The Immunological Basis for Immunization Series

Module 12: pneumococcal vaccines

Immunization, Vaccines and Biologicals

World Health Organization

The Immunological Basis for Immunization Series

Module 12: pneumococcal vaccines

Immunization, Vaccines and Biologicals

World Health Organization

WHO Library Cataloguing-in-Publication Data

The immunological basis for immunization series : module 12: pneumococcal vaccines / Helena Käyhty … [et al].

(Immunological basis for immunization series ; module 12)

1.Streptococcus pneumoniae - immunology. 2.Streptococcus pneumoniae - pathogenicity. 3.Pneumococcal infections - immunology. 4.Vaccines, Conjugate - standards. I.Käyhty, Helena. II.Nurkka, Anu. III.Soininen, Anu. IV.Väkeväinen, Merja. V.World Health Organization. VI.Series.

ISBN 978 92 4 159821 7 (NLM classification: WC 204)

The Department of Immunization, Vaccines and Biologicals thanks the donors whose unspecified financial support has made the production of this document possible.

This module was produced for Immunization, Vaccines and Biologicals, WHO, by:

Authors: Helena Käyhty, Ph.D; Anu Nurkka, Ph.D;
Anu Soininen, Ph.D; Merja Väkeväinen, Ph.D
Department of Vaccination and Immune Protection,
National Institute of Health and Welfare,
P.O. Box 30, 00271 Helsinki, Finland

The authors would like to thank
Dr David Goldblatt and Dr Carl Frasch, who reviewed the document.

Printed in September 2009

Copies of this publications as well as additional materials on immunization, vaccines and biological may be requested from:

World Health Organization
Department of Immunization, Vaccines and Biologicals
CH-1211 Geneva 27, Switzerland
• *Fax:* + 41 22 791 4227 • *Email:* vaccines@who.int •

Printed by the WHO Document Production Services, Geneva, Switzerland

Contents

Abbreviations and acronyms

AOM	acute otitis media
aP	acellular pertussis vaccine
CAP	community-acquired pneumonia
CI	confidence interval
C-PS	cell wall polysaccharide
CRM	nontoxic variant diphtheria toxin
DT	diphtheria toxoid
DTP	diphtheria, tetanus and pertussis combination vaccine
EIA	enzyme immunoassay
ELISPOT	enzyme linked immunospot assay
EPI	Expanded Programme on Immunization
FinOM	Finnish Otitis Media Vaccine Trial
GMC	geometric mean concentration
Hib	*Haemophilus influenzae* type b
HIV	human immunodeficiency virus
Ig	immunoglobulin, e.g. IgA, IgG, IgM
IPD	invasive pneumococcal disease
IPV	inactivated poliovirus vaccine
K	constant value
LXA	Luminex multiple analyte
MCV-C-CRM	meningococcal group C conjugate vaccine using CRM carrier
MMR	measles, mumps and rubella combination vaccine
NCKP	Northern California Kaiser Permanente
NVT	non-vaccine serotype
OMPC	outer membrane protein complex
OPA	opsonophagocytosis assay
PCV	pneumococcal conjugate vaccine
PCV7-CRM	7-valent PCV using CRM carrier
PCV9-CRM	9-valent PCV using CRM carrier

PCV7-OMPC	7-valent PCV using meningococcal OMPC carrier
PCV11-PD	11-valent PCV using *H. influenzae* protein D carrier
PCV4-T	4-valent PCV using TT carrier
PCV11-D-T	11-valent mixed carrier PCV using both DT and TT carrier
PD	protein D of *H. Influenzae*
POET	PCV11-PD efficacy trial conducted in Czeck Republic and Slovakia
PPSV	pneumococcal polysaccharide vaccine
PRP-CRM	Hib conjugate vaccine using CRM carrier
PRP-T	Hib conjugate vaccine using TT carrier
PS	polysaccharide
RIA	radio immunoassay
TT	tetanus toxoid
USA	the United States of America
VT	vaccine serotype
wP	whole cell pertussis vaccine
WHO	World Health Organization

Preface

This module is part of the series *The Immunological Basis for Immunization*, which was initially developed in 1993 as a set of eight modules focusing on the vaccines included in the Expanded Programme on Immunization (EPI)[1]. In addition to a general immunology module, each of the seven other modules covered one of the vaccines recommended as part of the EPI programme - diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. The modules have become some of the most widely used documents in the field of immunization.

With the development of the Global Immunization Vision and Strategy (GIVS) (2005-2015) (http://www.who.int/vaccines-documents/DocsPDF05/GIVS_Final_EN.pdf) and the expansion of immunization programmes in general, as well as the large accumulation of new knowledge since 1993, the decision was taken to update and extend this series.

The main purpose of the modules - which are published as separate disease/vaccine-specific modules - is to give immunization managers and vaccination professionals a brief and easily-understood overview of the scientific basis of vaccination, and also of the immunological basis for the World Health Organization (WHO) recommendations on vaccine use that, since 1998, have been published in the *Vaccine Position Papers* (http://www.who.int/immunization/documents/positionpapers_intro/en/index.html).

WHO would like to thank all the people who were involved in the development of the initial Immunological Basis for Immunization series, as well as those involved in its updating, and the development of new modules.

[1] This programme was established in 1974 with the main aim of providing immunization for children in developing countries.

1. The organism and the disease

Streptococcus pneumoniae (pneumococcus) is an encapsulated Gram-positive diplococcus that appears in pairs or short chains. At present, based on serotyping, over 90 different capsular types have been identified. Although the serotype distribution varies over time, by age and geographically, approximately 20 serotypes cover most of the infections worldwide. The Danish nomenclature defines serologically cross-reacting serotypes into serogroups, while the American nomenclature names the serotypes in order of their discovery. The Danish nomenclature is the one currently used most frequently.

Capsular polysaccharide (PS) is the most important virulence factor of pneumococcus. It protects pneumococci from phagocytosis by phagocytosing cells, like polymorphonuclear leukocytes, by shielding the inner structures of the bacterium from immunological defence mechanisms and by being a barrier for deposition of complement. Usually pneumococci that lack the capsule are avirulent. Antibodies to polysaccharides are protective and the present pneumococccal vaccines are PS-based. Other virulence factors include proteins that are associated with several stages of pneumococcal infection by, for example, inducing inflammation, promotion of adherence, damaging host tissue and inhibiting complement activation *(1,2)*. Many of the pneumococcal common proteins are regarded as promising vaccine candidates; the studies are in preclinical phase *(3,4)*. Pneumococci have been recently reported to have pili-like structures that enhance the adhesion and invasion of pneumococci in mice *(5)*.

Pneumococcus causes a wide variety of infections, from symptomless carriage or rather benign mucosal infections like otitis media, sinusitis and conjunctivitis, to invasive infections like pneumonia, bacteraemia and meningitis. Among pneumococci, antibiotic resistance has become a prevalent and threatening problem in the last twenty years *(6)*. The human nasopharynx is the only reservoir of pneumococci, which are transmitted by droplet spread between individuals. Carriage of pneumococci is most common during the first years of life, but varies between different geographical and socio-economic demographics. In developing countries or among indigenous populations of some industrialized countries, the carriage rate is much higher and the first acquisition happens at a much younger age than in industrialized countries. Multiple serotypes are carried concurrently in some populations *(7,8)*. Carriage is rare among adults, and especially in the elderly who are at high risk for pneumococcal disease. Mean prevalence of 4% to 10% has been reported for adults *(9,10)*. Healthy carriers occupy a central position in the pneumococcal infection, both as a reservoir of the bacteria, and the source of their transmission to others.

Pneumococcal colonization may sometimes spread to cause infection at contiguous sites such as the middle ear, paranasal sinuses or lung, leading to acute otitis media (AOM), sinusitis or pneumonia respectively. Much less frequently the bacteria may invade the bloodstream to cause sepsis, and spread to other secondary sites via the haematogeneous route to cause meningitis or other syndromes, collectively called invasive pneumococcal disease (IPD) whereby the pneumococcus is isolated from a normally sterile site.

The incidence of pneumococcal invasive disease varies greatly with age, time, geographic location, and population. The incidence is highest in early childhood and among the elderly, and in individuals with immune defects. Pneumonia is the leading cause of childhood mortality in developing countries. The etiology of pneumonia is difficult to establish, but recent vaccine trials *(11,12,13)* suggest that a substantial proportion of pneumonia in young children is caused by the pneumococcus, killing annually an estimated 700 000 to one million children less than five years of age. The incidence of pneumonia is also increased among the elderly population. Other risk factors include HIV-infection. The burden of vaccine-preventable pneumococcal disease is described in more detail in the review by the World Health Organization (WHO) Pneumococcal Conjugate Vaccine Working Group *(14)*.

2. Pneumococcal vaccines

At present two types of pneumococcal vaccines are commercially available — the 23-valent polysaccharide vaccine (PPSV) and conjugate vaccines (PCV). The first licensed 7-valent PCV uses nontoxic variant of diphtheria toxin (CRM) as a carrier protein (PCV7-CRM). The recently licensed 10-valent PCV is a mixed carrier vaccine and uses protein D (PD) of *H. influenzae* and diphtheria and tetanus toxoids as carriers.

In the first efficacy trials with PPSVs the focus was on pneumonia, but in a population in which a high proportion of the pneumonia patients were bacteraemic. The 4-valent PPSV was tested successfully in clinical studies in the 1940s among military recruits and the elderly in the United States of America (USA), and in the 1970s 6 to 14-valent PPSVs were evaluated among mineworkers in South Africa and also in Papua New Guinea *(15–19)*.

The 14-valent PPSV was licensed in 1977 and the 23-valent PPSV in 1983. There are now general recommendations in place for broad use of the vaccine to all those considered at increased risk of pneumococcal pneumonia or its complications; in the present recommendations this includes all those 65 years of age or older, plus younger individuals belonging to medically defined risk groups *(20)*. The PPSV is immunogenic among adults and older children, but not all serotypes are immunogenic among young children. This vaccine primarily prevents IPD in adults, but does not affect colonization *(21,22)*. The polysaccharides are T-cell independent antigens and thus do not induce immunologic memory, one of the corner stones of long-term protection following vaccination. The impact of the PPSV on pneumonia in the elderly is low, and in general, at present, the public-health benefit of PPSV in adults is thought to be limited *(23–26)*.

The immunological properties of the PPSV are changed dramatically when the polysaccharides are conjugated to protein carriers *(27,28)*. This finding led to the possibility of protecting young children and high-risk groups who do not respond to the polysaccharide vaccine. Active research over 20 years culminated in the licensure of the first pneumococcal conjugate vaccine (PCV7-CRM) in 2000. This vaccine contains seven capsular polysaccharides conjugated to a non-toxic diphtheria toxin variant — CRM 197. The second PCV containing serotypes 1, 5 and 7F in addition to those in the PCV7-CRM has been licensed in Canada and Europe in 2009. Several investigational pneumococcal conjugate vaccines (PCV) have also been tested.

Different proteins or protein complexes have been used as carriers in addition to CRM; diphtheria (DT) and tetanus (TT) toxoids *(29–32)*, meningococcal outer membrane protein complex (OMPC) *(33,34)*, and protein D (PD) of *H. influenzae (35,36)*. The seven polysaccharides included to the first registered vaccine, PCV7-CRM, were the ones which caused 80% of invasive disease in infants in the USA. The serotype distribution varies in different parts of the world, and so the next generation of PCVs have been formulated to contain 10 to 13 serotypes that are common worldwide *(37,38,39)*.

The PCV7-CRM has recently been adopted into the routine childhood immunization programme of several industrialized countries, and is expected to be introduced into national immunization programmes in developing countries starting as early as 2008. Most of the developed countries that have taken PCV7-CRM into their programmes use the American schedule of three doses in infancy and a booster in the second year of life (3+1 schedule). However, some countries (e.g. the United Kingdom, parts of Canada, the Scandinavian countries and Italy) use a schedule with two doses administered in infancy and a third dose in the second year of life (2+1 schedule).

3. Measurement of immunity

Protection against pneumococcal infections is mediated by concerted action of antibodies and complement components opsonizing bacteria for phagocytosis. Consequently, two essential serologic methods assessing immunity to pneumococcus measure quantity and functional activity of antibodies acquired by vaccination or after natural exposure to the pathogen. Currently, enzyme immunoassay (EIA) for quantitation of anti-pneumococcal capsular antibodies is the primary method recommended by WHO to evaluate immune responses to pneumococcal PS-based vaccines *(40)*. The EIA method has been well validated and standardized between laboratories. Furthermore, a bridge of IgG antibody concentration to efficacy data in infants has been established *(41,42,43)*. For further vaccine evaluation, demonstration of functionality of antibodies by opsonophagocytosis assay (OPA) is required. Although the OPA is considered the best biomarker of protective immunity in vivo, technical challenges have limited its use in vaccine evaluation. With methodological improvements, it has become a feasible tool in assessment of immune responses in large-scale vaccine trials. Intensive efforts are ongoing for OPA standardization.

EIA can also be used to quantitate antibodies to different pneumococcal virulence proteins. However, no vaccines including pneumococcal protein antigens are currently commercially available, and the protective value in humans of these anti-protein antibodies is unclear. Additional serologic tests, such as isotype or subclass analysis, and determination of relative antibody avidity, provide information about the quality of the immune response. Although these measurements do not seem to be informative with respect to predicting protective efficacy, development of antibody avidity to PS antigens has been observed to indicate with establishment of immunological memory following immunization of infants with conjugate vaccines *(44–47)*. Further information can be obtained by analyzing cellular and mucosal immune responses to the vaccination.

3.1 Quantitation of antibodies

3.1.1 Radioassay and enzyme immunoassay

The standard method used in the past to measure antibody responses to pneumococcal vaccines was radioimmunoassay (RIA) developed by Schiffman and colleagues *(48)*. The method used 14C labelled capsular PS antigens and the Farr technique *(49)* for precipitation of antibody-antigen complexes to determine the total amount of antigen bound. The technique was sensitive and reproducible, but required radio-labelled PS and was unable to distinguish between the different antibody isotypes and subtypes. The RIA may also have co-measured antibody to the pneumococcal cell wall PS (C-PS).

EIA replaced RIA because it consumed lower quantities of serum, provided isotype and subclass data, and did not require radioactive reagents. EIA for pneumococcal capsular antibodies involves binding of purified PS antigens to the wells of microtitre plates, and quantitation of bound antibodies in serum using secondary antibody with conjugated enzyme for detection. The early methods were not specific for quantitation of serotype-specific antibodies, as antibodies to the C-polysaccharide (C-PS) of pneumococcus were also detected. C-PS is found in PS preparations used as EIA antigens. Anti-C-PS antibodies have been found to be non-protective and abundant in almost all human sera *(50–53)*. The EIA protocols have since been modified and now include an adsorption step with a soluble C-PS preparation to remove the C-PS antibodies from the sera *(52)*. Recently, an additional adsorbent, a soluble PS of an irrelevant serotype, e.g. pneumococccal PS 22F, has been recommended to be added to all subject sera *(54–58)*.

Concentrations of serotype-specific IgG, IgA, and IgM antibodies, as well as IgG subclasses, can be determined by EIA using a standard reference serum (89SF). It is a pooled human serum with defined antibody assignments for several pneumococcal capsular PS antigens and C-PS *(59–62)*. The standard reference serum is currently available from the United States Food and Drug Administration for use in assessing pneumococcal vaccines in clinical trials *(40,57,63)*. The use of this serum enables comparisons between different vaccine formulations. Additionally, it can be used to compare serologic data from different time periods, different laboratories, and also using alternative methods. All major pneumococcal laboratories use this reference serum or an internal reference serum calibrated against standard serum 89SF. Although 89SF contains some level of non-serotype specific antibodies, addition of a second adsorbent (22F PS) is not recommended because the original antibody assignments were determined using the C-PS adsorption step only.

International collaboration on standardization of immunological assays for pneumococcal vaccines resulted in completion of a cross-laboratory standardization process of the EIA *(58)*. WHO sponsored an expert consultation on serological criteria for evaluation of pneumococcal conjugate vaccines, and agreed that the EIA protocol and reference serum described by Quataert and colleagues formed the foundation for future vaccine evaluations *(40)*. WHO has also published guidelines for quantitation of serotype-specific IgG antibodies to pneumococcus in human sera *(63)*, and designated two reference laboratories for pneumococcal antibody assays (at the Institute of Child Health, London, United Kingdom, and the University of Alabama, Birmingham, AL). Furthermore, a website with updated technical information enables access to the current recommended procedures and reagents *(57)*. Data generated in clinical trials by the standard EIA without 22F adsorption, have enabled establishment of a threshold pneumococcal anti-PS antibody concentration linked to the clinical efficacy against invasive disease in infants *(40,43)*. Antibody concentrations using an alternative method need to be bridged to the standard EIA method to derive an equivalent threshold concentration.

