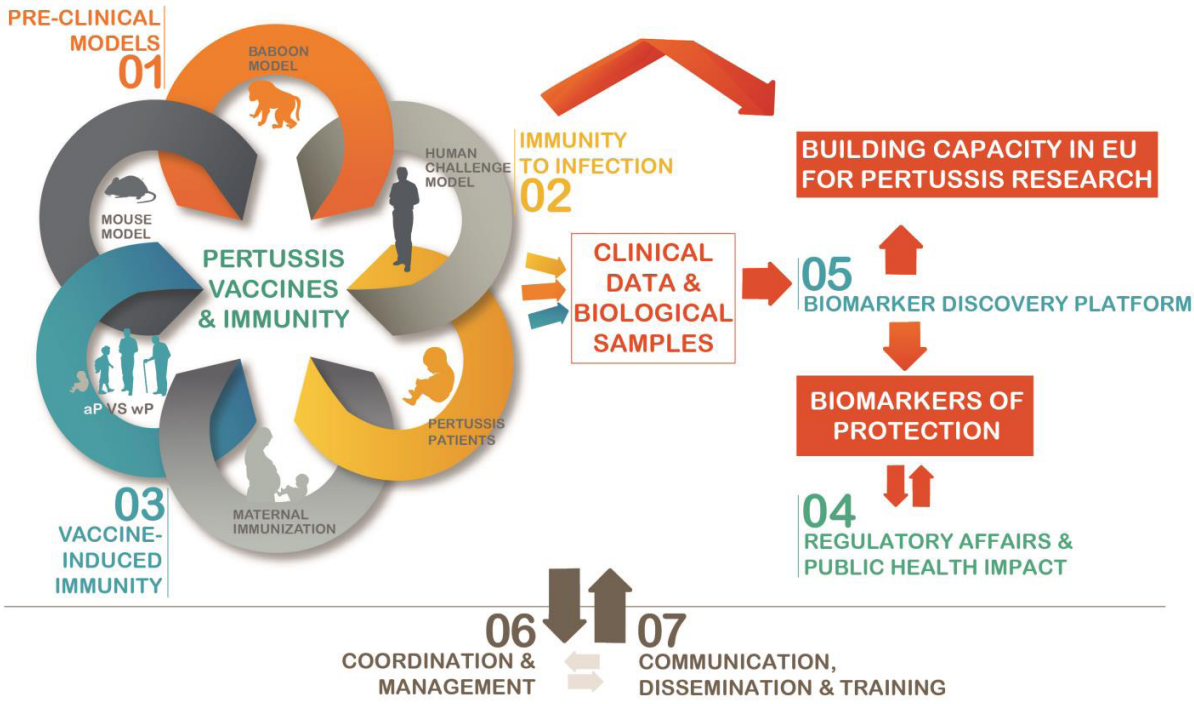
induction of pertussis antigen-specific Th1, Th2 and Th17 cells; the effect of maternally derived pertussis specific infant antibody response to aP versus wP vaccine; and the induction and persistence of pertussis specific antibodies up to 13 months of age following an aP booster. This data will generate insights with regard to the biomarkers for and the mechanisms of long-term protection. These will be the subject of subsequent work to be undertaken by collaborators within the PERISCOPE consortium who are using identical assays to study children with infection and are developing human and animal challenge models of pertussis infection. Such studies will provide scientific knowledge that will significantly facilitate the design and evaluation of more efficacious future vaccines with an acceptable level of reactogenicity.

The PERISCOPE consortium

This study is part of work-package-3 of the Innovative Medicines Initiative (IMI) funded PERISCOPE consortium (**PER**tuss**IS CO**rrelates of **P**rotection in **E**urope).

PERISCOPE is a unique public-private partnership between a large group of pertussis experts from 15 universities across Europe, 3 national public health institutes, 2 small enterprises and two partners from the European federation of pharmaceutical industries and associations (EFPIA), Sanofi Pasteur and GlaxoSmithKline. The PERISCOPE consortium aims to generate knowledge on immune responses to pertussis. Better understanding of human biomarkers of protective immune responses to *Bordetella pertussis*, and its waning immunity is needed to accelerate the design and testing of new pertussis vaccines with a longer duration of protection. In the figure below, we present an overview of the interactions between the 7 work packages (WPs) in PERISCOPE and the associated tasks. The current protocol is part of WP 3, which also contains a European maternal-infant vaccination study in the Gambia and in Europe (NL, UK, Finland). Through a number of carefully harmonised preclinical (WP1) and clinical (WP2&3) studies, we will be able to systematically compare immune responses to existing pertussis vaccines (acellular pertussis vaccine versus whole cell pertussis vaccine) as well as infection induced immunity (WP2).These studies will provide samples and clinical parameters for analysis in WP5, in the biomarker discovery platform. In order to facilitate a streamlined regulatory approval process for these biomarkers we will engage regulatory authorities from the onset of the project (WP4).To ensure adequate knowledge transfer, training of consortium partners, and optimal visibility of the consortium activities to external stakeholders, including the general public, we have designed a clear communication strategy (WP7). Finally, all activities will be carefully managed so that the partners will successfully deliver on the tasks and milestones set out in the project (WP6).

Figure 1. Overview of the inter-related work-packages (1 to 7) and their studies that constitute the PERISCOPE consortium



6. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

	Objectives	Endpoints
Primary	To investigate Pertussis Toxin (PT) specific antibody responses, as a marker of memory, following an aP booster given at 12 months of age to infants primed with aP versus wP vaccines	PT-specific antibody GMC at 13 months in aP versus wP groups
Secondary	1. To assess the effect of baseline PT, FHA, PRN-specific IgG antibody responses on the PT, FHA and PRN antibody responses in infants primed with aP versus wP vaccines 2. To compare pertussis antigen-specific memory B-cell frequencies at 5, 12, and 13 months of age in infants primed with aP versus wP vaccines	1.Percentage reduction in PT, FHA, PRN antibody GMC at 5, 12, and 13 months of age for each 2-fold higher baseline antibody concentration in the aP versus wP groups. 2. Pertussis antigen-specific memory B-cell geometric mean frequencies at 5, 12, and 13 months of age measured by ELISpot in the aP versus wP groups.

	3. To compare pertussis antigen specific T cell responses at 5 months of age, in infants primed with aP versus wP vaccines	3.Pertussis antigen-specific T cell responses at 5 months following antigen-specific restimulation
	4. To compare PT-specific antibody responses in infants primed with aP versus wP vaccine	4. PT-specific antibody GMC at 2, 5 and 12months of age in the aP versus wP groups
	5. To compare other pertussis specific antibody responses including FHA, PRN and FIM specific antibodies in infants primed with aP versus wP	5.FHA, PRN and FIM specific antibody GMCs prior to immunisation and at 2, 5, 12 and 13 months of age
	6. To compare serological responses to the non-pertussis vaccines (Hib, diphtheria, tetanus, pneumococcus, polio) in infants primed with aP versus wP vaccines	6.Hib, diphtheria, tetanus, and pneumococcal-specific antibody responses at 2, 5, 12 and 13 months in the aP versus wP groups
	7. Assessment of pertussis specific functional antibodies prior to and after immunisation with aP versus wP vaccines	7.Assays of pertussis specific functional antibodies may include: adherence inhibition; bacterial agglutination; bactericidal activity; bacterial opsonization and phagocytosis undertaken on serum or plasma samples taken at 2 (prior to immunisation), 5, 12 and 13 months of age
	8. To determine the induction and persistence of mucosal antibodies	8. The soluble factors in the eluate from nasosorption will be

	and / or cytokines in mucosal lining fluid before vaccination (2 months) and after vaccination (at 5 and 12 months) 9. To assess vaccine reactogenicity in those primed with aP versus wP vaccines	analysed by Luminex multiplex immunoassay 9. Proportion of individuals with local and systemic symptoms (solicited and unsolicited) after vaccine doses at 2, 4 and 12 months in infants primed with aP versus wP vaccine.
Exploratory	1. Assessment of pertussis specific T-cell responses to aP versus wP vaccination 2. Assessment of genetic and epigenetic determinants of the immune response to aP or wP immunisation	1.Exploratory T-cell assays (including, but not limited to measurement of intra-cellular and supernatant cytokines from ex-vivo stimulated PBMCs/) to assess T-cell responses at a variety of time-points around dose 1 (baseline), 5, 12 and 13 months. 2. Measurement of genetic variation (e.g. SNPs in candidate genes, B-cell repertoire in bulk and individual B-cells) and epigenetic variation (e.g. methylation and histone modification) and correlation with vaccine immunogenicity and reactogenicity 3. Continuous body temperature measurements in the first 24

	3. To assess vaccine reactogenicity in those primed with aP versus wP vaccines using novel methods	hours after the administration of the study vaccines
	4. To explore parental motivations and experiences participating in the trial.	4. Parent motivation and experience questionnaire sent between the 5th and 6th visit

7. Trial DESIGN

7.1 Trial design

This study will be conducted as a, randomised, open-label controlled trial comparing infants born, from aP immunised mothers, randomised to receive aP or wP vaccines as part of their primary immunisations.

7.1.1 Study population:

The study will recruit a total of 114 infants, and randomise 1:1 to receive either wP or aP at 2 and 4 months of age (primary immunisations). See **Figure 2** for trial flow chart. The target recruitment accounts for a possible 20% dropout rate in the study population.

7.1.2 Sample size calculation

Assumptions based on data from unpublished NL study of aP given at 3, 5 and 12m:

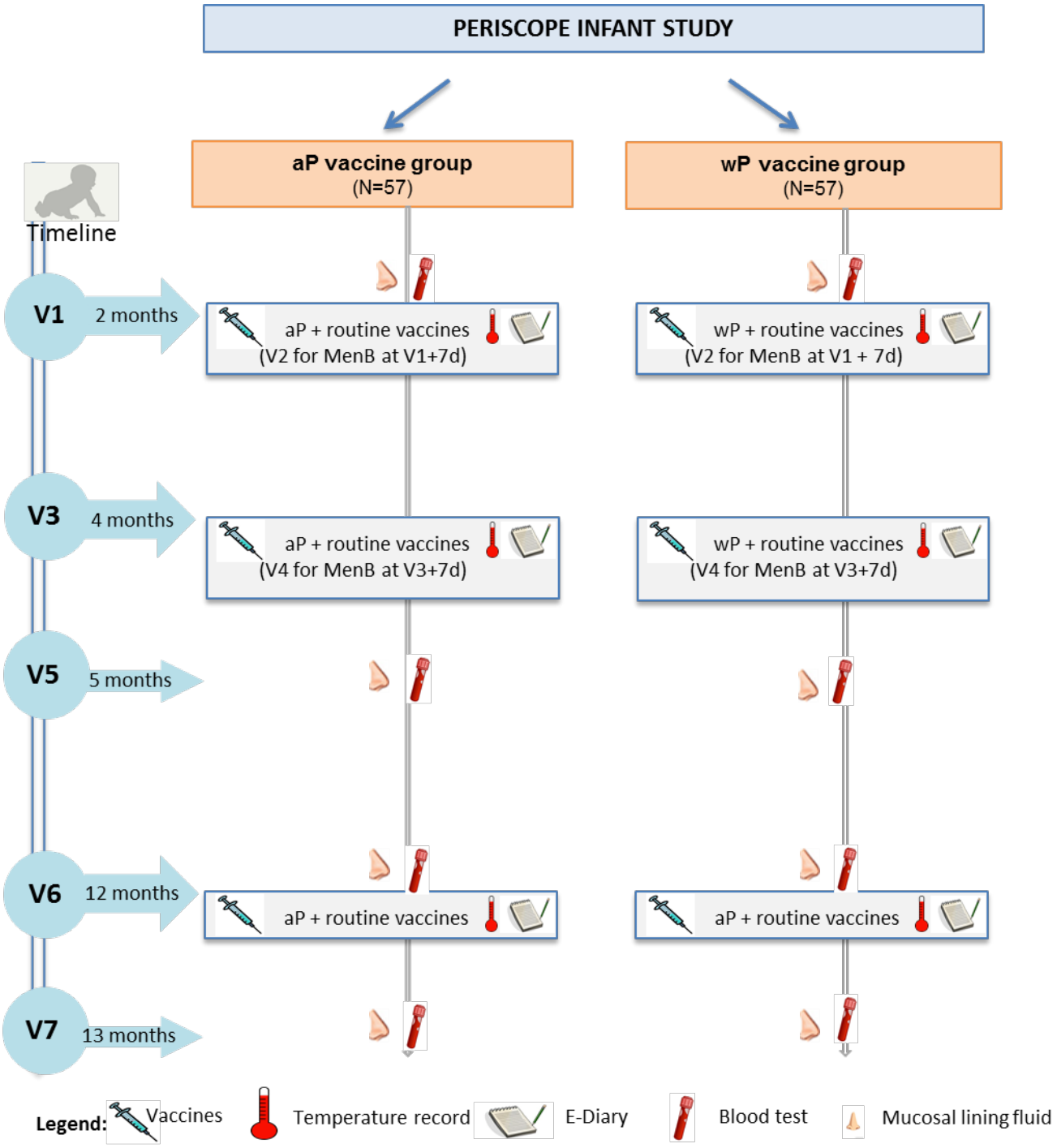
- Since the Netherland's vaccine schedule is 2 priming doses in the first 5 months of newborns + 1 booster at 11 months using aP, we will use the GMC of PT antibody at 12m in the unpublished data as our assumption to power the current study, which has a primary outcome at 1 months post ap-booster (13 months).

