Combination Vaccines in the South African Setting

A Visser
A Hoosen

Abstract:
The number of vaccines available and included as part of the national immunization schedules, has increased significantly over the past few decades. This impacts on patient/parent compliance and creates a challenge for health care providers for implementation of schedules necessitating training and infrastructure improvements. Use of combination rather than component vaccines offers advantages for compliance by single dose administration of various antigens, reducing stock costing as well as reducing cost of additional health care visits. Combination vaccines are often significantly more expensive than individual constituent vaccines. Concerns regarding an increased incidence of adverse events with use of combination vaccines have not been confirmed and rates may seem high as the adverse events seem to mimic the sum total of adverse event rates for each individual antigen used but may in fact be lower. Manufacturers typically advise against interchanging use of vaccine products. Despite this, health authorities advocate use of an alternative vaccine where the original vaccine in not available, to ensure continuity of vaccination. A notable exception is the acellular pertussis vaccine. Partly, because no serological correlates of immunity exist, but also a general lack of convincing follow up studies has prompted the recommendation for manufacturer fidelity for at least the first 3 vaccine doses. According to the South African Medicines Formulary, a variety of vaccines are available in South Africa. Although a large number are available in the private sector, the only true combination vaccine included in the current state EPI, modified in 2009, is the DTaP-IPV/Hib vaccine (Diphtheria, Tetanus, acellular Pertussis, inactivated Poliomyelitis virus and *Haemophilus influenzae* type b). There are many reasons justifying the use of combination vaccines rather that the individual constituent formulations. Implementation of use in the South African setting at this point is still limited, but may offer an exciting avenue of expanding the antigen repertoire without impacting on side-effects, efficacy or complexity of scheduling.

Introduction

The number of vaccines available and included as part of the national immunization schedule, has drastically increased over the past few decades[1, 2]. This not only impacts on patient/parent compliance, but also on the complexity of schedules, making implementation progressively more difficult[3]. Since the first combination vaccines became available in the 1940s in the form of DTP[4], there has been a drive to increasing the amount of antigens captured within single administration doses, with preservation of vaccine efficacy[5]. Initial efforts included reconstitution of various component just prior to administration[6, 7], dual-chambered syringes which is mixed just prior to administration[8] to present day true combination vaccines with individual components merged at time of production[9].

Development has been subject to many teething problems. Chemical incompatibility was the first major obstacle noted with use of thimerosal as

preservative in whole cell and some acellular pertussis vaccines, detrimentally impacting on the immune response to IPV. Although current Hib vaccines have not been subject to carrier-induced epitope suppression, this was a significant problem in previous formulations. The issue of carrier-induced epitope suppression is specifically noted in certain bacterial pathogens with multiple serotypes causing disease. This requires inclusion of a multitude of conjugates into the vaccine, leading to a diminished immune response, particularly upon booster doses[10]. Furthermore, antigenic competition in formulations containing more than one related live virus (notably OPV and MMR) required adjustment of titres to ensure adequate response to all strains[11].

Use of combination vaccines is advocated by the American Advisory Committee on Immunization Practices (ACIP) rather than component vaccines as they offer the possibility of reducing issues surrounding compliance by single dose administration of various antigens[5]. Furthermore, it has the potential advantage of reducing stock costing (syringes, disposables and disposal), reducing cost of additional health care visits and facilitates the introduction of novel vaccines into the vaccination schedule[5]. The indirect costs of deferral or delay of vaccination and storage cost are often not even included in cost-benefit analyses but well worth considering. These advantages are balanced against certain theoretical disadvantages. Most notably, conflicts in dosing schedules may paradoxically confuse current scheduling[5]. Any alteration of convention in vaccination timing, may have far-reaching effects to the detriment of vaccination. However, some authors feel that it will inevitably lead to marked simplification of schedules and consequently improved compliance[12]. In addition, chemical incompatibility and immunological interference is a theoretical risk which should be studied and overcome prior to implementation of use[13-15] in the pre-licensing phase.

Implementation Considerations

Compliance and Vaccination Timeliness

There is no doubt that the availability of vaccines has significantly reduced morbidity and mortality within most communities[1, 2]. This has been shown to be a shared sentiment amongst most parents; however, concern is often raised regarding adverse events associated with the high vaccine load administered in one injection[16]. It has been suggested that the process of reducing the amount of injections, while maintaining the amount of antigens administered will improve patient and parent compliance significantly[3, 17]. Meyerhoff and coworkers showed that up to 26% of patients deferred one vaccine dose when 3 or less injections were indicated, with the deferral rate increasing to 48% when the doses increased to 5 injections[18].

The most frequently cited reasons for vaccination deferral include the number of injections, complexity of the dosing schedule and pain or discomfort experienced[19]. Therefore, use of combination vaccines has shown to improve on timeliness of vaccination (decreasing deferrals), and therefore coverage rates per age[20, 21].

From the health care provider's perspective, combination vaccines are also well received, citing increased staff efficiency, ease of record keeping and improved relations with parents and patients, and therefore compliance as significant advantages to this practice[3]. From a health and safety perspective, handling of fewer sharps also reduces the risk of occupational exposures by staff and personnel[22].

