International Journal of Infectious Diseases 46 (2016) 71–78

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Comparison of the immunogenicity and safety of pentavalent vaccine Quinvaxem in a compact prefilled auto-disabled (cPAD) injection system versus single-dose vials in healthy infants: a phase 3, open-label, randomized, parallel-group, non-inferiority study

Maria Rosario Z. Capeding [a,*], Edison Alberto [a], Amanda Versteilen [b], Martina Rauscher [c], Partha Bagchi [d], Patricia Ibarra de Palacios [c]

[a] Research Institute for Tropical Medicine, 9002 Research Drive, Filinvest Corporate City, Alabang, Muntinlupa City 1781, Philippines
[b] Crucell Holland BV, Leiden, Netherlands
[c] Crucell Switzerland AG, Bern, Switzerland
[d] Janssen Research and Development, Raritan, New Jersey, USA

ARTICLE INFO

Article history:
Received 28 August 2015
Received in revised form 18 February 2016
Accepted 20 February 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Immunogenicity
Compact prefilled auto-disabled injection
Quinvaxem
Single-dose vials
Pentavalent vaccine

SUMMARY

Objective: To evaluate non-inferiority of three doses of Quinvaxem in a compact prefilled auto-disabled (cPAD) injection system versus Quinvaxem in a single-dose vial administered with conventional syringe in terms of seroconversion/seroprotection rates for all antibodies (anti-hepatitis B (HB), anti-*Haemophilus influenzae* type b polyribosylribitol phosphate (Hib PRP), anti-diphtheria, anti-tetanus, anti-*Bordetella pertussis*) at 1 month after primary vaccination.
Methods: Four hundred healthy infants aged 42–65 days were randomized (1:1) to receive Quinvaxem in cPAD or single-dose vial at 6, 10, and 12 weeks of age. Blood samples were collected before vaccination and at 1 month after the third dose to determine seroconversion/seroprotection rates. Safety was assessed from solicited and unsolicited adverse events and serious adverse events (SAEs).
Results: Of the 400 infants randomized, 395 (98.8%) received all three vaccine doses. In the cPAD vs. single-dose vial groups, seroprotection rates against Hib PRP (both 98.5%), HB (92.9% vs. 93.4%), diphtheria (100% vs. 99%), and tetanus toxoids (both 100%), and seroconversion against *B. pertussis* (95.4% vs. 97%) were ≥92% at 1 month after the third vaccination (lower limits of 95% confidence intervals simultaneously greater than −10%). Geometric mean concentrations exceeded seroprotection/seroconversion thresholds by large margins. The incidences of induration and erythema were comparable between the groups; tenderness was slightly higher in the cPAD group (85.5% vs. 76.5%). No vaccine-related SAEs occurred.
Conclusions: Quinvaxem in cPAD was non-inferior to single-dose vial with respect to seroprotection/seroconversion rates for all antibodies. Both presentations were well-tolerated.

1. Introduction

Immunization is a cost-effective and life-saving intervention that prevents an estimated two to three million deaths every year from vaccine-preventable diseases.[1,2] The aim of the World Health Organization (WHO) Expanded Programme on Immunization (EPI) is to protect children against life-threatening diseases like tuberculosis, diphtheria, neonatal tetanus, whooping cough, poliomyelitis, and measles, as well as hepatitis B[3] and *Haemophilus influenzae* type b (Hib).[4] According to the WHO, approximately 22.6 million children worldwide did not receive the full three doses of diphtheria–tetanus–pertussis (DTP3) vaccine in 2012.[2] Likewise, globally only 45% of children received three doses of Hib vaccine in 2012.[5]

Pentavalent vaccines remain the cornerstone of the EPI because of their numerous advantages over separate injections, including protection against five diseases in one injection (DTP, hepatitis B (HB), and Hib), better immunization coverage, simple, easy-to-administer fully liquid formulations, fewer injections and less distress for children, lower shipping and transport costs, fewer

* Corresponding author. Tel./Fax: +63 2 772 4916.
E-mail address: lerosecap@gmail.com (M.R.Z. Capeding).

http://dx.doi.org/10.1016/j.ijid.2016.02.015

syringes, and reduced environmental impact.[6] The Global Alliance for Vaccines and Immunisation (GAVI) aimed to increase the coverage level of pentavalent vaccine from 53% at the end of 2013 to 77% by the end of 2015.[7] To date, the WHO has prequalified six pentavalent vaccines for use in EPI programs, including Quinvaxem in 2006.[8]

Quinvaxem (DTwP–HepB–Hib) is a fully liquid pentavalent combination vaccine consisting of inactivated hepatitis B surface antigen (HBsAg), Hib conjugated to *Corynebacterium diphtheriae* cross-reacting material 197 (CRM_{197}–Hib), tetanus toxoid, diphtheria toxoid, and whole-cell pertussis antigens.[9,10] The vaccine is indicated for active immunization of infants and toddlers against diseases caused by five different pathogens: hepatitis B virus, *C. diphtheriae*, *Clostridium tetani*, *Bordetella pertussis*, and Hib. Quinvaxem has been shown to be protective, immunogenic, and well-tolerated in several clinical studies.[10–15]

The Becton Dickenson Uniject is a compact autodisposable, prefilled, single-use injection system, originally developed by PATH in 1987 to promote vaccination in developing countries in response to a recognized need for the development of single-use, auto-disable injection systems to reduce cross-contamination and the risk of blood-borne infections, vaccine wastage, and missed vaccination opportunities.[16–18] Currently, the WHO has prequalified both tetanus and HB vaccines in Uniject presentations.[16] A new presentation of Quinvaxem has also been developed in this compact prefilled auto-disabled (cPAD) injection system. The formulation for Quinvaxem in cPAD is the same as the fully liquid pentavalent vaccine that is available in a single-dose vial. The cPAD injection system is expected to have considerable advantages in the field of vaccination, particularly in difficult-to-reach settings, e.g., hard-to-reach communities[19–21] for which there are unique challenges in terms of resources and logistics. It also offers simplified vaccine administration, as well as additional benefits such as reduced vaccine and medical wastage, reduced possibility of contamination risk and disease transmission, and a removal of the need for vaccine reconstitution before use.

Quinvaxem injection is a ready-to-use, preservative-free, fully liquid pentavalent vaccine that gained WHO pre-qualification status in 2006. Quinvaxem was found to be highly immunogenic in each of the primary vaccination studies and was also shown to be suitable as a booster, with the advantage that it could be given concomitantly with measles vaccine, in four clinical trials and one post-marketing observational study.[22] Quinvaxem has been included in EPI vaccination programs to further support the needs of EPI vaccination processes and developing countries. A simple, all-in-one, compact, prefilled, auto-disabled Uniject injection system is a potentially optimized new presentation for Quinvaxem; the cPAD contains the same vaccine formulation as the approved and marketed product.