Pneumococcus invades the human body through mucosal membranes. Thus, immunological events on the mucosal surfaces are an important part of defence against pneumococcal infection. Antibody concentrations to pneumococcal PS and protein antigens can be measured from mucosal secretions e.g. saliva, nasopharyngeal secretions and bronchial lavage with EIA, in the same way as from serum *(64–71)*.

In most studies salivary fluid has been used to measure antibody concentrations against pneumococcus in the upper respiratory tract mucosa. Saliva can be collected relatively easily and there are different collection methods available *(72,73,74)*. However, saliva contains degradative enzymes produced by bacteria, for example IgA1-protease *(75)*. The function of these agents can be prevented by freezing or by enzyme inhibitors. An efficient method to hinder degradation of antibodies in the saliva samples is to use 50% glycerol and storage in – 70 °C *(76,77)*.

3.1.2 Luminex method

While the EIA is well suited for screening large numbers of specimens against a single analyte, a separate assay is required for each pneumococcal serotype. As more serotypes are included in the new conjugate vaccines, immunogenicity testing by EIA becomes laborious, time-consuming, and costly. To increase the efficiency of immunogenicity assessment and to reduce serum consumption, several laboratories have employed Luminex multiple analyte (LXA) profiling technology to quantitate pneumococcal capsular antibodies to up to 23 capsular PSs simultaneously *(78,79,80)*. The LXA technology appears to produce specific, sensitive, and reproducible data. The antibody concentrations obtained by LXA for multiple serotypes correlate well with assignments obtained by EIA when using sera from adults *(79,80)*. However, one needs to be cautious before transitioning from a well-validated method to a new system. Coupling of pneumococcal PS to microspheres may affect the epitope configuration of the antigen, causing different populations of antibodies to bind than in the EIA. Furthermore, the serological correlates of efficacy that have been proposed based on EIA may be invalid for LXA, and the LXA method needs to be validated for each serotype of interest and standardized across laboratories.

3.2 Functional assays (OPA)

Since opsonophagocytosis is the essential host defence mechanism against pneumococcus, the current pneumococcal vaccines are designed to induce opsonic antibodies. An ideal surrogate assay reflecting the protective immunity evoked by the vaccines would therefore directly measure functional i.e. opsonic activity, of antibodies. Although EIA for pneumococcal capsular antibodies is mostly adequate as a surrogate measure of immunity for young healthy children, alone it appears to be inadequate in the elderly, human immunodeficiency virus (HIV) infected individuals, and several other high-risk populations who seem to produce antibodies with low functional activity *(81–85)*. Results measured by the functional OPA method correlate better with clinical efficacy than EIA results, especially in these populations. The accumulating data from clinical trials clearly demonstrate the added value of OPA in vaccine evaluation *(86,87)*.

To be useable in large-scale vaccine trials, the OPA needs to be robust, efficient, standardized, and also use only small volumes of sera. A variety of techniques have been developed to replicate the biological process of opsonophagocytosis in vitro. In the classic killing type OPA, pneumococcal bacteria are exposed to antibodies, complement, and phagocytes, followed by enumeration of the surviving bacteria by counting colonies. The killing type OPA was first validated by Romero-Steiner and colleagues as a reference assay *(88)*. This method used cultivable phagocytes (HL-60), commercially available rabbit complement, and micro-colony counting. The method, with minor modifications, has since been validated by laboratories of major vaccine manufacturers *(89,90)*.

The original approach was laborious to perform, but recent development of multiplexed killing type OPA, and the use of automated colony counters, has made the technique more efficient *(91,92,93)*. At the same time, serum consumption has decreased. To overcome difficulties in colony counting, use of chromogenic *(94)* or fluorogenic *(95)* dyes, producing signals proportional to the number of bacteria, has also been introduced. Alternative OPA techniques for pneumococcus without requirement of colony counting include uptake assays of radiolabelled *(96,97)* or fluorescent bacteria *(97,98,99)* and chemiluminescence assay *(100)*. None of these assays has been as extensively evaluated as the killing type OPA, which has served as the reference assay *(101)*.

Based on the WHO criteria, demonstration of functional activity of antibodies as measured by OPA is the secondary end-point when evaluating new pneumococcal vaccines for licensing *(63)*. The use of OPA has even been encouraged as the primary analysis in studies in the elderly. To be able to compare the results from different laboratories, WHO is currently supporting extensive cross-laboratory standardization efforts. The first round of multi-laboratory standardization of the single serotype killing type OPA was led by investigators in the Centers for Disease Control and Prevention. The variation in OPA results between laboratories was found to be significant *(102)*. The current approach is to have several types of validated OPA methods in use *(89,90,92)* that would give comparable OPA results when compared to the reference assay.

Based on comparisons between opsonic titres and antibody concentrations from clinical studies in infants, an opsonophagocytic titre of eight has been found to correlate with the protective efficacy against pneumococcal invasive disease against all serotypes after three doses of conjugate vaccine *(40,89)*. The protective titre may vary from serotype to serotype, between different pneumococcal disease syndromes, and between populations. Additional and larger studies are required to better establish the protective titre.

3.3 Assessment of antibody avidity

Antibody avidity is the strength of the multivalent interaction between antibody and antigen. Methods based on both EIA and RIA have been developed for determining avidity of pneumococcal capsular antibodies. In EIA techniques, the binding of antibody to the coated antigen may be prevented by competitive inhibition *(103)* or by eluting the antibody from the antigen with a dissociating agent (47,85,104). Calculation of the avidity constant value (K), representing a median value for the antibody population in each specimen, is feasible with the former but not with the latter technique, which only ranks the antibodies by their relative avidity. With RIA, the avidity constant can be obtained by evaluating the capacity of antibodies to bind radiolabelled antigen at different molar concentrations *(105,106)*.

Avidity measurements provide qualitative data about immune responses to different vaccines but do not seem to be informative with respect to predicting protective efficacy. However, increases in avidity are believed to correlate with establishment of B-cell memory *(44–47)*. Measurement of antibody avidity may thus prove useful in assessing development of immunological memory.

3.4 Assessment of cell-mediated immunity

Immunogenicity of pneumococcal polysaccharide vaccines can be improved by conjugating PS to carrier protein, altering the nature of immune response from T-cell independent to T-cell dependent. The cellular reasons for the improved immunogenicity have been studied by analyzing T-lymphocyte proliferation and cytokine secretion upon in vitro antigen stimulation *(107,108,109)*. The importance of T-cell responses as determinants of immune response to conjugate vaccines has been demonstrated in mice *(110)*. Assessment of T-cell responses is essential also to understand cellular basis for the improved immune responses elicited by adjuvanted vaccines, especially when novel, non-aluminium adjuvants are being tested *(111,112)*.

3.5 Assessment of immunological memory

Pneumococcal conjugate vaccines are T-cell dependent antigens and induce immunological memory. It is not clear whether certain concentrations of circulating, functional antibodies, are required for protection against pneumococcal diseases after immunization, or whether memory alone is sufficient to confer long-term protection. Probably both are needed for optimal protection. Nevertheless, for the purposes of vaccine evaluation, it is important to show induction of immunological memory in response to vaccination. Immunological memory to a polysaccharide antigen is defined as a response that is present in otherwise non-responsive individuals (e.g. infants), characterized by a higher antibody response with IgG dominance on exposure to an antigen, and dominated by antibodies with increased avidity. Direct measurement and characterization of memory B-cells after booster immunization *(113)* using flow cytometric or enzyme-linked immunospot assay (ELISPOT) techniques, is laborious and rarely feasible.

4. Immunological response to natural infection

4.1 Host defence mechanisms

The main host immune mechanism against pneumococcal infection is opsonization of the bacteria in the presence of early complement components and specific antibodies, followed by phagocytic killing by polymorphonuclear leukocytes and macrophages *(114)*. The importance of these host mechanisms is indirectly demonstrated by the increased risk for infections in patients with hypogammaglobulinaemia, impaired phagocytic or complement systems, or splenic dysfunction, and the capacity of replacement immunoglobulin therapy to restore immunity *(115,116)*. Pneumococci have developed many virulence factors to avoid opsonization *(117,118)*, the most important being the polysaccharide capsule *(119)*. Pneumococci probably undergo phase variation in order to adapt and survive in ecological niches of the host; invasive isolates often have opaque colonies, recognized by a thick polysaccharide capsule, whereas transparent colonies with less capsule are found more often in mucosal sites. Transparent colonies express greater amounts of proteins associated with adherence *(120,121,122)*. In blood, the thick capsule shelters the subcapsular antigens from recognition by host opsonins, and antibodies that bind to the capsule are required to make the bacterium susceptible for phagocytosis.

Because asymptomatic nasopharyngeal carriage is the main reservoir for spread of pneumococci, and a precursor for infection, the factors of innate immunity, and both mucosal and systemic immunity, contribute in the defence against nasopharyngeal carriage and in the prevention of subsequent infections. Serum IgG transudates to mucosal surfaces, and together with locally produced secretory IgA can interfere with adherence to mucosal epithelium. Upon inflammation, phagocytes migrate into mucosal sites and augment in the clearance of the pathogen.

4.2 Development of immunity

In vaccine naive individuals, low concentrations of antibodies to pneumococcal PSs can be measured quite frequently, reflecting previous pneumococcal infection or immunization by cross-reacting antigens. Epidemiological data show a clear age-specific susceptibility to pneumococcal carriage and disease, suggesting that protective immunity develops with age. Indeed, the age-dependent ability to mount an antibody response to pneumococcal capsular polysaccharides coincides with the decline in the pneumococcal disease by age in childhood. On the other hand, the senescence of the immune system, most probably both innate and adaptive *(123)*, increases the risk of pneumococcal disease among the elderly. Asymptomatic pneumococcal carriage or disease, induce natural development of antibodies to pneumococcal PS and protein antigens *(124–134)*.

Antibodies recognizing the pneumococcal PS antigens in EIA develop through serotype-specific contact with pneumococci, and also through contact with other bacteria bearing cross-reactive antigens. However, based on animal experiments, the protective natural immunity can be antibody or cell mediated *(135,136)*, and thus at present it can be concluded that the role of natural antibodies induced by pneumococcal carriage or disease is still unclear. The majority of the anti-PS specific IgG is of IgG2 subclass in adults, while antibodies of young children are mainly of IgG1 subclass (*137,138)*. IgG antibodies interact with FcγRIIa receptor on the phagocytes. It has two functionally different allotypes. FcγRIIa-H131 receptor has high affinity to IgG2, while FcγRIIa-R131 receptor binds IgG2 poorly *(139)*. This functional polymorphism can have a role in the defence against pneumococcal infection *(140,141)*.

5. Response to vaccination

5.1 Polysaccharide vaccine

As described above, the first PPSV vaccine was licensed in 1977, and is now recommended for use among adults and older children at high risk for pneumococcal disease (see also Sections 2 and 7).

Several studies have evaluated the immunogenicity of the PPSV in elderly adults and found little or no diminution in IgG antibody concentrations with ageing *(85,142–145)*, so that the concentration of IgG antibodies to pneumococcal PSs in healthy elderly adults does not seem to offer a satisfactory explanation to the poor efficacy of PPSV in this population. Rather, the low functional activity and short duration of immunity compares to the inefficient protection *(84,85,146)*. High-risk groups e.g. patients with sickle-cell disease, bone marrow or solid organ transfer transplantation, and asplenia, tend to have lower and shorter-lasting antibody concentrations after vaccination than healthy adults *(147–152)*.

A randomized double-blind trial in HIV-infected adults in Uganda *(153)*, showed no benefit of vaccination with PPSV. In fact, rates of pneumonia and pneumococcal disease were increased in the vaccine recipients. This effect could not be associated with immune responses in the same population *(154)*. In an American retrospective case-control study *(155)*, overall effectiveness was 49% (95% confidence interval (CI) 12%-70%) against IPD among HIV-infected adults, but a stratified analysis showed no benefit in African Americans, effectiveness 24% (95% CI - 50% to 61%). Several immunogenicity studies suggest that PPSV is less immunogenic in HIV-infected adults than in healthy controls *(156–159)*. Anti-retroviral therapy has only had a minimal effect on the immune response to PPSV in this population.

Since young adults respond satisfactorily to PPSV, PPSV immunization of mothers has been considered as a protection for young infants against pneumococcal disease and colonization. Several studies show that infants of immunized mothers have elevated serum antibody concentrations up to four months after delivery *(160–164)*. In addition to the transfer of antibodies through placenta breast milk, antibodies can be important for protection.

5.2 Conjugate vaccines

The experience gained from efficacy trials with either PCV7-CRM or similar 9-valent PCV (PCV9-CRM) has been described in a detailed WHO position paper *(14)*. IPD was an end-point in four efficacy trials *(12,13,41,165)*. The first one was conducted in Northern California — Northern California Kaiser Permanente (NCKP) trial. Subsequent trials were conducted among American Indian, South African and Gambian infants. The vaccine efficacy against IPD caused by the vaccine serotypes (VT) varied between 76.8% and 97.4% with overlapping confidence intervals, and was lowest among American Indian, the Gambian and HIV-infected South African infants.

The efficacy of PCV7-CRM, PCV9-CRM or 11-valent mixed carrier PCV using TT and DT as carrier proteins (PCV11-D-T) against community-acquired pneumonia with radiological confirmation, has been evaluated among Northern Californian *(11)*, South African *(13)*, Gambian *(12)*, American Indian *(165)* and Philippine infants *(166)*. The NCKP, South African and Gambian trials showed significant efficacy (20%–35%) against radiologically confirmed pneumonia, while the trials with American Indian and Philippine infants showed comparable point estimates, but did not reach statistical significance.

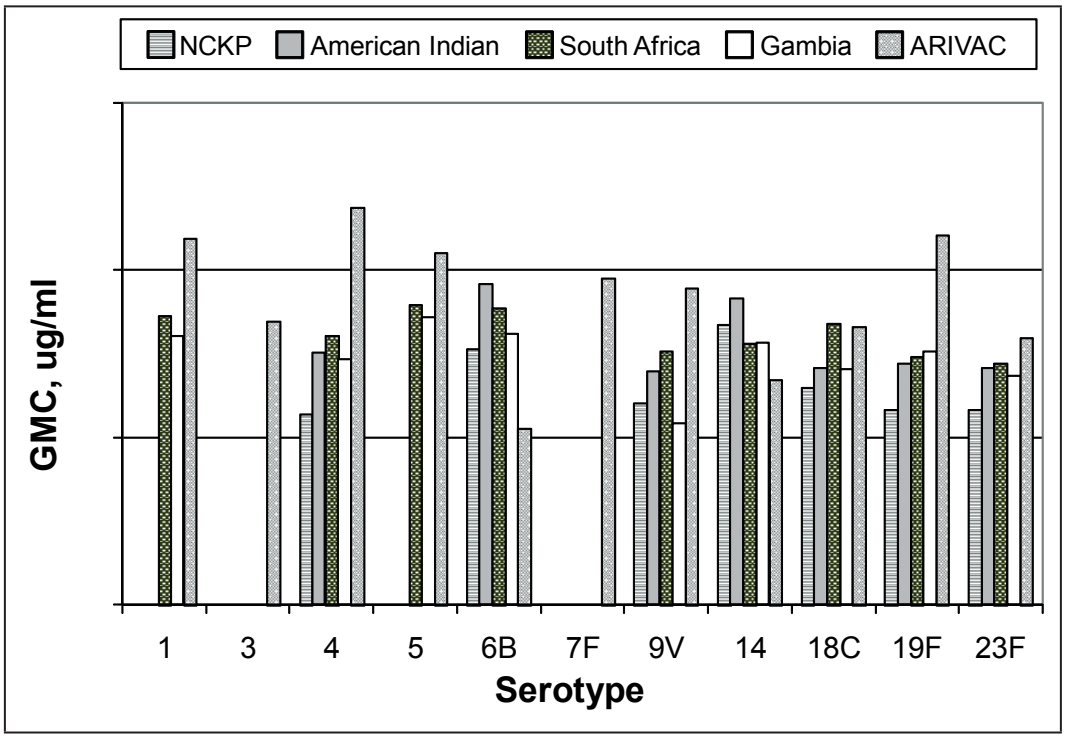
Two trials have looked at the efficacy of PCVs on culture-confirmed AOM in infants, and a third trial estimated the efficacy of PCV7-CRM against recurrent culture- confirmed AOM among older children *(167–170)*. Two vaccines, PCV7-CRM and PCV7-OMPC, were tested in parallel in Finland — Finnish Otitis Media (FinOM) Vaccine Trial. The second infant trial (POET) was conducted in the Czech and Slovak Republics and used a novel investigational PCV11-PD, in which the *Haemophilus influenzae*-derived PD was used as the carrier protein. The observed efficacy against pneumococcal AOM caused by vaccine serotypes (VT) was very similar (56%–57.6%) in all of the infant trials. The overall impact on AOM was 0% for PCV7-OMPC and 6% for PCV7-CRM in the FinOM Vaccine Trial *(167,168)*, while in the POET trial a 33.6% reduction in the overall incidence of AOM was reported *(169)*. The difference is most probably due to the prevention of both pneumococcal and *Haemophilus influenzae* AOM in the POET trial, and the replacement of VT AOM by non-vaccine serotype (NVT) AOM in the FinOM Vaccine Trial.