Financial implications

Vaccines are considered the most cost effective tool for prevention of infectious disease[23]. Therefore, the true issue surrounding vaccination is not whether or not to implement immunization, but rather acquisition of the most cost-effective formulations. Combination vaccines are often more expensive than individual constituent vaccines[20]. In fact, pricing has evoked multiple studies leading to pricing algorithms to ensure preferential implementation in vaccination schedules[24]. At present, vaccines other than the 6 original Expanded Programme on Immunization (EPI) formulations as stipulated by the World Health Organization (WHO), are distributed at much higher prices as compared to the EPI vaccines[25]. In light of all these controversies, bulk procurement by Governments, with or without external financial aid by organizations like Global Alliance for Vaccines and Immunization (GAVI) etc, may negate the issues surrounding cost of these vaccines[1]. South Africa is not included amongst the 75 countries receiving support from GAVI[1, 26] as the annual per capita gross national income is more than $1 000[27].

Some US based studies place some emphasis on the impact of fewer injections translating in lower administrative costs. This leads to lower charges to the patient but consequently lower income to the clinician[20]. South Africa shows some similarity in that a clear delineation exists between public and private health care, with full vaccination cost being R1 275[28] and R4 396 (whole sale) respectively. The financial impact of combination vaccines in the South African setting has not been studied.

Despite the enormous success that has been attained by global use of vaccines[29-31], more than 80 million cases of vaccine preventable disease and 1.5 million deaths are reported annually, worldwide[31]. This is by and large due to inadequate delivery and lack of infrastructure and communication within the developing world[31]. Despite this, marked improvement in vaccine coverage has already been attained – in1974 less than 5% of children worldwide had access to the 6 major vaccines targeting poliomyelitis, tuberculosis, pertussis, measles, tetanus and whooping cough[32]. Since initiation of the World Health Organization's (WHO) Expanded Programme on Immunization (EPI) in 1974[33], DTP3 rates have increased to 81% by 2006[24], preventing an estimated 3 million deaths annually[2]. Combination vaccines offer an additional avenue to facilitate global distribution of multiple antigens simultaneously[30], also improving administration safety and relative reduction in biohazardous waste[34].

Safety and Adverse Events

Concerns regarding an increased incidence of adverse events with use of combination vaccines have to date not been confirmed. These rates may seem higher as the adverse events seem to mimic the sum total of adverse event rates for each individual antigen used[35-37] but may even have lower rates[11]. However, the reduction in amount of vaccine preparations will lead to decrease in cumulative exposure to stabilizers and preservatives contained in vaccines[35, 38], a benefit well worth considering.

Questions surrounding immune system overload by exposure to multiple antigens arose both due to the observation of carrier- associated immunosuppression[15] as well as occasional transient delayed-type hypersensitivity reactions to certain antigens in the MMR vaccine[39, 40]. This requires inclusion of a multitude of conjugates into the vaccine, leading to a diminished immune response, particularly upon booster doses[10]. Despite these findings, the effects caused by combination vaccines seem to have a shorter duration of immune modification as compared to individual vaccines administered separately, and should be considered a major advantage[41]. It should also be considered that immunization leads to exposure to a significantly lower number of antigens as compared to infection with the pathogen itself. Hib vaccine typically contains 2 antigens, as opposed to the more than 50 antigens associated with invasive disease[11]. The same can be said for Hepatitis B vaccine containing 1 antigen as opposed to the 4 or more antigens associated with this viral infection[11, 42]. Considering that the immune system has been estimated to be capable of responding to >10 million antigens[11, 43], immune overstimulation is highly unlikely through the practice of vaccination[11].

Efficacy

Evaluation of the efficacy of combination vaccines is typically conducted as a non-inferiority-based study format, thereby proving similar efficacy to individual component vaccines[44, 45]. These studies need to be interpreted with clear consideration of what the final endpoint of evaluation is[46] as it can reflect in vivo models of antibody geometric mean concentrations[46, 47], or epidemiological disease rates[41, 48]. Epidemiological proof of effectiveness is defined by the US Code of Federal Regulations as proof through controlled investigations, of clinically significant prevention of disease in a significant proportion of the target population[49].

Licensing of combination products are required when either unlicensed components are added to existing vaccines or when licensed vaccines are combined[50]. Licensing procedures aim at ensuring that the act of combining antigens does not negatively impact on purity, potency, safety or efficacy of individual components[51]. Once efficacy has been established to be non-inferior to individual components through preclinical phase 1, 2 and 3 trials by manufacturers and licensing has been procured, vaccines use can be implemented[52]. This is followed by extensive post-licensing surveillance during which time the epidemiological impact can be thoroughly investigated[46].

Combination vaccine formulations currently available not only have extensive research backing from manufacturers and independent researchers, but also through independent evaluation of combination vaccines compared to individual components[53-56] as well as effect of administration with additional vaccines at varying time intervals[57, 58]. Through this rigorous process, vaccine efficacy is therefore by and large proven, and thereby immunogenicity established.

Interchangeability of Vaccine Products
Manufacturers typically advise against interchanging use of vaccine products. Despite this, the ACIP still recommends administration of vaccines from various manufacturers if original vaccine is not available, to ensure continuity of vaccination[5]. A notable exception is the acellular pertussis vaccine. Partly, because no serological correlates of immunity exist, but also a general lack of convincing follow up studies[5, 59] has prompted the recommendation for manufacturer fidelity for at least the first 3 vaccine doses[5]. Interchangeability studies are not typically conducted formally, but rather derived from either known correlates for protection or post-licensing surveillance data[60, 61].

Antigen redundancy
Inclusion of combination vaccines into a vaccination programme may lead to over-administration of certain antigens, as these vaccines are less adaptable. Additionally, administration of extra doses of many live-virus vaccines, Hib and Hepatitis B vaccine has not been associated with harmful events[5]. However, certain vaccines, most notably tetanus toxoid[62-68] and pneumococcal polysaccharide vaccine, may cause adverse events if additional doses are administered[69, 70] and this practise is therefore not advocated.