The GMC of PT on $\log_{10}$ scale= 1.9 (80 mcg/ml)

- Standard deviation of GMC at 13m on $\log_{10}$ scale = 0.324
- Expected difference in post-boost PT-antibody concentration on $\log_{10}$ scale = 0.23 (135 v 80 mcg/ml) derived as a conservative assumption from two previous studies (see above) (Kitchin and Englund)
- Expected withdrawal proportion 20%

A total of 57 infants will be recruited to each arm. With a total of 90 infants in the final analysis, the study will have a 90.8% power to detect the difference between a GMC of 135 and 80 mcg/ml (0.2 on $\log_{10}$ scale) in the wP and aP cohort at a significance level of 0.05. The sample size calculation was done as two-sided hypothesis test using R "pwr" package and validated using PASS v15.0.6.

Figure 2: Trial flow chart



Blood collection at V1 (before the 2 months immunisations) will be performed in order to allow an adjustment for baseline antibody levels. In order to mitigate any possible cases of infants with previous contact with pertussis and consequently detectable antibodies, infants with previous history of pertussis disease/whooping cough confimed by a diagnostic laboratory test would be excluded.

Written informed consent will be obtained from the mothers of the potential study participants prior to enrolment of each infant participant in order to have access to their immunisation status records before enrolment.

Prior to the infant's enrolment, informed consent will be obtained from parent(s) or the legal guardian (detailed in section 9 (Study procedures), recruitment (9.1) and informed consent (9.3)), after which eligibility against the inclusion and exclusion criteria documented here in section 8.1.1 will be confirmed.

If they are eligible to continue, the participants will have blood samples collected and will be vaccinated by the study team under the schedule set out in the table below. If the participant is not eligible, they will not be enrolled in the study and the infants will need to be vaccinated through the NIP.

Parents/legal guardians will report local and systemic reactions using a diary (an ediary system will be provided to the parents and alternatively, in case of any failure in this system, an identical paper diary will be provided) for the 7 days that follow the 2, 4 and 12 months vaccinations. In addition, temperature will be recorded after the study vaccines have been administered at 2, 4 and 12 months of age, for a 24 hour period using a validated device for continuous temperature monitoring (such as the Ibutton® system). The chosen device will be CE marked, commercially available and unmodified. In addition, axillary temperatures will be collected by parents using an axillary thermometer at 4, 8, and 24 hours after vaccination and then once daily for 7 days post vaccination at 2,4 and 12 months of age**.**

The samples (serum, plasma PBMC, mucosal secretions, and DNA) of the participants in this study will be stored in the central biobank from the PERISCOPE consortium, located at the Radboud university medical centre in Nijmegen, the Netherlands, until further distribution to partners for further laboratory analysis.

We are also keen to explore and understand the motivations and experiences of parents who choose for their children to participate in this trial. We will send out an online questionnaire between the V5 and V6 visit. The findings will help us to better understand trial participation,

especially in paediatric trials this wil inform the way in which future paediatric studies are designed.

7.2 Trial Schedule

Table 1: Study design documenting: **visits** (V1-7); **vaccines** to be given (6:1, Rota, PCV, IPV, 5:1, MenB, MenC, MMR) and **blood sampling** B.

		aP vaccine groups N=57	wP vaccine groups N=57
V1 (baseline) 2 months	**e-diary** Temperature record	**B / MLF** 6:1, Rota, PCV	**B / MLF** 5:1, IPV, Rota, PCV
V2	V1+7d	Men B	Men B
V3 4 months	**e-diary** Temperature record	6:1, Rota, PCV	5:1, IPV, Rota, PCV
V4	V3+7d	Men B	Men B
V5 5 months		**B / MLF**	**B / MLF**
	Parent motivation and experiences questionnaire		
V6 12 months	**e-diary** Temperature record	**B / MLF** 6:1, PCV	**B / MLF** 6:1, PCV
V7 13 months		**B/ (MLF)** Men C, MMR, Men B	**B/ (MLF)** Men C, MMR, Men B

Legend:

B: infant blood

MLF: Mucosal lining fluid

Vaccine abbreviations: 6:1: DTaP-IPV-Hib- HepB (diphtheria (D), tetanus (T), pertussis (acellular component) (Pa), hepatitis B (rDNA) (HBV), poliomyelitis (inactivated (IPV) and *Haemophilus influenza* type b (Hib) conjugated vaccine (adsorbed). Infanrix hexa ®; **5:1:** DTwP-Hib- HepB; (diphtheria (D), tetanus (T), pertussis (whole cell) (w), hepatitis B (rDNA) (HBV), and *Haemophilus influenza* type b (Hib) conjugated vaccine (adsorbed) – COMVAC 5 ® *;* **IPV**: Inactivated poliomyelitis vaccine- Imovax ® polio; **Men B:** meningococcal serogroup B vaccine adsorbed rDNA vaccine Bexsero ® ; **Men C**: Meningococcal Group C Polysaccharide Conjugated Vaccine adsorbed- Neisvac ® **PCV**: pneumococcal conjugate vaccine - Prevenar13. **Rota***:* live attenuated vaccine rotavirus vaccine- Rotarix ®: will be administered at 2 and 4 months of age; **MMR**: live attenuated vaccine against measles, mumps and rubella - Priorix ® ® or M-M-RVAXPRO ® in UK.

Vaccine products to be used	Commercial name
DTaP-IPV-Hib- HepB	Infanrix hexa ® (GSK)
DTwP- Hib-HepB	Comvac 5 ® (Bharat Biotech)
IPV	Imovax ® polio (Sanofi Pasteur Ltd)
Men B	Bexsero ® (GSK)
Men C	Neisvac ® (Pfizer Ltd)
PCV 13	Prevenar 13 ® (Pfizer Ltd)
Rota	Rotarix ® (GSK)
MMR	Priorix ® (GSK): UK or M-M-RVAXPRO ® (Merck,Sharp and Dohme) UK

Primary immunisations:

- DTaP-IPV-Hib- HepB into UPPER RIGHT antero-lateral thigh
- DTwP- Hib-HepB into UPPER RIGHT antero-lateral thigh
- PCV into UPPER LEFT antero-lateral thigh
- IPV into LOWER LEFT antero-lateral thigh
- Bexsero into LEFT antero-lateral thigh
- Rotavirus vaccine orally

Subsequent immunisations at 12 and 13 months:

- DTaP-IPV-Hib- Hep B into UPPER RIGHT antero-lateral thigh

- PCV into UPPER LEFT antero-lateral thigh
- MMR UPPER RIGHT antero-lateral thigh
- Men C into LOWER RIGHT antero-lateral thigh
- MenB into LEFT antero-lateral thigh

8. PARTICIPANT IDENTIFICATION

8.1 Study Participants

Infants must meet all inclusion criteria and none of the exclusion criteria in order to be eligible to participate.

8.1.1 Inclusion and exclusion criteria

a) Inclusion Criteria

Infants

- Infants due to receive their primary immunisations, aged up to 10 weeks at first vaccinations.
- Infants born at ≥ 37 weeks of gestational age
- Written informed consent given by parent(s) or legal guardian(s) who is aged ≥18 years
- Parent(s) or legal guardian(s) willing and able to comply with the requirements of the protocol for the duration of the study.
- Maternal immunisation: received dtap vaccine during the current pregnancy

b) Exclusion Criteria

Mothers

- Any condition which in the opinion of the investigator may interfere with the ability to fulfil study requirements (this may include plans to move house and language comprehension)
- Receipt of immunosuppressive treatment during pregnancy or known HIV positive.

Infants

- Child in care (with safeguarding in place)
- Children of parents who are on the delegation log for this study
- Prior or planned receipt of any other investigational vaccine/drug or if current participation in other research study, at investigator discretion
- Major congenital defects or serious chronic illness
- Presence of an evolving or changing neurological disorder
- Presence of central nervous system disease or convusions in the infant or another family member
- Bleeding disorder
- Confirmed or suspected immunodeficiency
- A family history of congenital or hereditary immunodeficiency
- Receipt of more than 1 week of immune-suppressants or immune modifying drugs (e.g. oral prednisolone >0.5ml/kg/day or intravenous glucocorticoid steroid). Nasal, topical or inhaled steroids are allowed.
- Administration of immunoglobulin and/or any blood products since birth or planned administration during the study period
- History of allergy to any component of the vaccines
- History of pertussis disease/whooping cough confirmed by laboratory analysis (serology, culture or other available methods)
- History of encephalopathy of unknown aetiology, occurring within 7 days following receipt of a previous pertussis containing vaccine

8.1.2 Temporary Exclusion Criteria (infants)

Visits where vaccines are administered should be delayed:

- In the presence of an acute illness or the presence of fever ≥38ºC, until 72 hours after resolution
- For at least 6 hours since last dose of ibuprofen/ paracetamol

- For 48 hours after finishing an antibiotic treatment.

All the treatments should be documented in the CRF at the time of the visit (name, dose, duration of treatment).

9. STUDY PROCEDURES

9.1 Recruitment

Potential participants will be identified and approached using the following recruitment strategies:

- Mail-outs: age appropriate children may be identified via the Child Health Information Service (CHIS) or other National Health Service equivalent databases. Initial contact to potential participants will not be made by the study team. Instead the invitation letter with a reply slip and possibly the study information booklet will be sent out by an external company, CFH Docmail Ltd, in order to preserve the confidentiality of potential participants. CFH Docmail Ltd is accredited with 100% approval under the Department of Health Information Governance Toolkit scheme. It is anticipated that the study information booklet will always be included in the mail-out except under exceptional circumstances, for example if unexpected printing problems occur. However parents/guardians will always have access to the study information booklet on the study website and if they express an interest in the study, a copy can also be sent via email/post by the study team before screening. If a response has not been received within a few weeks a reminder postcard will be sent out as a reminder of the study and the information previously provided.

