Lot-to-lot consistency study of the fully liquid pentavalent DTwP-HepB-Hib vaccine Quinvaxem® demonstrating clinical equivalence, suitability of the vaccine as a booster and concomitant administration with measles vaccine

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This double-blind, randomized study evaluated the immunogenicity and safety of three production lots of the fully liquid combination DTwP-Hep-Hib vaccine, Quinvaxem® (Cruell, The Netherlands) in 360 healthy infants aged 42–64 d old given at 6, 10 and 14 weeks of age (Core Study). The Core Study was followed by an open-label Booster Phase evaluating immunogenicity and safety of a booster dose of Quinvaxem® given with either concomitant or deferred measles vaccine in 227 infants who completed the Core Study. One month after the third dose of Quinvaxem® immune responses reflecting seroprotection or seroconversion were observed in more than 90% of infants for all three vaccine lots. Quinvaxem® elicited a strong booster response as demonstrated by a large increase in antibodies against all antigens, which appeared to be unaffected by concomitant administration of the measles vaccine. Safety results were in line with previous reports for Quinvaxem® with no unexpected adverse events (AEs) being reported. In the Core Study and Booster Phase, Quinvaxem® was well tolerated. No study vaccine-related serious AEs were reported. Thus, we conclude Quinvaxem® was immunogenic and well-tolerated when administered to infants according to a 6–10–14 week vaccination schedule. The three production lots had consistent reactogenicity and immunogenicity profiles. The booster dose of Quinvaxem® was also immunogenic and safe, regardless of whether a monovalent measles vaccine was administered concomitantly or one month later.

Introduction

Combination vaccines against diphtheria, tetanus, and pertussis (DTP) are the cornerstone of childhood immunization programs, particularly in developing countries. Since 1990, the global coverage for DTP vaccine has been around 80%. The basic course consists of three doses of DTP vaccine given during the first year of life. In addition, a booster dose should be added, preferably during the second year of life, and in several countries further boosters are recommended in older children/adolescents.^{1–3}

Vaccines against hepatitis B virus (HBV) have been in use since the 1980s. Discovery of the virus, development of plasma-derived and then DNA-recombinant vaccines, and reductions in vaccine prices have made the wide use of this vaccine possible.⁴ Hepatitis B vaccine given in infancy is highly effective in preventing HBV infection.⁵ The WHO has recommended hepatitis B vaccination in the Expanded Programme on Immunization (EPI) since 1992, aiming to reduce the overall incidence of HBV infection and chronic carriage in HBV-endemic areas.⁶

Since 1997, vaccination against *Haemophilus influenzae* type b (Hib) has also been recommended by the WHO for inclusion into the EPI.^{7,8} Hib conjugate vaccines are highly efficacious in preventing invasive Hib disease in infants and young children.^{7,9} Invasive Hib disease has been virtually eliminated in those countries where Hib conjugate vaccine has been introduced for routine immunization of children.^{9,10}

Adequate supplies of combined DTP, hepatitis B, and Hib vaccines are essential for the continuing success of these programs, with their availability for childhood use being an essential requirement for increasing vaccine coverage in infant vaccination programs. Pediatric combination vaccines reduce the number of injections that children receive during vaccination sessions, the number of clinic visits and logistical requirements, and could ultimately increase parental compliance.^{11,12}

Until the development of Quinvaxem® the only licensed, internationally available, pentavalent combination vaccine containing DTwP-HepB-Hib was formulated with a DTwP-HepB component and a separate lyophilized Hib component

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reconstituted before injection.¹³⁻¹⁵ Quinvaxem®, a fully liquid pentavalent DTwP-HepB-Hib combination vaccine, was developed to eliminate the need for reconstitution of lyophilized components, decreasing the time needed to complete vaccination and reducing contamination and handling errors.^{16,17} The vaccine has been a WHO pre-qualified vaccine since 2006. Quinvaxem® contains recombinant hepatitis B surface antigen (HBsAg; produced in *Hansenula polymorpha* using the surface antigen coding sequence from human hepatitis B virus subtype adr), Hib conjugated to *Corynebacterium diphtheriae* cross-reacting material 197 (CRM197-Hib), tetanus toxoid, diphtheria toxoid, and whole-cell pertussis antigens. The vaccine is indicated in infants for protection against diseases caused by HBV, *C. diphtheriae*, *Clostridium tetani*, *Bordetella pertussis*, and Hib.