Local Availability and use of Combination Vaccines in the South African EPI
The South African Medicines Formulary (SAMF) lists a variety of combination vaccines that are available in South Africa (table 1). Although these are all available in the private sector, the only true combination vaccine included in the current EPI as modified in 2009, is the DTaP-IPV/Hib vaccine (Pentaxim™) by Sanofi Pasteur (Diphtheria, Tetanus, acellular Pertussis, inactivated Poliomyelitis virus and *Haemophilus influenzae* type b)[71, 72]. Safety and immunogenicity data has been produced in abundance, including within South African cohorts[73, 74]. These studies seem to suggest a favourable safety profile with acceptable rates of adverse reactions[73]. As to be expected, booster doses seemed to show a slightly higher incidence of adverse reactions as compared to primary vaccinations. Local efficacy data is also promising. Recent work by Madhi and coworkers showed significant protection extending throughout the vaccination period. Following a single vaccine, protective responses could be demontrated just prior to booster dosing at 18-19 months in more than 97% of patients for tetanus, diphtheria, polio virus and Hepatitis B virus. Although it seemed as though titres for the Haemophilus influenzae type B component PRP had waned, a booster increased titres by more than 400% in more than 95% of cases[74].

South Africa was declared to be free of wild-type poliovirus in 2006 by the Africa Region Certification Commission (ARCC), based on adequate surveillance showing no local cases since 1989[75]. South Africa is unique in utilizing both IPV and OPV vaccines within the EPI[76]. Since the eventual move to IPV, a more expensive vaccine, has been shown to be cost-effective, the cost of complications of OPV like vaccine-associated paralytic poliomyelitis (VAPP) needs to be considered[77]. Complications like VAPP are very rare, however, certain risk factors may predispose to its development. These include specific immunosuppressive states, most notably congenital agammaglobulinaemia, which has been associated with a single case of VAPP in South Africa in 2011[78]. Although no other cases have been confirmed in South Africa, numerous cases have been described in other African countries (Nigeria and Ethiopia)[79]. For these reasons, the inevitable change to IPV may be prudent to prevent further cases of VAPP.

A significant reduction in maternal and neonatal tetanus has also been demonstrated and the WHO declared South Africa to be free of disease in these populations in 2002[75].

Although invasive infection by *Haemophilus influenzae* type b still occurs, incidence has dramatically declined[80]. Hib vaccine was introduced as part of the South African EPI in 1999[81]. In a subseqent evaluation looking at the rates of invasive disease caused by *Haemophilus influenzae* type b from 1999 to 2004, a 65% reduction could be demonstrated[80]. Unfortunately, the impact pre-and post-vaccination cannot be determined reliably as the national laboratory-based surveillance system was introduced in conjuction with Hib vaccination[82]. However, a survey performed in Cape Town in 1994 cited rates of invasive Hib disease amongst <1 year olds, to be as high as 169 cases per 100 000 population[83]. This stand in contrast to 1999-2000 rates of 55 cases per 100 000 population amongst <1 year olds[80]. Efficacy has been shown locally in both outcome-related- as well as behaviour-related productivity gains, and use is therefore advocated[84], however contradictory views do exist[85, 86].

Use of MMR vaccines is currently not included in the EPI and only measles vaccine is utilized at 9 and 18 months of age[87-89]. The decision to not include rubella vaccine was based on the premise that if sustained high coverage of vaccine cannot be guaranteed, a paradoxical increase the number of susceptible young females could occur. This in turn would lead to an increase in congenital rubella syndrome (CRS)[90, 91]. This issue is currently compounded by the lack of formal surveillance for both primary rubella infection and CRS[90]. The MMR vaccine, however, is available in the private sector[90]. Although not formally studied over the greater South Africa, a study conducted in Gauteng revealed private sector vaccination to account for as much as 21% of all cases. Ironically, rates of complete vaccination seems to have been higher amongst attendees of public sector immunization clinics (83%) as compared to private clinics (75%)[92]. It is therefore clear that the private sector should not be neglected in consideration, as it both constitutes a significant portion of the population and current practices are clearly not optimal.

At present, there are some variations offered by the private sector. In terms of combination vaccines, the major difference lies in the availability of Infanrix® hexa (GlaxoSmithKline) to private patients. In addition to the antigens contained in Pentaxim (Sanofi Pasteur), it also contains Heptitis B virus surface antigen. The implication is administration of one less injection at both the 6-8 week, 10-12 week and 14-16 week intervals. With regard to other vaccines, OPV and Hepatitis B virus vaccine are not given at birth in the private sector. Furthermore, Varicella-, Hepatitis A virus- and MMR vaccines are included in the private schedule as opposed to the public sector which only offers Measles vaccine[93]. It is estimated that approximately 14% of the South African population makes use of private health insurance[94]. Therefore, an estimated 40 million South Africans make use of the Government EPI. The variability of National (Government) versus Extended (Private) EPI vaccines and the impact on herd immunity has not been studied.