Uniject is a cPAD injection system that was developed by PATH and subsequently marketed by Becton Dickinson.[23] It was initially developed in response to the WHO and other organizations recognizing a great need for the development of single-use, auto-disable injection systems to reduce cross-contamination and the risk of blood-borne infections (due to syringe/needle re-use), to reduce vaccine wastage, and to reduce missed vaccination opportunities.[23,24] Uniject is well-established as a successful system for the delivery of injectable contraceptives, vaccines, antibiotics, and uterotonic drugs, and around nine million doses of tetanus toxoid vaccine and 75 million doses of HB vaccine have been distributed using the Uniject presentation.[23–26]

This study on Quinvaxem in cPAD was conducted to demonstrate non-inferiority of three doses of Quinvaxem in cPAD to three doses of Quinvaxem in single-dose vials with respect to antibody seroprotection/seroconversion rates (anti-hepatitis B surface antigen (anti-HBs), anti-Hib polyribosylribitol phosphate (Hib PRP), anti-diphtheria toxoid, anti-tetanus toxoid, and anti-*B. pertussis*) at 1 month after completion of the 6–10–14 week primary vaccination course. The safety profile was also evaluated.

2. Methods

2.1. Study population

Healthy infants of both sexes aged between 42 and 64 days (at the time of first vaccination), immunized with the HB vaccination within 48 h after birth, with no obvious health problems as established by medical history and/or clinical examination, and eligible for the local EPI program, were enrolled in the study.

Exclusion criteria included known/suspected immune function impairment, known HIV positivity, systemic immunosuppressive therapy at the time of screening or within 1 month before study entry (except inhaled and topical steroids), previous treatment with a parenteral immunoglobulin preparation and/or blood products, vaccination against Hib and/or DTP, history of anaphylaxis or hypersensitivity to any vaccine ingredient, or presence of clinically significant acute infection or acute illness.

The institutional review board approved the protocol and the study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practice and applicable regulatory requirements. Parents/legal guardians gave written informed consent before study enrollment. The study is registered at ClinicalTrials.gov (NCT01349283).

2.2. Study design and vaccination schedule

This was a phase 3, open-label, randomized, comparator-controlled, single-center non-inferiority study conducted between September 13, 2013 and March 21, 2014 at the Research Institute for Tropical Medicine, Alabang, Muntinlupa City, Philippines.

Infants were randomized 1:1 to receive three intramuscular doses of Quinvaxem presented either in cPAD or in single-dose vials into the anterolateral region of the thigh at 6, 10, and 14 weeks of age. Randomization was performed using computer-generated randomization codes, with each infant receiving a sealed booklet containing the randomization ID. A blocked randomization scheme was adopted to achieve balance between the two study groups. All infants were vaccinated using a conventional needle (23 gauge × 1" (25 mm) length) at visits 1, 2, and 3. The active study phase lasted for 12 weeks for all infants. Four vaccinators performed the vaccinations throughout the study (approximately 100 infants each), and all three doses were administered by the same trained and qualified vaccinator once an infant had been randomized.

Each 0.5-ml dose of Quinvaxem in cPAD (lot number X9161009; Berna Biotech Korea Corporation) and in single-dose vials (lot number 1453138; Berna Biotech Korea Corporation) contained ≥30 IU diphtheria toxoid, ≥60 IU tetanus toxoid, ≥4 IU inactivated *B. pertussis*, 10 μg Hib polysaccharide conjugated to CRM_{197} protein (~25 μg), and 10 μg HBsAg.

2.3. Study assessments

2.3.1. Primary immunogenicity endpoint

The primary objective was to demonstrate non-inferiority of three doses of Quinvaxem in cPAD to three doses of Quinvaxem in single-dose vials with respect to antibody seroprotection/seroconversion rates (anti-HBs, anti-Hib PRP, anti-diphtheria toxoid, anti-tetanus toxoid, and anti-*B. pertussis*) at 1 month after completion of the primary vaccination course.

2.3.2. Secondary endpoints

The major secondary immunogenicity endpoints were (1) the percentage of infants with anti-Hib PRP titers ≥1.0 μg/ml and (2) geometric mean concentrations (GMCs) for anti-HBs, anti-diphtheria toxin, anti-tetanus toxin, anti-*B. pertussis*, and anti-Hib PRP at 1 month after the third vaccination.

2.3.3. Serology evaluations

A 3-ml blood sample was drawn by venous puncture immediately prior to the first vaccine dose (visit 1) and at 1 month after the third vaccine dose (visit 4). HB antibodies were analyzed using an indirect ELISA (Enzygnost; Siemens Diagnostics at Novartis Vaccines and Diagnostics GmbH, Marburg, Germany), with seroprotection defined as an HB antibody concentration ≥10 IU/ml. A competitive ELISA was used to measure antibodies against Hib PRP (VaccZyme; The Binding Site Ltd, UK), with seroprotection rates determined using two cut-off levels (≥0.15 μg/ml and ≥1.0 μg/ml). An indirect ELISA was used for diphtheria and tetanus toxoid antibodies (Virotech; Sekisui Diagnostics or Sekisui Virotech GmbH (Russelsheim, Germany)), with seroprotection defined as a titer level ≥0.1 IU/ml. *B. pertussis* antibodies were analyzed using a whole cell IgG ELISA (University of Turku, Finland). There is no international standard definition of seroprotection for *B. pertussis*. Therefore, to determine a significant immune response to vaccination, seroconversion was defined as either a concentration ≥20 EU/ml or a ≥4-fold increase from the pre-vaccination level.

2.3.4. Safety and tolerability

Each infant's parent/legal guardian documented solicited local adverse events (AEs) (tenderness, erythema, and induration) and body temperature in a diary for 5 days following vaccination (i.e., on the day of vaccination and for 4 days thereafter), along with any unsolicited AEs. Fever was defined as a body temperature ≥38 °C. Any other unsolicited AEs were also recorded at each visit from responses to non-leading questions by the investigator. Serious adverse events (SAEs) were collected throughout the study period.

2.4. Statistical methods

The non-inferiority of Quinvaxem in cPAD compared with Quinvaxem in single-dose vials was demonstrated using the Newcombe–Wilson score method if the lower limits of all two-sided 95% confidence intervals (CIs) of the differences in seroprotection/seroconversion rates between the two groups were simultaneously greater than −10%. The GMCs and corresponding 95% CIs (normal approximation) were calculated from $\log_{10}$-transformed concentrations. GMC fold-increases (within-group comparisons) and GMC ratios (between-group comparisons) are presented together with the corresponding two-sided 95% CIs (normal approximation) for each antigen. The immunogenicity analysis was performed on the according-to-protocol (ATP) analysis set, defined as the set of all infants who received all three doses and did not have any major protocol violations. The intention-to-treat (ITT) analysis set was defined as the set of all infants who had at least one vaccination and had a post-baseline measurement for at least one antigen.