- Poster advertising: posters with brief details of the study and contact details for further information may be displayed in , nurseries, schools and other suitable public places with the permission of the owner/proprietor.

- Media advertising: local media, newspaper, radio, website and social media advertisements that are relevant to the target population may be used, which include brief details of the study and contact details for further information.

- Email: representatives from local employers, schools, nurseries or other establishments relevant to the target population may be contacted to request that they circulate posters, study information sheets or emails containing links to the study website.

- Volunteer databases: the study may be advertised on the electronic newsletter sent out to parents signed up to the Oxford Vaccine Group's Children and Young Peoples Database. Members

of the public who have registered on this secure database have given their consent to be contacted when studies open for recruitment and understand that there is not a commitment to participate.

Those parents/legal guardians who are interested in their child taking part will be able to contact the study team via telephone, email, postal reply slips or the online registration form on the study website. Once an expression of interest has been received the study team will contact the parent/legal guardian to provide further information. If they haven't already received the study information booklet it will be emailed or posted out to them to read at their leisure or they will be directed to the OVG website where it is also available. If the parent/legal guardian is willing for their child to participate an appointment will be made for the first study visit. If there are no concerns regarding eligibility and a parent is interested in participating, a suitable time will be arranged for the first study visit. Eligible children whose parents are interested in the study will be visited at their home address (SOP OVG011: Safety of Research Staff whilst Travelling), by the paediatric study team.

In light of the current COVID-19 pandemic in the UK, the government has advised reducing social interaction between people to help reduce the transmission of the virus in the community. To enable us to follow these recommendations as much as possible during our visits, and therefore minimise the risk to participants and their family/household, we will be following the infection control precautions, as outlined in the Clinical Study Plan.

9.2 Screening and Eligibility assessment

Potentially eligible children whose parents/legal guardians are interested in the study will be visited at their home. Given the importance of maternal immunisation status to inclusion in the study, (i.e. whether an aP vaccine was received during the pregnancy) formal confirmation of receipt of the vaccine will be sought before the first study visit. In order to minimise any delay in infant immunisation, the team defined two strategies to gain confirmation of the maternal immunisation status.

In order to confirm maternal immunisation status one of two approaches will be used.

Either:

1) The mothers will be given access to an online form to take to the GP surgery in order to document their pertussis immunisation status, which will then be provided to the study site.

2) Alternatively, after discussion of the study details, the mothers will be sent a consent form (paper or electronic) giving permission for the study team to access their medical records to obtain this information (this includes consent to access their vaccination history either using their electronic patient record (EPR) or through their GP); Mothers should then return a copy of the signed consent form (paper or electronic). A countersigned form will be provided at the initial study visit.

This consent form is only for collection of the information regarding their immunisation status and not the final study consent for the infant enrolment, and a separate consent is going to be taken for inclusion of the infant in the trial.

The first visit will be delayed until the mother`s immunisation status has been confirmed. If the mother's immunisation status cannot be confirmed in the recommended time for the participants to be immunised (between 8 and 10 weeks of age as per inclusion criteria) the participant will be excluded from the study.

In order to assess eligibility of the infant for the study, a qualified doctor will perform the first visit that would include a complete medical history (including review of the red book) and a complete physical examination.

The participants GP and the child health department will be notified of all immunisations administered in the study.

9.3 Informed Consent

Written and verbal versions of the Participant Information and Informed Consent will be presented to the infants parents or legal guardians detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. The participants parent/legal guardian will be allowed as much time as wished 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. It will clearly state that the parent/guardian is free to withdraw their infant from the study at any time for any reason without prejudice to future care, and with no obligation to disclose the reason. All study staff who will take consent are

appropriately trained and experienced in obtaining informed consent for clinical trials and will be delegated to do so by the CI.

A written informed consent form for the infant must be completed by the parent/legal guardian before any study procedures are performed. A copy of the signed informed consent will be given to the participants parent/guardian and the original will be retained at the study site. GPs will be informed in writing of the participation of the infant registered at their surgery who joins the study.

Maternal consent will be taken for access to medical records where required and as described in section 9.2, above.

9.4. Randomisation, blinding and code-breaking

In this, open label, study there will be no blinding of study staff or families, so no system for un-blinding or code-breaking is required. Laboratory staff will have no knowledge of the group assignment of the individual.

Prior to V1, the participants will be randomised to either aP or wP on a 1:1 basis using a electronic randomisation system. The randomisation lists will be generated by the study statistician using block randomisation with a random block size 2 or 4. In case a participant cannot be enrolled for the study after randomisation, the current allocation will not be reused and the randomisation list will be followed until all participants are recruited.

9.5 Infant study (summary of procedures in table 1)

9.5.1 Visit 1: 8-10 weeks of age

1. Provide explanation of study

2. Obtain written informed consent from parent(s)/ legal guardian(s)

3. Perform thorough check of inclusion and exclusion criteria and record findings, including medical and vaccination history, of relevance to the inclusion/ exclusion criteria

4. Details and indications for any prescription medications for infant

5. Confirm maternal history of pertussis vaccination in pregnancy

6. Physical examination by study doctor or through the well-baby clinic, in line with the well-baby check and will include examination of the cardiovascular, respiratory, abdominal and neurological systems.

7. Record date of birth and gender for subsequent data analysis

8. Measure and record the participant's temperature.

9. If participant meets all inclusion and exclusion criteria, enrol in study.

10. Open allocation envelope that corresponds to participant number

11. Apply topical analgesia (cream or spray) according to SOP instructions (if parents allow, this could be applied at the beginning of the visit to avoid delays when doing the blood test)

12. Blood sampling: Up to 4mls (0.8-0.9 mls/kg)

13. Collect Mucosal Lining Fluid (MLF) using synthetic absorptive matrices (SAM)

Then:

1.Administer 0.5ml DTaP-IPV-Hib-Hep B by intra-muscular injection into the upper right antero-lateral thigh (aP group) record in source document and in red book

2. Administer 0.5ml DTwP-Hib-Hep B by intra-muscular injection into the upper right antero-lateral thigh (wP group), record in source document and in red book

3. Administer 0.5ml PCV by intra-muscular injection into the upper left antero-lateral thigh record in source document and in red book (both aP and wP groups)

4. Administer 0.5ml IPV by intra-muscular injection into the lower left antero-lateral thigh (wP group), record in source document and in red book

5. Administer Rotavirus vaccine oral drops

6. Issue eDiary login details to parent (or give paper diary), ask parent to login and change password. Demonstrate eDiary (or paper diary) to parents for recording AEs and paracetamol +/- ibuprofen use and check understanding.

7. Issue a digital thermometer and ruler. Explain how to measure and record temperature, local and systemic reactions, AEs (for 7 days post any vaccination) and concomitant medications (for 28 days post vaccination).

8. Issue the parents/ legal guardian with a continuous temperature-monitoring device together with instructions. Fit on the child and demonstrate how to fit, check function and remove device

9. Observe the participant for 15 minutes (or for 30 minutes for participants who have received Comvac5) after vaccination for any significant acute reactions, with appropriate medical treatment readily available in case of anaphylactic reaction. Any AEs occurring during the observation period should be recorded in the source document

10.Issue (only if parent's do not have any) and provide instructions on the use of paracetamol prophylaxis to those whose infant is randomised to receive wP

11. Instruct parent/legal guardians to contact the study team immediately should the infant manifest any signs/ symptoms they perceive as serious or if the infant is admitted to the hospital.

12. Leave with parents/guardians anaesthetic cream (unless spray is to be used), dressing pads and instructions for blood test visit (V5, at 5months).

13. Schedule next visit

14. Study staff complete red book, paperwork in case of no direct data entry and enter onto database

9.5.2 Visit 2: 7 days after V1 (range 7-14 days)

1. Check inclusion and exclusion criteria if still valid

2. Review eDiary or paper diary for AEs/SAEs since last visit

3. Record and report AEs/SAEs that occurred since last visit

4. Record paracetamol and/or ibuprofen use in 72h prior to immunisation.

5. Measure and record the participant axillary temperature

6. Administer 0.5ml dose of 4CMenB by intra-muscular injection into the left antero-lateral thigh, record site and time of vaccination and batch number in source document and in red book

7. After explanation according to JCVI recommendations, parents would be advised to give 2.5 mls of paracetamol (120mg/5ml) orally immediately after vaccination, and 2 other doses 4-6hours apart after last dose.

8. Observe the participant for 15 minutes after vaccination for any significant acute reactions, with appropriate medical treatment readily available in case of anaphylactic reaction. Any AEs occurring during the observation period should be recorded in the source document

9. Instruct parent/legal guardians to contact the study team immediately should they manifest any signs/ symptoms they perceive as serious or if the child is admitted overnight to hospital.

10. Leave with parents/guardians anaesthetic cream (unless spray to be used), dressing pads and instructions for blood test visit if not given previously

11. Schedule next visit

12. Study staff complete red book, paperwork in the case of no direct data entry and enter onto database

9.5.3 Visit 3: 56 days post V1 (+14 days)

1. Check Inclusion/ exclusion criteria are still valid

2. Review eDiary or paper diary for AEs/SAEs and prescription medicine since the last visit

3. Record and report any AEs/SAEs that have occurred since the last visit.

4. Issue new eDiary or paper diary and remind parents to measure and record temperature, local and systemic reactions, AEs (for 7 days post any vaccination) and concomitant medications (for 28 days post vaccination).

5. Measure and record axillary temperature

6. Administer 0.5ml DTaP-IPV-Hib-Hep B by intra-muscular injection into the upper right antero-lateral thigh (aP group), record in source document and in red book

7. Administer 0.5ml DTwP-Hib-Hep B by intra-muscular injection into the upper right antero-lateral thigh (wP group), record in source document and in red book

8. Administer 0.5ml PCV by intra-muscular injection into the upper left antero-lateral thigh record in source document and in red book

9. Administer 0.5ml IPV by intra-muscular injection into the lower left antero-lateral thigh (wP group), record in source document and in red book

10. Administer Rotavirus vaccine oral drops

11. Issue the parents/ legal guardian with a continuous temperature-monitoring device, together with instructions. Fit on the child and demonstrate how to fit, check function

12. Check parent familiar with how to measure and record temperature, local and systemic reactions, AEs (for 7 days post any vaccination) and concomitant medications (for 28 days post vaccination).

13. Observe the participant for 15 minutes after vaccination for any significant acute reactions, with appropriate medical treatment readily available in case of anaphylactic reaction. Any AEs occurring during the observation period should be recorded in the source document

14. Issue (only if parent's do not have any) and provide instructions on the use of paracetamol prophylaxis to those whose infant is randomised to receive wP

15. Instruct parent/legal guardians to contact the study team immediately should they manifest any signs/ symptoms they perceive as serious or if the child is admitted to the hospital.

16. Leave with parents/guardians anaesthetic cream (unless spray to be used), dressing pads and instructions for blood test visit if not given previously.