Table 1. Summary of combination vaccines currently available in South Africa

Pharmaceutical name	**Target pathogens**	**Formulation**	**Constituents**	**Adjuvant(s)**	**Primary administration**	**Special instructions**
Infanrix® DTPa GlaxoSmithKline	Diphtheria, Tetanus, Pertussis	Prefilled syringe	Diphtheria toxoid ≥30 IU Tetanus toxoid ≥ 40 IU Pertussis toxoid (acellular) 25 mcg FHA 25 mcg Outer membrane protein 8 mcg	None	3 doses of 0.5mL 4 weeks apart Starting age 6 weeks	Booster at 18 months
Diftavax® Aventis Pasteur	Diphtheria (reduced dosage), Tetanus	Prefilled syringe	Diphtheria toxoid ≥2 IU Tetanus toxoid ≥ 20 IU	Aluminium hydroxide	3 doses of 0.5mL 4 weeks apart After 12 years of age	Can be utilized as booster from age 6 Repeated boosting every 5-10 years Only available in public sector
TdPolio Sanofi Pasteur	Diphtheria, Tetanus, Polio	Prefilled syringe	Purified diphtheria toxin ≥ 2 IU Tetanus toxoid ≥ 20 IU Inactivated poliovirus types 1-3 at 40, 8, 32 D-antigen units	Aluminium hydroxide	N/A	0.5mL booster dose every 10 years
Tritanrix-HB® GlaxoSmithKline	Diphtheria, Tetanus, Pertussis, Hepatitis B	Single dose vial	Diphtheria toxoid ≥ 30 IU Tetanus toxoid ≥ 60 IU Inactivated pertussis bacteria (whole cell) ≥ 4 IU Recombinant HBsAg 10mcg	Aluminium salts	3 doses of 0.5mL 4 weeks apart From age 6 weeks	Babies born as carriers of HBV should also receive Hepatitis B immunoglobulin at a different injection site
COMBACT-HIB® Sanofi-Pasteur	*Haemophilus influenzae* type b, Diphtheria, Tetanus, Pertussis	Freeze-dried preparation for reconstitution	Haemophilus b polysaccharide 10mcg Diphtheria toxoid ≥ 30 IU Tetanus toxoid ≥ 60 IU Inactivated *B pertussis* ≥ 4 IU	None	3 doses of 0.5mL 4 to 8 weeks apart From age 6 weeks	Booster at age 15-18 months

PENTAXIM® Sanofi-Pasteur	Diphtheria, Tetanus, Pertussis (acellular), *Haemophilus influenzae* type b, Inactivated Polio	Two formulations produced with only suspension available in South Africa	Haemophilus b polysaccharide 10 mcg Diphtheria toxoid ≥ 30 IU Tetanus toxoid ≥ 40 IU Pertussis toxoid (acellular) 25 mcg Inactivated poliovirus 1-3 at 40, 8, 32 D-antigen units	None	3 doses of 0.5mL 4 weeks apart From age 6 weeks	Booster dose at 18 months
Infanrix hexa® GlaxoSmithKline	Diphtheria, Tetanus, Pertussis (acellular), *Haemophilus influenzae* type b, Hepatitis B, Inactivated Polio	Prefilled syringe	Diphtheria toxoid ≥30 IU Tetanus toxoid ≥40 IU Pertussis toxoid (acelllular) 25mcg FHA 25mcg Pertactin 8mcg Recombinant HBsAg ≥10mcg Inactivated poliovirus 1-3 at 40, 8, 32 D-antigen units Purified capsular polysaccharide of Hib 10mcg	None	3 doses of 0.5mL at 2, 3 and 4 months OR If HBV vaccine is given at birth: Administered at 6, 10 and 14 weeks	Booster dose at 18 months
Twinrix® GlaxoSmithKline	Hepatitis A and B	Prefilled syringe	Hepatitis A antigen 720 ELISA units Recombinant HBsAg 20 mcg	None	Adults: 3 doses of 1mL at 0, 1 and 6 months Paediatric: (1-15yrs) 2 doses of 1mL at 0 and 6 months	None
Morupar® Biovac	Measles, Mumps, Rubella	Prefilled syringe	Measles virus (Schwarz strain in chick embryo cell line) Mumps virus (Urabe AM9 strain in chick embryo cell line) Rubella virus (Wistar RA27/3 strain)	None	Single dose of 0.5mL	Booster at 4-6 year follow up improves protection

Priorix® GlaxoSmithKline	Measles, Mumps, Rubella	Single dose vial	Measles virus (Schwarz strain) Mumps virus (RIT4385 strain) Rubella virus (Wistar RA 27/3)	None	Single dose of 0.5mL	Booster at 4-6 year follow up improves protection
Trimovax® Sanofi-Pasteur	Measles, Mumps, Rubella	Single dose vial	Measles virus (Schwarz strain in chick embryo cell line) Mumps virus (Urabe AM9 strain in embryonated hen eggs) Rubella virus (Wistar RA 27/3 strain in human diploid cell line)	None	Single dose of 0.5mL	Booster at 4-6 year follow up improves protection

Effecting Change in the South African EPI

The South African National Advisory Group on Immunization (NAGI) was established in 1993 under instruction of the Ministry of Health. Subsequently, the National EPI was established in 1995. Prior to this, immunization programmes varied between various regions and local governing bodies[75]. This group consists of 14 members, of which 9 are regular members, representing the disciplines of paediatrics, neurology, community health, virology, microbiology, infectious diseases, pulmonology, vaccinology and medicines regulation. In addition, 3 ex officio members from the Department of Health EPI programme is included, as well as a WHO and UNICEF representative[95].