2.4.1. Sample size

Assuming a 95% seroprotection/seroconversion rate for each antigen in each group and a clinically significant non-inferiority limit of −10%, a sample size of 360 evaluable infants (randomized in a 1:1 ratio) was required to demonstrate non-inferiority of Quinvaxem in cPAD compared to Quinvaxem single-dose vials with an overall power of >90% and a one-sided significance level of 2.5%.

Assuming a dropout rate of approximately 10%, 400 infants in total were randomized 1:1 (200 infants in each group). The sample size was determined using the Newcombe–Wilson score method to construct the CI (nQuery statistical software version 6.0).

3. Results

3.1. Study disposition and clinical characteristics

Of the 400 infants randomized, 200 were vaccinated with Quinvaxem in cPAD and 200 with Quinvaxem in single-dose vials using a conventional syringe. Altogether 395 (98.9%) infants completed the entire study; 197 (98.5%) infants in the cPAD group and 198 (99%) infants in the single-dose vials group received all three vaccine doses. Five infants discontinued the study because their parents withdrew consent (three (1.5%) in the cPAD group and one (0.5%) in the single-dose group) or due to migration from the study area (one (0.5%) in the single-dose group) (Figure 1).

A total 393 (98.3%) infants were included in the ATP analysis set, 196 (98%) in the cPAD group and 197 (98.5%) in the single-dose vials group. Four infants from the cPAD group and three from the single-dose vials group were excluded from the ATP analysis set as they did not receive all three doses, or had a missing post-baseline serum level, or due to major protocol violations. Overall, 51.4% of the infants were male and 48.6% were female, and all were Asian (Table 1). The median weight in both groups was 4.65 kg. Median age was 6.5 weeks in the cPAD group and 6.4 weeks in the single-dose group. In total, 15.2% of infants had received a prior bacillus Calmette–Guérin (BCG) vaccination; none had previously used immunosuppressants or corticosteroids.

3.2. Immunogenicity analysis

3.2.1. Primary immunogenicity endpoint

One month after the third dose, the two groups exhibited similar seroprotection/seroconversion rates for tetanus toxoid (100%), and 92.9% of cPAD infants and 93.4% of single-dose infants achieved seroprotection against HB. Seroconversion against *B. pertussis* was achieved in 95.4% of infants in the cPAD group and 97% in the single-dose group. For diphtheria, 100% of cPAD infants and 99% of single-dose infants were seroprotected at 1 month after the third dose. The percentage of infants with anti-Hib PRP titers ≥0.15 μg/ml was 98.5% in both groups (Table 2). Since the lower limits of all these 95% CIs were simultaneously greater than −10%, non-inferiority of the cPAD group compared with the single-dose vial group was demonstrated. To assess the robustness of these results, the same analysis was performed on the ITT analysis set (Table 3). The results were similar in the ITT analysis set, assuring consistency of the results between the two analysis sets.

3.2.2. Secondary immunogenicity endpoint

One month after the third vaccine dose (day 85), GMCs for all antibodies in both groups exceeded the seroprotection/seroconversion thresholds markedly (Table 4). Anti-Hib PRP and anti-diphtheria

Table 1
Demographics and baseline characteristics (ITT population)

Characteristics	cPAD (*N* = 197)	Single-dose (*N* = 198)	Total (*n* = 395)
Sex, *n* (%)			
Male	103 (52.3)	100 (50.5)	203 (51.4)
Female	94 (47.7)	98 (49.5)	192 (48.6)
Age (weeks), mean (SD)	6.93 (0.94)	6.93 (1.0)	6.93 (0.96)
Weight (kg), mean (SD)	4.64 (0.60)	4.66 (0.65)	4.65 (0.62)
Prior BCG vaccination, *n* (%)			
Yes	29 (14.7)	31 (15.7)	60 (15.2)
No	168 (85.3)	167 (84.3)	335 (84.8)

BCG, bacillus Calmette–Guérin; cPAD, compact prefilled auto-disabled system; ITT, intention-to-treat; SD, standard deviation.