Several studies have confirmed that PCVs have an effect on pneumococcal colonization. The decrease of VT pneumococcal colonization is offset by an increased carriage of NVTs with no resultant effect on the overall pneumococcal carriage-rate *(171)*. Since the asymptomatic carriers are a reservoir and source of the spread of pneumococcal infection, studies on colonization may provide tools to predict the overall effects of PCV vaccination.

5.2.1 Immunogenicity of PCVs used in the efficacy trials

Serotype-specific serum antibody concentrations have been estimated with a standard EIA without 22F adsorption in nested immunogenicity trials among the IPD and pneumonia efficacy trial populations *(43,172,173)*, and in trials preceding the efficacy trials *(174,175)* (Figure 1). All studies show a robust antibody response. After the third dose the geometric mean antibody concentrations (GMC) tended to be lowest among the Californian infants. Serological data for HIV-uninfected and HIV-infected infants have been reported for a small number of South African vaccinees *(83)*. The GMCs of antibodies were not significantly different in the two groups, but the functional activity of antibodies seemed to be lower among HIV-infected infants. This is in accordance with the vaccine efficacy that appears to be lower and shorter- lasting in HIV-infected children than in HIV-uninfected children *(13,176)*. In general, the antibody concentrations in different trials did not vary much. The observed differences in the efficacy against pneumonia in some of the trials in different populations, seem to be dependent on other factors such as the variability in case- ascertainment and the predictive value of the clinical case definitions for pneumonia that were used in the trials.

Figure 1: Geometric mean concentrations (GMC) of antibodies to vaccine serotypes reported in connection with the PCV efficacy trials with invasive pneumococcal disease and/or radiologically confirmed pneumonia as end-points. (Modified from Käyhty et al. Immunogenicity and reactogenicity of pneumococcal conjugate vaccines in infants and children. In: Siber et al., eds. *Pneumococcal vaccines: the impact of conjugate vaccine*. Washington, DC. ASM Press, 2008).



Based on meta-analysis *(43)* of three efficacy trials (NCKP, South African, American Indians), WHO experts agreed on the use of the threshold of 0.35 µg/ml in non-inferiority evaluation when comparing new PCVs or PCV formulations to registered PCV *(63)*. It should be noted that this threshold does not necessarily predict protection in an individual subject. None of the trials has sufficient IPD cases to provide serotype-specific thresholds (see also Section 6).

Antibody concentrations in the FinOM Vaccine Trial were determined with EIA without 22F PS adsorption *(45)* and in the POET trial with 22F PS adsorption *(169)*, which should be kept in mind when comparing the results (Table 1). The serotype-specific significant protection could be determined for serotypes 6B, 14, and 23F in all three studies. Even though the GMCs for serotype 19F were high in all studies, the point estimates for clinical efficacy were low, and significant protection could be shown only for PCV7-OMPC and PCV11-PD, but not for PCV7-CRM. PCV7-CRM induced higher antibody concentrations to serotype 6B than the other PCVs, but the efficacy was 80% to 90% in all three trials, suggesting that even the lower antibody concentrations to serotype 6B evoked by PCV7-OMPC or PCV11-PD were sufficient to provide good protection.

The FinOM Vaccine Trial showed an inverse association between the risk of AOM and serotype-specific antibody concentration *(42)*. The mean antibody concentration predicting protection after vaccination varied between serotypes, so that lower concentration of antibodies was needed to protect against serotype 6B AOM than against AOM caused by other studied serotypes. In the POET trial the mean antibody concentration and opsonophagocytic activity against serotype 19F were lower in post- primary course samples of the breakthrough cases as compared to those who did not develop AOM caused by serotype 19F *(87)* .

The functional activity of anti-pneumococcal antibodies was measured by OPA in FinOM *(86)* and POET trials *(87)*. Due to the methodological differences, the data from the two studies cannot be compared directly. In general, the antibody concentration and the functional activity seem to correlate well when each serotype is evaluated separately. However the OPA shows differences between serotypes, and might be better associated with the protection, at least in the case of PCV7-CRM and PCV11-PD *(86,87,89)*, than the antibody concentration. In all three studies, less antibody was needed for 50% killing of the serotype 6B strain than for killing of the serotype 19F strain, which is in accordance with the efficacy of the three PCVs against these serotypes.

5.2.2 Mucosal antibody responses to PCVs

PCVs prevent mucosal infections (AOM and colonization), and some groups have made efforts to characterize the mucosal immune response after vaccination, in the hope of finding serological correlates of mucosal protection. Such studies have addressed IgG and secretory IgA antibody concentrations in salivary fluid *(64–66,68,177–179)*. Lymphocytes encountered by antigens in the respiratory tract migrate back to the mucosal tissues after maturation to antibody production in local lymph nodes. Thus another way to demonstrate mucosal immune response to pneumococcal vaccines has been the measurement of circulating antibody secreting cells by ELISPOT from the blood, on their way back to the effector sites in mucosa *(69,180)*. The PCVs induce the production of local antibodies but thus far no direct link has been established between the salivary antibody concentration and vaccine- induced protection.

Table 1: The geometric means of antibody concentrations to PSs of vaccine serotypes in efficacy trials in infants with an end-point of culture- confirmed AOM caused by the vaccine serotypes. [4]

Trial (ref)[1]	Sample	Geometric mean of antibody concentration (µg/ml) for indicated serotypes										
		1	3	4	5	6B	7F	9V	14	18C	19F	23F
FinOM PCV7-CRM (Unpublished)[3]	Post 3 N=376	NA[2]	NA	2.55	NA	2.68	NA	2.85	7.58	4.05	3.63	2.83
	Post 4 N=470	NA	NA	3.53	NA	10.45	NA	4.2	9.31	5.98	4.82	6.13
FinOM PCV7-OMPC *(168)*	Post 3 N=376	NA	NA	3.45	NA	0.35	NA	1.79	3.23	1.02	3.19	0.67
	Post 4 N=374	NA	NA	6.50	NA	2.34	NA	4.06	5.96	3.45	8.65	2.41
POET *(169)*	Post 3 N=140–143	1.58	3.78	2.16	1.92	0.62	2.34	1.60	3.00	1.49	2.60	0.9
	Post 4 N=118–143	2.55	2.83	2.50	3.11	2.17	4.66	3.63	6.48	2.71	4.88	3.55

1 The FinOM trials used EIA without 22F adsorption, while the POET study used 22F adsorption.

2 NA= not applicable; serotype not included in the vaccine.

3 The samples differ from those published *(45,167)*.

4 Modified from Käyhty et al. Immunogenicity and reactogenicity of pneumococcal conjugate vaccines in infants and children. In: Siber et al., eds. *Pneumococcal vaccines: the impact of conjugate vaccine*. Washington, DC. ASM Press, 2008.

5.2.3 Duration of immunity after vaccination with PCVs

The persistence of antibodies has been followed for PCV9-CRM in South Africa *(176,181)*, for PCV7-CRM in Finland *(182)*, and for PCV11-PD in the Czech Republic *(183)*. After 6.2 years of follow-up in South Africa, the proportion of children with IgG antibodies ≥0.35 µg/ml was similar for HIV-infected children in the placebo and PCV groups for most of the serotypes, while the proportions were significantly higher in HIV-uninfected PCV recipients compared with the placebo group. This is consistent with the long-term persistence of protection; among the HIV-uninfected children the efficacy was still 77.8% after 6.2 years of follow-up, but declined from 65% to 38.8% (95% CI - 7.8–65.2%) among HIV-infected children *(176)*. Huebner and colleagues report (Table 2) the antibody concentrations up to 18 months of age in a cohort of South African vaccinees *(181)*. Their conclusion is that even without a booster dose, the antibody concentrations remain significantly higher in the PCV9-CRM recipients until the age of 18 months, as compared with the placebo group.

The persistence of antibodies was determined in the FinOM vaccine trial at the age of four to five years for the PCV7-CRM and control arms (Table 2). Antibody concentrations had declined, but less for the frequently carried serotypes 6B, 14, 19F and 23F *(182)*. The follow-up of a subset of children that previously participated in the POET study up to their fourth year of life showed a similar trend (Table 2) *(183)*.

Table 2: Persistence of antibodies after immunization of South African, Czech or Finnish infants with PCVs. Geometric mean antibody concentrations are given for each vaccine serotype at indicated ages in children who had received PCV or a control vaccine in infancy. The frequently carried serotypes are in bold type. [1]

Country (reference)	South Africa *(181)*		Czech Republic *(183)*		Finland *(182)*	
Age	18 months		3.6 years		4–5 years	
Vaccine (N)	PCV9-CRM (211–214)	Control group (211–217)	PCV11-PD (50)	Control group (49)	PCV7-CRM 382	Control group 341
Serotype: 1	0.27	0.11	0.12	0.04	NA	NA
3	NA	NA	0.34	0.20	NA	NA
4	0.19	0.07	0.13	0.05	0.51	0.33
5	0.58	0.36	0.20	0.07	NA	NA
6B	**1.07**	**0.13**	**1.59**	**0.13**	**3.33**	**0.59**
7F	NA	NA	0.31	0.04	NA	NA
9V	0.39	0.28	0.23	0.06	0.95	0.58
14	**1.1**	**0.17**	**2.05**	**0.17**	**1.94**	**0.58**
18C	0.22	0.11	0.16	0.04	0.76	0.34
19F	**0.83**	**0.40**	**3.59**	**0.33**	**4.66**	**1.38**
23F	**0.24**	**0.05**	**0.92**	**0.08**	**1.19**	**0.36**

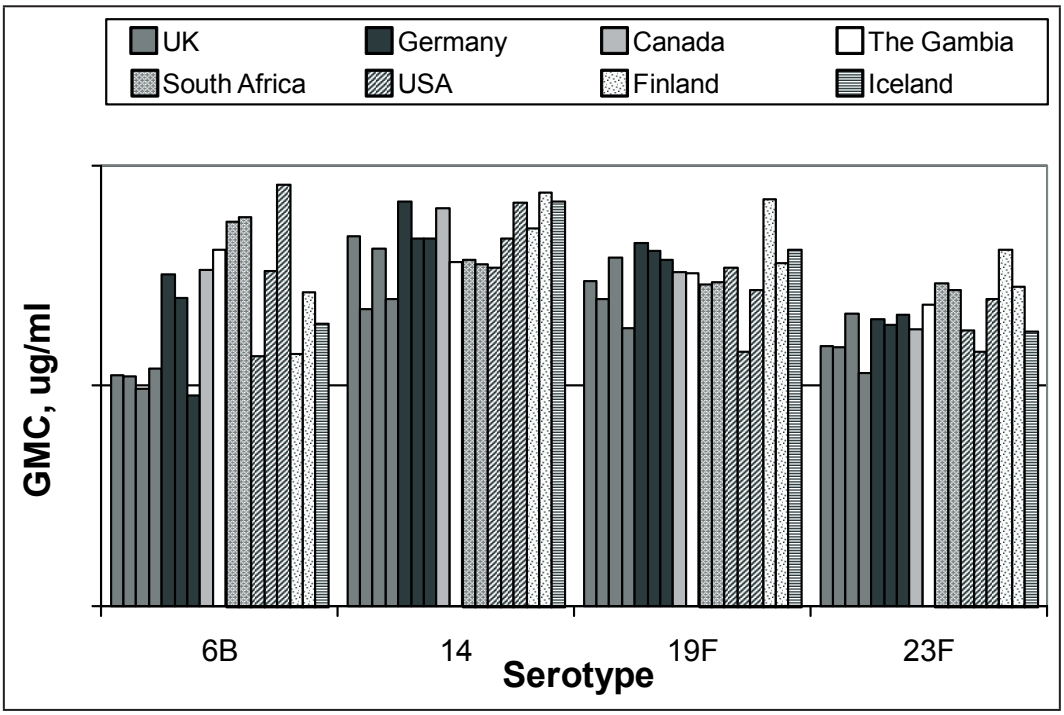
[1] Modified from Käyhty et al. Immunogenicity and reactogenicity of pneumococcal conjugate vaccines in infants and children. In: Siber et al., eds. *Pneumococcal vaccines: the impact of conjugate vaccine*. Washington, DC. ASM Press, 2008.

5.2.4 Immunogenicity of PCVs in different populations and risk groups

Populations

The licensed or investigational PCV7-CRM or similar PCV9-CRM has now been used in several populations. Figure 2 compares antibody concentrations for four vaccine serotypes (6B, 14, 19F and 23F) in 17 studies among African *(174,184)*, American *(43,172,185)*, German *(186,187,188)*, British *(47,189,190,191)*, Canadian *(192)*, Finnish *(178)* (Ekström et al. unpublished), and Icelandic *(193)* infants, after immunization with three doses of PCV7-CRM or PCV9-CRM in infancy. The EIAs were performed in different laboratories and the vaccines given with different schedules and with different concomitant vaccines. Serotype 6B seems to show the greatest variation between studies, but in general the PCV-CRM evokes a robust immune response in different populations.

Figure 2. Geometric mean concentrations (GMC) of antibodies after three primary doses of PCV7-CRM or PCV9-CRM in studies in the United Kingdom *(47,189–191)*, Germany *(186–188)*, Canada *(192)*, the Gambia *(175)*, South Africa *(174,184)*, USA *(43,172,185)*, Finland (unpublished, *178*) and Iceland *(193)*. The PCV was given at 2, 3 and 4 months in the United Kingdom, Germany and the Gambia; at 2, 4 and 6 months in Canada, the USA and Finland; at 3, 4 and 5 months in Iceland and at 6, 10 and 14 weeks in South Africa. (Modified from Käyhty et al. Immunogenicity and reactogenicity of pneumococcal conjugate vaccines in infants and children. In: Siber et al., eds. *Pneumococcal vaccines: the impact of conjugate vaccine.* Washington, DC. ASM Press, 2008).



The investigational mixed carrier aluminium adjuvanted PCV11-D-T was given concomitantly with whole-cell pertussis vaccine (wP) containing diphtheria, tetanus and pertussis combination vaccine (DTP) to Philippine, Israeli, and Finnish infants in two separate studies *(194)*. After the third dose given at 14 weeks in the Philippines and at six months in Israel and Finland, or a booster dose given at nine months in the Philippines and at 12 months in Israel and Finland, the Philippine infants showed higher antibody concentrations to most of the 11 VTs. The difference was most notable for serotypes conjugated to TT. Possible reasons for the higher responses among Philippine infants could be infant priming by tetanus vaccination of mothers in the Philippines, natural boosting by earlier carriage acquisition in the Philippines than in Finland or Israel, genetic factors, antibodies elicited by cross- reacting antigens or the vaccination schedule.

Published data on the immunogenicity of the PCV11-PD are available from the immunogenicity studies in Finland *(36)*, the Philippines *(35)*, and from the phase III study in the Czech and Slovak Republics *(169)*. Again, even though the PCV was given with different schedules and with different concomitant vaccines, the mean concentrations after the third dose were similar in the three populations and also after the fourth dose given in Finland and the Czech and Slovak Republics.

Risk groups

Studies with PCVs among HIV-infected children show adequate antibody responses *(83,195–198)* with the trend towards a higher response in those with less advanced disease. The South African trial *(13)* has been, thus far, the only efficacy trial among HIV-infected children. In that trial, the immunogenicity was lowest among those infants with the most severe condition, especially with respect to the functional activity of antibodies. Furthermore, while both the immunogenicity and protection waned during the several years follow-up in HIV-infected children, it was sustained in healthy controls *(83)*.

Studies in the USA, Malawi and Uganda show that adult HIV-infected PCV7-CRM recipients mount high antibody concentrations and OPA titres *(199–202)*. The Malawian study also reported significant increases in salivary IgA *(200)* and IgG in in lung fluid *(67)*, both in the HIV-infected and HIV-uninfected group.