A previous study showed that vaccination with Quinvaxem® was as immunogenic as separate administration of the licensed vaccines Quattvaxem® (DTwP-Hib) and Hepavax-Gene® (hepatitis B antigen) at 2, 3 and 4 mo of age.¹⁸ One study indicated that Quinvaxem® was immunogenic when given to infants regardless of whether they had or had not received a hepatitis B vaccine at birth.¹⁹ Another study showed that Quinvaxem® could be administered as a booster to infants who have received a primary vaccination course with another combination vaccine.²⁰

Hib antibodies induced by vaccination during the first year of life tend to wane progressively over the 2–3 y following primary vaccination.²¹ In most countries a booster dose is recommended for children < 4 y of age.²² Furthermore, the EPI recommends approximately a 12 mo interval between the primary course of Hib vaccination and the booster injection. The availability of pentavalent vaccines simplifies addition of a Hib booster (in the second year of life) in national immunization schedules that do not already recommend it, at the same time allowing the appropriate interval between hepatitis B vaccination and booster dose. In our study, the Quinvaxem® booster dose was scheduled to coincide with the measles vaccination as per the local EPI recommendations. The influence of measles vaccine on the antibody responses to all components of Quinvaxem® was evaluated and was a main aim of the booster phase.

We report a phase III lot-consistency study with a booster phase to assess the effect of a Quinvaxem® booster given at 18 mo of age with a monovalent measles vaccine given either concomitantly or one month after the booster.

Results

Study populations. A total of 360 (190 male; mean age 6.7 weeks) were enrolled in the core study and randomized to receive one of three lots of Quinvaxem® (Lots A, B and C, Fig. 1). There were no significant differences in demographic characteristics between the three groups (data not shown). A total of 320 infants were included in the primary immunogenicity analyses based on the according-to-protocol (ATP) population one month after the third vaccination (Table 1). All infants (360) were included in the safety analyses. Of 331 infants who completed the core study, 227 infants were enrolled in the booster vaccination phase (Fig. 1). Of these, 225 infants (121 male; mean age at enrolment 16.7

mo) were included in the safety analysis and 212 infants were included in the immunogenicity analyses (ATP population one month after the booster) (Table 2).

In the core study, the second and third vaccinations had to be administered at least 28 d and no more than 35 d after the previous vaccination. The mean time of vaccination for the second vaccine dose was 28.7 d (range: 21–42 d) for the pooled population, 28.8 d (21–35 d) for Lot A, 28.9 d (28–42 d) for Lot B, and 28.5 d (21–42 d) for Lot C. The mean time of vaccination for the third vaccine dose was 29.1 d (range: 23–36 d) for the pooled population, 29.3 d (25–36 d) for Lot A, 28.9 d (23–35 d) for Lot B, and 29.0 d (24–35 d) for Lot C.

Immunogenicity. Core study. Clinical equivalence of the three vaccine lots was demonstrated with respect to seroprotection/seroconversion rates for the anti-HB antigen (pairwise comparison data not shown). Table 3 presents the seroprotection/seroconversion rates at one month post-third vaccination. High seroprotection rates were achieved for all antigens, with 100% of infants achieving seroprotective titers against tetanus, 99% against diphtheria, 99% against Hib (anti-PRP ≥ 0.15 $\mu\text{g/mL}$) and 91.4% against hepatitis B (pooled lot data). At the higher anti-PRP cut-off level of ≥ 1.0 $\mu\text{g/mL}$, a slightly lower seroprotective rate was observed (88.2%, pooled data) than for ≥ 0.15 $\mu\text{g/mL}$, and more variability between lots (data not shown) was observed for the Hib component. The immunogenic response to the *B. pertussis* component was also high with a seroconversion rate of 97.4% (pooled data).

The GMCs at one month post-third vaccination (week 12) are shown in Table 3. Some variation in anti-HBs GMCs were observed between groups. However, the ratios of anti-HBs GMCs at Week 12 to GMCs at baseline were similar for each treatment group with a mean 19.9-fold increase over baseline concentration levels. GMCs for anti-diphtheria toxoid (pooled: 1.0 IU/mL), anti-tetanus toxoid (pooled: 4.2 IU/mL), anti-PRP (pooled: 7.9 $\mu\text{g/mL}$) and anti-*B. pertussis* (pooled: 47.4 EIU/mL) were similar between the three treatment groups after completion of the primary vaccination course.