17. Schedule next visit

18. Study staff complete red book, paperwork in case of no direct data entry and enter onto database

9.5.4 Visit 4: 7 days after V3 (range 7-14 days*)*

1. Check inclusion and exclusion criteria if still valid

2. Review eDiary or paper diary for AEs/SAEs since last visit

3 Record and report AEs/SAEs that occurred since last visit

4. Record paracetamol and/or ibuprofen use in 72h prior to immunisation.

5. Measure and record the participants axillary temperature

6. Administer 0.5ml dose of 4CMenB by intra-muscular injection into the left antero-lateral thigh, record site and time of vaccination and batch number in source document and in red book

7. After explanation according to JCVI recommendations, parents would be advised to give 2.5 mls of paracetamol (120mg/5ml) orally immediately after vaccination, and 2 other doses 4-6 hours apart after last dose.

8. Observe the participant for 15 minutes after vaccination for any significant acute reactions, with appropriate medical treatment readily available in case of anaphylactic reaction. Any AEs occurring during the observation period should be recorded in the source document

9. Instruct parent/legal guardians to contact the study team immediately should they manifest any signs/ symptoms they perceive as serious or if the child is admitted to the hospital.

10. Leave with parents/guardians anaesthetic cream (unless spray to be used), dressing pads and instructions for blood test visit if not previously given.

11. Schedule next visit

12. Study staff complete, red book, paperwork and enter onto database

9.5.5 Visit 5: 28 days post V4 (+ 14 days) (all participants)

1. Check Inclusion/ exclusion criteria are still valid

2. Review eDiary or paper diary for AEs/SAEs and prescription medicine since the last visit

3. Record and report any AEs/SAEs that have occurred since the last visit.

4. Measure and record axillary temperature

5. Blood sampling: obtain up to 4 mls

6. Collect Mucosal Lining Fluid (MLF) using synthetic absorptive matrices (SAM)

7. Instruct parent/legal guardians to contact the study team immediately should they manifest any signs/ symptoms they perceive as serious or if the child is admitted to the hospital.

8. Parents informed of parental motivation and satisfaction survey questionnaire link that will be sent to them before the V6 visit.8. Schedule next visit

9. Leave with parents/guardians anaesthetic cream (unless spray to be used), dressing pads and instructions for blood test visit (V6).

10. Complete paperwork in case of no direct data entry and enter onto database

9.5.6 Visit 6: 12 months +14 days (all participants)

1. Check Inclusion/ exclusion criteria are still valid

2. Review eDiary or paper diary for AEs/SAEs and prescription medicine since the last visit

3. Record and report any AEs/SAEs that have occurred since the last visit.

4. Issue new eDiary or paper diary and remind parents to measure and record temperature, local and systemic reactions, AEs (for 7 days post any vaccination) and concomitant medications (for 28 days post vaccination).

5. Measure and record axillary temperature

6. Blood sampling: obtain up to 6 mls

7. Collect Mucosal Lining Fluid (MLF) using synthetic absorptive matrices (SAM)

8. Administer 0.5ml DTaP-IPV-Hib-Hep B by intra-muscular injection into the upper right antero-lateral thigh record in source document and in red book

9. Administer 0.5ml PCV by intra-muscular injection into the upper left antero-lateral thigh record in source document and in red book

10. Explain how to measure and record temperature, local and systemic reactions, AEs (for 7 days post any vaccination) and concomitant medications (for 28 days post vaccination).

11. Issue the parents/ legal guardian with a continuous temperature-monitoring device, together with instructions. Fit on the child and demonstrate how to fit, check function and remove device

12. Observe the participant for 15 minutes after vaccination for any significant acute reactions, with appropriate medical treatment readily available in case of anaphylactic reaction. Any AEs occurring during the observation period should be recorded in the source document

13. Instruct parent/legal guardians to contact the study team immediately should they manifest any signs/ symptoms they perceive as serious or if the child is admitted to the hospital.

14. Leave with parents/guardians anaesthetic cream (unless spray to be used), dressing pads and instructions for next blood test visit (V7).

15. Schedule next visit

16. Study staff complete, paperwork in case of no direct data entry and enter onto database

9.5.7 Visit 7: 28 days post V6 (+14 days)

Applicable for all sub-groups

1. Check Inclusion/ exclusion criteria are still valid

2. Review eDiary or paper diary for AEs/SAEs and prescription medicine since the last visit

3. Record and report any AEs/SAEs that have occurred since the last visit.

4. Measure and record axillary temperature

5. Blood sampling: obtain up to 6 mls

6. Collect Mucosal Lining Fluid (MLF) using synthetic absorptive matrices (SAM)

7. Administer 0.5ml MMR by intra-muscular injection into the upper right antero-lateral thigh record in source document and in red book

8. Administer 0.5ml Men C by intra-muscular injection into the lower right antero-lateral thigh record in source document and in red book.

9. Administer 0.5ml dose of 4CMenB by intra-muscular injection into the left antero-lateral thigh record site and time of vaccinations and batch number in source document and in red book

10. Observe the participant for 15 minutes after vaccination for any significant acute reactions, with appropriate medical treatment readily available in case of anaphylactic reaction. Any AEs occurring during the observation period should be recorded in the source document

11. Instruct parent/legal guardians to contact the study team immediately should they manifest any signs/ symptoms they perceive as serious or if the child is admitted to the hospital.

12. Complete paperwork in case of no direct data entry and enter onto database

13. Give letter of “end of the study” to the parents

9.6 Blood sampling

Blood sampling will be carried out in line with SOPs. A local anaesthetic cream will be applied for an appropriate period of time prior to each venepuncture. The maximum blood volumes requested

for each sample are in accordance with the NIHR Medicines for Children Research Network[57], that states that “per individual, the trial-related blood loss should not exceed **3%** of the total blood volume during a period of **four weeks** and should **not exceed 1% at any single time**. **The total volume of blood is estimated at 80 to 90 ml/kg body weight”**; **3% is 2.7 ml blood per kg body weight** considering a total estimated volume of 90 ml/kg body weight.

For the calculations, we considered the WHO centile curves for girls and boys on the 5th centile as representative of the smallest participant and the visit schedule one-month period. Based on these estimates a maximum of 4 ml of blood could be safely obtained during blood visits for laboratory analysis in participants up to 6 months of age (V1 (baseline), and V5), and a maximum of 6 mls in participants at 12 and 13 months of age (V7, V8). Blood volumes and assays for each group are shown in **table 3**

If the initial attempt at venepuncture is unsuccessful, verbal consent will be sought from the parents for a further attempt at that visit. No more than two attempts at venepuncture will be made.

9.7 Mucosal lining fluid sampling

Nasal absorption is performed by manoeuvring a strip of synthetic absorptive matrices (SAM) up the lumen of the nostril, avoiding rubbing against the nasal mucosa. The outside of the nose is then pressed with a finger to cause apposition of the SAM against the mucosa. The procedure may tickle slightly but is painless, and MLF can be obtained even from non-inflamed noses at frequent intervals, without the need for local anaesthetic. Imperial College London has performed successful studies with nasal strips in adults after nasal allergen challenge (NAC), in babies and in young children, showing the applicability of this procedure to this age group. There is minimal protein binding to the SAM strip, and fluid can be eluted by spin filtration. High levels of mediators of inflammation can then be measured in the MLF: higher than detectable by nasal lavage.

9.8 Recording symptoms electronic diary card (RedCap)

The parents of participants will be asked to maintain a diary card detailing all (solicited and unsolicited) reactions in the 7 days following the 2, 4, and 12 months vaccinations (V1, V3 & V6), as well as prescription medications that are given in the 28 days that follow these time-points.

The parents will be issued an electronic diary (using RedCap). In case the parents cannot access the electronic diary, they can record the requested information on a paper diary, which has the same exact structure as the ediary.

The electronic diary will include space to record the following reactions at these time points:

1. **Local reactions** (erythema, induration and swelling) and tenderness. Parents will be issued with a ruler to measure any local reactions, and tenderness severity will be graded as shown in **table 2**

2. **Temperature**. Parents will be asked to record axillary temperatures at four and eight hours post vaccine and once daily from then on using the digital thermometer provided. The axillary temperature will also be measured if, at any time, the parent feels that the child may have developed a fever.

3. **Solicited systemic reactions**. The electronic and paper diary will include a grid for parents to record the presence or absence of a number of systemic symptoms that have previously been reported following vaccination: change in eating habits, drowsiness, irritability, and change in activity, vomiting and diarrhoea. Parents will grade severity according to the descriptions in **Table 2**.

4. **Unsolicited adverse events**. There will be space to record any other symptoms that occur in the 7days post vaccination.

The electronic or paper diary will be reviewed at each post vaccination visit. If symptoms persist after 7 days, an end-date should be entered if available. If concomitant medication persists beyond day 28, an end date will be entered if available. Parents/ legal guardians will be provided with a 24-hour phone number to access a member of the study team should they require urgent advice

Table 2: Grading local and systemic reactions

	Grading of severity		
Solicited Reactions	Mild (1)	Moderate (2)	Severe (3)
Tenderness	Minor reaction to touch	Cries/protests on touch	Cries when limb is moved / spontaneously painful
Change in feeding habit	Feeding less than usual / no effect on normal activity	Feeding less than usual / interferes with normal activity	Not feeding at all
Drowsiness	Drowsiness easily tolerated	Drowsiness that interferes with normal	Drowsiness that prevents normal activity

		activity	
Reduction in normal activity	Less interested in surroundings, toys etc	No interest in above and sleeping through feeds	Sleeping most of the time
Irritability/fussiness	Crying more than usual / no effect on normal activity	Crying more than usual / interferes with normal activity	Crying that cannot be comforted / prevents normal activity
Vomiting	1-2 episodes without interfering with routine	Several episodes & cannot keep any food down	Frequent episodes & taking nothing by mouth
Diarrhoea	More loose stools than usual	Frequent runny stools without much solid material	Multiple liquid stools without much solid material

9.9 Concomitant Medication

Prescription medication taken within 28 days following the 2 or 4 month and 12 month vaccinations will be recorded by parents on the electronic diary system (or paper diary), together with the reason for starting it, and the start and stop date if applicable. Ibuprofen and paracetamol use within 72 hours prior to immunisation and within 7 days following immunisation at these visits will be recorded. Antibiotic use in the 7 days preceding a visit will be recorded. Electronic (or paper) diary entries will be reviewed with the clinical staff at the next visit.

9.10 Continuous temperature monitoring

A skin sensor temperature monitoring device with data logger will be issued to the parent/legal guardian for use in the first 24 hours following the 2, 4 and 12 months vaccinations (V1, V3 and V6). Parents will be given instructions in how to fit the temperature-monitoring device and how to check it remains in place, and what to do with the device at the end of the monitoring period. If the device fails or is not tolerated, the child will remain enrolled in the study and any data already collected will be used for descriptive analysis. If no continuous data is generated, the axillary temperatures alone will be analysed. Temperature monitoring devices can be collected either by a

member of a study team if the next visit occurs within 7 days and/or the device can be returned via post by the parents, using pre-paid envelops provided by the study team. The rational for using this system in this study, is based on previous studies that have shown that intermittent temperature measurements recorded less episodes of fever when compared with the continuous temperature monitoring.

9.11 Potential risks for participants and mitigation strategies

1) **Immunisation schedule:** In this study we will be changing the times of the primary immunisations, for a different schedule when compared with the UK routine schedule. Currently the UK primary immunisation schedule is at 2, 3 and 4 months of age, and in this study it will be at 2 and 4 months. This schedule change, only affects the timing of administration for a few of the immunisations, **but the number of total doses administered will not change**. The pertussis vaccine, will be discussed separately in the next section.