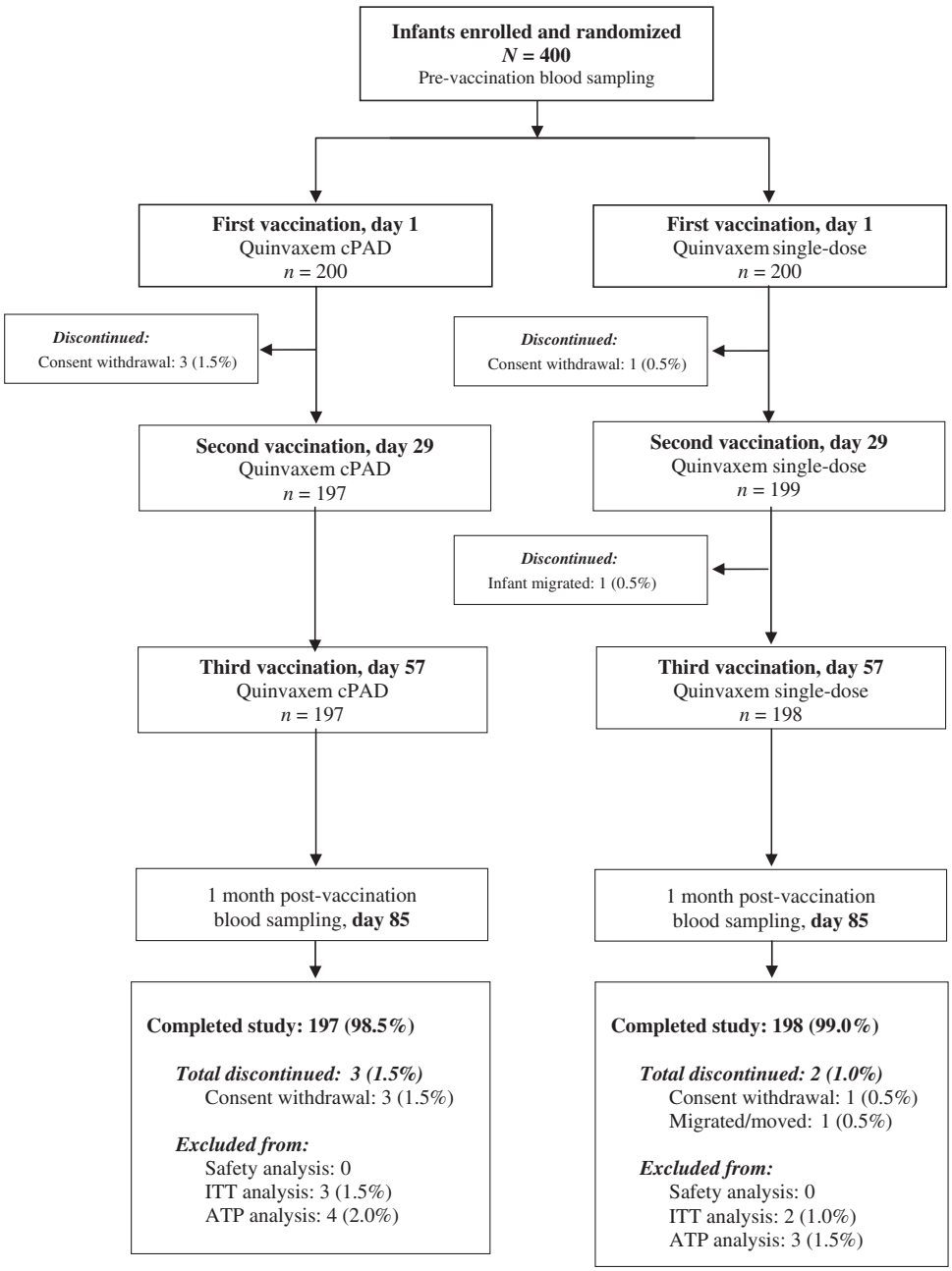

Figure 1. Disposition of the infants (ATP, according-to-protocol; ITT, intention-to-treat; *N* is the number of subjects in the specified category; percentages are based on the total number of randomized infants in each group).

toxoid GMCs were similar after vaccination with Quinvaxem in cPAD and Quinvaxem in single-dose vials (GMC ratios 1.04 and 0.99, respectively). For tetanus and pertussis, antibody GMCs in the single-dose group were numerically higher (both GMC ratios were 0.9). For HB, antibody GMCs were numerically higher in the cPAD group (GMC ratio 1.1). Fold-increases in antibody GMCs from day 1 to day 85 were numerically higher in the single-dose group for all antigens except tetanus. For the ATP population, there were slightly more responders in the single-dose group (176; 89.3%) than in the cPAD group (172; 87.8%). The logistic regression analysis on responder rates adjusted for vaccinator showed no influence due to vaccinator (Table 5).

3.3. Safety

The overall frequency of AEs was similar in the two groups (Table 6). A total of 175 (87.5%) infants in the cPAD group and 169 (84.5%) infants in the single-dose vial group exhibited at least one solicited local AE when events after all three doses were combined. Tenderness was the most common solicited local AE and was experienced by more infants in the cPAD group (171; 85.5%) than in the single-dose group (153; 76.5%). The frequency of induration was similar in the cPAD and single-dose groups (74 (37.0%) vs. 76 (38.0%) infants); erythema was observed more frequently in the single-dose group (61; 30.5%) than in the cPAD group (52; 26.0%). Frequencies for all local solicited AEs in both groups were highest after the first dose and decreased after the second and third doses (data not shown). Fever based on diary-recorded body temperature was reported in 42.5% of infants in the cPAD group and 41.5% of infants in the single-dose group.

Overall, 131 (65.5%) infants in the cPAD group and 138 (69.0%) infants in the single-dose group experienced unsolicited AEs. The most commonly experienced unsolicited AEs, irrespective of causal

Table 2
Seroprotection/seroconversion rate: ATP population[a,b,c]

Seroprotection/ seroconversion	cPAD (N = 196) n (%)	Single-dose (N = 197) n (%)	Diff. (95% CI[d])
Hib			
Baseline (day 1)	137 (69.9)	153 (77.7)	
Visit 4 (day 85)	193 (98.5)	194 (98.5)	0.0 (−3.05, 3.02)
Diphtheria			
Baseline (day 1)	46 (23.5)	39 (19.8)	
Visit 4 (day 85)	196 (100)	195 (99.0)	1.0 (−1.04, 3.63)
Tetanus			
Baseline (day 1)	181 (92.3)	189 (95.9)	
Visit 4 (day 85)	196 (100)	197 (100)	0.0 (−1.92, 1.91)
Hepatitis B			
Baseline (day 1)	54 (27.6)	51 (25.9)	
Visit 4 (day 85)	182 (92.9)	184 (93.4)	−0.5 (−5.78, 4.66)
Pertussis			
Visit 4 (day 85)	187 (95.4)	191 (97.0)	−1.5 (−5.78, 2.51)

ATP, according-to-protocol; CI, confidence interval; cPAD, compact prefilled auto-disabled system; Hib, *Haemophilus influenzae* type b.
[a] ATP population: The set of all intention-to-treat infants who received all three vaccinations, who had at least one blood sample with one post-baseline measurement for at least one antigen, and who had no major protocol violations.
[b] N is the total number of infants in each group and n is the total number of infants meeting the event. Percentages are based on the total number of infants in each vaccine group (N).
[c] Non-inferiority of the cPAD group with respect to the single-dose vials group was demonstrated, as the lower limits of the CI of the differences in seroconversion/seroprotection rates between the two groups were simultaneously greater than −10% for the five parameters.
[d] The 95% two-sided CIs for the difference in seroconversion/seroprotection rates are constructed based on the Newcombe–Wilson score method.

relationship, were pyrexia (43.5%, cPAD; 42.5%, single-dose vial), upper respiratory tract infection (24.0%, cPAD; 26.5%, single-dose vial), rhinitis (7.5% each in the cPAD and single-dose vial groups), and nasopharyngitis (7.0%, cPAD; 6.0%, single-dose vial). In total, 85 (42.5%) infants in the cPAD group experienced unsolicited AEs assessed as related to study vaccine, compared with 84 (42.0%) infants in the single-dose group. The most common unsolicited AE considered vaccine-related was pyrexia (42.5%, cPAD; 41.5%, single-dose vial).

Table 3
Seroprotection/seroconversion rate: ITT population[a,b]

Seroprotection/ seroconversion	cPAD (N = 197) n (%)	Single-dose (N = 198) n (%)	Diff. (95% CI[c])
Hib			
Baseline (day 1)	138 (70.1)	153 (77.3)	
Visit 4 (day 85)	194 (98.5)	195 (98.5)	0.0 (−3.04, 3.01)
Diphtheria			
Baseline (day 1)	46 (23.4)	39 (19.7)	
Visit 4 (day 85)	197 (100)	196 (99.0)	1.0 (−1.04, 3.61)
Tetanus			
Baseline (day 1)	182 (92.4)	190 (96.0)	
Visit 4 (day 85)	197 (100)	198 (100)	0.0 (−1.91, 1.90)
Hepatitis B			
Baseline (day 1)	54 (27.4)	51 (25.8)	
Visit 4 (day 85)	183 (92.9)	184 (92.9)	0.0 (−5.31, 5.23)
Pertussis			
Visit 4 (day 85)	188 (95.4)	192 (97.0)	−1.5 (−5.75, 2.50)

CI, confidence interval; cPAD, compact prefilled auto-disabled system; Hib, *Haemophilus influenzae* type b; ITT, intention-to-treat.
[a] ITT population: The set of all randomized infants who received at least one injection of the study vaccine and had a post-baseline measurement for at least one antigen.
[b] N is the total number of infants in each group and n is the total number of infants meeting the event. Percentages are based on the total number of infants in each vaccine group (N).
[c] The 95% two-sided CIs for the difference in seroconversion/seroprotection rates are constructed based on the Newcombe–Wilson score method.