NAGI functions in an advisory capacity and has effected inclusion of Hib vaccine in 1999[80] as well as the introduction of rotavirus and pneumococcal vaccines[96]. These decisions are based on various factors, including (in decreasing order of importance): mortality, disability-adjusted life years or quality-adjusted life years lost, hospitalizations, equity, overall morbidity and epidemic potential. Economic issues are also taken into considerations, to not only ensure affordability, but also sustainability. This does not include formal economic evaluations by the group, but rather use of data generated from local research units[95]. In addition, issues like burden of disease and equity also play a major role in decisions the EPI.

The final decision is taken by the Department of Health, and NAGI therefore simply acts as an advisory board. Of note, over 75% of suggestions formulated by NAGI has been implemented in the local EPI[95].

Conclusion

South Africa shows great diversity in terms of socio-economic development and infrastructure. These factors have been shown to directly impact on timeliness of vaccination, where poorer communities classically show lower coverage rates with reduced timeliness[97]. This being said, combination vaccines have been proposed as a cost-effective alternative that owing to its relative ease of administration, should theoretically improve on both timeliness and therefore coverage rates[98]. Despite this, implementation of use in the South African setting at this point is still limited, but it may offer an exciting avenue of expanding the antigen repertoire without impacting on side effects, efficacy or complexity of scheduling.

References

1. Global Alliance for Vaccines and Immunization. Available at: http://www.gavialliance.org/about/mission/impact/ [Accessed 20 October 2011]
2. UNICEF. Available at: www.childinfo.org/hiv_aids.html [Accessed 28 December 2011]
3. Koslap-Petraco M, Judelsohn R. Societal Impact of Combination Vaccines: Experiences of Physicians, Nurses, and Parents. *J Pediatric Health Care* 2008,**22**:300-309.
4. CDC. Pertussis. In: *Epidemiology and prevention of vaccine-preventable diseases*. Edited by Atkinson W, Hamborsky J, Wolf C. Washington DC: Public Health Foundation; 2006. pp. 1-44.
5. CDC. Combination vaccines for childhood immunization: Recommendations of the Advisory Committee on Immunization Practices (ACIP), the American Academy of Pediatrica (AAP) and the American Academy of Family Physicians (AAFP). In: *MMWR*: Centres of Disease Control and Prevention; 1999. pp. 1-15.
6. Dagan R, Botujansky C, Watemberg N. Safety and immunogenicity in young infants of Haemophilus b-tetanus protein conjugate vaccine, mixed in the same syringe with diphtheria-tetanus-enhanced inactivated poliovirus vaccine. *Pediatr Infect Dis J* 1994,**13**:356-.
7. Decker M, Edwards K. Combination vaccines: problems and promise [editorial]. *J Pediatr* 2000,**137**:291-295.
8. Halsey N, Blatter M, Bader G. Inactivated poliovirus vaccine alone or sequential inactivated and oral poliovirus vaccine in 2-, 4-, and 6-month-old infant with combination Haemophilus influenzae type b/hepatitis B vaccine. *Pediatr Infect Dis J* 1997,**16**:675-679.
9. Marcy S. Pediatric Combination Vaccines: Their Impact on Patients, Providers, Managed Care Organizations, and Manufacturers. *Am J Manag Care* 2003,**9**.
10. Decker M, Edwards K, Bogaerts H. Chapter 38 Combination Vaccines. In: *Vaccines*. Edited by Plotkin S, Orenstein W, Offit P. 5th ed: Elsevier; 2008. p.1069-1101
11. Halsey N. Safety of combination vaccines: perception versus reality. *Pediatr Infect Dis J* 2001,**20**:S40-44.
12. Woodkin K, Rodewald L, Humiston S, Carges M, Schaffer S, Szilagyi P. Physician and parent opinions: are children becoming pincushions from immunizations? *Arch Pediatr Adolesc Med* 1995,**149**:845-849.
13. Corbel M. Control testing of combined vaccines: A considerations of potential problems and approaches. *Biologicals* 1994,**22**:353-360.
14. Anthony B. FDA perspective on regulatory issues in vaccine development. In: *Combined vaccines and simultaneous administration: current issues and perspectives*. Edited by Williams J, Goldenthal K, Burns D, Lewis B. New York: Annals of the New York Academy of Sciences; 1995. pp. 10-16.
15. Insel R. Potential alterations in immunogenicity by combining or simultaneous administering vaccine components. In: *Combined vaccines and simultaneous administration: current issues and perspectives*. Edited by Williams J, Goldenthal K, Burns D, Lewis B. New York: Annals of the New York Academy of Sciences; 1995. pp. 35-47.
16. Gellin B, Maibach E, Marcuse E. Do parents understand immunizations? A national telephone survey. *Pediatrics* 2000,**106**:1097-1102.