The vaccines that would have variations from the routine schedule are:

- Rotavirus vaccine (rotarix®): currently given in the UK at 2 and 3 months of age. In our study it would be given at 2 and 4 months of age. This schedule remains in the recommended timelines [58], i.e. the first dose provided after 6 weeks of age and last dose before 6 months of age. The study schedule is similar to other European countries and we do not anticipate that this alteration would have any negative impact on the study population.

- Meningitis B vaccine (Bexsero®): This vaccine is going to be administered at the recommended ages according to the UK routine schedule (2 and 4 months). A small variation compared with the routine procedures will occur. The vaccine will be given separately at 7-14 days after the 2 and 4 month immunisations. The main reason for this slight variation is that the Meningitis B vaccine is considered very reactogenic and in order to better assess possible side effects/reactogenecity of the study vaccine, we think it is preferable to separate the administration of those 2 vaccines. We do not anticipate that this change would have any negative impact since children are normally vaccinated in the routine schedule in a time frame of 2 weeks, from the first day that they are eligible.

- Pneumococcal vaccine, PCV (Prevenar13®): the UK schedule is changing during early 2020 from that used in the current study, 2 and 4 months and booster at 12 months (2+1) to a single dose of PCV to be given at 3 months followed by the booster dose at 12 months (1+1 schedule). Given this is the first 1+1 schedule to be used globally and the current study has no visits at 3 months the

current study will keep a 2+1 PCV schedule. There is not thought to be a significant difference in the protection from pneumococcal disease in UK infants using either schedule and the two dose schedule will have the benefit of avoiding an extra visit at 3 months of age.

2) Pertussis vaccines: In this study, the main focus is going to be the pertussis vaccine that would be administered in two different formulations:

- aP group would receive the DTaP-IPV-Hib- Hep B (Infanrix hexa ®), a licensed vaccine in Europe. This vaccine was introduced into the UK routine schedule for babies born from 1st of August 2017, replacing the previous vaccine, a similar formulation that didn't contain the hepatitis B vaccine. Since the vaccine is the same as the current vaccine on the schedule, we do not anticipated any differences in side effects or any increased risk. This vaccine will also be the booster vaccine (12 months) for both control and test groups.

-wP group: The participants will receive the DTwP- Hib-Hep B (Comvac 5 ®) + Imovax ® polio at 2 and 4 months of age. The Imovax ® polio is licensed in Europe and Comvac 5® has manufacturing approval in India. Whole cell pertussis vaccines are given routinely in low and middle income countries throughout the world, and were in use in the UK until 2004. In general wP vaccines are more reactogenic than acellular vaccines, in terms of fever, irritability, and local reactions, although the symptoms are normally short lived and more common after booster doses. Other side effects, such as hypotonic hyporesponsive episodes, although rare are considered to occur at higher frequency (see Introduction: Adverse events after pertussis immunisation – aP and wP). In keeping with UK practice for MenB vaccines, which are considered relatively reactogenic, prophylactic paracetamol will be suggested for those infants given wP. This will follow the recommendation of the the UK department of health guidance for MenB given in the "Green Book" chapter on meningococcal disease (https://www.gov.uk/government/collections/immunisation-against-infectious-disease-the-green-book). "*A 2.5ml dose of liquid paracetamol (infant paracetamol 120mg/5ml) should be given orally as soon as possible after vaccination, followed by a second 2.5 ml dose after 4-6 hours and a third 2.5 ml dose 4-6 hours after the second dose. Should fever persist following the third dose and provided that the child appears otherwise well, additional doses of paracetamol may be administered at intervals of four to six hours for up to 48 hours. Parents should be advised to seek medical advice if their child is noticeably unwell with a fever present, or if the fever occurs at other times*"

This vaccine has thimerosal in the composition as many of the other current vaccines. Thimerosal is used in vaccines as a preservative because of its anti-fungal and anti-septic properties. US Food and Drug administration (FDA) reported that approved vaccines that contain thimerosal as a

preservative have been proven to be safe under the regulatory requirements of this entity and the same position on safety was announced by the WHO and CDC. Although ComVac5 is not on the list of FDA approved vaccines, the amount of thimerosal and, consequently, mercury is similar to the vaccines listed (0.029 mg/0.5 ml of thimerosal; 29µg/0.5ml of mercury). WHO recommend the whole cell vaccines as the preferred vaccine for protection against Bordetella pertussis and it is used in the majority of the infants globally

All the adverse events are going to be recorded and evaluated by the DSMC.

3) Pertussis vaccines schedule: In our study the above described vaccines are going to be administrated at 2, 4 and 12 months of age (2+1). The rational for this change was that a minimum of two doses are required for protection according to WHO. The 2+1 schedule is part of NIP's in several European countries, especially Nordic, including Finland. If this schedule is performed at 2, 4 and 12 months, it is considered to combine the advantages of early protection in the most vulnerable age group with the advantages of a booster, when compared with the 3+0 schedules.. Although the 1st immunisation at 2 months of age might not induce antibody levels that would confer high level of protection in this population before the 2nd immunisation at 4 months of age, the infants will be already protected due to passive immunisation through their mothers. Since 2012, the UK Department of Health did the first recommendation to immunise all pregnant women with the pertussis vaccine since previous studies using tetanus, diphtheria and aP-containing (TdaP) combination vaccines during pregnancy have shown to reduce pertussis-related infant mortality and morbidity. All the infants recruited, would have this protection since it is an inclusion criterion for the study. Based on all this information and in the herd immunity, we do not believe that we are increasing any risk to the study population

4) Blood samples: All the children will need to have blood taken as part of the study. We do not anticipate any main issues with this procedure, since all the maximum volumes were calculated according to the NIHR Medicines for Children Research Network guidelines, and all the study staff carrying out the procedure are trained to do so. One of the main side effects is the risk of bruising after the procedure, which is normally not associated to any sequelae and short lived.

9.12 Discontinuation/Withdrawal of Participants from Study

The parent/ legal guardian has the right to withdraw their infant from the study at any time without having to provide a reason for doing so. 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 undisclosed at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or study requirements
- An adverse event, which requires discontinuation of the study medication or results in inability to continue to comply with study procedures.
- Lost to follow up- this will be declared when 3 attempts to contact by phone/email at different times of the day have been unsuccessful and a letter has been sent to the address with no reply within 2 weeks
- Participants who are withdrawn will not be replaced but data collected prior to discontinuation or withdrawal may still be used for analysis. The reason for withdrawal will be recorded in the source document and eCRF. 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. If a participant were to withdraw early, his/her GP and the Child Health Computer Department will be informed of any vaccinations that have been given, as well as outstanding routine immunisations.

9.13. Definition of End of Study

The end of study defined as the when laboratory analysis of samples for primary and secondary end-points has been completed for all biological samples.

10 INTERVENTIONS

Vaccine products to be used	Commercial name	IMP/non-IMP
DTaP-IPV-Hib- Hep B	Infanrix hexa ® (GSK)	IMP
DTwP- Hib-Hep B	Comvac 5 ® (Bharat Biotech)	IMP

IPV	Imovax ® polio (Sanofi Pasteur Ltd)	IMP
PCV 13	Prevenar 13 ® (Pfizer Ltd)	IMP
Men B	Bexsero ® (GSK)	non-IMP
Men C	Neisvac ® (Pfizer Ltd)	non-IMP
Rota	Rotarix ® (GSK	non-IMP
MMR	Priorix ® (GSK) or M-M-RVAXPRO ® (Merck,Sharp and Dohme) UK	non-IMP

10.1 Study vaccines

The following vaccines will be used in this study:

Investigational products:

- **DTaP-IPV-Hib- Hep B**: Infanrix hexa ® (GSK): is diphtheria (D), tetanus (T), pertussis (acellular component) (Pa), hepatitis B (rDNA) (HBV), poliomyelitis (inactivated (IPV) and *Haemophilus influenza* type b (Hib) conjugated vaccine (adsorbed). The vaccine contains 3 *Bordetella pertussis* antigens: Pertussis Toxoid (PT), filamentous Haemagglutinin (FHA) and pertactin (PRN). The vaccine is indicated for primary and booster to prevent diseases described above and is licensed to be used in the European Union, in children between 6 weeks and 36 months of age. Infanrix-hexa will be administered intramuscular as a 2-dose primary (2 and 4 months) and a booster at 12 months of age as described in the SMPC section 4.2. Infanrix-hexa will also be used as a booster at 12 months for the children primed with DTwP-Hib-Hep B an immovax-IPV. The vaccine will be purchased commercially in single dose prefilled syringe and a vial containing Hib powder, preparation will be done according to section 6.6 of the SmpC.

- **DTwP- Hib-Hep B:** Comvac 5 ® (Bharat Biotech) diphtheria (D), tetanus (T), pertussis (whole cell) (w), hepatitis B (rDNA) (HBV), and *Haemophilus influenza* type b (Hib) conjugated vaccine (adsorbed). Will be given intramuscular as a 2-dose primary (2 and 4 months).

- **IPV:** Imovax ® polio (Sanofi Pasteur Ltd): Inactivated poliomyelitis vaccine, containing 3 types of the poliovirus: type 1 (Mahoney), type 2 (MEF1) and type 3 (Saukett). Is indicated for infants from 2 months of age, children and adults for both primary and booster doses. This vaccine is licensed in 60 countries including France and Finland. Imovax-polio will be given intramuscular as a 2-dose primary (2 and 4 months). The vaccine will be purchased commercially in single dose prefilled syringes, preparation will be done according to section 6.6 of the SmpC
- **PCV13**: Prevenar13® (Pfizer Limited): a pneumococcal conjugate vaccine that contains 13 different pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) and is licensed in Europe. It is part of the UK routine schedule at 2, 4 and 12 months of age. This vaccine is only going to be administered at 2, 4 and 12 months of age

Non- Investigational products:

The routine NIP vaccines are not part of this study, but will be given to all infants in the study by the study team for practical reasons.

- **Men B:** Bexsero® (Novartis): a meningococcal serogroup B vaccine adsorbed rDNA vaccine. This vaccine is licensed in Europe and currently given as part of the UK routine schedule (2, 4 and 12 months).
- **Men C:** Neisvac-C ® (Pfizer Ltd): Meningococcal Group C Polysaccharide Conjugated Vaccine adsorbed. Is recommended for active immunisation in children from 2 months of age, adolescents and adults for prevention of the *Neisseria meningitidis* serogroup C disease and is licensed in Europe. MenC is currently part of the UK immunization schedule at 12 months of age (Menitorix: MenC and Hib combined vaccine). We intend to administer the monovalent MenC vaccine (Neisvac-C ®) because the Hib component is contained in infanrix-hexa.
- **Rotavirus:** Rotarix® (GSK): a live attenuated vaccine (RIX4414 strain) that is administered orally as a suspension in a pre-filled oral applicator. It protects against gastroenteritis caused by rotavirus. This vaccine is licensed and is part of UK routine schedule at 2 and 3 months. This vaccine is only going to be administered at 2 and 4 months of age

- **MMR:** Priorix® (GSK): a live attenuated vaccine that protects against measles (Schwarz strain), mumps (RIT 4385 strain) and rubella (Wistar RA 27/3 strain) or M-M-RVAXPRO ® (Merck,Sharp and Dohme) UK. Is indicated for active immunisation in children more than 9 months of age, adolescents and adults, to prevent the diseases above described. This vaccine is licensed in Europe and is given in the UK as part of the routine schedule at 12 months and 3 ½ years of age. This vaccine is going to be administered in UK at 13 months of age

Where needles are not supplied with the vaccine a 25mm, 23 gauge (0.6mm) needle will be used.