Table 4
Ratio of geometric mean concentration and geometric mean concentration fold-increases for the antibody titers: ATP population[a,b]

Antigen	cPAD (N = 196) n (%)	Single-dose (N = 197) n (%)	Ratio, cPAD/ single-dose
Anti-Hib PRP concentration			
Pre-vaccination GMC	0.38	0.43	
Post-vaccination GMC	4.42	4.24	
GMC fold-increase	11.51	9.86	
Ratio of GMC fold-increase[c]			1.167
GMC ratio[d]			1.042
Anti-diphtheria concentration			
Pre-vaccination GMC	0.067	0.066	
Post-vaccination GMC	1.53	1.545	
GMC fold-increase	22.79	23.31	
Ratio of GMC fold-increase[c]			0.978
GMC ratio[d]			0.992
Anti-tetanus concentration			
Pre-vaccination GMC	1.230	1.266	
Post-vaccination GMC	1.11	1.234	
GMC fold-increase	0.902	0.974	
Ratio of GMC fold-increase[c]			0.925
GMC ratio[d]			0.899
Anti-hepatitis B concentration			
Pre-vaccination GMC	5.4	4.9	
Post-vaccination GMC	149.4	138.5	
GMC fold-increase	27.5	28.5	
Ratio of GMC fold-increase[c]			1.0
GMC ratio[d]			1.1
Anti-*Bordetella pertussis* concentration			
Pre-vaccination GMC	4.6	4.6	
Post-vaccination GMC	38.6	42.7	
GMC fold-increase	8.3	9.4	
Ratio of GMC fold-increase[c]			0.9
GMC ratio[d]			0.9

ATP, according-to-protocol; CI, confidence interval; cPAD, compact prefilled auto-disabled system; GMC, geometric mean concentration; Hib, *Haemophilus influenzae* type b; PRP, polyribosylribitol phosphate.
[a] N is the number of infants with non-missing antibody titer results at both pre- and post-vaccination.
[b] GMCs and 95% CIs were calculated by taking the anti-$\log_{10}$ of the means and 95% CIs of the log-transformed pre- and post-vaccination hemagglutination inhibition titers. The GMC fold-increases and 95% CIs were calculated by taking the anti-$\log_{10}$ of the means and 95% CIs of the log-transformed fold-increases in post-vaccination antibody titer over pre-vaccination antibody titer.
[c] Ratio of GMC fold-increase = GMC fold-increase for cPAD/GMC fold-increase for single-dose.
[d] GMC ratio = post-vaccination GMC for cPAD/post-vaccination GMC for single-dose.

No deaths were reported during the study. Eight infants experienced eight SAEs: pneumonia (n = 3), measles pneumonia (n = 3), idiopathic thrombocytopenic purpura (n = 1), and an umbilical abscess (n = 1). Five occurred in the cPAD group and three in the single-dose group; none were considered related to the study vaccine. No infants were withdrawn from the study due to an AE/SAE.

4. Discussion

Preventing bacterial and viral infections in infants via vaccination remains an ultimate public health goal. Quinvaxem in a new presentation – the cPAD injection system – is expected to reduce procedural steps, vaccination process time, and storage needs, and to minimize vaccine wastage as compared to conventional administration with a syringe. In the present study, the immunogenicity of Quinvaxem in cPAD was shown to be non-inferior to single-dose vials in infants aged 42 to 64 days, in terms of seroprotection/seroconversion rates for all antibodies at 1 month after the primary vaccination course. Both presentations were well-tolerated. Previous clinical studies have shown that Quinvaxem, as a combination of five antigens, can be administered

Table 5
Responder rate differences and 95% confidence intervals: ATP population[a]

	cPAD (*N* = 196) *n* (%)	Single-dose (*N* = 197) *n* (%)
Unadjusted for vaccinator		
Visit 4 (day 85)		
Number of responders	172 (87.76)	176 (89.34)
Difference in rates	−1.585	
95% CI[b]	(−8.01, 4.81)	
Adjusted for vaccinator		
Visit 4 (day 85)		
Number of responders	172 (87.75)	176 (89.34)
Difference in rates	−1.594	
95% CI[c]	(−7.86, 4.68)	

ATP, according-to-protocol; CI, confidence interval; cPAD, compact prefilled auto-disabled system.
[a] *N* is the total number of infants in each group; *n* is the total number of infants meeting the event. Percentages are based on the total number of infants in each group (*N*).
[b] Calculated using the Newcombe–Wilson score method.
[c] Adjusted rates are derived from the odds ratios from the logistic regression analysis with vaccinator as a factor; the 95% CI for the difference in rates is based on the normal approximation.

without an impact on immunogenicity of the individual components and has a good safety profile.[27–29] However, the safety and immunogenicity of two different presentations of Quinvaxem had not been studied to date.

In this study, the seroconversion/seroprotection rates were similar in the cPAD and single-dose vial groups. The percentages of infants with seroprotection against Hib PRP, HB, diphtheria, and tetanus toxoids, and seroconversion against *B. pertussis*, were greater than 92% for the cPAD and single-dose vial presentations. GMCs for all antibodies in both groups exceeded the seroprotection/seroconversion thresholds by large margins.

In the current study, the baseline antibody concentrations to Hib were high, with 70.1% of cPAD infants and 77.3% of single-dose infants seroprotected already above the short-term protective threshold (≥0.15 μg/ml). These high antibody titers are thought to be related to high maternal anti-Hib PRP concentrations, as previously reported from countries with low coverage for Hib vaccination.[14,15,30] This is further supported by data from a study in which cord blood anti-Hib IgG concentrations in the infants of Hib–T recipient mothers were found to be 61% of the maternal concentrations, and geometric mean anti-Hib antibody concentrations in the vaccinated infants of vaccinated mothers at birth and at 2 and 5 months of age were markedly higher than those in the vaccinated infants of unvaccinated mothers. At the age of 2 months, 60% of the infants of the vaccinated mothers and 26% of those of unvaccinated mothers had anti-Hib antibody concentrations considered to be protective (>0.15 μg/ml).[31] In the current study, post-vaccination there was a robust increase in Hib antibody titers in both vaccine groups, indicating a good immune response to the Hib component of the Quinvaxem vaccine.