17. Meyerhoff A, Jacoba R, Greenberg D, Yagoda B, Castles C. Clinicians satisfaction with vaccination visits and the role of multiple injections, results from the COVISE study (Combination Vaccines Impact on Satisfaction and Epidemiology). *Clinical Pediatrics* 2004,**43**:87-93.
18. Meyerhoff A, Jocobs R. Do too many shots due lead to missed vaccination opportunies? Does it matter? *Prevnatitive Medicine* 2005,**41**:540-544.
19. Hull B, McIntyre P. Timeliness of childhood immunisation in Australia. *Vaccine* 2006,**24**:4403-4408.
20. Freed G, Cowan A, Clark S, Santoli J, Bradley J. Use o fa New Combined Vaccine in Pediatric Practices. *Pediatrics* 2006,**118**:e251-257.
21. Kalies H, Grote V, Verstaeten V, HEssel L, Schmitt H, Kries Rv. The use of combination vaccines had improved timeliness of vaccination in children. *Pediatr Infect Dis J* 2006,**25**:507-512.
22. Goldfarb N, Patel N, Clarke J. Improving quality by encouraging providers to use pediatric combination vaccines. *Manag Care* 2005,**6**:3-12.
23. World Development Report 1993. In. Edited by Development IBoRa. Washington DC: International Band of Reconstruction and Development; 1993.
24. World Health Organization. State of the World's vaccines and immunization. Available at: http://whqlibdoc.who.int/publications/2009/9789241563864_eng.pdf [Accessed 28 February 2012]
25. Mahoney R, Ramachandran S, Xu Z. The introduction of new vaccines into developing countries II. Vaccine financing. *Vaccine* 2000,**18**:2625-2635.
26. Blecher M, Meheus F, Kollipara A, Hecht R, Cameron N, Pillay Y. Financing vaccinations - the South African experience. *Vaccine* 2012:Current Issue.
27. Lydon P, Levine R, Makinen M, Brenzel L, Mitchell V, Milstien J*, et al.* Introducing new vaccines in the poorest countires: What did we learn from the GAVI experience with financial sustainability? *Vaccine* 2008,**26**:6706-6716.
28. Phoshoko S. Vaccine costing in SA. In. Edited by Visser A. Pinelands; 2012.
29. Hersh B, Tambini G, Nogueira A, Carrasco P, Quadros Cd. Review of regional measles surveillance data in the Americas, 1996-99. *Lancet* 2000,**355**:1943-1948.
30. Edwards K, Decker M. Combination vaccines: hopes and challenges. *Pediatr Infect Dis J* 1994,**13**.
31. Fabio JD, Quadros Cd. Considerations for Combination Vaccine Development and Use in the Developing World. *Clin Infect Dis* 2001,**33**:S340-345.
32. Hadler S, Dietz V, Okwo-Bele J, Cutts F. Immunization in developing countries. In: *Vaccines*. Edited by Plotkin S, Orenstein W, Offit P. 5th edition ed: Saunders Elsevier; 2008. pp. 1542.
33. World Health Organization. Expanded Programme on Immunization (EPI). Available at: http://www.who.int/immunization_delivery/en/ [Accessed on 25 January 2012]
34. Dicko M, Oni A, Ganivet S, Kone S, Pierre L, Jacquet B. Safety of immunization in Africa: not simply a problem of logistics. *Bull World Health Organ* 2000,**78**:163-169.
35. Halsey N. Limiting infant exposure to thimerosal in vaccines and other sources of mercury. *JAMA* 1999,**282**:1763-1766.
36. Pichichero M, Latiolais T, Bernstein D. Vaccine antigen interaction after a combination diphtheria-tetanus toxoid-acellular pertussis/purified capsular

polysaccharide of *Haemophilus infulenzae* type b-tetanus toxoid vaccine in two-, four- and six-month-old infants. *Pediatr Infect Dis J* 1997,**16**:863-870.
37. Schmitt H, Zepp F, Muschenborn S. Immunogenicity and reactogenicity of a *Haemophilus influenzae* type b tetanus conjugate vaccine when administred separately or mixed with concomittant diphtheria-tetanus-toxoid and aceullar pertussis vaccine for primary and for booster immunization. *Eur J Pediatr* 1998,**157**:208-214.
38. Nakayama T, Aizawa C, Kuno-Sakai H. A clinical analysis of gelatin allergy and determination of its causal relationship to the previous administration of gelatin-containing acellular pertussis vaccine combined with diphtheria and tetanus toxoids. *J Allergy Clin Immunol* 1999,**103**:321-325.
39. Berkovich S, Starr S. Effects of live type 1 poliovirus vaccine and other viruses on the tuberculin test. *N Engl J Med* 1966,**274**:67-72.
40. Brickman H, Beardry P, Marks M. The timing of tuberculin tests in relation to immunization with live viral vaccines. *Pediatrics* 1975,**55**:392-396.
41. Halsey N. Combination Vaccines: Defining and Addressing Current Safety Concerns. *Clin Infect Dis* 2001,**33**:S312-318.
42. Intitute of Medicine, Division of Health Promotion and Disease Prevention. Immunologic reactions. In: *Adverse events associated with childhood vaccines: evidence bearing on causality*. Edited by Stratton K, Howe C, Johnston R. Washington DC: National Academy Press; 1993. pp. 59-66.
43. Pickering L, Siegelman M. Lymphoid tissues and organs. In: *Fundamental immunology*. Edited by Paul W. 4th ed. Philedelphia: Lippincott-Raven; 1999. pp. 479-532.
44. Center for Biologics Evaluation and Research, US Food and Drug Administration. Guidance for industry for the evaluation of combination vaccines for preventable disease: production, testing and clinical studies. *Fed Reg* 1997,**62**:17624-17625.
45. Horne A, Lachenbruch P, Getson P, Hsu H. analysis of studies to evaluate the immune response to combination vaccines. *Clin Infect Dis* 2001,**33**:S306-311.
46. Ball L, Falk L, Horne A, Finn T. Evaluating the Immune Response to Combination Vaccines. *Clin Infect Dis* 2001,**33**:S299-305.
47. Halsey N. A perspective on combination vaccines. *Pediatr Infect Dis J* 1998,**17**:653-654.
48. Harrison L, Tajkowski C, Croll J. Postlicensure effectiveness of the *Haemophilus influenzae* type b polysaccharide-*Neisseria meningitidis* outer membrane protein comjplex conjugate vaccine among Navajo children. *J Pediatr* 1994,**125**:571-576.
49. US Code of Federal Regulations, title 21, Part 601.25(d)(2). In. Washington DC: US Government Printing Office; 2001.
50. US Code of Federal Regulations, title 21, part 610.17. In. Washington DC: US Government Printing Office; 2001.
51. US Code of Federal Regulations, title 21, part 601.25(d)(4). In. Washington DC: US Government Printing Office; 2001.
52. US Code of Federal Regulations, title 21, part 312.21. In. Washington DC: US Government Printing Office; 2001.
53. Kuter B, Brown M, Hartzel J, Williams W, EvesiKaren A, Black S*, et al.* Safety and immunogenicity of a combination measles, mumps, rubella and varicella vaccine (ProQuad). *Hum Vaccin* 2006,**2**:205-214.
54. Shinefield H, Black S, Staehle B, Adelman T, Ensor K, Ngai A*, et al.* Safety, tolerability and immunogenicity of concomitant injections in separate locations