Booster phase. The proportions of infants in each treatment group with antibody titers above the cut-off levels for protection post-booster vaccination are presented in Table 4. Seroprotection rates fell during the period between the end of the core study and the pre-vaccination time point for the booster phase (12 ± 3 mo). Respective seroprotection rates (based on pooled data) at these two time points were 99.0% vs. 45.3% for diphtheria, 100% vs. 94.3% for tetanus, 99% vs. 66.7% (≥ 0.15 $\mu\text{g/mL}$ cut-off) and 88.2% vs. 22.4% (≥ 1.0 $\mu\text{g/mL}$ cut-off) for Hib, and 91.4% vs. 76.4% for hepatitis B.

One month after the Quinvaxem® booster dose, seroprotection rates against hepatitis B in the group with concomitant administration of measles vaccine were 97.1% vs. 96.4% in the group with delay measles immunization, 100% in both groups for tetanus, 96.0% vs. 94.5% for Hib (anti-PRP ≥ 1.0 $\mu\text{g/mL}$), 100% vs. 99.1% for diphtheria, and 98.0% vs. 94.5% for pertussis (seroconversion). Thus, not only was a good booster response seen (Fig. 2), but the responses suggest a lack of interference by concomitant measles vaccination. Most infants demonstrated

Figure 1. Consort diagram of patient disposition through core study and booster phase

antibody levels associated with seroprotection or seroconversion against all vaccine antigens (94.5% in the delayed measles group and 96.0% in the concomitant measles group).

The GMCs at one month after the booster dose are shown in Table 4. GMCs fell during the period between the end of the core study to the pre-vaccination time point for the booster phase (12 ± 3 mo). For diphtheria, GMCs at these two timepoints were 1.0 IU/mL vs. 0.1 IU/mL (pooled), respectively, for tetanus 4.2 IU/mL vs. 0.5 IU/mL, for pertussis were 47.4 EIU/mL vs.

7.8 EIU/mL, for Hib were 7.9 μ g/mL vs. 0.3 μ g/mL, and for hepatitis B were 107.8 mIU/mL vs. 37.6 mIU/mL.

One month after the Quinvaxem® booster dose, antibody GMCs for diphtheria, tetanus and Hib were similar in the two groups, but anti-HBsAg and anti-*B. pertussis* antibody concentrations were higher in infants who had received a concomitant measles vaccine. However, GMCs were well above the seroprotective levels in both groups for anti-HBsAg and anti-*B. pertussis* suggesting that the differences had no clinical relevance. These

Table 1. Core study safety population and according-to-protocol population with reasons for exclusion

CORE STUDY	Enrolled and randomized n = 360			
	Lot A	Lot B	Lot C	Pooled
Safety population	119 (100%)	120 (100%)	121 (100%)	360 (100%)
ATP population	106 (89.1%)	106 (88.3%)	108 (89.3%)	320 (88.9%)
Excluded from ATP ^a :	13 (10.9%)	14 (11.7%)	13 (10.7%)	13 (10.7%)
Infant did not receive 2nd vaccination	3 (2.5%)	3 (2.5%)	6 (5.0%)	12 (3.3%)
Infant did not receive 3rd vaccination	8 (6.7%)	7 (5.8%)	8 (6.6%)	23 (6.4%)
No post baseline titer measurement	10 (8.4%)	9 (7.5%)	10 (8.3%)	29 (8.1%)
Age outside window	1 (0.8%)	1 (0.8%)	0	2 (0.6%)
Violation of exclusion criteria	1 (0.8%)	1 (0.8%)	0	2 (0.6%)
Contraindication criteria	0	1 (0.8%)	0	1 (0.3%)
Second vaccination performed outside window	1 (0.8%)	1 (0.8%)	2 (1.7%)	4 (1.1%)
Third vaccination performed outside window	0	3 (2.5%)	2 (1.7%)	5 (1.4%)
Positive HIV status	1 (0.8%)	0	1 (0.8%)	2 (0.6%)

ATP, according-to-protocol; ^aInfants could have been excluded from the ATP population for more than one reason.

Table 2. Booster phase safety population and according-to-protocol population with reasons for exclusion

Booster phase	Enrolled and randomized n = 227		
	Booster plus Rouvax (1 mo later)	Booster plus Rouvax (concomitantly)	Pooled
ATP population	110 (94.8%)	102 (91.9%)	212 (93.4%)
Excluded from ATP:	6 (5.2%)	9 (8.1%)	15 (6.6%)
No post-baseline titer for any antigen	6 (5.2%)	9 (8.1%)	15 (6.6%)
Safety population	115 (99.1%)	110 (99.1%)	225 (99.1%)
Excluded from safety:	1 (0.9%)	1 (0.9%)	2 (0.9%)
Follow-up safety data missing	1 (0.9%)	1 (0.9%)	2 (0.9%)

ATP, according to protocol.

data confirm a good booster response not affected by concomitant measles vaccination.