Pertussis seroconversion rates were comparable in the two vaccine groups, with more than 95% achieving at least a 4-fold increase in antibody titers in the cPAD and single-dose vial groups; similar results have been obtained in other studies, but with significantly lower geometric mean titers.[14,28] In many industrialized countries, the DTP-based combination vaccines incorporate acellular pertussis (aP). For several decades, inactivated whole-cell pertussis (wP) vaccines have been part of the national childhood vaccination programs, dramatically reducing the considerable public health impact of pertussis. Frequent, but usually mild adverse reactions, and a fear of rare but serious acute or chronic neurological events associated with wP vaccination, prompted the development of a new generation of pertussis vaccines, the aP vaccines, which are believed to have fewer such reactions than wP vaccines.[27,32] However, evidence suggests that licensed aP vaccines have lower initial efficacy, faster waning of immunity, and possibly a reduced impact on transmission compared with current internationally available wP vaccines.[33,34] Mathematical modeling studies and baboon models support the hypothesis that transition from wP to shorter duration of protection aP may be associated with disease resurgence.[35,36]

The tetanus toxoid antibody pre/post-vaccination ratios were 0.90 and 0.97 for the cPAD and single-dose groups, respectively. For both groups, 100% long-term tetanus seroprotection was obtained. Long-term seroprotection is indicated by an antibody concentration of ≥0.1 IU/ml; the concentrations for cPAD and single-dose groups post-vaccination were 1.11 and 1.23 IU/ml, respectively, with a post-vaccination concentration of 1.11 IU/ml, well above the cut-off of 0.1 IU/ml as recommended by the WHO.[37]

Table 6
Solicited and unsolicited adverse events (after first, second, and third vaccine doses combined)[a,b]

	cPAD (*N* = 200) *n* (%)	Single-dose (*N* = 200) *n* (%)	Risk difference,[c] cPAD − single-dose difference
Solicited AEs			
Infants with at least one solicited AE	185 (92.5)	184 (92.0)	0.5
Infants with at least one local event	175 (87.5)	169 (84.5)	3.0
Infants with erythema	52 (26.0)	61 (30.5)	−4.5
Infants with induration	74 (37.0)	76 (38.0)	−1.0
Infants with tenderness	171 (85.5)	153 (76.5)	9.0
Infants with at least one systemic event	85 (42.5)	83 (41.5)	1.0
Infants with fever (≥38 °C)	85 (42.5)	83 (41.5)	1.0
Infants with high fever (≥39.5 °C)	1 (0.5)	8 (4.0)	−3.5
Unsolicited AEs			
Infants with at least one unsolicited AE	131 (65.5)	138 (69.0)	−3.5
Infants with at least one unsolicited AE assessed as related to study vaccine	85 (42.5)	84 (42.0)	−0.5
Crying	0	1 (0.5)	-0.5
Pyrexia	85 (42.5)	83 (41.5)	1.0
Infants with at least one serious AE	5 (2.5)	3 (1.5)	1.0

AE, adverse event; cPAD, compact prefilled auto-disabled system.
[a] *N* is the total number of infants in each group; *n* is the total number of infants meeting the event. Percentages are based on the total number of infants in the vaccine group (*N*).
[b] All solicited and unsolicited AEs observed after the first vaccine dose and up to the last safety follow-up are included. Solicited AEs include data collected 30 min after each vaccination.
[c] The risk differences are presented in percentages.

These high baseline antibodies were presumably due to maternal immunization. These concentrations are known to vary depending on the characteristics of the population in which the study is performed and have an impact on the observed pre/post-vaccination GMC increase.[38,39] Tetanus toxin maternal antibodies decay with an average half-life of 48 days.[40,41] At an age of 4 months, an infant has around 10% of the original maternal antibody concentration circulating in the blood. From the half-life, it can be concluded that a GMC fold-increase of $\geq$0.10 is derived from antibodies produced by the infants indicative of a vaccine response. Even if a mathematical correction model was used to correct for the maternal antibody concentrations, the vaccine response of the infants in the present study was still well above the required antibody concentration level for long-term protection.

As per the WHO recommendation, four vaccinators were included in this study to assess whether any differences in immunogenicity were observed when various vaccinators administered Quinvaxem in cPAD. Logistic regression analysis on seroconversion/seroprotection rates at visit 4 adjusted for vaccinator did not reveal any effect of the vaccinator. Moreover, the unadjusted seroprotection/seroconversion rates for each antigen were the same as the rates adjusted for the vaccinator.

Overall, both vaccines were well-tolerated and most of the local and systemic reactions were mild-to-moderate. The incidence of solicited or unsolicited AEs was largely consistent with previous studies and similar in the two groups.[14,15,28,29] Tenderness, the most common solicited local AE, was reported at 9% higher incidences in the cPAD group than in the single-dose group. However, injection site induration and erythema were observed at similar or lower frequencies in the cPAD group compared with the single-dose group. Overall, both presentations were well tolerated.

Quinvaxem in cPAD is a widely used fully liquid pentavalent vaccine that is available in a single-dose vial, with the only difference being its compact, prefilled, auto-disable injection system and secondary packaging. The new presentation, cPAD, might help to increase vaccine coverage, especially in hard-to-reach situations,[15,42] because it simplifies transport and logistics by being lightweight,[43] with a small volume,[43,44] safe disposal,[29,42] easy to use presentation simplifying vaccine administration,[15,20,21,42] and reducing handling errors.[15,19,45,46] It also offers several other benefits including reduced missed vaccination opportunities due to mismatch between vaccine doses and consumables (needles and syringes) as all required components are present at the same place and time,[29,42,43] increased vaccine coverage by reducing vaccination time (six times quicker administration versus a single-dose lyophilized vial),[21] and less vaccine wastage[45,47–49] as the single-dose presentation ensures opened multi-dose vials are not discarded. The contamination risk is also expected to be minimized as vaccine sterility is maintained right up to the point of administration,[42,45] and the auto-disable feature prevents needle and syringe reuse.[29] These benefits of cPAD can potentially improve vaccination program performance by facilitating increased vaccination coverage in many countries.

This study aimed to demonstrate non-inferiority of three doses of Quinvaxem in cPAD to three doses of Quinvaxem in single-dose vials with respect to immunogenicity. The study was performed at a single research site where the vaccinators were properly trained. It will be important to assess the acceptability and continue to collect further safety data when the new presentation is administered by health workers as part of the EPI program.

In conclusion, non-inferiority of three doses of Quinvaxem in cPAD to three doses of Quinvaxem in single-dose vials was demonstrated with respect to seroprotection/seroconversion rates for all antibodies (anti-HBs, anti-Hib PRP, anti-diphtheria, anti-tetanus, and anti-*B. pertussis*) at 1 month after completion of the 6–10–14 week schedule. The overall safety profiles of the two presentations were similar.