10.2 Storage of IMP and other vaccinations

The IMP and other vaccines on the trial will be stored at the study site following the manufactured recommendation and as per existing SOPs. Vaccines will be shipped at +2ºC to +8ºC to the study site. Upon receipt at the study site, vaccines will be immediately transferred to a +2ºC to +8ºC temperature monitored refrigerator for storage.

The refrigerator will be secure and have controlled access. Cool boxes with an attached thermometer will be used while transporting the study treatment during scheduled visits.

The temperature of the refrigerator is remotely monitored and the site will be informed of any excursions in temperature. In case of temperature deviations, the study vaccines cannot be used and should be quarantined until authorisation to use the vaccine is received from the manufacturers.

10.3 Compliance with Trial Treatment

All vaccines will be administered by study staff during the visits therefore compliance will not be an issue.

10.4 Accountability of the Trial Treatment

The study vaccine (Comvac 5®) will be manufactured and sourced from Bharat Biotech, based in India.

Other study vaccines will be purchased commercially and distributed to the study sites through OTC Direct (http://www.otcdirectltd.co.uk/) or obtained as part of the usual supply from the respective national immunisation programs.

A vaccine accountability log will be used to check that supplies used and remaining vaccine numbers matched at all times. All used packaging and any unused or damaged vaccines will be destroyed locally at the end of the study depending on the manufacturer's instructions.

11. OTHER NON-INVESTIGATIONAL PRODUCTS

All infants will be offered local anaesthetic cream to numb the skin before venepuncture.

11.1 Name and description of non-investigational product(s)

A local anaesthetic cream (EMLA®/ AMETOP®) will be applied for an appropriate period of time prior to each venepuncture. Written instructions about how the cream should be applied will be given and explained to the parents/guardians.

EMLA 5%® contains Lidocaine 2.5% w/w (25 mg/g) and Prilocaine 2.5% w/w (25 mg/), with marketing authorisation number PL 39699/0088, first approved 16th May 1996 and renewaled on 5th of July 2002.

AMETOP® contains Tetracaine base 4.0% w/w with marketing authorisation number PL 14038/0001 on 09/10/2006.

EMLA® will be the first anaesthetic cream to be supplied to the parents for this population, that can be replaced by AMETOP® in case of previous or new allergy to EMLA®. In order not to increase the length of the visit, the study team can also apply AMETOP® in case the parents/guardians didn't apply the cream before the beginning of the visit, considering that the time for action for AMETOP ® is less (30 minutes) than EMLA® (60 minutes).

11.2 Summary of known and potential risks and benefits

Summary of known and potential risks and benefits can be found in the SmPC of each product.

12 LABORATORY

12.1 Sample collection

Blood samples collection:

Blood samples will be collected according to SOPs agreed across all Periscope partner sites and transported to the laboratory for further processing within 4 hours. Samples taken from the participant will be labelled with a participant, visit and site-specific number, according to the sample management plan.

Mucosal samples collection

Mucosal samples, from mucosal lining fluid (MLF) will be collected using synthetic absorptive matrices (MAP), according to SOPs agreed across all Periscope partner sites and transported to the laboratory until further testing.

Table 3: Summary of the type of assays which may be undertaken according to visits and sub-groups

	V1 Baseline Vaccines	**V2** Men B vaccine	**V3** Vaccines	**V4** Men B vaccine	**V5**	**V6** Vaccines	**V7** Vaccines
	2 M	2M +7d	4M	4M+d7	5M	12M	**13M**
aP	**4.0 mls**				**4.0 mls**	**6.0 mls**	**6.0 mls**
wP subgroups	**4.0 mls**				**4.0 mls**	**6.0 mls**	**6.0 mls**

Indication of typical blood volumes required for proposed assays as an explanation of total volumes needed (S=serum; Bpc=Plasma cellls T=T-cell assay; Bm=B-cell (memory); GEX: gene expression; Ef: Euroflow; CyT: CyTOF)

Serum : up to 1.0ml

Bm/Bpc : up to 3.0ml

T-cell assay : up to 3.0ml for whole blood assay and up to 6.0ml for 'in-depth' T-cell assay

PBMC storage: for exploratory assays (e.g. antibody repertoire, antigen-specific single-cell sorting)

Plasma, for use in some of the serological assays, will be collected from samples processed for cellular assays (e.g. B-cell, T-cell). For each time-point, in the event of lower volumes of blood being obtained than the maximal for that time-point, there will be a prioritisation of assays and the volumes to be used for each assay. The volumes stated above are an ideal volume to allow testing of the broadest array of antigens and phenotypes and a minimum volume will be defined to ensure that the primary and secondary end-points are achieved. This prioritisation will be stated clearly in the laboratory analysis plan.

12.2 Sample processing

Samples will be received, processed and stored in the local laboratory for further distribution to the central PERISCOPE Consortium laboratory in batches. Samples will be labelled with a barcoded label including the participant number, study visit and study site. The codes will be provided by the Consortium for uniformisation and easy access to the samples.

Some assays will be undertaken within the local laboratory from fresh and stored samples. Samples of serum, plasma, mucosal lining fluid, cellular material, RNA and DNA not to be used at the local laboratory will be shipped for storage at the PERISCOPE consortium Biobank at Radboud University Medical Centre, Geert Grooteplein-Zuid 10, 6525 GA Nijmegen, the Netherlands for subsequent use by investigators of the PERISCOPE consortium to address the primary, secondary and exploratory end-points of this study and also within the other studies of the Consortium. The laboratories that may be involved in these analyses are listed in Appendix A and B. These analyses will be according to a laboratory analysis plan, which will be developed as part of the study. Within PERISCOPE, a number of core assays will be developed in a standardized manner to allow comparison of immune responses to *B. pertussis* across the different clinical studies.

A more detailed analysis plan, including the PERISCOPE-wide strategy for biomarker identification, will be drafted by the Clinical Study Teams according to the Description of Action of the PERISCOPE consortium.

Blood sample processing:

The blood from the heparinised tubes will be spun and separated in the laboratory to provide plasma, peripheral blood mononuclear cells (PBMCs) and DNA isolation.

Samples will be used directly for immune analysis, or stored in freezers at -80°C and -135°C respectively, until further testing.

Table 3 describes the different assays, which are planned

Mucosal sample processing:

Freshly collected MLF samples will be used directly for immune analysis, or processed and stored in freezers at -20ºC/-80ºC, until further testing.

12.3 Sample storage

To ensure uniform handling and processing of samples the ones that can be frozen without loss of quality will be stored in the PERISCOPE biobank, which is located at the Radboud University Medical Centre, Nijmegen, the Netherlands. This includes storage for short periods of samples that will be analysed at labs other than where the samples were obtained and material left over after analyses are done. The labs where the analyses will be done during the PERISCOPE project are all part of the PERISCOPE consortium. The PERISCOPE biobank also includes storage of left over samples for maximally 10 years after the end of the PERISCOPE project. Any samples from the Biobank will only be used for the study purposes and objectives of the PERISCOPE project. This includes samples that are left after the duration of the project, as we anticipate analysis of samples will continue after the project has finished. The types of analyses that will be done on the samples during the project are described in the research proposal. Samples that are left over after the project will only be used to answer the research questions of the PERISCOPE project. To ensure this, we will install a group of senior PI's of PERISCOPE to oversee and decide on the use of the samples after PERISCOPE has ended.

The Radboud university medical center is listed in the Commercial Register of the Chamber of Commerce under file number 41055629.

12.4 Laboratory tests (primary objective)

Analysis of Serum/plasma antibody responses

The concentration of PT, other pertussis specific antibodies and antibodies relating to the non-pertussis vaccine antigens will be measured from plasma using a flow-cytometric based method with antigen-coated fluorescent beads (BioPlex/Luminex) at the National Institute for Public Health and the Environment – RIVM, Centre for Infectious Disease Control (CIb), Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, the Netherlands. In the fluorescent-bead-based multiplex immunoassay (MIA), each antigen of interest is coupled to beads with a distinct fluorescence. Combination of these beads enables the detection of antibodies directed against multiple antigens

in one single serum sample, using Luminex technology. IgG-PT antibody concentrations as primary outcome will be measured in duplicate in two dilutions using an in-house standard, calibrated on the WHO reference serum for pertussis as standard expressed in IU/ml.
Using different conjugates, IgG-total, IgG-subclasses and -avidity, and IgA-specific antibody concentrations can be measured in serum with the MIA. Besides anti-pertussis specific antibody concentrations, the other vaccine-specific IgG-antibody concentrations of diphtheria and tetanus will be measured with MIA in two dilutions using the national reference serum (IU/ml) as standard, which has been calibrated against the WHO standard [59-61].

12.5 Other laboratory tests (secondary and exploratory objectives)

Analysis of B-cell and T-cell responses **(secondary and exploratory objectives)**

One of the most studied potential correlates of protection in pertussis are serum antibodies. There is also evidence that B and T cells can have a relevant role. Studies have shown that the type of vaccine used in priming (aP vs wP) can effect the response of B and T cells to the booster vaccination [33, 62].
The rationale for this analysis is to increase our knowledge in how antigen specific B and T cells can be induced and maintained after different priming immunisation schedules (aP vs wP).
Memory B cell responses to Bp will be analysed by enzyme-linked immunospot assay (ELISpot) following B-cell culture with polyclonal stimulants. The ELISpot is a sensitive immunoassay to enumerate antigen specific antibody-secreting cells (ASCs). This assay will be undertaken using PBMCs that have previously been frozen following collection.
T-cell responses to Bp will be performed to determine the quantity, quality and persistence of antigen-specific T cell responses in the blood, allowing a comparison of cellular immunity in different groups of participants. This may involve a whole-blood stimulation assay which will be used and undertaken in the laboratory on fresh blood. Whole blood/PBMCs will be stimulated with antigens according to a protocol developed as part of the PERISCOPE consortium. The stimulated cells and supernatants will then be frozen pending subsequent transfer to collaborators in the PERISCOPE consortium for analysis by flow-cytometry and cytokine detection in supernatants.

Functional antibody responses

To better understand the functionality of pertussis vaccine-induced antibodies, that have a relevant role in protection against the disease, a variety of assays will be used in this study. The assays, as

an essential part of the PERISCOPE consortium, will be established as core standardised immunoassays. Although not limited to these assays alone, the *in vitro* assays may include: 1) adherence inhibition, to measure the ability of antibodies to inhibit the adherence of Bp to respiratory epithelial cells; 2) bacterial agglutination, to measure the ability of the antibodies to induce bacterial aggregation 3) pertussis toxin neutralization 4) opsonisation and 5) phagocytosis, to measure the uptake and killing by phagocytic cells.

Dynamics of the immune response to pertussis vaccination

Analysis of the genomic variation (SNP's candidates, B cell repertoire); epigenetic variation (methylation and histone modification) may be undertaken as part of exploratory analyses and linked to measure of Bcell and T cell response to better understand variation between individuals.

Analysis of mucosal antibody and/ or cytokine responses

The concentration of PT and other pertussis specific antibodies against vaccine antigens, as well as the concentration of T cell cytokines (e.g. IL17, IFNg and IL4/5/13), will be measured in mucosal samples, obtained from MLF. This will allow us to determine the induction and persistence of those antibodies and / or cytokines before (2 months) and after vaccination (5 and 12 months). Cytokine concentrations will be determined using methods such as Luminex. The determination of antibody responses will be determined using similar methods to serum and plasma samples (MIA, Bacterial adherence inhibition assay (BAI) and Serum bactericidal antibodies assays (SBA)).