Safety. Core study. The extent of exposure to Quinvaxem® in the core study was as follows: 360 infants randomized between the three treatment groups (receiving lots A to C) received the first vaccination; 348 infants received the second vaccination and 337 received the third vaccination. For the analysis, the safety data generated after each of the three vaccinations was pooled giving overall data.

Table 5 presents the overall incidence rates of solicited local and systemic adverse events (AE) regardless of relationship to vaccination. Induration occurred in 22.5% of infants. There were no marked differences between the lots, though slightly more events of induration were reported for lot C (25.6%) than lot B (24.2%) and lot A (17.6%). The frequency of induration decreased with successive injections: 17.2% after first, 11.4% after second and 7.5% after third injection (pooled data).

Solicited systemic AEs were experienced by 76.4% of the infants. The most frequently reported solicited systemic AEs (based on pooled data) were unusual crying (45.8%), sleepiness (37.8%), fever (34.2%) and irritability (31.9%) (**Table 5**). There were no marked differences between the lots. After successive injections, the frequency of solicited systemic AEs decreased,

mostly after the first injection (data not shown), although for rash slightly more children experienced more rash after the third injection (11.3%) than the second injection (9.2%).

The majority of unsolicited AEs were mild in intensity. Only two vaccine-related unsolicited local AEs were reported across all three lots. Injection site pain/tenderness was reported in 34 infants (9.4%, pooled data), mostly after the second vaccination (5.8%). Injection site redness/erythema (> 5 mm) was reported only after the third vaccine by six infants (1.7%). No other vaccine-related unsolicited AEs were reported across all three lots.

Eight serious AE (SAE), including two deaths, were reported during the core study. All were assessed as unrelated to vaccination. The two fatal cases, both three-month old female infants three weeks after the second vaccination, involved suspected pneumonia in one and diarrhea and dehydration in the other. The other SAEs involved viral bronchopneumonia, respiratory syncytial virus, pneumonia, bronchiolitis and a viral infection.

Booster phase. The overall incidence rates of solicited local and systemic AEs (regardless of relationship to vaccination) after booster vaccination are presented in **Table 5**. Induration was the only injection site reaction that was solicited, and showed comparable incidence rates in both groups (plus delayed measles vaccine 21.8%; Quinvaxem® plus concomitant measles vaccine 23.8%).

Table 3. Observed seroprotection and seroconversion rates and geometric mean concentrations (GMC) at one month after the third vaccination in the core study based on the according-to-protocol population

Antigen	Unit	Post-third vaccination seroprotection/seroconversion rate % [95% CI]				Post-third vaccination GMC [95% CI]			
		Lot A n = 106	Lot B n = 106	Lot C n = 108	Lots Pooled 320	Lot A n = 106	Lot B n = 106	Lot C n = 108	Lots Pooled 320
Diphtheria	IU/mL	98.1 [93.3,99.8]	100 [96.5,100]	99.0 [94.8,100]	99.0 [97.2,99.8]	0.9 [0.8,1.1]	0.9 [0.8,1.1]	1.0 [0.9,1.2]	1.0 [0.9,1.1]
Tetanus	IU/mL	100 [96.5,100]	100 [96.5,100]	100 [96.5,100]	100 [98.8,100]	4.2 [3.4,5.3]	4.6 [3.9,5.4]	3.9 [3.2,4.7]	4.2 [3.8,4.7]
Pertussis ^a	EIU/mL	97.1 [91.8,99.4]	98.1 [93.2,99.8]	97.1 [91.8,99.4]	97.4 [95.0,98.9]	46.3 [42.0,51.0]	51.8 [47.8,56.0]	44.5 [40.8,48.4]	47.4 [45.1,49.9]
Hib (anti-PRP ≥ 0.15 µg/mL)	µg/mL	98.1 [93.2,99.8]	99.0 [94.8,100]	100 [96.5,100]	99.0 [97.2,99.8]	8.4 [6.2,11.5]	9.7 [7.1,13.1]	6.0 [4.4,8.2]	7.9 [6.6,9.4]
Hib (anti-PRP ≥ 1.0 µg/mL)	µg/mL	91.3 [84.2,96.0]	91.4 [84.4,96.0]	81.7 ^b [72.9,88.6]	88.2 [84.1,91.5]	-	-	-	-
Hepatitis B	mIU/ mL	90.5 [83.2,95.3]	90.5 [83.2,95.3]	93.3 [86.6,97.3]	91.4 [87.7,94.3]	141.6 [103.9,192.9]	103.8 [76.7,140.5]	85.0 [63.6,113.6]	107.8 [90.6,128.2]

CI, confidence interval; GMC, geometric mean concentration; Hib, *Hemophilus influenzae* type b; ^aSeroconversion rates; ^bStatistically significantly lower than lots A and B by (pairwise comparison).