Good immunogenicity of PCV7-CRM in different risk groups has now been shown in several studies. These studies suggest that immunocompromised patients with, for example, sickle-cell disease *(203,204)*, bone-marrow transplant *(205)* and asplenia *(206)* can benefit from vaccination with PCV, whereas PPSV might not give protective immunity. Bone-marrow or stem-cell recipients have satisfactory responses, but the optimal immunization includes three doses after transplant. Immunization of the donor one month before the harvest of bone marrow or before harvesting the stem cells, augments the response *(207,208)*.

5.2.5 Schedule issues

Efficacy studies among infants have used either 3+1 *(41,165,167,168,169)* or three- dose schedules — the local infant immunization schedule *(12,13,166)*. A number of countries have, however, different schedules for routine infant immunization, and many now use a 2+1 dose schedule for PCV7-CRM. In addition, to be able to adopt the PCVs in resource-poor countries, an immunization series of fewer than three doses would offer the possibility to lower the costs of immunization. Studies comparing alternative schedules of PCV7-CRM in different populations in Africa, Asia, and Europe are ongoing. The data from those studies on antibody concentrations, development of memory, functional activity of antibodies, and the effect on carriage, will be available in the near future.

Three types of studies have addressed the schedule issue. Kinetics studies, randomized studies comparing schedules, and studies using published data for comparison. Studies on the kinetics of antibodies from Finland, the Philippines and South Africa, and the USA *(45,174,185,209)* show very similar trends in spite of different PCVs, different schedules, and concomitant vaccines used. Typically, serotypes 6B and 23F induce antibody increases only after the second or third dose, while for serotypes 4 and 14, increases can already be found after the first dose.

Leach and colleagues compared randomized groups that received PCV5-CRM at 2, 3 and 4, or at 2 and 4 months of age *(210)*. Higher antibody concentrations were noted in the group that had received three doses compared to those receiving two doses, but due to the small numbers of vaccinees the difference was significant only for serotype 14. In an Icelandic study, infants were randomized to receive PCV9-CRM combined with group C meningococcal conjugate vaccine (MCV-C-CRM) either at 3 and 5, or at 3, 4 and 5 months with a booster dose at 12 months. The mean antibody concentrations after the primary courses were similar in the two groups (Table 3), with the exception of serotypes 6B and 23F *(193)*). Similar results have been reported from an Israeli study using PCV7-CRM *(211)*. After a booster dose of either PCV9-CRM or PPSV, no differences were noted in the Icelandic study.

Goldblatt and colleagues compared data from two studies using PCV9-CRM that had either a 2, 3, 4 and 12 months, or a 2, 4 and 12 months schedule respectively *(47)*. The mean antibody concentrations were similar at five months of age (Table 3). The similar avidity maturation (studied for serotypes 6B, 14 and 19F) and the booster responses to PPSV or PCV at 12 months, suggest that the 2-dose schedule had induced similar immunological memory compared to the 3-dose schedule. Esposito and colleagues, and Käyhty and colleagues reported antibody concentrations for the 2+1 dose schedule (at 3, 5, and 11 or 12 months) of PCV7-CRM in Italian and Swedish infants respectively *(212,213)*. Comparison with historical controls i.e. studies that have used the same vaccine with the 3+1 dose schedule *(41,45,167)* showed that the GMCs for serotypes 6B and 23F remained lower after the primary 2+1 course, but no clear-cut differences were noted for other serotypes (Table 3). After the booster at 11 or12 months, the GMCs were similar *(212,213)* to those found after boosting in previous studies using three doses in infancy. In general, the comparative studies suggest that the 2+1 dose schedule induces a satisfactory immune response with the possible exception of serotypes 6B and 23F.

Table 3: Geometric means of antibody concentrations to serotype 4, 6B, 14 and 23F PSs after primary series and booster dose in studies that have directly or indirectly compared schedules including two or three primary doses in early infancy with PCV boosting at 12 months of age. [1]

Vaccine	Country (ref)	Schedule (N)	Serotype 4		Serotype 6B		Serotype 14		Serotype 23F	
			Post prim	Post boost	Post prim	Post boost	Post prim	Post boost	Post prim	Post boost
PCV9-CRM	Iceland *(193)*	3-5-12 mths (108) 3-4-5-12 mths (110)	2.34 2.97	3.87 4.30	0.69 1.94	9.42 14.01	4.69 6.95	8.75 10.15	0.91 1.77	2.83 4.42
PCV9-CRM	UK (*47*)	2-4-12 mths (82) 2-3-4-12 mths (73)	2.05 1.82	5.55 5.19	1.01 1.12	9.68 11.11	3.20 4.78	13.88 18.48	1.15 1.52	6.11 6.40
PCV7-CRM	Sweden/Finland (*167,213*)	3-5-12 mths (99) 2-4-6-12 mths (57)	4.43 1.70	9.43 2.56	0.30 2.00	4.92 9.05	3.37 6.28	11.67 10.82	0.88 2.51	4.59 6.25

[1] Modified from Käyhty et al. Immunogenicity and reactogenicity of pneumococcal conjugate vaccines in infants and children. In: Siber et al., eds. *Pneumococcal vaccines: the impact of conjugate vaccine*. Washington, DC. ASM Press, 2008.

A Philippine study showed similar antibody concentrations at nine months of age in children immunized with three doses of PCV11-DT at 6, 10 and 14 weeks of age, or with a single dose at 18 weeks *(214)*. The relative avidity was tested for four serotypes and was lower in the group that had received one dose at 18 months for serotypes 5, 6B and 23F, but not for serotype 19F *(215)*.

5.2.6 A booster dose of PCV or PPSV

Independently from the PCV formulation or the primary immunization schedule, a booster dose of either PCV or PPSV is able to induce higher or similar antibody concentrations compared with the primary immunization. In many cases the PPSV boosting induces higher mean antibody concentrations than the PCV boosting *(29,31,32,36,47,86,168,178,181,193,216)*. The development of B-cell memory during the primary course can be studied by comparing antibody response after PPSV or PCV booster in age-matched previously unimmunized versus PCV-immunized children. The booster response to PPSV has been used, for example for testing the persistence of memory *(47,183)*, or the induction of memory by different schedules *(47)*, different PCV doses *(32)*, different vaccine combinations *(190)*, or when comparing responses of pre-term and full-term infants *(191)*.

British experience with *Haemophilus influenzae* type b (Hib) and meningococcal group C conjugate vaccines suggests that there is a need for a booster dose of conjugate vaccine in the developed world; the 3-dose schedule in early infancy without boosting did not provide satisfactory long-lasting protection *(217,218)*, especially when these vaccines are co-administered with acellular pertussis vaccine (aP) containing DTP. Furthermore, the South African follow-up study suggests that HIV-infected children would benefit from a booster immunization, while HIV-uninfected children might have persistent protection due to natural boosting via pneumococcal colonization or cross-reacting antigens *(176)*.

5.2.7 Interactions between PCV and simultaneously administered other vaccines

Four trials have addressed the effect of concomitant vaccines on the responses to PCV. In three trials, differences in concomitant vaccines had no effect on the response to PCV7-CRM *(192,219,220)*. A fourth study in Israel compared two groups, one receiving investigational PCV11-D-T concomitantly with DTwP-IPV/PRP-T, and the other with DTaP-IPV/PRP-T. The response to TT-based conjugates after the third and fourth dose, was significantly lower among infants who had received aP-containing combination vaccine *(221)*.

Antibody response to the DT has been measured in a number of PCV7-CRM studies. The response to a primary series of DTP alone has been compared to the response to DTP co-administered with PCV7-CRM. The GMCs of antibodies against DT were generally higher in the group given PCV7-CRM than in studies with both wP [190] and aP-containing combination vaccines *(186,187,188,190)*.

PCV7-CRM has not been administered alone to determine the immunizing or boosting effect of the carrier, CRM. PCV11-D-T given at 18 weeks of age was able to boost the DT and TT responses of Philippine infants who had received DTwP at 6, 10 and 14 weeks *(222)*. Similarly, vaccination of toddlers with PCV11-D-T *(223)* was able to boost responses to, respectively, DT and TT.

The administration PCV can either increase or decrease the antibody response to other conjugate vaccines using the same carrier protein. When PCV7-CRM was administered at the same time as a CRM-based Hib conjugate vaccine (PRP-CRM), the mean antibody concentration to Hib PS was doubled compared with subjects not receiving concomitant PCV7-CRM *(190)*. A similar effect was observed when CRM- based group C meningococcal conjugate vaccine was co-administered with PRP-CRM *(224)*. Although PCV7-CRM in general showed evidence of enhancement of response to concomitant CRM-based conjugate vaccines, there is also some evidence of carrier-mediated suppression with CRM *(189,220)*.

Reduced antibody responses were reported in a Finnish dose-ranging study of a 4-valent TT-conjugated PCV (PCV4-T), in which infants also received TT-conjugated Hib vaccine (PRP-T) and DTwP combination vaccine *(32,225)*. After the three primary vaccinations, both anti-Hib PS and -TT GMCs were inversely related to PCV4-T dose *(225*). A similar placebo-controlled study of a single dose level of PCV4-T in the State of Israel, also showed reduced anti-Hib PS responses in the subjects given concomitant PCV4-T *(225,226)*.

The immunogenicity of immunologically unrelated vaccines administered with and without concomitant PCV has been examined in several studies. These include studies with DTaP or DTwP-containing combination vaccines, including also Hepatitis B vaccine (Hep B) PRP-T and/or IPV *(36,186,187,188,192,227,228)*, Hep B *(186,188,192,220)*, oral polio vaccine *(220)*, IPV and PRP-T *(187,188,192,228,229)*, MMR *(229)* and monovalent attenuated human rotavirus vaccine *(230)*. Further studies have been conducted with PCV9-CRM *(174,175)*, PCV9CRM-MCV-C-CRM *(189*) and PCV11-PD *(169)*. The studies show minor and variable differences between the groups for particular antigens in vaccines given concomitantly with PCVs, but in general these are unlikely to be of clinical significance.

6. Serological correlates for protection

Due to the various infections caused by pneumococci, and the multiplicity of important disease-causing serotypes, a definitive serological correlate of protection is hard to establish. Similarly, the paucity of individual serotypes captured in the context of a vaccine efficacy trial with IPD as an end-point, means that clinical serotype- specific efficacy estimates have not been established *(40,41)*. The etiology of non-bacteraemic pneumonia cannot be specifically defined with current diagnostic tools, thereby making it unlikely that a serological correlate for protection against the major pneumococcal disease burden can be achieved.

The protective effect of systemic circulating antibodies that bind to the capsular polysaccharide of pneumococci, enabling the killing of the bacteria by phagocytes, has been well established for invasive pneumococcal disease. In 2003, data was available from three randomized controlled efficacy trials against invasive pneumococcal disease with the licensed PCV7-CRM or its closely related PCV9-CRM *(12,13,41)*. On this basis WHO undertook a series of consultations to develop serological criteria for evaluation of pneumococcal conjugate vaccines for licensure purposes *(63)*. The approach adopted was to define a threshold concentration that would best predict protection at a population level. A protective threshold serum IgG antibody concentration of 0.35µg/ml measured one month after vaccination by EIA, was defined by extrapolating to pooled immunogenicity data from the pooled point-estimate of clinical efficacy against invasive pneumococcal disease from three different clinical trials. This threshold antibody concentration is applicable for evaluation of new vaccine formulations, vaccination schedules, or combination vaccines in head-to-head comparisons with the already licensed PCV7-CRM for non-inferiority, when efficacy trial is not feasible. In addition to showing non-inferiority in antibody concentrations, direct measurement of opsonophagocytosis by an OPA assay, and markers of immunologic memory, are recommended in order to confirm good immunogenicity.

The antibody data used for the calculation of this threshold was generated by a standardized EIA. As many laboratories are moving to an improved EIA with 22F PS adsorption to increase assay specificity, the threshold has been under discussion *(43,231)*. The approach chosen for the definition of this correlate for protection required several simplifying assumptions that should be considered in the application of this estimate. Firstly, it is only applicable for IPD. The currently recommended serologic criteria cannot be used to evaluate efficacy against other clinical end-points; higher antibody thresholds appear to be needed for protection from acute otitis media (AOM), pneumonia, or nasopharyngeal carriage *(42,232,233,234)*. Secondly, this relatively low protective antibody concentration is a threshold meant to establish non-inferiority of a new product as compared with a product of proven clinical efficacy aggregated across all the vaccine serotypes, and should not be used to predict protection

at an individual level. Because of immunological memory, vaccinated individuals can mount a rapid and vigorous immune response upon contact, and protection can be achieved in spite of a low antibody concentration at the time of infection. Therefore, a similar low antibody concentration in unvaccinated individuals may be insufficient to confer protection. Thirdly, it is an aggregate-level estimate for all the serotypes in the vaccine and does not necessarily reflect the differences between individual serotypes in its capacity to mediate opsonophagocytosis. Fourthly, caution must be applied in using thresholds derived from a population of infants, to other populations differing by age and/or health status.

The protective effect of the PCV7-CRM as well as other PCVs, against mucosal infections such as serotype-specific AOM, has been lower than for IPD, and different for the individual serotypes *(167,168,169)*. A different approach was undertaken by investigators who defined mean serum antibody concentrations in vaccinated infants associated with the risk of serotype-specific AOM *(42)*. On the basis of this association, they showed that the mean serotype-specific antibody concentrations that predicted vaccine efficacy, varied by serotype. For 6B a constant efficacy was found with a low mean concentration of ≥0.5µg/ml, whereas mean concentrations as high as >10µg/ml were not able to protect against AOM caused by serotype 19F. Thus, a single threshold concentration may underestimate clinical efficacy for some serotypes and overestimate it for others. Alternatively, the lower level of protection against mucosal infections, may be due to the limits of protection that systemic antibodies to capsular polysaccharides are able to mediate *(136)*.

Accumulating data from efficacy trials against AOM suggest that opsonophagocytic activity, currently recommended as a secondary end-point in PCV evaluation, could predict serotype-specific efficacy better than the concentration of antibodies *(86,87)*. For example, serotype 19F conjugates induce high concentrations of antibodies, but their functional activity, and the clinical efficacy of the conjugate is low. By contrast, serotype 6B conjugates induce lower concentrations of antibodies, but their functional activity and clinical efficacy is high *(86,87,167,168,169)*. Immune responses at mucosal sites and the persistence of immunity, may be as important as the concentration of circulating antibody, in protection against pneumonia and less invasive mucosal infections such as AOM and nasopharyngeal carriage.

7. Vaccine effectiveness

The 23-valent PPSV is widely recommended for persons ≥ 65 years old and for persons 2–65 years old with high risk for pneumococcal disease e.g. underlying disease or splenectomy *(20)*. PPSV has proven effectiveness against IPD in the healthy target group *(24,235–240)*. However, its effectiveness in risk groups is arguable. For example, it reduces IPD in indigenous Australian children *(241)*, but not in Alaskan natives ≥ 55 years old *(242)*. Furthermore, the PPSV has not provided a significant effect on morbidity or mortality of patients suffering from chronic obstructive pulmonary disease *(243)* or other immunocompromising conditions e.g. sickle-cell disease or leukemia *(238)*. The impact of PPSV on pneumonia is also controversial *(23,26,235,244,245,246)*. In contrast to the PCVs, the PPSV does not reduce carriage *(15,22,247)*, so it is not expected to have any effect on the transmission of pneumococci, and accordingly no indirect effect (herd immunity).

Most of the information regarding the effectiveness of the PCV7-CRM has been derived from the USA, where it has been in the routine vaccination schedule since 2000. In the USA, the PCV is recommended for all children younger than two years of age, and high-risk children aged 2–4 years *(248,249,250)*. After six years routine use, the overall effectiveness of the PCV7-CRM against IPD (irrespective of the serotype) in children under five years old, has been estimated to be 70% *(251)*, and highest among healthy children against the VT IPD (96%). The effectiveness against IPD has also been recorded in other countries that have introduced PCV7-CRM in their national childhood immunization programmes. Data is available from the Barcelona and Navarra districts in Spain *(252,253)*, Australia *(254)*, and the Greater Vancouver area in Canada *(255)*.

According to post-licensure surveillance in the USA, PCV7-CRM has reduced the rates of community-acquired pneumonia hospital admissions (CAP) in infants 0 to 2 years old *(256,257)*, as well as in adults in the age group 18–39 years; reduction in other age groups did not achieve statistical significance however. The decline in hospital admissions due to all-cause pneumonia has been estimated to be 39% in the target population *(256)*. The routine use of PCV has also diminished the incidence of AOM *(258)*, the number of persistent AOM cases, and the prevalence of penicillin-resistant pneumococcal strains *(259)*. The vaccination has also decreased the recovery of pneumococci in acute maxillary sinusitis *(260)*.