of M-M-RII, VARIVAX and TETRAMUNE in healthy children vs. concomittant injections of M-M-RII and TETRAMUNE followed six weeks later by VARIVAX. *Pediatr Infect Dis J* 1998,**17**:980-985.
55. Schmitt H, Steul K, Borkowski A, Ceddia F, Ypma E, Knuf M. Two versus three doses of a meningococcal C conjugate vaccine concomitantly administred with a hexavalent DTaP-IPV-HBV/Hib vaccine in healthy infants. *Vaccine* 2008,**26**:2242-2252.
56. Tejedor J, Moro M, Ruiz-Contreras J, Castro J, Gómez-Campderá J, Navarro M*, et al.* Immunogenicity and reactogenicity of primary immunization with a hexavalent diphtheria-tetanus-acellular pertussis-hepatitis B-inactivated polio-Haemophilus influenzae type B vaccine coadministered with two doses of a meningococcal C-tetanus toxoid conjugate vaccine. *Pediatr Infect Dis J* 2006,**25**:713-720.
57. Reisinger K, Brown M, Xu J, Sullivan B, Marshall G, Nauert B*, et al.* A combination measles, mumps, rubella, and varicella vaccine (ProQuad) given to 4- to 6-year-old healthy children vaccinated previously with M-M-RII and Varivax. *Pediatrics* 2006,**117**:265.
58. Arístegui J, Dal-Ré R, Díez-Delgado J, Marés J, Casanovas J, García-Corbeira P*, et al.* Comparison of the reactogenicity and immunogenicity of a combined diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated polio (DTPa-HBV-IPV) vaccine, mixed with the Haemophilus influenzae type b (Hib) conjugate vaccine and administered as a single injection, with the DTPa-IPV/Hib and hepatitis B vaccines administered in two simultaneous injections to infants at 2, 4 and 6 months of age. *Vaccine* 2003,**21**:3593-3600.
59. CDC. Pertussis vaccination: use of acellular pertussis vaccines among infants and young children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 1997,**46**:1-25.
60. Granoff D, Rappuoli R. Are serological responses to acellular pertussis antigens sufficient criteria to ensure that new combination vaccines are effective for prevention of disease? *Dev Biol Stand* 1997,**89**:379-389.
61. Clements-Mann M. Lessons for AIDS vaccine development from non-AIDS vaccines. *AIDS Res Hum Retroviruses* 1998,**14**:S197-203.
62. Edsall G, Elliott M, Peebles T, Panaro R, Eldred M. Excessive use of tetanus toxoid boosters. *JAMA* 1967,**202**:111-113.
63. Peebles T, Levine L, Eldred M, Edsall G. Tetanus-toxoid emergency boosters: a reappraisal. *N Engl J Med* 1969,**280**.
64. Jacobs R, Lowe R, Lanier B. Adverse reactions to tetanus toxoid. *JAMA* 1982,**247**:40-42.
65. Baraff L, Cody C, Cherry J. DTP-associated reactions: an analysis by injection site, manufacturer, prior reactions, and dose. *Pediatrics* 1984,**73**:31-36.
66. Jones A, Melville-Smith M, Watkins J, Seagroatt V, Rice L, Sheffield F. Adverse reactions in adolescents to reinforcecing dose of plain and adsorbed tetanus vaccines. *Community Med* 1985,**7**:99-106.
67. American Academy of Pediatrics. Tetanus (Lockjaw). In: *1997 Red book: report of the Committee on Infectious Diseases*. Edited by Peter G. 24th ed. Elk Grove Village, Il: American Academy of Pediatrics; 1997. pp. 518-523.
68. CDC. Diphtheria, tetanus, and pertussis: recommendations for vaccine use and other preventative measures. Recommendations of the Immunization Practice Advisory Committee (ACIP). *MMWR* 1991,**40**:1-28.