Table 4. Observed seroprotection and seroconversion rates and geometric mean concentrations (GMC) at one month after the booster vaccination in the booster phase based on the according-to-protocol population

Antigen	Unit	Post-booster seroprotection/seroconversion rate % [95% CI]			Post-booster GMC [95% CI]		
		Booster plus Rouvax (1 mo later) n = 110	Booster plus Rouvax (concomitantly) n = 102	Pooled n = 212	Booster plus Rouvax (1 mo later) n = 110	Booster plus Rouvax (concomitantly) n = 102	Pooled n = 212
Diphtheria	IU/mL	99.1 [95.0,100]	100 [96.4,100]	99.5 [97.4,100]	2.1 [1.7,2.6]	2.0 [1.7,2.4]	2.1 [1.8,2.4]
Tetanus	IU/mL	100 [96.7,100]	100 [96.4,100]	100 [98.3,100]	12.3 [10.5,14.5]	13.3 [11.4,15.5]	12.8 [11.4,14.3]
Pertussis ^a	EIU/mL	94.5 [88.5,98.0]	98.0 [93.1,99.8]	96.2 [92.7,98.4]	48.0 [42.8,53.7]	57.4 [52.7,62.5]	52.3 [48.7,56.2]
Hib (anti-PRP ≥ 0.15 µg/mL)	µg/mL	100 [96.7,100]	100 [96.4,100]	100 [98.3,100]	25.1 [18.8,33.4]	25.3 [18.7,34.4]	25.2 [20.5,31.0]
Hib (anti-PRP ≥ 1.0 µg/mL)	µg/mL	94.5 [88.5,98.0]	96.0 [90.2,98.9]	95.3 [91.5,97.7]	-	-	-
Hepatitis B	mIU/mL	96.4 [91.0,99.0]	97.1 [91.6,99.4]	96.7 [93.3,98.7]	1318 [868,2001]	2204 [1553,3126]	1688 [1283,2221]

CI, confidence interval; GMC, geometric mean concentration; Hib, *Hemophilus influenzae* type b; ^aSeroconversion rates.

The frequencies of the unsolicited local reactions pain/tenderness and redness/erythema were low with only one infant in the delayed measles group receiving Quinvaxem® (0.9%) reporting tenderness at the injection site.

After the booster dose more infants who received Quinvaxem® with concomitant measles vaccine (65.7%) showed solicited

systemic AEs than infants who received the measles vaccine one month later (54.5%). The most commonly solicited systemic AE was unusual crying with an overall incidence of 30.7%. Other frequently reported systemic AEs included change in eating habits (26.5%), sleepiness (25.6%) and irritability (22.8%). Fever was reported for 16.7% of infants overall and was slightly more

Figure 2. Seroprotection and seroconversion rates (%) at one month following the third vaccination in the core study and the booster phase. Data are based on the according-to-protocol population. Pooled lot data for the Core Study is presented. Seroprotection rates are shown for all antigens except anti-*B. pertussis* for which the seroconversion rate is shown.

common after vaccination with Quinvaxem® with concomitant measles vaccine, however, there was a general trend toward more reporting of AEs in this group. Diarrhea, vomiting, rash, and persistent crying were reported for ≤ 10% of infants in each group.

There were only five vaccine-related systemic AEs reported in the booster phase, all were mild cases of insomnia. One SAE was reported during the booster phase involving glomerulonephritis which was judged as unlikely related to vaccination. No deaths were reported. No vaccine-related SAEs were reported during either the core study or the booster phase.

Discussion

Immunogenicity. At one month after the third dose of Quinvaxem®, administered according to a 6–10–14 week schedule, over 90% of infants were seroprotected against all five immunogenic components for all three vaccine lots. Values for the three vaccine lots were very similar. Pairwise comparison of the seroprotection rates for all markers showed equivalence with the exception of Hib at the higher cut off of ≥ 1.0 µg/mL.