PCVs reduce the pneumococcal carriage and thus are able to prevent the transmission of VT pneumococci. The results of post-licensure surveillance confirm this as the carriage of vaccine-type pneumococci has been decreasing in both vaccinated and unvaccinated individuals *(261,262)*. Routine vaccination with PCV7-CRM has reduced the incidence of IPD significantly in the target group, in unvaccinated younger infants *(263)*, young adults, and in elderly people aged 65 or older (264,265,266). Pneumococcal infections have also decreased among HIV-infected adults living with children immunized with PCV7-CRM *(267)*.

The disease reduction gains from the large-scale use of the PCV7-CRM has been offset to varying degrees by increase of infections caused by NVT. The overall effect of the vaccine on nasopharyngeal carriage has been estimated as zero due to the replacement of VTs by NVTs *(257,261,262,268)*. The replacement effect has also been seen with pneumococcal disease *(266,269–273)*. As with nasopharyngeal carriage, the increase in AOM caused by NVT substantially offsets the gain from reduction in VT AOM, especially for PCV-CRM and PCV-OMP. However, increase in NVT IPD has been marginal compared with the reduction in VT IPD.

PCV has also been shown to cause reduction in disease due to related serotypes, i.e. serotypes within the same serogroup, with 43% effectiveness against the IPD due to the vaccine-related serotypes being reported *(251)*. The effect is most notable for serotype 6A. On the other hand however, 19A disease has increased, and it is one of the major replacement serotypes *(271,273–277)*. Worryingly, the increase of NVT disease, especially of serotype 19A, is due to an increase of antimicrobial-resistant pneumococcal clones *(275–279)*. However, it has to be noted that 19A disease has also increased in some countries that have not introduced PCV7-CRM via the spread of a 19A clone *(280,281)*.

8. Future prospects

There is currently one licensed PCV (PCV7-CRM) which has proved effective in preventing VT disease and colonization. However, the vaccine is fairly new, and the serotype coverage is not optimal globally. Experience of large-scale use in different developed countries is accumulating steadily. The use of PCV in resource-poor countries is expected to begin starting in 2008. For optimal and feasible adoption of the PCV, ongoing and future studies (together with the post-licensure follow-up) need to show how many doses would be needed for optimal protection in the target population, as well as in the unvaccinated population. The need for and feasibility of a booster dose needs to be clarified. Furthermore, the PCVs might need to be given in combination with new vaccines. The possible interactions should be studied.

New infant formulations with wider serotype coverage are expected to come to the market within the next five years. Their effectiveness is expected to be better than that of the 7-valent vaccine, especially in populations where serotypes 1, 5 and 7F are prevalent. Furthermore, inclusion of serotype 19A to the vaccine will hopefully decrease not only 19A infections but also the spread of antimicrobial resistance.

Studies on pneumococcal colonization will improve the understanding of the biological mechanisms, kinetics of transmission, and the effects of PCVs. Studies on colonization can provide tools to predict both direct and indirect effects of pneumococcal vaccines. It is therefore possible that optimally-designed clinical trials with colonization end-points will offer an alternative and/or additional way to evaluate the efficacy of new pneumococcal vaccines, vaccine formulations, and vaccination schedules.

Experience with PCVs in adults and high-risk groups is still sparse. Studies addressing the optimal vaccine serotype coverage, formulations, adjuvants and the number of doses needed, will provide guidance on how to proceed in the prevention of pneumococcal disease in these important groups.

Finally, the new third-generation pneumococcal vaccines containing pneumococcal proteins, both PCVs and protein antigens, or pneumococcal PSs conjugated to pneumococcal proteins, will be tested in clinical trials in the next 5–10 years. Such trials will determine the feasibility of these approaches, and hopefully provide more information on the protective mechanisms and immunological basis of protection evoked by pneumococcal protein vaccines.

References

1. Bergmann S, Hammerschmidt S. Versatility of pneumococcal surface proteins. *Microbiology*, 2006, 152:295–303.
2. Hammerschmidt S. Adherence molecules of pathogenic pneumococci. *Current Opinion in Microbiology*, 2006, 9:12–20.
3. Ogunniyi AD et al. Development of a vaccine against invasive pneumococcal disease based on combinations of virulence proteins of *Streptococcus pneumoniae*. *Infection and Immunity*, 2007, 75:350–357.
4. *Searching for a new pneumonia vaccine* (http://www.path.org/projects/pneumococcal_protein_vaccine_project.php).
5. Barocchi MA et al. A pneumococcal pilus influences virulence and host inflammatory responses. *Proceedings of the National Academy of Sciences of the United States of America*, 2006, 103:2857–2862.
6. Jacobs MR, Dagan R. Antimicrobial resistance among pediatric respiratory tract infections: clinical challenges. *Seminars in Pediatric Infectious Diseases*, 2004, 15:5–20.
7. Gratten M et al. Colonization of *Haemophilus influenzae* and *Streptococcus pneumoniae* in the upper respiratory tract of neonates in Papua New Guinea: primary acquisition, duration of carriage, and relationship to carriage in mothers. *Biology of the Neonate*, 1986, 50:114–120.
8. Obaro SK et al. Carriage of pneumococci after pneumococcal vaccination. *Lancet*, 1996, 348:271–272.
9. Hussain M et al. A longitudinal household study of *Streptococcus pneumoniae* nasopharyngeal carriage in a UK setting. *Epidemiology and Infection*, 2005, 133:891–898.
10. Regev-Yochay G et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* by adults and children in community and family settings. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 2004, 38:632–639.
11. Black SB et al. Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. *The Pediatric Infectious Disease Journal*, 2002, 21:810–815.
12. Cutts FT et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomized, double-blind, placebo-controlled trial. *Lancet*, 2005, 365:1139–1146.

13. Klugman KP et al. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *The New England Journal of Medicine*, 2003, 349:1341–1348.

14. *Detailed review paper on pneumococcal conjugate vaccine*. Geneva, WHO Pneumococcal Conjugate Vaccine Working Group. Presented to the WHO Strategic Advisory Group of Experts (SAGE) on immunization, November 2006 (http://www.who.int/immunization/SAGE_wg_detailedreview_pneumoVaccine.pdf, 2006).

15. MacLeod M et al. Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. *The Journal of Experimental Medicine*, 1945, 82:445–465.

16. Kaufman P. Pneumonia in old age. Active immunization against pneumonia with pneumococcus polysaccharide, results of a six year study. *Archives of Internal Medicine*, 1947, 79:518–531.

17. Austrian R et al. Prevention of pneumococcal pneumonia by vaccination. *Transactions of the Association of American Physicians*, 1976, 89:184–194.

18. Smit P et al. Protective efficacy of pneumococcal polysaccharide vaccines. *JAMA: the journal of the American Medical Association*, 1977, 238:2613–2616.

19. Riley ID et al. Immunization with a polyvalent pneumococcal vaccine. Reduction of adult respiratory mortality in a New Guinea Highlands community. *Lancet*, 1977, 1:1338–1341.

20. Centers for Disease Control and Prevention. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR. Morbidity and Mortality Weekly Report*, 1997, 46:1–24.

21. Douglas RM et al. Pneumococcal carriage and type-specific antibody. Failure of a 14-valent vaccine to reduce carriage in healthy children. *American Journal of Diseases of Children (1960)*, 1986, 140:1183–185.

22. Herva E et al. The effect of polyvalent pneumococcal polysaccharide vaccine on nasopharyngeal and nasal carriage of *Streptococcus pneumoniae*. *Scandinavian Journal of Infectious Diseases*, 1980, 12:97–100.

23. Honkanen PO et al. Incremental effectiveness of pneumococcal vaccine on simultaneously administered influenza vaccine in preventing pneumonia and pneumococcal pneumonia among persons aged 65 years or older. *Vaccine*, 1999, 17:2493–2500.

24. Jackson LA et al. Effectiveness of pneumococcal polysaccharide vaccine in older adults. *The New England Journal of Medicine*, 2003, 348:1747–1755.

25. Simberkoff MS et al. Efficacy of pneumococcal vaccine in high-risk patients. Results of a Veterans Administration Cooperative Study. *The New England Journal of Medicine*, 1986, 315:1318–1327.

26. Örtqvist Å et al. Randomized trial of 23-valent pneumococcal capsular polysaccharide vaccine in prevention of pneumonia in middle-aged and elderly people. Swedish Pneumococcal Vaccination Study Group. *Lancet*, 1998, 351:399–403.

27. Avery OT, Goebel WF. Chemo-immunological studies on conjugated carbohydrate-proteins. V. The immunological specificity of an antigen prepared by combining the capsular polysaccharide of type III pneumococcus with foreign protein. *The Journal of Experimental Medicine*, 1931, 54:437–447.

28. Schneerson R et al. Serum antibody responses of juvenile and infant rhesus monkeys injected with *Haemophilus influenzae* type b and pneumococcus type 6A capsular polysaccharide-protein conjugates. *Infection and Immunity*, 1984, 45:582–591.

29. Sigurdardottir ST et al. Immune response to octavalent diphtheria- and tetanus-conjugated pneumococcal vaccines is serotype- and carrier-specific: the choice for a mixed carrier vaccine. *The Pediatric Infectious Disease Journal*, 2002, 21:548–554.

30. Wuorimaa T et al. Tolerability and immunogenicity of an eleven-valent pneumococcal conjugate vaccine in healthy toddlers. *The Pediatric Infectious Disease Journal*, 2001, 20:272–277.

31. Åhman H et al. *Streptococcus pneumoniae* capsular polysaccharide-diphtheria toxoid conjugate vaccine is immunogenic in early infancy and able to induce immunologic memory. *The Pediatric Infectious Disease Journal*, 1998, 17:211–216.

32. Åhman H et al. Dose dependency of antibody response in infants and children to pneumococcal polysaccharides conjugated to tetanus toxoid. *Vaccine*, 1999, 17:2726–2732.

33. Käyhty H et al. Pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine is immunogenic in infants and children. *The Journal of Infectious Diseases*, 1995, 172:1273–1278.

34. Anderson EL et al. Immunogenicity of heptavalent pneumococcal conjugate vaccine in infants. *The Journal of Pediatrics*, 1996, 128:649–653.

35. Gatchalian SR et al. A randomized, placebo-controlled study to evaluate the immunogenicity of an 11-valent pneumococcal protein D conjugate vaccine administered as primary vaccination to infants at 6, 10 and 14 weeks of age. In: *Proceedings of the nineteenth annual meeting of the European Society for Paediatric Infectious Diseases (ESPID), Istanbul, Turkey*. Geneva, European Society for Paediatric Infectious Diseases, 2001:18.

36. Nurkka A et al. Immunogenicity and safety of the eleven valent pneumococcal polysaccharide-protein D conjugate vaccine in infants. *The Pediatric Infectious Disease Journal*, 2004, 23:1008–1014.

37. Vesikari T et al. Immunogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) compared to the licensed 7vCRM vaccine. *The Pediatric Infectious Disease Journal*, 2009, 28:S66-S76.

38. Chevallier B et al. Safety and reactogenicity of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) when coadministered with routine childhood vaccines. *The Pediatric Infectious Disease Journal*, 2009, 28:S109-S118.

39. Kieninger DM et al. Safety & immunologic non-inferiority of 13-valent pneumococcal conjugate vaccine compared to 7-valent pneumococcal conjugate vaccine given with routine vaccines in healthy infants. *Proceedings of the Forty-Eight Annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Washington, DC.* 2008:abstract G-2117

40. Jodar L et al. Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. *Vaccine*, 2003, 21:3265–3272.

41. Black S et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *The Pediatric Infectious Disease Journal*, 2000, 19:187–195.

42. Jokinen J et al. Concentration of anti-pneumococcal antibodies as a serological correlate of protection: an application to acute otitis media. *The Journal of Infectious Diseases*, 2004, 190:545–550.

43. Siber G et al. Estimating the protective concentration of anti-pneumococcal capsular polysaccharide antibodies. *Vaccine*, 2007, 25:3816–3826.

44. Anttila M et al. Avidity of IgG for *Streptococcus pneumoniae* type 6B and 23F polysaccharides in infants primed with pneumococcal conjugates and boosted with polysaccharide or conjugate vaccines. *The Journal of Infectious Diseases*, 1998, 177:1614–1621.

45. Ekström N et al. Kinetics and avidity of antibodies evoked by heptavalent pneumococcal conjugate vaccines PncCRM and PncOMPC in the Finnish Otitis Media Vaccine Trial. *Infection and Immunity*, 2005, 73:369–377.

46. Goldblatt D, Vaz AR, Miller E. Antibody avidity as a surrogate marker of successful priming by *Haemophilus influenzae* type b conjugate vaccines following infant immunization. *The Journal of Infectious Diseases*, 1998, 177:1112–1115.

47. Goldblatt D et al. Immunogenicity and boosting after a reduced number of doses of a pneumococcal conjugate vaccine in infants and toddlers. *The Pediatric Infectious Disease Journal*, 2006, 25:312–319.

48. Schiffman G et al. A radioimmunoassay for immunologic phenomena in pneumococcal disease and for the antibody response to pneumococcal vaccines. I. Method for the radioimmunoassay of anticapsular antibodies and comparison with other techniques. *Journal of Immunological Methods*, 1980, 33:133–144.

49. Farr RS. A quantitative immunochemical measure of the primary interaction between I BSA and antibody. *The Journal of Infectious Diseases*, 1958, 103:239–262.

50. Briles DE et al. Naturally occurring antibodies to phosphocholine as a potential index of antibody responsiveness to polysaccharides. *The Journal of Infectious Diseases*, 1987, 155:1307–1314.

51. Goldblatt D, Levinsky RJ, Turner MW. Role of cell wall polysaccharide in the assessment of IgG antibodies to the capsular polysaccharides of *Streptococcus pneumoniae* in childhood. *The Journal of Infectious Diseases*, 1992, 166:632–634.

52. Koskela M. Serum antibodies to pneumococcal C polysaccharide in children: response to acute pneumococcal otitis media or to vaccination. *The Pediatric Infectious Disease Journal*, 1987, 6:519–526.

53. Musher DM, Watson DA, Baughn RE. Does naturally acquired IgG antibody to cell wall polysaccharide protect human subjects against pneumococcal infection? *The Journal of Infectious Diseases*, 1990, 161:736–740.

54. Concepcion NF, Frasch CE. Pneumococcal type 22f polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. *Clinical and Diagnostic Laboratory Immunology*, 2001, 8:266–272.

55. Feikin DR et al. Specificity of the antibody response to the pneumococcal polysaccharide and conjugate vaccines in human immunodeficiency virus-infected adults. *Clinical and Diagnostic Laboratory Immunology*, 2004, 11:137–141.

56. Hu BT et al. Some sera from infant and child population may require addition of PnPs22F absorbent in the anti-PnPs ELISA. In: *The third international symposium on Pneumococci and Pneumococcal Diseases*. Anchorage, Alaska, 2002.

57. Nahm M. *Training manual for enzyme-linked immunosorbent assay for the quantitation of Streptococcus pneumoniae serotype-specific IgG (Pnc PS ELISA)*. Respiratory Pathogen Reference Laboratory, University of Alabama (http://www.vaccine.uab.edu).

58. Wernette CM et al. Enzyme-linked immunosorbent assay for quantitation of human antibodies to pneumococcal polysaccharides. *Clinical and Diagnostic Laboratory Immunology*, 2003, 10:514–519.

59. Quataert SA et al. Assignment of weight-based antibody units to a human antipneumococcal standard reference serum, lot 89-S. *Clinical and Diagnostic Laboratory Immunology*, 1995, 2:590–597.

60. Quataert SA et al. Assignment of weight-based antibody units for 13 serotypes to a human antipneumococcal standard reference serum, lot 89-S(f). *Clinical and Diagnostic Laboratory Immunology*, 2004, 11:1064–1069.

61. Sikkema DJ et al. Assignment of weight-based immunoglobulin G1 (IgG1) and IgG2 units in antipneumococcal reference serum lot 89-S(F) for pneumococcal polysaccharide serotypes 1, 4, 5, 7F, 9V, and 18C. *Clinical and Diagnostic Laboratory Immunology*, 2005, 12:218–223.

62. Soininen A et al. Assignment of immunoglobulin G1 and G2 concentrations to pneumococcal capsular polysaccharides 3, 6B, 14, 19F, and 23F in pneumococcal reference serum 89-SF. *Clinical and Diagnostic Laboratory Immunology*, 1998, 5:561–566.

63. *Pneumococcal conjugate vaccines. Recommendations for the production and control of pneumococcal conjugate vaccines*. Geneva, World Health Organization, 2005 (WHO Technical Report Series No. 927, Annex 2) (http://www.who.int/biologicals/publications/trs/areas/vaccines/pneumo/en/index.html).

64. Choo S et al. Primary and booster salivary antibody responses to a 7-valent pneumococcal conjugate vaccine in infants. *The Journal of Infectious Diseases*, 2000, 182:1260–1263.