13. SAFETY REPORTING

13.1 Premature termination or suspension of a trial

In accordance to sections 5.16 and 5.21 of the international committee on harmonisation (ICH) guidance on good clinical practice (GCP), the sponsor will suspend the study if there are sufficient grounds to believe that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited research ethics committee (REC) and competent authority (CA), according to local requirements. The study will be suspended pending a further positive decision by the accredited MREC. The investigator will take care that all subjects are kept informed.

Nevertheless, the local sponsor is entitled to terminate the study prematurely if this is beneficial to the health or welfare of the subjects and after advice from the DSMC.

The REC and CA will be informed about such a decision, according to local requirements.

In case of premature study termination, there will be no consequences for a participant other than that further blood sampling is suspended and any ongoing SAEs will be followed up.

13.2 Adverse events, serious adverse events and suspected unexpected serious adverse reactions - Definitions

13.2.1 Adverse events (AE)

a) Definition:

An AE or adverse event 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).

An AE can therefore 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.

13.2.2 Serious adverse events (SAE)

a) Definition:

A SAE is any untoward medical occurrence or affect that at any dose:

-results in death;

-is life threatening (at the time of the event);

-requires hospitalisation or prolongation of existing inpatients' hospitalisation;

-results in persistent or significant disability or incapacity;

-is a congenital anomaly or birth defect; or

-any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been, based upon appropriate judgement by the investigator.

A hospital admission for an elective procedure will not be considered as a SAE.

Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. For this specific case, hypotonic hyporesponsive episodes, even if they do not require hospitalisation, would be considered a medically relevant event and reported as an SAE if occuring within 48hours after immunisation. Although this event is considered rare it has been described to occur more commonly after wP vaccination compared to aP. Pertussis confirmed cases would be reported as an SAE, due to a reduced primary schedule (2+1) when compared with the current UK schedule (3+0).

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.

13.2.3 Adverse Reactions (AR)/ Suspected unexpected serious adverse reactions (SUSAR)

a) Definition:

ARs are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected ARs are suspected unexpected serious adverse reactions (SUSARs) if the following three conditions are met:

- the event must be serious (see section 9.2.2);
- there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
- the AR must be unexpected, that is to say, the nature and severity of the AR are not in agreement with the product information as recorded in the SmPC for an authorised medicinal product.

13.3 Adverse events, serious adverse events and suspected unexpected serious adverse reactions: Procedures

13.3.1.Procedures for Recording Adverse Events:

For the first 7 days after immunisation at visits 1, 3 and 6 (Day 0 to Day 7), all AEs observed by the study team or reported by the participant's parent/ legal guardian, whether or not attributed to study medication, will be recorded in the RedCap or paper diary. Reactions occurring in the first 7 days after immunisation (including day of immunisation) will be divided up into: solicited reactions, which will be considered related to study vaccine unless stated otherwise; and unsolicited adverse events, which will be assessed for relatedness and graded for severity by a medically qualified member of the study team. There will be space in the electronic or paper diary to record the end date of adverse events that continue beyond Day 7. The RedCap system will allow review by clinical staff of grade 3 AEs in real-time.

Medical events not considered SAEs that occur 7 days after immunisation do not need to be recorded. Because of the possibility that medication may interfere with the evolution of an immune response, the details of any prescription medication that is given within 28 days following the 2, 4 or 12 month vaccinations will be recorded on the eDiary or paper diary. Information on paracetamol/ ibuprofen use within 72 hours prior to immunisation and in the 7 days following immunisation will be specifically asked for.

AEs will be recorded using the following guidance:

- Pre-existing medical conditions (present before start of the AE collection period) are considered "concurrent medical conditions" and should not be recorded as AEs. However, if the participant experiences a worsening or complication of such a condition, the

worsening or complication should be recorded as an AE. Investigators should ensure that the AE term recorded captures the change in the condition (e.g. “worsening of”)

- Each AE should be recorded to represent a single diagnosis. Accompanying signs or symptoms (including abnormal laboratory values) should not be recorded as additional AEs.

All AEs that are considered related and result in a participant’s withdrawal from the study or are present at the end of the study should be followed up until the end of symptoms or the condition becomes stable.

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. A parent/ legal guardian may also voluntarily withdraw their child due to what he or she perceives as an intolerable AE.

13.3.2. Procedures for recording serious adverse events

a) SAE and SAR reporting:

All SAEs and severe adverse reactions (SAR) occurring for each participant from taking informed consent for their participation until the participant’s last visit will be reported. All SAEs must be reported on the SAE reporting form by a member of the clinical research team and reported to the PI within 24 hours. Relatedness and expectedness will be assessed by the clinical research team in discussion with the PI, and determined according to the Investigator’s Brochure for Comvac5 and approved SMPC for the other IMPs.

13.3.3 Procedures for Recording SUSAR’s

All SUSARs will be reported by the UK Chief investigator to the MHRA and the REC. Fatal and life-threatening SUSARS will be reported no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

13.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported until the end of the study, as defined in the protocol.

13.5 Data safety monitoring committee

For neonatal/maternal clinical trials performed by the PERISCOPE consortium, a DSMC is installed. The aims of the committee are to safeguard the interests of the trial participants and monitor the safety outcomes of the trials including SAEs. The DSMC for the maternal-neonatal studies is fully independent.

A separate DSMC charter will be prepared detailing the composition, roles and responsibilities of the DSMC. A summary of all the SAE's will be sent to the DSMC after recruitment of 50 and then 100 infants and following completed enrollment and all SAR's will be reported to the DSMC chair within 7 days of acknowledgement of the event.

Safety data will be collected by the study team and also reported by parents/legal guardians using the eDiary or paper diary or other communication with the investigator.

13.6 Temporary halt for reasons of participant safety

In accordance to sections 5.16 and 5.21 of the international committee on harmonisation (ICH) guidance on good clinical practice (GCP), the sponsor will suspend the study if there are sufficient grounds to believe that continuation of the study will jeopardise participants' health or safety. The sponsor will notify the accredited REC and CA, according to local requirements without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited REC. The investigator will take care that all participants are kept informed.

13.7 Development Safety Update Reports (DSUR)

The CI will submit (in addition to the expedited reporting of SUSARs) DSURs once a year

throughout the clinical trial, or on request, to the Competent Authority (MHRA in the UK).

14. STATISTICS AND ANALYSIS

14.1 Statistical methods and analysis plan

Responsibilities

Statistical analysis will be undertaken by the trial statsitican at the Oxford Vaccine Group, Oxford for the primary and secondary endpoints. Data will also be generated using samples sent to the Radboud University Medical Centre Biobank, Nijmegen, Netherlands for a variety of exploratory end-points. Responsibility for the analysis of these data will be with the individual collaborators undertaking the assays (see Appendices A and B) and will be co-ordinated by the PERISCOPE consortium lead for Data Analysis and Management.

Analysis for primary endpoint

The primary statistical analysis will be carried out on the basis of modified intention-to-treat (mITT). That is, participants who have received two doses of the vaccine will be included in the analysis. We will endeavour to obtain full follow-up on every participant to allow full mITT analysis, but we will inevitably experience the problem of missing data due to withdrawal and loss to follow-up.

The primary outcome is PT-specific antibody GMC concentration at 13 months of age. The GMC of PT-specific antibody will be compared between wP and aP under the hypothesis:

H_0: $GMC_{wP} / GMC_{aP} = 1$ or $log_{10}GMC_{wP} - log_{10}GMC_{aP} = 0$;

H_1: $GMC_{wP} / GMC_{aP} \neq 1$ or $log_{10}GMC_{wP} - log_{10}GMC_{aP} \neq 0$.

The antibody concentration will be transferred using logarithmic transformations (base 10) to render a normal distribution. We will test the above hypothesis using mixed effects model, adjusting for pre-specified covariates. Estimates of PT-specific antibody concentration with the corresponding 95% confidence intervals (CI) will be delivered from the model at 13 months to compare the antibody response after an aP booster between the wP and aP groups. Interactions with time of blood samples will be included in the models for each comparison as appropriate (e.g. time x Bp vaccination). The results from the trial will be presented as comparative summary statistics

(difference in log_{10}GMC) with 95% CIs, which will be calculated as the mean of log_{10}GMC in wP group compared with that in aP group.

An unstructured correlation matrix will be used to model the within-participant error correlation structure. We will also perform various sensitivity analyses using other imputation methods, e.g. multiple imputation and pattern mixture model, to test whether the results are robust to different assumptions about the missing data. The study results will be reported in accordance with the CONSORT (Consolidated Standards of Reporting Trials) 2010 statements.

A per-protocol analysis will also be carried out as secondary analysis, allowing the comparison between participants and groups that completed the study, since the main interest of the study is in understanding the biology of the vaccine. Analysing only participants with complete information would allow us to describe better the immune mechanisms associated to each intervention.

Reactogenicity and safety data will be analysed based on participants who received each primary immunisation schedule (aP vs wP) and for each dose of vaccine.

Understanding the immune mechanisms of aP and wP vaccination

To better understand the immune mechanisms associated with each intervention we will do comparative analyses between the cellular, molecular and immunological parameters. These analyses, which will be done under supervision of the consortium member Prof. Martijn Huynen of the Radboud UMC Nijmegen, will determine how the immunological assays correlate with each other, and which of the molecular (gene expression data) and cellular parameters (cell concentrations) show the most consistent and highest correlation with the immunogenicity data. Specific attention will be given to deconvoluting the cell-type specific gene expression data from the cellular concentrations and gene expression data to determine whether shifts in whole blood gene expression are mainly a reflection of shifts in cell concentrations or whether there are also significant shifts in gene expression per cell type indicative of activation of the immune response.

Combining the data from this study with other studies in the IMI Periscope project, we will perform cross-study analyses by comparing gene expression data, cell concentrations and immunological data of aP-vaccinated and wP-vaccinated groups with the aP and wP vaccinated Baboons and the human challenge model. These cross study analyses will delineate: 1) Whether there are consistent patterns across the data types: i.e. whether the molecular or immunological parameters that separate wP from aP vaccinated infants are also the ones that separate aP from wP vaccinated baboons. 2) Whether, as expected, molecular and immunological differences between aP and wP

vaccination correlate with parameters of protection in the human challenge model. Specific attention will be given to the alignment of the data from the various studies: i.e. to compare the timing of the various aspects of the immune response between aP and wP vaccination in infants and in Baboons to allow comparisons that are more meaningful.

14.2 The Level of Statistical Significance

Differences will be shown at a two-sided 5% significance level.

15. DATA MANAGEMENT

15.1 Source Data

In this study, the CRF and eDiary or paper diary entries will be considered source data, as these will be the site of the original recording for all assessments and measurements made during visits. Data for analysis will be entered onto an electronic database or eCRF for monitoring and analysis purposes. Other documents such as medical notes and GP letters may be used as source documents if required. All documents will be stored securely in confidential conditions. On all study-specific documents, other than the initial response form, participant contact sheet, the signed consent form, GP notification letters and unscheduled vaccination forms the participant will be referred to by a study participant number, not by name.

15.2 Access to Data

Direct access will be granted to authorised representatives from the Sponsor and/or host institution for monitoring, PERISCOPE Consortium members/partners and/or audit of the study to ensure compliance with regulations. Pseudonymised data will be made available to the PERISCOPE consortium members through the consortium's Data Management Team. This is in order to allow the use of samples in assays undertaken in the laboratories of the consortium (Appendices A and B) and to facilitate the integrate analysis of data from a range of studies using similar laboratory assays across the Consortium. This team is based at Radboud University Medical Centre, Geert Grooteplein-Zuid 10, 6525 GA Nijmegen, the Netherlands. This data management team at Radboud University are under the direction of prof. dr. Gert Vriend a co-investigator of the PERISCOPE consortium..