69. Sackett D, Rosenberg W, Gray J, Haynes R, Richardson W. Evidence based medicine: what it is and what it isn't. *BMJ* 1996,**312**:71-72.
70. American Academy of Pediatrics. Pneumococcal infections. In: *1997 Red Book: report of the Committee on Infectious Diseases*. Edited by Peter G. 24th ed. Elk Grove Village, Il: american Academy of Pediatrics; 1997. pp. 410-419.
71. World Health Organization. Available from: http://www.int/countries/en/ [Accessed on 25 April 2012]
72. Ntombenhle J, Cameron N. The Decision Making Process on New Vaccine Introduction in South Africa. *Vaccine* 2012:Current issue.
73. Madhi S, Cutland C, Jones S, Groome M, Ortiz E. One-year post-primary antibody persistence and booster immune response to a DTaP-IPV//PRP-T vaccine (Pentaxim) given at 18-19 months of age in South African children primed at 6, 10 and 14 weeks of age with the same vaccine. *S Afr J Med* 2011,**101**:879-883.
74. Madhi S, Cutland C, Jones S, Groome M, Ortiz E. Immunogenicity and safety of an acellular pertussis, diphtheria, tetanus, inactivated poliovirus, Hib-conjugate combined vaccine (Pentaxim) and monovalent hepatitis B vaccine at 6, 10 and 14 weeks of age in infants in South Africa. *S Afr J Med* 2011,**101**:126-131.
75. Ngcobo N. The impact of the immunisation programme on vaccine-preventable diseases in South Africa: A review of progress over a 10- to 15-year period. *S Afr J Epidemiol Infect* 2008,**23**:9-13.
76. Schoub B. Introduction of inactivated poio vaccine (IPV) into the routine immunization in South Africa. *Vaccine* 2012:Current issue.
77. Griffiths U, Botham L, Schoub B. The cost-effectiveness of alternative polio immunization policies in South Africa. *Vaccine* 2006,**24**:5670-5678.
78. National Institutes for Communicable Diseases. Polio Paralyses Child. Available from: www.nicd.ac.za/?page=alerts&id [Accessed on 12 April 2012]
79. National Institute for Communicable Diseases - Annual Review 2010-2011. Available from: www.nicd.ac.za/assets.files/ [Accessed on 12 April 2012]
80. Von Gottberg A, De Gouveia I, Madhi S. Impact of conjugate *Haemophilus influenzae* type b (Hib) vaccine introduction in South Africa. *Bull World Health Organ* 2006,**84**:811-818.
81. Von Gottberg A, Cohen C, Whiteloaw A, Chhagan M, Flannery B, Cohen A*, et al.* Invasive disease due to *Haemophilus influenzae* serotype b ten years after routine vaccination, South Africa, 2003-2009. *Vaccine* 2012,**30**:565-571.
82. Huebner R, Klugman K, Matai U, Eggers R, Hussey G. Laboratory surveillance for Haemophilus influenzae type b, meningococcal and pneumococcal disease. Haemophilus Surveillance Working Group. *S Afr Med J* 1999,**89**:924-925.
83. Hussey G, Hitchcock J, Schaaf H, Hanslo GC, Schalkwyk Ev, Pitout J*, et al.* Epidemiology of invasive Haemophilus influenzae infections in Cape Town, South Africa [Abstract]. *Ann Trop Paediatr* 1994,**14**:97-103.
84. Barnighausen T, Bloom D, Canning D, Friedman A, Levine O, O'Brien J*, et al.* Rethinking the benefits and costs of childhood vaccination: The example of the *Haemophilus influenzae* type b vaccine. *Vaccine* 2011,**29**:2371-2380.
85. John T, Muliyil J. Introducing pentavalent vaccine in EPI in India: issues involved. *Indian j Med Res* 2010,**132**:450-455.
86. Madhavi Y, Raghuram N. Pentavalent & other new combination vaccines: solution in search of problems. *Indian J Med Res* 2010,**132**:456-457.
87. SAVIC. Available from: www.savic.ac.za/news/newsarticles.php [Accessed on 28 December 2011]

88. Du Toit G, Weinberg E. Egg allergy and MMR vaccination. *S Afr J Med* 2003,**93**:113-114.
89. Cameron N. When should be introduce a combination measles-mumps- (MMR) vaccine into the national childhood expanded immunization programme in South Africa? *Vaccine* 2012:Current issue.
90. Corcoran C, Hardie D. Seroprevalence of rubella antibodies among antenatal patients in the Western Cape. *S Afr J Med* 2005,**95**:688-690.
91. Robertson S, Cutts F, Samuel R, Diaz-Ortega J. Control of rubella and congenital rubella syndrome (CRS) in developing countries. Part 2:Vaccination against rubella. *Bull World Health Org* 1997,**75**:69-80.
92. Fonn S, Sartorius B, Levin J, Likibi M. Immunisation coverage estimates by cluster sampling survey of children (aged 12-23 months) Gauteng province, 2003. *S Afr J Epidemiol Inf* 2006,**21**:164-169.
93. Paediatric Group. Available from: www.paediatrician.co.za [Accessed on 21 April 2012]
94. Mail And Guardian. Forty million South African without medical aid. Available frrom: www.mg.co.za/article/2009-07-07-forty-million-south-africans-without-medical-aid [Accessed 21 April 2012]
95. Schoub B, Ngcobo N, Madhi S. The National Advisory Group on Immunization (NAGI) of the Republic of South Africa. *Vaccine* 2010,**28S**:A31-34.
96. Madhi S, Puren A, Kuwanda L, Jassat W, Jones S, Little T*, 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* 2007,**25**:2451-2457.
97. Fadnes L, Jackson D, Engebretsen I, Zembe W, Sanders D, Sommerfelt H*, et al.* Vaccination coverage and timeliness in three South African areas: a prospective study. *BMC Public Health* 2011,**11**:404-414.
98. Gentile A, Bhutta Z, Bravo L, Samy A, Garcia R, Hoosen A*, et al.* Pediatric disease burden and vaccination recommendations: understanding local differences. *Int J Infect Dis* 2010,**14**:e649-658.