Author contributions

P. Ibarra de Palacios, M.R.Z. Capeding, A. Versteilen, P. Bagchi, and M. Rauscher were involved in the study design and analysis. P. Ibarra de Palacios provided significant intellectual contributions, A. Versteilen was involved in the analysis of the immunology data, M. Rauscher was involved in the analysis of safety data, and P. Bagchi was the project statistician. M.R.Z. Capeding was the principal investigator and E. Alberto co-investigator, and both were involved in data collection and the critical review. All authors critically reviewed the manuscript and approved the final version for publication.

Acknowledgements

The authors wish to thank the study volunteers and their parents/guardians for their contributions and the investigational staff for the medical care. We thank Ashwini Patil (SIRO Clinphgroup Pvt. Ltd) for medical writing assistance (funded by Janssen Research and Development, LLC) and Bradford Challis (Janssen Research and Development, LLC) for additional editorial support. The authors would also like to thank Max Gough for his work in the development of the protocol and study report and Youssef Chebihi-Quadouri for his work in the coordination of the conduct of the clinical trial.

Study support: This study was sponsored by Crucell Switzerland (Janssen Infectious Diseases and Vaccines). The sponsor also provided formal review of this manuscript.

Conflict of interest: P. Ibarra de Palacios, P. Bagchi, and M. Rauscher are employees of Crucell Switzerland. A. Versteilen is an employee of Crucell Holland BV. E. Alberto has no conflicts of interests to declare. M.R.Z. Capeding has received speaker honoraria, travel and research grants from Pfizer Inc., GlaxoSmithKline, Sanofi Pasteur, and Novartis, and a research grant from Crucell Switzerland AG for this clinical study.

References

1. World Health Organization. Global immunization vision and strategy 2014. Geneva: WHO; Available at: http://whqlibdoc.who.int/hq/2005/WHO_IVB_05.05.pdf?ua=1 (accessed March 4, 2015).
2. World Health Organization. Global immunization data, February 2014. Geneva: WHO; Available at: http://www.who.int/immunization/monitoring_surveillance/Global_Immunization_Data.pdf?ua=1 (accessed March 4, 2015).
3. Klein NP, Bartlett J, Fireman B, Rowhani-Rahbar A, Baxter R. Comparative effectiveness of acellular versus whole-cell pertussis vaccines in teenagers. *Pediatrics* 2013;**131**:1716–22.
4. Keja K, Chan C, Hayden G, Henderson RH. Expanded programme on immunization. *World Health Stat Q* 1988;**41**:59–63.
5. World Health Organization. WHO global immunization data 2014. Geneva: WHO; Available at: http://www.who.int/immunization/monitoring_surveillance/global_immunization_data.pdf (accessed March 4, 2015).
6. GAVI Alliance. Pentavalent vaccine infographic, April 2013. GAVI; Available at: http://www.gavialliance.org/support/nvs/pentavalent/infographic/ (accessed March 18, 2015).
7. Vaccine News Daily; Available at: http://vaccinenewsdaily com/vaccine_development/331301-pentavalent-vaccine-now-available-in-73-countries/2014 (accessed March 5, 2015).
8. World Health Organization. WHO prequalified vaccines. Geneva: WHO; Available at: http://www.who.int/immunization_standards/vaccine_quality/PQ_vaccine_list_en/en/. Last updated April 2014 (accessed 3 June, 2015).
9. Quinvaxem® summary of product characteristics. Available at: http://www.who.int/immunization_standards/vaccine_quality/quinvaxem_PI_eng.pdf (accessed March 5, 2015).
10. Schmid DA, Macura-Biegun A, Rauscher M. Development and introduction of a ready-to-use pediatric pentavalent vaccine to meet and sustain the needs of developing countries—Quinvaxem®: the first 5 years. *Vaccine* 2012;**30**:6241–8.
11. World Health Organization. Safety of Quinvaxem (DTwP–HepB–Hib) pentavalent vaccine. Geneva: WHO; Available at: http://www.who.int/