65. Nurkka A et al. Salivary antibodies induced by the seven-valent PncCRM conjugate vaccine in the Finnish Otitis Media Vaccine Trial. *Vaccine*, 2004, 23:298–304.

66. Nurkka A et al. Salivary antibodies induced by the seven-valent PncOMPC conjugate vaccine in the Finnish Otitis Media Vaccine Trial. *BMC Infectious Diseases*, 2005, 5:41.

67. Gordon S et al. Pneumococcal conjugate vaccine is immunogenic in lung fluid of HIV infected and immunocompetent adults. *The Journal of Allergy and Clinical Immunology*, 2007, 120:208–210.

68. Korkeila M et al. Salivary anti-capsular antibodies in infants and children immunized with *Streptococcus pneumoniae* capsular polysaccharides conjugated to diphtheria or tetanus toxoid. *Vaccine*, 2000, 18:1218–1226.

69. Nieminen T et al. Circulating antibody secreting cell response to parenteral pneumococcal vaccines as an indicator of a salivary IgA antibody response. *Vaccine*, 1998, 16:313–319.

70. Virolainen A et al. Antibodies to pneumolysin and pneumococcal capsular polysaccharides in middle ear fluid of children with acute otitis media. *Acta Oto-laryngologica*, 1995, 115:796–803.

71. Virolainen A et al. Nasopharyngeal antibodies to pneumococcal capsular polysaccharides in children with acute otitis media. *The Journal of Infectious Diseases*, 1995, 172:1115–1118.

72. Hodinka RL, Nagashunmugam T, Malamud D. Detection of human immunodeficiency virus antibodies in oral fluids. *Clinical and Diagnostic Laboratory Immunology*, 1998, 5:419–426.

73. Judd A et al. Evaluation of a modified commercial assay in detecting antibody to hepatitis C virus in oral fluids and dried blood spots. *Journal of Medical Virology*, 2003, 71:49–55.

74. Vyse AJ, Cohen BJ, Ramsay ME. A comparison of oral fluid collection devices for use in the surveillance of virus diseases in children. *Public Health*, 2001, 115:201–207.

75. Kilian M, Mestecky J, Schrohenloher RE. Pathogenic species of the genus Haemophilus and *Streptococcus pneumoniae* produce immunoglobulin A1 protease. *Infection and Immunity*, 1979, 26:143–149.

76. Butler JE et al. Humoral immunity in root caries in an elderly population. 2. *Oral Microbiology and Immunology*, 1990, 5:113–120.

77. Nurkka A et al. Effects of sample collection and storage methods on antipneumococcal immunoglobulin A in saliva. *Clinical and Diagnostic Laboratory Immunology*, 2003, 10:357–361.

78. Biagini RE et al. Method for simultaneous measurement of antibodies to 23 pneumococcal capsular polysaccharides. *Clinical and Diagnostic Laboratory Immunology*, 2003, 10:744–750.

79. Lal G et al. Development and validation of a nonaplex assay for the simultaneous quantitation of antibodies to nine *Streptococcus pneumoniae* serotypes. *Journal of Immunological Methods*, 2005, 296:135–147.

80. Pickering JW et al. A multiplexed fluorescent microsphere immunoassay for antibodies to pneumococcal capsular polysaccharides. *American Journal of Clinical Pathology*, 2002, 117:589–596.

81. de Roux A et al. Opsonophagocytic responses to pneumococcal conjugate vaccine in the elderly. *The programme and abstract book of the fifth international symposium on Pneumococci and Pneumococcal Diseases*. Alice Springs, Australia, 2006:212.

82. Devaster J et al. Inferior humoral response in elderly versus young adults to the 23-valent polysaccharide vaccine. *The programme and abstract book of the fifth international symposium on Pneumococci and Pneumococcal Diseases*. Alice Springs, Australia, 2006:244.

83. Madhi SA et al. Quantitative and qualitative antibody response to pneumococcal conjugate vaccine among African human immunodeficiency virus-infected and uninfected children. *The Pediatric Infectious Disease Journal*, 2005, 24:410–416.

84. Parkkali T et al. Opsonophagocytic activity against *Streptococcus pneumoniae* type 19F in allogeneic BMT recipients before and after vaccination with pneumococcal polysaccharide vaccine. *Bone Marrow Transplantation*, 2001, 27:207–211.

85. Romero-Steiner S et al. Reduction in functional antibody activity against *Streptococcus pneumoniae* in vaccinated elderly individuals highly correlates with decreased IgG antibody avidity. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 1999, 29:281–288.

86. Ekström N et al. Functional activity of antibodies elicited by heptavalent pneumococcal conjugate vaccines PncCRM and PncOMPC in the Finnish Otitis Media Vaccine Trial. *Infection and Immunity*, 2007, 75:1794–1800.

87. Schuerman L et al. ELISA IgG concentrations and opsonophagocytic activity following pneumococcal protein D conjugate vaccination and relationship to efficacy against acute otitis media. *Vaccine*, 2007, 25:1962–1968.

88. Romero-Steiner S et al. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against *Streptococcus pneumoniae* using differentiated HL-60 cells. *Clinical and Diagnostic Laboratory Immunology*, 1997, 4:415–422.

89. Henckaerts I et al. Validation of a routine opsonophagocytosis assay to predict invasive pneumococcal disease efficacy of conjugate vaccine in children. *Vaccine*, 2007, 25:2518–2527.

90. Hu BT et al. Approach to validating an opsonophagocytic assay for *Streptococcus pneumoniae*. *Clinical and Diagnostic Laboratory Immunology*, 2005, 12:287–295.

91. Bogaert D et al. Multiplex opsonophagocytosis assay (MOPA): a useful tool for the monitoring of the 7-valent pneumococcal conjugate vaccine. *Vaccine*, 2004, 22:4014–4020.

92. Burton RL, Nahm MH. Development and validation of a fourfold multiplexed opsonization assay (MOPA4) for pneumococcal antibodies. *Clinical and Vaccine Immunology: CVI*, 2006, 13:1004–1009.

93. Kim KH, Yu J, Nahm MH. Efficiency of a pneumococcal opsonophagocytic killing assay improved by multiplexing and by coloring colonies. *Clinical and Diagnostic Laboratory Immunology*, 2003, 10:616–621.

94. Lin JS, Park MK, Nahm MH. Chromogenic assay measuring opsonophagocytic killing capacities of antipneumococcal antisera. *Clinical and Diagnostic Laboratory Immunology*, 2001, 8:528–533.

95. Bieging KT et al. Fluorescent multivalent opsonophagocytic assay for measurement of functional antibodies to *Streptococcus pneumoniae*. *Clinical and Diagnostic Laboratory Immunology*, 2005, 12:1238–1242.

96. Vitharsson G et al. Opsonization and antibodies to capsular and cell wall polysaccharides of *Streptococcus pneumoniae*. *The Journal of Infectious Diseases*, 1994, 170:592–599.

97. Väkeväinen M et al. Are the opsonophagocytic activities of antibodies in infant sera measured by different pneumococcal phagocytosis assays comparable? *Clinical and Diagnostic Laboratory Immunology*, 2001, 8:363–369.

98. Jansen WT et al. Use of highly encapsulated *Streptococcus pneumoniae* strains in a flow-cytometric assay for assessment of the phagocytic capacity of serotype-specific antibodies. *Clinical and Diagnostic Laboratory Immunology*, 1998, 5:703–710.

99. Martinez JE et al. A flow cytometric opsonophagocytic assay for measurement of functional antibodies elicited after vaccination with the 23-valent pneumococcal polysaccharide vaccine. *Clinical and Diagnostic Laboratory Immunology*, 1999, 6:581–586.

100. Bortolussi R et al. Serum antibody and opsonic responses after immunization with pneumococcal vaccine in kidney transplant recipients and controls. *Infection and Immunity*, 1981, 34:20–25.

101. Romero-Steiner S et al. Use of opsonophagocytosis for serological evaluation of pneumococcal vaccines. *Clinical and Vaccine Immunology: CVI*, 2006, 13:165–169.

102. Romero-Steiner S et al. Multilaboratory evaluation of a viability assay for measurement of opsonophagocytic antibodies specific to the capsular polysaccharides of *Streptococcus pneumoniae*. *Clinical and Diagnostic Laboratory Immunology*, 2003, 10:1019–1024.

103. Musher DM et al. Antibody to capsular polysaccharide of *Streptococcus pneumoniae* at the time of hospital admission for Pneumococcal pneumonia. *The Journal of Infectious Diseases*, 2000, 182:158–167.

104. Anttila M et al. Differences in the avidity of antibodies evoked by four different pneumococcal conjugate vaccines in early childhood. *Vaccine*, 1999, 17:1970–1977.

105. Griswold WR et al. Functional affinity of antibody to the *Haemophilus influenzae* type b polysaccharide. *The Journal of Infectious Diseases*, 1989, 159:1083–1087.

106. Sun Y, Hwang Y, Nahm MH. Avidity, potency, and cross-reactivity of monoclonal antibodies to pneumococcal capsular polysaccharide serotype 6B. *Infection and Immunity*, 2001, 69:336–344.

107. Guttormsen HK et al. Cognate stimulatory B-cell-T-cell interactions are critical for T-cell help recruited by glycoconjugate vaccines. *Infection and Immunity*, 1999, 67:6375–6384.

108. Kamboj KK et al. Significant variation in serotype-specific immunogenicity of the seven-valent *Streptococcus pneumoniae* capsular polysaccharide-CRM197 conjugate vaccine occurs despite vigorous T-cell help induced by the carrier protein. *The Journal of Infectious Diseases*, 2003, 187:1629–1638.

109. Wuorimaa T et al. Activation of cell-mediated immunity following immunization with pneumococcal conjugate or polysaccharide vaccine. *Scandinavian Journal of Immunology*, 2001, 53:422–428.

110. Jakobsen H et al. Early life T-cell responses to pneumococcal conjugates increase with age and determine the polysaccharide-specific antibody response and protective efficacy. *European Journal of Immunology*, 2006, 36:287–295.

111. Lynch JM, Briles DE, Metzger DW. Increased protection against pneumococcal disease by mucosal administration of conjugate vaccine plus interleukin-12. *Infection and Immunity*, 2003, 71:4780–4788.

112. Sen G, Chen Q, Snapper CM. Immunization of aged mice with a pneumococcal conjugate vaccine combined with an unmethylated CpG-containing oligodeoxynucleotide restores defective immunoglobulin G antipolysaccharide responses and specific CD4+-T-cell priming to young adult levels. *Infection and Immunity*, 2006, 74:2177–2186.

113. Clutterbuck EA et al. The kinetics and phenotype of the human B-cell response following immunization with a heptavalent pneumococcal-CRM conjugate vaccine. *Immunology*, 2006, 119:328–337.

114. Brown EJ, Hosea SW, Frank MM. The role of antibody and complement in the reticuloendothelial clearance of pneumococci from the bloodstream. *Reviews of Infectious Diseases*, 1983, 5(Suppl. 4):S797–S805.

115. Siber GR et al. Evaluation of bacterial polysaccharide immune globulin for the treatment or prevention of *Haemophilus influenzae* type b and pneumococcal disease. *The Journal of Infectious Diseases*, 1992, 165(Suppl. 1):S129–S133.

116. Shurin PA et al. Bacterial polysaccharide immune globulin for prophylaxis of acute otitis media in high-risk children. *The Journal of Pediatrics*, 1993, 123:801–810.

117. Jedrzejas MJ. Pneumococcal virulence factors: structure and function. *Microbiology and Molecular Biology Reviews: MMBR*, 2001, 65:187–207.

118. Cheng Q, Finkel D, Hostetter MK. Novel purification scheme and functions for a C3-binding protein from *Streptococcus pneumoniae*. *Biochemistry*, 2000, 39:5450–5457.

119. Moxon ER, Kroll JS. The role of bacterial polysaccharide capsules as virulence factors. *Current Topics in Microbiology and Immunology*, 1990, 150:65–85.

120. Overweg K et al. Differential protein expression in phenotypic variants of *Streptococcus pneumoniae*. *Infection and Immunity*, 2000, 68:4604–4610.

121. Rosenow C et al. Contribution of novel choline-binding proteins to adherence, colonization and immunogenicity of *Streptococcus pneumoniae*. *Molecular Microbiology*, 1997, 25:819–829.

122. Cundell DR et al. Relationship between colonial morphology and adherence of *Streptococcus pneumoniae*. *Infection and Immunity*, 1995, 63:757–761.

123. Kovaiou RD, Herndler-Brandstetter D, Grubeck-Loebenstein B. Age-related changes in immunity: implications for vaccination in the elderly. *Expert Reviews in Molecular Medicine*, 2007, 9:1–17.

124. Rapola S et al. Natural development of antibodies to pneumococcal surface protein A, pneumococcal surface adhesin A, and pneumolysin in relation to pneumococcal carriage and acute otitis media. *The Journal of Infectious Diseases*, 2000, 182:1146–1152.

125. Rapola S et al. Antibody response to the pneumococcal proteins pneumococcal surface adhesin A and pneumolysin in children with acute otitis media. *The Pediatric Infectious Disease Journal*, 2001, 20:482–487.

126. Soininen A et al. Natural development of antibodies to pneumococcal capsular polysaccharides depends on the serotype: association with pneumococcal carriage and acute otitis media in young children. *The Journal of Infectious Diseases*, 2001, 184:569–576.

127. Soininen A et al. Antibody response to pneumococcal capsular polysaccharides in children with acute otitis media. *The Pediatric Infectious Disease Journal*, 2002, 21:186–192.

128. Simell B et al. Pneumococcal carriage and otitis media induce salivary antibodies to pneumococcal surface adhesin A, pneumolysin, and pneumococcal surface protein A in children. *The Journal of Infectious Diseases*, 2001, 183:887–896.

129. Simell B, Kilpi TM, Käyhty H. Pneumococcal carriage and otitis media induce salivary antibodies to pneumococcal capsular polysaccharides in children. *The Journal of Infectious Diseases*, 2002, 186:1106–1114.

130. Bogaert D et al. Development of antibodies against the putative proteinase maturation protein A in relation to pneumococcal carriage and otitis media. *FEMS Immunology and Medical Microbiology*, 2006, 46:166–168.

131. Holmlund E et al. Development of natural antibodies to pneumococcal surface protein A, pneumococcal surface adhesin A and pneumolysin in Filipino pregnant women and their infants in relation to pneumococcal carriage. *Vaccine*, 2006, 24:57–65.

132. Holmlund E et al. Serum antibodies to the pneumococcal surface proteins PhtB and PhtE in Finnish infants and adults. *The Pediatric Infectious Disease Journal*, 2007, 26:447–449.

133. Simell B et al. Serum antibodies to pneumococcal neuraminidase NanA in relation to pneumococcal carriage and acute otitis media. *Clinical and Vaccine Immunology: CVI*, 2006, 13:1177–1179.

134. Simell B et al. Antibodies to pneumococcal surface protein A families 1 and 2 in serum and saliva of children and the risk of pneumococcal acute otitis media *The Journal of Infectious Diseases*, 2007, 196:1528–1536.

135. Malley R et al. CD4+ T-cells mediate antibody-independent acquired immunity to pneumococcal colonization. *Proceedings of the National Academy of Sciences of the United States of America*, 2005, 102:4848–4853.

136. McCool TL, Weiser JN. Limited role of antibody in clearance of *Streptococcus pneumoniae* in a murine model of colonization. *Infection and Immunity*, 2004, 72:5807–5813.

137. Anttila M et al. Contribution of serotype-specific IgG concentration, IgG subclasses and relative antibody avidity to opsonophagocytic activity against *Streptococcus pneumoniae*. *Clinical and Experimental Immunology*, 1999, 118:402–407.

138. Soininen A et al. IgG subclass distribution of antibodies after vaccination of adults with pneumococcal conjugate vaccines. *Vaccine*, 1999, 17:1889–1897.

139. Warmerdam PA et al. A single amino acid in the second Ig-like domain of the human Fc gamma receptor II is critical for human IgG2 binding. *Journal of Immunology (Baltimore, Md.: 1950)*, 1991, 147:1338–1343.

140. Sanders LA et al. Fc gamma receptor IIa (CD32) heterogeneity in patients with recurrent bacterial respiratory tract infections. *The Journal of Infectious Diseases*, 1994, 170:854–861.

141. Wiertsema SP et al. Pneumococcal vaccine efficacy for mucosal pneumococcal infections depends on Fcgamma receptor IIa polymorphism. *Vaccine*, 2006, 24:792–797.

142. Musher DM et al. The lack of association between aging and postvaccination levels of IgG antibody to capsular polysaccharides of *Streptococcus pneumoniae*. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 1996, 22:165–167.