15.3 Data Recording and Record Keeping

All study files (paper and electronic) with demographic and clinical details on the participants will be kept in a locked research office at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM). The study data will subsequently be entered on to a computer with an electronic database protected by a password and encrypted transfer. All stored blood samples will be identified by study number only and will have no personal identifiers.

Information on study participants will be recorded on hard copy source documents held locally, and information will be entered into a web based electronic CRF

A dataset on each participant will also be entered on a secure electronic database designed (TRanSmart) and managed by the PERISCOPE consortium. This will include participant's gender, date of birth, ethnicity, name of study, vaccination dates and sample dates.

Following completion of the study, the trial master file, CRFs and all personal data will be kept until 3 years after the last participant has turned 18 years old, at Ardington Archives storage (Faringdon, Oxford) according to the relevant OVG/OVC SOP. Storage of this data will be reviewed every 5 years and files will be confidentially destroyed if storage is no longer required. Electronic data will be stored securely for the same period in University of Oxford electronic archives.

15.4 Data management

Data will be managed using clinical trials software for electronic data capture (EDC) and clinical data management (CDM), which enables compliance, with regulatory guidelines such as 21 CFR Part 11, as previously described. This system will allow the management of the data specific for this study, and will be hosted in the UK site.

Pseudonymised, processed data of the core, pre-core and exploratory assays that will be performed on the samples from the volunteers, will be also made available to the PERISCOPE consortium members by the consortium's data management team. PERISCOPE for this propose will use the tranSMART database system.

Using the system will enforce universal annotation of the data to allow sharing, comparison and dissemination. The data in tranSMART system will also contain pseudonymised patient IDs, including relevant clinical information imported from the CRFs that are entered in the database (e.g. gender and date of birth). Via the tranSMART system, those data will remain available after the conclusion of the PERISCOPE project. Universal annotation of the data allows the comparison of samples in assays undertaken in the laboratories of the consortium and the integrative analysis of

data from a range of studies using similar laboratory assays across the consortium. The data management team is based at Radboud University Medical Centre, Geert Grooteplein-Zuid 10, 6525 GA Nijmegen the Netherlands. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

A monitoring plan (risk based) detailing the monitoring activities will be finalised before the study is initiated and may include (but not be limited to) the following activities:

For the purpose of compliance with GCP, it may be necessary to conduct a site audit performed by authorised representatives of the sponsor(s) and/or a regulatory authority and/or the MREC and/or the site themselves. This may occur at any time from start to after conclusion of the study

16. SERIOUS BREACHES

A ‘serious breach’ means a breach likely to affect to a significant degree the safety and rights of a subject or the reliability and robustness of the data generated in the clinical trial.

A “serious breach” is defined as “A breach of GCP or the trial protocol” is a breach which is likely to affect to a significant degree:

(a) The safety and rights of a subject;

(b) The reliability and robustness of the data generated in the clinical trial.

Any serious breach of: (a) The Regulation (EU) No 536/2014 (b) The version of the protocol applicable at the time of the breach, should be reported within 7 days of the sponsor becoming aware of the breach (Appendix C details the entities that the study site will report to). The report should also be sent to the Trial Steering Committee and DSMC. (*adapted from reference:* Guideline for the notification of serious breaches of Regulation (EU) No 536/2014 or the clinical trial protocol, EMA/430909/2016)

17. ETHICAL AND REGULATORY CONSIDERATIONS

17.1 Declaration of Helsinki

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

17.2 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 and guideline clinical investigation of medicinal products in the paediatric population, document CPMP/ICH/2711/99, effective since July 2000.

NL only: This is also in accordance to the rules of the Dutch medical research involving human subjects act (WMO), under the general ruling of the clinical trial directive of the EU (2001/20/EU).

17.3 Good distribution practices

The Investigator will ensure that this study is conducted in full conformity with current relevant regulations and with the ICH Guidelines for Good Distribution Practice

17.4 Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), Competent Authority, and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

17.5 Reporting

The CI shall submit once a year throughout the study or on request, an Annual Progress report to the REC, competent authority, host organisation and Sponsor(s). Information will be provided on the date of inclusion of the first participant, numbers of participants included and numbers of participants that have completed the trial, SAEs/ SARs, other problems, and amendments. In addition, an End of Study notification and final report will be submitted to the same parties.

17.6 Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participants ID number and the participants initials on the CRF and any electronic database. All documents will be stored securely and only accessible by study staff and authorised personnel.

17.7 General data protection regulation

The investigator(s) will ensure that this study is conducted in full conformity with the general data protection regulation. The PERISCOPE steering committee is responsible for the privacy impact assessment of the Periscope project.

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participants ID number on the CRF and any electronic database. All documents and human materials will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the General Data Protection Regulation, which requires data to be de-identified as soon as it is practical to do so.

18 FINANCE AND INSURANCE

18.1 Funding

The study is funded by the Innovative Medicines Initiative (European Union Funding for Research and Innovation) and by the Bill and Melinda Gates Foundation.

18.3 Insurance

UK

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London, policy numbered :WD1200463).

19 TRIAL STEERING COMMITTEE

All significant operational matters relating to the research will be decided upon by the trial steering committee that would have as main objectives:

- provide advice, through its chair, to the investigators, the trial sponsor, the authority, the collaborators on all appropriate aspects of the trial
- concentrate on progress of the trial, adherence to the protocol, patient safety and the consideration of new information of relevance to the research question
- ensure that the rights, safety and well-being of the trial participants are the most important considerations and should prevail over the interests of science and society
- ensure appropriate ethical and other approvals are obtained in line with the project plan

- agree proposals for substantial protocol amendments and provide advice to the sponsor and funder regarding approvals of such amendments

The trial steering committee will meet monthly by teleconferences or at any time when reasonably considered necessary

20 PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the Innovative Medicines Initiative (European Union Funding for Research and Innovation) and by the Bill and Melinda Gates Foundation. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. A more detailed publication policy will be drafted by the Steering Committee of the PERISCOPE consortium and implemented according to the governance principles defined in the PERISCOPE Grant Agreement.

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22. APPENDIX A: Laboratories involved in analysis of samples from trial

List of assays which may be undertaken as part of the laboratory work related to primary, secondary and exploratory outcomes. Institutions listed are involved with either assay development or undertaking the assays on samples from the study. The list of abbreviations for the institutions is in Appendix B

Assay description	Lead institution	Partner institutions
T5.1: Serological anti-Bp antibody levels (MIA and ELISA)	RIVM	UTU, MRC, CEA
T5.2: Serum bactericidal assay (SBA)	IPL	PHE, SP, GSK, CEA
T5.3: Opsonophagocytosis assay (OPA)	PHE	SP, GSK, CEA
T5.4: Bacterial adherence inhibition assay(BAI)	RUMC	GSK, CEA
T5.5: Pertussis toxin neutralization assay (PTNA)	IPL	UTU, SP, GSK
T5.6: EuroFlow-based flowcytometric immunophenotyping of leukocyte	USAL	LUMC, ULB, RIVM, RUMC
T5.7: Bp-specific T-cell assay	ULB	RIVM, RUMC, TCD, ICL, CEA, USAL, LUMC, MRC
T5.8: Standardization of Bp-specific B-cell ELISpot assays	RIVM	UOXF, ICL, UTU, CEA
T5.9: Flowcytometric detection and quantitation of Bp-specific B-cell subsets	LUMC	USAL, IPL, RUMC, SP, RIVM, GSK
T5.10: IGH and IGK/IGL gene repertoire of B-cell subsets	LUMC	UOXF, USAL, RIVM, SP
T5.11: Innate immune response to Bp vaccination and infection	RUMC	CHUV, LUMC, SP
T5.12: Innovative methods for Bp-related T-cell biomarker discovery	RIVM	TCD, RUMC, CHUV, CEA, ULB, USAL, LUMC, UTU, IMIC
T5.13: Dissection of mucosal immunity against Bordetella pertussis	RUMC	PHE, USAL, GSK, RIVM
T5.14: Microbiological studies	UB	RIVM, IPL,IMIC

23. APPENDIX B: List of institutions and abbreviations for APPENDIX A

Institutions which may be involved in undertaking or developing assays related to the primary, secondary and exploratory end-points

LIST OF PARTICIPANTS

Participant No		Participant organisation name	Country
1	RUMC	Stichting Katholieke Universiteit (Radboud University Medical Center)	NL
2	UOXF	The Chancellor, Masters and Scholars of the University of Oxford	UK
3	PHE	Department of Health (Public Health England)	UK
4	IPL	Institut Pasteur de Lille Fondation	FR
5	TCD	The Provost, Fellows, Foundation Scholars and the other members of Board, of the College of the Holy and Undivided Trinity of Queen Elizabeth near Dublin (Trinity College Dublin)	IE
6	UTU	Turun Yliopisto (Turku University)	FI
7	CEA	Commissariat à l'énergie atomique et aux energies alternatives	FR
8	ICL	Imperial College of Science Technology and Medicine	UK
9	RIVM	Rijksinstituut voor Volksgezondheid en Milieu part of the Dutch State	NL
10	IMIC	Mikrobiologicky USTAV - AVCR, V.V.I. (Institute of Microbiology of the ASCR, v. v. i.)	CZ
11	UNIBAS/UKBB	Universitaet Basel	CH
12	UB	University of Bath	UK
13	LUMC	Leiden University Medical Center	NL
14	USAL	Universidad de Salamanca	ES
15	ULB	Université libre de BruxellesEmC	BE
16	CHUV	Hospices Cantonaux CHUV	CH
17	US	University of Southampton	UK
18	QB	Q-Biologicals NV	BE
19	MRC	Medical Research Council (The Gambia)	GM
20	EURICE	European Research and Project Office GmbH	DE
21	SP	Sanofi Pasteur SA	FR
22	GSK	GSK Biologicals SA	UK

24. APPENDIX C: SERIOUS BREACH* reporting: specific procedures

	UK
Entities to be informed	MHRA Sponsor REC

25. APPENDIX D: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
Substantial Amendment 1	V2.0	07-Nov-2019	Sarah Rhead/Dominic Kelly	Clarification of PCV13 schedule that will be followed for this study Clarification that either paper or electronic diaries can be used
Substantial Amendment 2	V2.1	27-Jan-2020	Sarah Rhead/Rachel White/Nelly Owino	Clarification of provision of prophylactic paracetamol to infants randomised to receive whole cell vaccine Clarification that mothers will be sent either a paper or an electronic consent form

				giving permission for the study team to access their medical records to confirm their pertussis immunisation status Addition of a sentence to clarify study visits will take into account infection control recommendations and measures in place in response to the COVID-19 outbreak.
Substantial Amendment 3	V3.0	18-Aug-2021	Kushalinii Hilson/Dominic Kelly/Bryn Horsington	Addition of a new document - Parent Participation Survey. The survey will be emailed to participants to understand their motivations for enrolling their child in the AWARE study.
Substantial Amendment 4	V4.0	18-Jan-22	Stanislava Koleva/Nelly Owino/Rachel White	Change of vaccination site for the MMR and Men-C vaccines Clarification that for the Measles,Mumps and Rubella (MMR) vaccine, either Priorix®

				or MMRVXPRO® can be used
Non Substantial Amendment 1	V4.1	08-Dec-23	Sophie Vernon	Change to planned study period, to extend trial to June 2024. Change of sponsor and monitor name from CTRG to RGEA.