immunization_standards/vaccine_quality/quinvaxem_pqnote_may2013/en/ (accessed March 5, 2015).
12. Asturias EJ, Contreras-Roldan IL, Ram M, Garcia-Melgar AJ, Morales-Oquendo V, Hartman K, et al. Post-authorization safety surveillance of a liquid pentavalent vaccine in Guatemalan children. *Vaccine* 2013;**31**:5909–14.
13. Capeding MR, Jica C, Macura-Biegun A, Rauscher M, Alberto E. Interchangeability of Quinvaxem during primary vaccination schedules: results from a phase IV, single-blind, randomized, controlled, single-center, non-inferiority study. *Vaccine* 2014;**32**:888–94.
14. Kanra G, Kara A, Demiralp O, Contorni M, Hilbert AK, Spyr C, et al. Safety and immunogenicity of a new fully liquid DTPw–HepB–Hib combination vaccine in infants. *Hum Vaccin* 2006;**2**:155–60.
15. Suarez E, Asturias EJ, Hilbert AK, Herzog C, Aeberhard U, Spyr C. A fully liquid DTPw–HepB–Hib combination vaccine for booster vaccination of toddlers in El Salvador. *Rev Panam Salud Publica* 2010;**27**:117–24.
16. PATH. Vaccines in the cPAD™ injection system, March 2015. PATH; Available at: http://www.who.int/immunization/programmes_systems/service_delivery/InfoBulletin_Uniject_March2015_FINAL_ENG.pdf (accessed April 10, 2015).
17. Lorenson K, Mendoza M, Zaffran M. Stakeholder perceptions of pentavalent vaccine (DTwP–HepB–Hib) in the Uniject™ injection system. PATH;. Available at: http://www.path.org/publications/files/TS_dtwp_hepb_hib.pdf (accessed March 6, 2015).
18. Steinglass R, Boyd D, Grabowsky M, Laghari AG, Khan MA, Qavi A, et al. Safety, effectiveness and ease of use of a non-reusable syringe in a developing country immunization programme. *Bull World Health Organ* 1995;**73**:57–63.
19. PATH. The Uniject injection system: multi-country experience and evidence, February 2011. PATH;. Available at: http://www.path.org/publications/files/RH_depo_subq_experience.pdf (accessed July 2, 2015).
20. Colombini A, Guillermet E, Dicko HM, Mai LT, Hane F, Jaillard P, Gessner BD. Optimal approaches for the use of DTP–HepB–Hib vaccine in a compact prefilled auto disable (cPAD) syringe in resource-poor settings. Poster presentation. 16th International Congress on Infectious Diseases, Cape Town, South Africa, April 2–5, 2014.
21. Drain PK, Nelson CM, Lloyd JS. Single-dose versus multi-dose vaccine vials for immunization programmes in developing countries. *Bull World Health Organ* 2003;**81**:726–31.
22. Schmid DA, Macura-Biegun A, Rauscher M. Development and introduction of a ready-to-use pediatric pentavalent vaccine to meet and sustain the needs of developing countries. Quinvaxem: the first 5 years. *Vaccine* 2012;**30**:6241–8.
23. PATH. The radically simple Uniject™ injection system. PATH; Available at: http://path.org/projects/uniject.php (accessed February 3, 2016).
24. PATH. Vaccines in the Uniject™ injection system. PATH;. Available at: http://www.path.org/publications/files/TS_update_uniject_vaccine.pdf (accessed February 3, 2016).
25. PATH. Status report on the Uniject™ prefilled injection device. PATH;. Available at: http://www.path.org/publications/files/Uniject-Status-Report.pdf (accessed February 3, 2016).
26. PATH. The Uniject injection system: multi-country experience and evidence. PATH;. Available at: http://www.path.org/publications/files/RH_depo_subq_experience.pdf (accessed February 3, 2016).
27. Chatterjee S, Rego SJ, D'Souza F, Bhatia BD, Collard A, Datta SK, et al. The immunogenicity and safety of a reduced PRP-content DTPw–HBV/Hib vaccine when administered according to the accelerated EPI schedule. *BMC Infect Dis* 2010;**10**:298.
28. Eregowda A, Lalwani S, Chatterjee S, Vakil H, Ahmed K, Costantini M, et al. A phase III single arm, multicenter, open-label study to assess the immunogenicity and tolerability of a pentavalent DTwP–HepB–Hib vaccine in Indian infants. *Hum Vaccin Immunother* 2013;**9**:1903–9.
29. Gentile A, Umido V, Czerniuk P, Nacul J, Seigelchifer M, Hilbert AK, et al. Immunogenicity and reactogenicity of a combined fully liquid DTPw–HepB–Hib pentavalent vaccine in healthy infants: no clinically relevant impact of a birth dose of hepatitis B vaccine. *Int J Infect Dis* 2011;**15**:24–9.
30. Esposito S, Bosis S, Morlacchi L, Baggi E, Sabatini C, Principi N. Can infants be protected by means of maternal vaccination? *Clin Microbiol Infect* 2012;**18**:85–92.
31. Mulholland K, Hilton S, Adegbola R, Usen S, Oparaugo A, Omosigho C, et al. Randomised trial of *Haemophilus influenzae* type-b tetanus protein conjugate vaccine [corrected] for prevention of pneumonia and meningitis in Gambian infants. *Lancet* 1997;**349**:1191–7.
32. World Health Organization. Pertussis vaccines. WHO position paper, October 2010. Geneva: WHO; Available at: http://www.who.int/immunization/pertussis_grad_safety.pdf (accessed June 2, 2015).
33. World Health Organization. WHO Information Sheet: Observed rate of vaccine reactions—diphtheria, pertussis, tetanus vaccines, May 2014. Geneva: WHO;. Available at: http://www.who.int/vaccine_safety/initiative/tools/DTP_vaccine_rates_information_sheet.pdf?ua=1 (accessed June 8, 2015).
34. World Health Organization. WHO SAGE pertussis working group. Background paper, April 2014. Geneva: WHO; Available at: http://www.who.int/immunization/sage/meetings/2014/april/1_Pertussis_background_FINAL4_web.pdf?ua=1 (accessed June 15, 2015).
35. Warfel JM, Merkel TJ. *Bordetella pertussis* infection induces a mucosal IL-17 response and long-lived Th17 and Th1 immune memory cells in nonhuman primates. *Mucosal Immunol* 2013;**6**:787–96.
36. Warfel JM, Merkel TJ. Reply to Domenech de Celles et al.: Infection and transmission of pertussis in the baboon model. *Proc Natl Acad Sci U S A* 2014;**111**:718.
37. World Health Organization. Tetanus vaccine: WHO position paper. *Wkly Epidemiol Rec* 2006; Available at: http://www.who.int/immunization/wer8120tetanus_May06_position_paper.pdf?ua=1 (accessed March 6, 2015) 81:198–207.
38. Panpitpat C, Thisyakorn U, Chotpitayasunondh T, Furer E, Que JU, Hasler T, et al. Elevated levels of maternal anti-tetanus toxin antibodies do not suppress the immune response to a *Haemophilus influenzae* type b polyribosylphosphate–tetanus toxoid conjugate vaccine. *Bull World Health Organ* 2000;**78**:364–71.
39. Van Der Meeren O, Kuriyakose S, Kolhe D, Hardt K. Immunogenicity of Infanrix hexa administered at 3, 5 and 11 months of age. *Vaccine* 2012;**30**:2710–4.
40. Borrow R, Balmer P, Roper MH. The immunological basis for immunization series, module 3: tetanus (update 2006). Geneva, Switzerland: World Health Organization; 2007.
41. Sarvas H, Seppala I, Kurikka S, Siegberg R, Makela O. Half-life of the maternal IgG1 allotype in infants. *J Clin Immunol* 1993;**13**:145–51.
42. PATH. Vaccines in Uniject™ injection system, August 2013. PATH;. Available at: http://www.path.org/publications/files/TS_update_uniject_vaccine.pdf (accessed February 22, 2015).
43. Quinvaxem injection DTwP–HepB–Hib fully liquid combination vaccine. Prescribing information.; 2010. Available at: http://www.who.int/immunization_standards/vaccine_quality/quinvaxem_PI_eng.pdf (accessed May 5, 2015).
44. Guichard S, Hymbaugh K, Burkholder B, Diorditsa S, Navarro C, Ahmed S, et al. Vaccine wastage in Bangladesh. *Vaccine* 2010;**28**:858–63.
45. World Health Organization. Monitoring vaccine wastage at country level: guidelines for programme managers. Geneva: WHO; 2005, Available at: http://whqlibdoc.who.int/hq/2005/WHO_V&B_03.18.Rev.1_eng.pdf (accessed June 12, 2015)
46. Parmar D, Baruwa EM, Zuber P, Kone S. Impact of wastage on single and multi-dose vaccine vials: Implications for introducing pneumococcal vaccines in developing countries. *Hum Vaccin* 2010;**6**:270–8.
47. PATH. Logistics and waste management benefits of depo-subQ in Uniject. PATH; 2011. Available at: http://www.path.org/publications/files/RH_depo_subq_logistics.pdf (accessed May 30, 2015).
48. PATH. The Uniject injection system: multi-country experience and evidence, February 2011. PATH;. Available at: http://www.path.org/publications/files/RH_depo_subq_experience.pdf (accessed May 30, 2015).
49. World Health Organization, Department of Vaccines and Biologicals. WHO policy statement. The use of opened multi-dose vials of vaccine in subsequent immunization sessions, March 2000. Geneva: WHO;. Available at: http://apps.who.int/iris/bitstream/10665/66278/1/WHO_V-B_00.09_eng.pdf?ua=1 (accessed May 30, 2015).