143. Rubins JB et al. Determination of antibody responses of elderly adults to all 23 capsular polysaccharides after pneumococcal vaccination. *Infection and Immunity*, 1999, 67:5979–5984.

144. Rubins JB et al. Magnitude, duration, quality, and function of pneumococcal vaccine responses in elderly adults. *The Journal of Infectious Diseases*, 1998, 178:431–440.

145. Sankilampi U et al. Antibody response to pneumococcal capsular polysaccharide vaccine in the elderly. *The Journal of Infectious Diseases*, 1996, 173:387–393.

146. Sankilampi U et al. Persistence of antibodies to pneumococcal capsular polysaccharide vaccine in the elderly. *The Journal of Infectious Diseases*, 1997, 176:1100–104.

147. Johansen KS, Pedersen FK. Antibody response and opsonization after pneumococcal vaccination in splenectomized children and healthy persons. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica. Section C, Immunology*, 1982, 90:265–270.

148. Kazancioglu R et al. Immunization of renal transplant recipients with pneumococcal polysaccharide vaccine. *Clinical Transplantation*, 2000, 14:61–65.

149. Overturf GD et al. Pneumococcal polysaccharide immunization of children with sickle cell disease. II. Serologic response and pneumococcal disease following immunization. *The American Journal of Pediatric Hematology/ Oncology*, 1982, 4:25–35.

150. Parkkali T et al. IgG subclasses and avidity of antibodies to polysaccharide antigens in allogeneic BMT recipients after vaccination with pneumococcal polysaccharide and *Haemophilus influenzae* type b conjugate vaccines. *Bone Marrow Transplantation*, 1999, 24:671–678.

151. Pedersen FK, Nielsen JL, Ellegaard J. Antibody response to pneumococcal vaccine in splenectomized adults and adolescents. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica. Section C, Immunology*, 1982, 90:257–263.

152. Shatz DV et al. Immune responses of splenectomized trauma patients to the 23-valent pneumococcal polysaccharide vaccine at 1 versus 7 versus 14 days after splenectomy. *The Journal of Trauma*, 1998, 44:760–765; discussion 765–766.

153. French N et al. 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults: double-blind, randomized and placebo controlled trial. *Lancet*, 2000, 355:2106–2111.

154. French N et al. A case control study to investigate serological correlates of clinical failure of 23-valent pneumococcal vaccine in HIV-1-infected Ugandan adults. *The Journal of Infectious Diseases*, 2004, 190:707–712.

155. Breiman RF et al. Evaluation of effectiveness of the 23-valent pneumococcal capsular polysaccharide vaccine for HIV-infected patients. *Archives of Internal Medicine*, 2000, 160:2633–2638.

156. Carson PJ et al. Antibody class and subclass responses to pneumococcal polysaccharides following immunization of human immunodeficiency virus-infected patients. *The Journal of Infectious Diseases*, 1995, 172:340–345.

157. French N et al. Pneumococcal vaccination in HIV-1-infected adults in Uganda: humoral response and two vaccine failures. Aids 1998;12:1683–9

158. Kroon FP et al. Antibody response to influenza, tetanus and pneumococcal vaccines in HIV-seropositive individuals in relation to the number of CD4+ lymphocytes. *AIDS (London, England)*, 1994, 8:469–476.

159. Vandenbruaene M et al. Equal IgG antibody response to pneumococcal vaccination in all stages of human immunodeficiency virus disease. *The Journal of Infectious Diseases*, 1995, 172:551–553.

160. Lehmann D et al. Maternal immunization with pneumococcal polysaccharide vaccine in the highlands of Papua New Guinea. *Vaccine*, 2002, 20:1837–1845.

161. Munoz FM et al. Maternal immunization with pneumococcal polysaccharide vaccine in the third trimester of gestation. *Vaccine*, 2001, 20:826–837.

162. O'Dempsey TJ et al. Immunization with a pneumococcal capsular polysaccharide vaccine during pregnancy. *Vaccine*, 1996, 14:963–970.

163. Quiambao BP et al. Immunogenicity and reactogenicity of 23-valent pneumococcal polysaccharide vaccine among pregnant Filipino women and placental transfer of antibodies. *Vaccine*, 2007, 25:4470–4477.

164. Shahid NS et al. Serum, breast milk, and infant antibody after maternal immunization with pneumococcal vaccine. *Lancet*, 1995, 346:1252–1257.

165. O'Brien KL et al. Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomized trial. *Lancet*, 2003, 362:355–361.

166. Lucero MG et al. Efficacy of an 11-valent pneumococcal conjugate vaccine against radiologically confirmed pneumonia among children less than two years of age in the Philippines: a randomized, double-blind, placebo-controlled trial. *The Pediatric Infectious Disease Journal*, 2009, in press

167. Eskola J et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *The New England Journal of Medicine*, 2001, 344:403–409.

168. Kilpi T et al. Protective efficacy of a second pneumococcal conjugate vaccine against pneumococcal acute otitis media in infants and children: randomized, controlled trial of a 7-valent pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine in 1666 children. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 2003, 37:1155–1164.

169. Prymula R et al. Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae*: a randomized double-blind efficacy study. *Lancet*, 2006, 367:740–748.

170. Veenhoven R et al. Effect of conjugate pneumococcal vaccine followed by polysaccharide pneumococcal vaccine on recurrent acute otitis media: a randomized study. *Lancet*, 2003, 361:2189–2195.

171. Käyhty H et al. Nasopharyngeal colonization: a target for pneumococcal vaccination. *Expert Review of Vaccines*, 2006, 5:651–667.

172. O'Brien KL et al. Predictors of conjugate pneumococcal vaccine immunogenicity among infants and toddlers in the American Indian PnCRM7 efficacy trial. *The Journal of Infectious Diseases*, 2007, 196:104–114.

173. Soininen A et al. IgG antibody concentrations after immunization with 11-valent mixed-carrier pneumococcal conjugate vaccine in efficacy trial against pneumonia among Filipino infants. *Vaccine*, 2009, in press.

174. Huebner RE et al. Immunogenicity after one, two or three doses and impact on the antibody response to coadministered antigens of a nonavalent pneumococcal conjugate vaccine in infants of Soweto, South Africa. *The Pediatric Infectious Disease Journal*, 2002, 21:1004–1007.

175. Obaro SK et al. Safety and immunogenicity of pneumococcal conjugate vaccine in combination with diphtheria, tetanus toxoid, pertussis and *Haemophilus influenzae* type b conjugate vaccine. *The Pediatric Infectious Disease Journal*, 2002, 21:940–947.

176. Madhi SA et al. Long-term immunogenicity and efficacy of a 9-valent conjugate pneumococcal vaccine in human immunodeficient virus infected and non-infected children in the absence of a booster dose of vaccine. *Vaccine*, 2006, 25:2451–2457.

177. Nurkka A et al., and the Finnish Otitis Media Study Group. Salivary antibodies 3 to 4 years after the vaccination with pneumococcal conjugate vaccine (PncCRM) in infancy. In: *Proceedings of the third international symposium on Pneumococci and Pneumococcal Diseases (ISPPD)*. 2002:86.

178. Nurkka A et al. Serum and salivary anti-capsular antibodies in infants and children immunized with the heptavalent pneumococcal conjugate vaccine. *The Pediatric Infectious Disease Journal*, 2001, 20:25–33.

179. Nurkka A et al. Serum and salivary anti-capsular antibodies in infants and children vaccinated with octavalent pneumococcal conjugate vaccines, PncD and PncT. *Vaccine*, 2001, 20:194–201.

180. Nieminen T et al. Pneumococcal conjugate vaccination in toddlers: mucosal antibody response measured as circulating antibody-secreting cells and as salivary antibodies. *The Pediatric Infectious Disease Journal*, 1999, 18:764–772.

181. Huebner RE et al. Long-term antibody levels and booster responses in South African children immunized with nonavalent pneumococcal conjugate vaccine. Vaccine, 2004, 22:2696–2700.

182. Åhman H et al. Persistence of anti-pneumococcal antibodies 3 to 4 years after immunization with the 7-valent pneumococcal conjugate vaccine PncCRM in infancy. In: *Proceedings of the third international symposium on Pneumococci and Pneumococcal Diseases (ISPPD)*. 2002:70.

183. Schuerman L et al. Kinetics of the immune response following pneumococcal PD conjugate vaccination. Vaccine, 2007, 25:1953–1961.

184. Mbelle N et al. Immunogenicity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. *The Journal of Infectious Diseases*, 1999, 180:1171–1176.

185. Rennels MB et al. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM197 in United States infants. *Pediatrics*, 1998, 101:604–611.

186. Knuf M et al. Immunogenicity, reactogenicity and safety of a 7-valent pneumococcal conjugate vaccine (PCV7) concurrently administered with a DTPa-HBV-IPV/Hib combination vaccine in healthy infants. *Vaccine*, 2006, 24:4727–4736.

187. Schmitt HJ et al. The safety, reactogenicity and immunogenicity of a 7-valent pneumococcal conjugate vaccine (7VPnC) concurrently administered with a combination DTaP-IPV-Hib vaccine. *Vaccine*, 2003, 21:3653–3662.

188. Tichmann-Schumann I et al. Immunogenicity and reactogenicity of four doses of diphtheria-tetanus-three-component acellular pertussis-hepatitis B-inactivated polio virus-*Haemophilus influenzae* type b vaccine coadministered with 7-valent pneumococcal conjugate vaccine. *The Pediatric Infectious Disease Journal*, 2005, 24:70–77.

189. Buttery JP et al. Immunogenicity and safety of a combination pneumococcal-meningococcal vaccine in infants: a randomized controlled trial. *JAMA: the journal of the American Medical Association*, 2005, 293:1751–1758.

190. Choo S et al. Immunogenicity and reactogenicity of a pneumococcal conjugate vaccine administered combined with a *Haemophilus influenzae* type B conjugate vaccine in United Kingdom infants. *The Pediatric Infectious Disease Journal*, 2000, 19:854–862.

191. Ruggeberg JU et al. Immunogenicity and induction of immunological memory of the heptavalent pneumococcal conjugate vaccine in preterm UK infants. *Vaccine*, 2007, 25:264–271.

192. Scheifele DW et al. Assessment of the compatibility of co-administered 7-valent pneumococcal conjugate, DTaP.IPV/PRP-T Hib and hepatitis B vaccines in infants 2–7 months of age. *Vaccine*, 2006, 24:2057–2064.

193. Sigurdardottir ST et al. Safety and immunogenicity of CRM197 conjugated pneumococcal-meningococcal C combination vaccine (9vPnc-MnCC) whether given in 2 or 3 primary doses. *Vaccine*, 2008, 26:4178-4186.

194. Puumalainen T et al. Greater antibody responses to an eleven-valent mixed carrier diphtheria- or tetanus-conjugated pneumococcal vaccine in Filipino than in Finnish or Israeli infants. *The Pediatric Infectious Disease Journal*, 2003, 22:141–149.

195. Abzug MJ et al. Immunogenicity, safety, and predictors of response after a pneumococcal conjugate and pneumococcal polysaccharide vaccine series in human immunodeficiency virus-infected children receiving highly active antiretroviral therapy. *The Pediatric Infectious Disease Journal*, 2006, 25:920–929.

196. Nachman S et al. Safety and immunogenicity of a heptavalent pneumococcal conjugate vaccine in infants with human immunodeficiency virus type 1 infection. *Pediatrics*, 2003, 112:66–73.

197. Spoulou VI et al. Immunogenicity and immunological memory induced by a 7-valent pneumococcal CRM197 conjugate vaccine in symptomatic HIV-1 infected children. *Vaccine*, 2005, 23:5289–5293.

198. Tarrago D et al. Assessment of antibody response elicited by a 7-valent pneumococcal conjugate vaccine in pediatric human immunodeficiency virus infection. *Clinical and Diagnostic Laboratory Immunology*, 2005, 12:165–170.

199. Feikin DR et al. Randomized trial of the quantitative and functional antibody responses to a 7-valent pneumococcal conjugate vaccine among HIV-infected adults. *Vaccine*, 2001, 20:545–553.

200. French N et al. Salivary and serum responses to 7-valent pneumococcal conjugate vaccine (PCV) in HIV-infected adults. In: *Proceedings of the fifth international symposium on Pneumococci and Pneumococcal Disease (ISPPD), Alice Springs, Australia*. 2006:256.

201. Miiro G et al. Conjugate pneumococcal vaccine in HIV-infected Ugandans and the effect of past receipt of polysaccharide vaccine. *The Journal of Infectious Diseases*, 2005, 192:1801–1805.

202. Käyhty H et al. Opsonophagocytic activity of antibodies against type 6B and 19F *Streptococcus pneumoniae* after vaccination of Malawian adults with 7-valent pneumococcal conjugate vaccine (PCV). In: *Proceedings of the fifth international symposium on Pneumococci and Pneumococcal Disease (ISPPD), Alice Springs, Australia*. 2006:258.

203. Nowak-Wegrzyn A et al. Serum opsonic activity in infants with sickle-cell disease immunized with pneumococcal polysaccharide protein conjugate vaccine. The Pneumococcal Conjugate Vaccine Study Group. *Clinical and Diagnostic Laboratory Immunology*, 2000, 7:788–793.

204. O'Brien KL et al. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM(197) among infants with sickle-cell disease. Pneumococcal Conjugate Vaccine Study Group. *Pediatrics*, 2000, 106:965–972.

205. Meisel R et al. Pneumococcal conjugate vaccine provides early protective antibody responses in children after related and unrelated allogeneic hematopoietic stem cell transplantation. *Blood*, 2007, 109:2322–2326.

206. Smets F et al. Randomized revaccination with pneumococcal polysaccharide or conjugate vaccine in asplenic children previously vaccinated with polysaccharide vaccine. *Vaccine*, 2007, 25:5278–5282.

207. Antin JH et al. Protective antibody responses to pneumococcal conjugate vaccine after autologous hematopoietic stem cell transplantation. *Biology of Blood and Marrow Transplantation: journal of the American Society for Blood and Marrow Transplantation*, 2005, 11:213–222.

208. Molrine DC et al. Donor immunization with pneumococcal conjugate vaccine and early protective antibody responses following allogeneic hematopoietic cell transplantation. *Blood*, 2003, 101:831–836.

209. Puumalainen T et al. Antibody response to an eleven-valent diphtheria- and tetanus- conjugated pneumococcal conjugate vaccine in Filipino infants. *The Pediatric Infectious Disease Journal*, 2002, 21:309–314.

210. Leach A et al. Pilot trial of a pentavalent pneumococcal polysaccharide/protein conjugate vaccine in Gambian infants. *The Pediatric Infectious Disease Journal*, 1996, 15:333–339.

211. Givon-Lavi N et al. Inferior immunogenicity after primary immunization with conjugated pneumococcal vaccine at 2 doses (4, 6m) versus 3 doses (2, 4, 6m). In: *Proceedings of the twenty-fifth annual meeting of the European Society for Paediatric Infectious Diseases (ESPID), Porto, Portugal*. Geneva, The European Society for Paediatric Infectious Diseases, 2007:60.

212. Esposito S et al. Immunogenicity, safety and tolerability of heptavalent pneumococcal conjugate vaccine administered at 3, 5 and 11 months post-natally to pre- and full-term infants. *Vaccine*, 2005, 23:1703–1708.

213. Käyhty H et al. Immunogenicity and tolerability of a heptavalent pneumococcal conjugate vaccine administered at 3, 5 and 12 months of age. *The Pediatric Infectious Disease Journal*, 2005, 24:108–114.

214. Lucero MG et al. Similar antibody concentrations in Filipino infants at age 9 months, after 1 or 3 doses of an adjuvanted, 11-valent pneumococcal diphtheria/tetanus-conjugated vaccine: a randomized controlled trial. *The Journal of Infectious Diseases*, 2004, 189:2077–2084.

215. Käyhty H et al. Concentration and avidity of antibodies determined by 22F EIA after immunization by 1 or 3 doses of 11-valent pneumococcal conjugate vaccine (11PCV) in early infancy. In: *Proceedings of the twenty-third annual meeting of the European Society for Paediatric Infectious Diseases (ESPID), Valencia, Spain*. Geneva, The European Society for Paediatric Infectious Diseases, 2005:107.

216. Zangwill KM et al. Safety and immunogenicity of a heptavalent pneumococcal conjugate vaccine in infants. *Vaccine*, 2003, 21:1894-1900.

217. Trotter CL et al. Effectiveness of meningococcal serogroup C conjugate vaccine 4 years after introduction. *Lancet*, 2004, 364:365–367.

218. Ramsay ME et al. Estimating *Haemophilus influenzae* type b vaccine effectiveness in England and Wales by use of the screening method. *The Journal of Infectious Diseases*, 2003, 188:481–485.

219. Pichichero M et al. Immunogenicity and safety of a combination diphtheria, tetanus toxoid, acellular pertussis, hepatitis B, and inactivated poliovirus vaccine coadministered with a 7-valent pneumococcal conjugate vaccine and *Haemophilus influenzae* type b conjugate vaccine. *The Journal of Pediatrics*, 2007, 151:43–49.

220. Shinefield HR et al. Safety and immunogenicity of heptavalent pneumococcal CRM197 conjugate vaccine in infants and toddlers. *The Pediatric Infectious Disease Journal*, 1999, 18:757–763.

221. Dagan R et al. Reduction of antibody response to an 11-valent pneumococcal vaccine coadministered with a vaccine containing acellular pertussis components. *Infection and Immunity*, 2004, 72:5383–5391.

222. Ocampo AF et al. Response and persistence of antibodies to PRP-T and DTwP vaccines with concomitant administration of conjugate vaccines. *Vaccine*, 2007, 25:605–611.

223. Ölander RM et al. Booster response to the tetanus and diphtheria toxoid carriers of 11- valent pneumococcal conjugate vaccine in adults and toddlers. *Vaccine*, 2001, 20:336–341.

224. English M et al. A randomized, double-blind, controlled trial of the immunogenicity and tolerability of a meningococcal group C conjugate vaccine in young British infants. *Vaccine*, 2000, 19:1232–1238.

225. Dagan R et al. Reduced response to multiple vaccines sharing common protein epitopes that are administered simultaneously to infants. *Infection and Immunity*, 1998, 66:2093–2098.

226. Dagan R et al. Safety and immunogenicity of tetravalent pneumococcal vaccines containing 6B, 14, 19F and 23F polysaccharides conjugated to either tetanus toxoid or diphtheria toxoid in young infants and their boosterability by native polysaccharide antigens. *The Pediatric Infectious Disease Journal*, 1997, 16:1053–1059.

227. Prymyla R et al. Co-administration of hexa-valent diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated polio and *Haemophilus influenzae* type B (DTaP-HBV-IPV/Hib) vaccine with either 11-valent pneumococcal conjugate (11Pn-PD) or hepatitis A vaccines: immunogenicity results from POET. In: *Proceedings of the twenty-fourth annual meeting of the European Society for Paediatric Infectious Diseases (ESPID), Basel, Switzerland*. Geneva, The European Society for Paediatric Infectious Diseases, 2006.

228. Reinert P et al. The safety and immunogenicity of an heptavalent pneumococcal polysaccharide conjugate vaccine (Prevenar) administered in association with a whole-cell pertussis-based pediatric combination vaccine (DTP-IPV/PRP-T) to French infants with a two-, three-, and four-month schedule. *Archives of Pediatrics*, 2003, 10:1048–1055.

229. Black SB et al. Immunogenicity and safety of measles-mumps-rubella, varicella and *Haemophilus influenzae* type b vaccines administered concurrently with a fourth dose of heptavalent pneumococcal conjugate vaccine compared with the vaccines administered without heptavalent pneumococcal conjugate vaccine. *The Pediatric Infectious Disease Journal*, 2006, 25:306–311.

230. Schuster V et al. Rotarix ™ (RIX4414), an oral human Rotavirus vaccine, is highly immunogenic when co-administered with *Streptococcus pneumoniae* conjugate vaccine (Prevenar™) in healthy infants from France and Germany. In: *Proceedings of the twenty-fourth annual meeting of the European Society for Paediatric Infectious Diseases (ESPID), Basel, Switzerland*. Geneva, The European Society for Paediatric Infectious Diseases, 2006.

231. Henckaerts I et al. Critical differences between pneumococcal polysaccharide enzyme-linked immunosorbent assays with and without 22F inhibition at low antibody concentrations in pediatric sera. *Clinical and Vaccine Immunology: CVI*, 2006, 13:356–360.

232. Goldblatt D et al. Antibody responses to nasopharyngeal carriage of *Streptococcus pneumoniae* in adults: a longitudinal household study. *The Journal of Infectious Diseases*, 2005, 192:387–393.

233. Dagan R et al. Serum serotype-specific pneumococcal anticapsular immunoglobulin g concentrations after immunization with a 9-valent conjugate pneumococcal vaccine correlate with nasopharyngeal acquisition of pneumococcus. *The Journal of Infectious Diseases*, 2005, 192:367–376.

234. Millar EV et al. Anticapsular serum antibody concentration and protection against pneumococcal colonization among children vaccinated with 7-valent pneumococcal conjugate vaccine. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 2007, 44:1173–1179.

235. Andrews RM et al. Effectiveness of a publicly funded pneumococcal vaccination programme against invasive pneumococcal disease among the elderly in Victoria, Australia. *Vaccine*, 2004, 23:132–138.

236. Bolan G et al. Pneumococcal vaccine efficacy in selected populations in the United States. *Annals of Internal Medicine*, 1986, 104:1–6.

237. Broome CV, Facklam RR, Fraser DW. Pneumococcal disease after pneumococcal vaccination: an alternative method to estimate the efficacy of pneumococcal vaccine. *The New England Journal of Medicine*, 1980, 303:549–552.

238. Butler JC et al. Pneumococcal polysaccharide vaccine efficacy. An evaluation of current recommendations. *JAMA: the journal of the American Medical Association*, 1993, 270:1826–1831.

239. Melegaro A, Edmunds WJ. The 23-valent pneumococcal polysaccharide vaccine. Part I. Efficacy of PPV in the elderly: a comparison of meta-analyses. *European Journal of Epidemiology*, 2004, 19:353–363.

240. Shapiro ED et al. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. *The New England Journal of Medicine*, 1991, 325:1453–1460.

241. Hanna JN, Humphreys JL, Murphy DM. Invasive pneumococcal disease in indigenous people in north Queensland, 1999–2004. *The Medical Journal of Australia*, 2006, 184:118–121.

242. Singleton RJ et al. Invasive pneumococcal disease epidemiology and effectiveness of 23-valent pneumococcal polysaccharide vaccine in Alaska native adults. *Vaccine*, 2007, 25:2288–2295.

243. Granger R et al. Injectable vaccines for preventing pneumococcal infection in patients with chronic obstructive pulmonary disease. *Cochrane Database of Systematic Reviews*, 2006:CD001390.

244. Koivula I et al. Clinical efficacy of pneumococcal vaccine in the elderly: a randomized, single-blind population-based trial. *The American Journal of Medicine*, 1997, 103:281–290.

245. Lipsky BA, Hirschmann JV. Pneumococcal polysaccharide vaccines do not protect the elderly from pneumococcal infections. *The Netherlands Journal of Medicine*, 2004, 62:33–35.

246. Musher DM et al. Effect of pneumococcal vaccination: a comparison of vaccination rates in patients with bacteremic and nonbacteremic pneumococcal pneumonia. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 2006, 43:1004–1008.

247. Riley ID, Lehmann D, Alpers MP. Pneumococcal vaccine trials in Papua New Guinea: relationships between epidemiology of pneumococcal infection and efficacy of vaccine. *Reviews of Infectious Diseases*, 1991, 13 (Suppl. 6):S535–S541.

248. Preventing pneumococcal disease among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recommendations and Reports: Morbidity and Mortality Weekly Report. Recommendations and Reports / Centers for Disease Control*, 2000, 49:1–35.

249. American Academy of Pediatrics. Committee on Infectious Diseases. Policy statement: recommendations for the prevention of pneumococcal infections, including the use of pneumococcal conjugate vaccine (Prevnar), pneumococcal polysaccharide vaccine, and antibiotic prophylaxis. *Pediatrics*, 2000, 106:362–366.

250. Pneumococcal conjugate vaccine for childhood immunization—WHO position paper. *Weekly Epidemiological Record*, 2007, 82:93–104.

251. Whitney CG et al. Effectiveness of seven-valent pneumococcal conjugate vaccine against invasive pneumococcal disease: a matched case-control study. *Lancet*, 2006, 368:1495–1502.

252. Calbo E et al. Invasive pneumococcal disease among children in a health district of Barcelona: early impact of pneumococcal conjugate vaccine. *Clinical Microbiology and Infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, 2006, 12:867–872.

253. Barricarte A et al. Effectiveness of the 7-valent pneumococcal conjugate vaccine: a population-based case-control study. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 2007, 44:1436–1441.

254. Roche PW et al. Invasive pneumococcal disease in Australia, 2004. *Communicable Diseases Intelligence*, 2006, 30:80–92.

255. Bjornson G et al. Effectiveness of pneumococcal conjugate vaccine in Greater Vancouver, Canada: 2004–2005. *The Pediatric Infectious Disease Journal*, 2007, 26:540–542.

256. Grijalva CG et al. Decline in pneumonia admissions after routine childhood immunization with pneumococcal conjugate vaccine in the USA: a time-series analysis. *Lancet*, 2007, 369:1179–1186.

257. Nelson JC et al. Impact of the introduction of pneumococcal conjugate vaccine on rates of community-acquired pneumonia. In: *Proceedings of the fifth international symposium on Pneumococci and Pneumococcal Disease (ISPPD), Alice Springs, Australia*. 2006:72.

258. Fletcher MA, Fritzell B. Brief review of the clinical effectiveness of PREVENAR® against otitis media. *Vaccine*, 2007, 25:2507–2512.

259. Casey JR, Pichichero ME. Changes in frequency and pathogens causing acute otitis media in 1995–2003. *The Pediatric Infectious Disease Journal*, 2004, 23:824–828.

260. Brook I, Gober AE. Frequency of recovery of pathogens from the nasopharynx of children with acute maxillary sinusitis before and after the introduction of vaccination with the 7-valent pneumococcal vaccine. *International Journal of Pediatric Otorhinolaryngology*, 2007, 71:575–579.

261. Huang SS et al. Post-PCV7 changes in colonizing pneumococcal serotypes in 16 Massachusetts communities, 2001 and 2004. *Pediatrics*, 2005, 116:e408–413.

262. Moore MR et al. Impact of a conjugate vaccine on community-wide carriage of nonsusceptible *Streptococcus pneumoniae* in Alaska. *The Journal of Infectious Diseases*, 2004, 190:2031–2038.

263. Poehling KA et al. Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. *JAMA: the journal of the American Medical Association,* 2006, 295:1668–1674.

264. Black S et al. Postlicensure surveillance for pneumococcal invasive disease after use of heptavalent pneumococcal conjugate vaccine in Northern California Kaiser Permanente. *The Pediatric Infectious Disease Journal*, 2004, 23:485–489.

265. Whitney CG et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *The New England Journal of Medicine*, 2003, 348:1737–1746.

266. Albrich WC et al. Changing characteristics of invasive pneumococcal disease in Metropolitan Atlanta, Georgia, after introduction of a 7-valent pneumococcal conjugate vaccine. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 2007, 44:1569–1576.

267. Flannery B et al. Changes in invasive Pneumococcal disease among HIV-infected adults living in the era of childhood pneumococcal immunization. *Annals of Internal Medicine*, 2006, 144:1–9.

268. Hennessy TW et al. Impact of heptavalent pneumococcal conjugate vaccine on invasive disease, antimicrobial resistance and colonization in Alaska Natives: progress towards elimination of a health disparity. *Vaccine*, 2005, 23:5464–5473.

269. Byington CL et al. Temporal trends of invasive disease due to *Streptococcus pneumoniae* among children in the intermountain west: emergence of nonvaccine serogroups. *Clinical Infectious Diseases: an official publication of the Infectious Diseases Society of America*, 2005, 41:21–29.

270. McEllistrem MC et al. Epidemiology of acute otitis media caused by *Streptococcus pneumoniae* before and after licensure of the 7-valent pneumococcal protein conjugate vaccine. *The Journal of Infectious Diseases*, 2003, 188:1679–1684.

271. Messina AF et al. Impact of the pneumococcal conjugate vaccine on serotype distribution and antimicrobial resistance of invasive *Streptococcus pneumoniae* isolates in Dallas, TX, children from 1999 through 2005. *The Pediatric Infectious Disease Journal*, 2007, 26:461–467.

272. Hicks LA et al. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998–2004. *The Journal of Infectious Diseases*, 2007, 196:1346–1356.

273. Singeton RJ et al. Invasive pneumococcal disease caused by nonvaccine serotypes among Alaska native children with high levels of 7-valent pneumococcal conjugate vaccine coverage. *JAMA: the journal of the American Medical Association,* 2007, 297:1784–1792.

274. Pai R et al. Postvaccine genetic structure of *Streptococcus pneumoniae* serotype 19A from children in the United States. *The Journal of Infectious Diseases*, 2005, 192:1988–1995.

275. Farrell DJ, Klugman KP, Pichichero M. Increased antimicrobial resistance among nonvaccine serotypes of *Streptococcus pneumoniae* in the pediatric population after the introduction of 7-valent pneumococcal vaccine in the United States. *The Pediatric Infectious Disease Journal*, 2007, 26:123–128.

276. Hanage WP et al. Diversity and antibiotic resistance among nonvaccine serotypes of *Streptococcus pneumoniae* carriage isolates in the post-heptavalent conjugate vaccine era. *The Journal of Infectious Diseases*, 2007, 195:347–352.

277. Pelton SI et al. Emergence of 19A as virulent and multidrug resistant Pneumococcus in Massachusetts following universal immunization of infants with pneumococcal conjugate vaccine. *The Pediatric Infectious Disease Journal*, 2007, 26:468–472.

278. Klugman KP, McGee L. Resurgence of the multiresistant pneumococcus in the United States: a commentary. *The Pediatric Infectious Disease Journal*, 2007, 26:473–474.

279. Pichichero M, Casey JR. Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. *JAMA: the journal of the American Medical Association,* 2007, 298:1772–1778.

280. Choi E et al. Single clonal expansion of *Streptococcus pneumoniae* serotype 19A in Korean children before the introduction of 7-valent pneumococcal conjugate vaccine. In: *Proceedings of the forty-fifth annual meeting of the Infectious Diseases Society of America (IDSA), San Ciego, CA*. The Infectious Diseases Society of America, 2007:abstract 202.

281. Dagan R et al. Increased importance of antibiotic-resistant (AR) *S. pneumoniae* serotype 19A (Pn 19A) in acute otitis media (AOM) occurring before introduction of 7-valent pneumococcal conjugate vaccine (PCV7) in Southern Israel. In: *Proceedings of the Forty-Seventh Annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Chicago, IL*. 2007:abstract G-1001.

The World Health Organization has provided technical support to its Member States in the field of vaccine-preventable diseases since 1975. The office carrying out this function at WHO headquarters is the Department of Immunization, Vaccines and Biologicals (IVB).

IVB's mission is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. The Department covers a range of activities including research and development, standard-setting, vaccine regulation and quality, vaccine supply and immunization financing, and immunization system strengthening.

These activities are carried out by three technical units: the Initiative for Vaccine Research; the Quality, Safety and Standards team; and the Expanded Programme on Immunization.

The Initiative for Vaccine Research guides, facilitates and provides a vision for worldwide vaccine and immunization technology research and development efforts. It focuses on current and emerging diseases of global public health importance, including pandemic influenza. Its main activities cover: i) research and development of key candidate vaccines; ii) implementation research to promote evidence-based decision-making on the early introduction of new vaccines; and iii) promotion of the development, evaluation and future availability of HIV, tuberculosis and malaria vaccines.

The Quality, Safety and Standards team focuses on supporting the use of vaccines, other biological products and immunization-related equipment that meet current international norms and standards of quality and safety. Activities cover: i) setting norms and standards and establishing reference preparation materials; ii) ensuring the use of quality vaccines and immunization equipment through prequalification activities and strengthening national regulatory authorities; and iii) monitoring, assessing and responding to immunization safety issues of global concern.

The Expanded Programme on Immunization focuses on maximizing access to high quality immunization services, accelerating disease control and linking to other health interventions that can be delivered during immunization contacts. Activities cover: i) immunization systems strengthening, including expansion of immunization services beyond the infant age group; ii) accelerated control of measles and maternal and neonatal tetanus; iii) introduction of new and underutilized vaccines; iv) vaccine supply and immunization financing; and v) disease surveillance and immunization coverage monitoring for tracking global progress.

The Director's Office directs the work of these units through oversight of immunization programme policy, planning, coordination and management. It also mobilizes resources and carries out communication, advocacy and media-related work.

Department of Immunization, Vaccines and Biologicals

Family and Community Health

World Health Organization
20, Avenue Appia
CH-1211 Geneva 27
Switzerland
E-mail: vaccines@who.int
Web site: http://www.who.int/immunization/en/

ISBN 978 92 4 159821 7