Over 90% of infants had pre-existing antibodies to tetanus, probably as a result of maternal vaccination. Irrespective of whether or not they had pre-existing antibodies, all infants had seroprotective levels of tetanus antibodies one month after the third vaccination with no apparent difference in GMCs.

As evidenced in this study and by others,²⁹ after primary vaccination with a pentavalent vaccine seroprotection rates and titers of associated antibodies decrease with time, emphasizing the need for a booster vaccination. The relatively low pre-booster seroprotection rates against HBV, Hib and diphtheria in this study might also be due to the shorter primary vaccination schedule applied (6–10–14 week) compared with the 2–4–6 mo schedule applied in other studies. Schedule-dependent lower

pre-booster seroprotection against HBV has also been observed with a similar DTwP-HepB-based vaccine when used at two different vaccination schedules.^{29,30} However, the large increase in seroprotection rates at post-booster levels demonstrates that immune memory was successfully induced by primary vaccination using the shorter 6–10–14 week schedule.

At one month after the booster dose of Quinvaxem®, a high proportion of infants were seroprotected/seroconverted against all antigens included in the vaccine (94.5% for all antigens in the delayed measles group and 96% in concomitant measles group) indicating that the booster dose of Quinvaxem® was immunogenic regardless of concomitant or deferred administration of a monovalent measles vaccine.

Before administration of the booster, there was a statistically significantly higher GMC of anti-HBs in the concomitant measles group compared with the delayed measles group ($p=0.022$). Although the GMC in the concomitant measles group was still higher than the delayed measles group after administration after administration of the Quinvaxem® booster, this difference was not statistically significant. Furthermore, the fold increases in GMC compared with baseline were similar irrespective of whether or not infants were given measles vaccine concurrently or sequentially (44.2-fold and 45.7-fold, respectively).

Safety. The incidence of AEs were consistent with previous Quinvaxem® reports and other pentavalent vaccines in infants.^{18–20,30} All three vaccine lots demonstrated similar AE profiles; the majority of AE were mild in severity. The incidence of solicited AEs tended to decrease from the first to the third vaccination as seen in earlier studies with Quinvaxem®.¹⁹

There were no marked differences in the overall incidence of AEs between the two study groups after booster dose administration. The incidence of solicited systemic vaccination-related AEs was slightly higher with concomitant than with deferred measles

Table 5. Solicited local and systemic adverse events reported within five days of each vaccination in the core study and booster phase based on the safety population

Adverse events	Core study n (%) [95% CI] ^a				Booster phase n (%) [95% CI] ^a		
	Lot A n = 119	Lot B n = 120	Lot C n = 121	Pooled n = 360	Booster plus Rouvax (1 mo later) n = 110	Booster plus Rouvax (concomitantly) n = 105	Pooled n = 215
Local							
Induration	21 (17.6) [11.3,25.7]	29 (24.2) [16.8,32.8]	31 (25.6) [18.1,34.4]	81 (22.5) [18.3,27.2]	24 (21.8) [14.5,30.7]	25 (23.8) [16.0,33.1]	49 (22.8) [17.4,29.0]
Systemic							
≥ 1 event reported	96 (80.7) [72.4,87.3]	96 (80.0) [71.7,86.7]	83 (68.6) [59.5,76.7]	275 (76.4) [71.7,80.7]	66 (62.9) [52.9,72.1]	52 (47.3) [37.7,57.0]	118 (54.9) [48.0,61.7]
Fever ^b	44 (37.0) [28.3,46.3]	41 (34.2) [28.8,43.4]	38 (31.4) [23.3,40.5]	123 (34.2) [29.3,39.3]	20 (19.0) [12.0,27.9]	12 (10.9) [5.8,18.3]	32 (14.9) [10.4,20.4]
Rash	28 (23.5) [16.2,32.2]	31 (25.8) [18.3,34.6]	29 (24.0) [16.7,32.6]	88 (24.4) [20.1,29.2]	4 (3.8) [1.0,9.5]	5 (4.5) [1.5,10.3]	9 (4.2) [1.8,6.3]
Change in eating habits	28 (23.5) [16.2,32.2]	32 (26.7) [19.0,35.5]	34 (28.1) [20.3,37.0]	94 (26.1) [21.6,31.0]	24 (22.9) [15.2,32.1]	20 (18.2) [11.5,26.7]	44 (20.5) [15.3,26.5]
Sleepiness	47 (39.5) [30.7,48.9]	45 (37.5) [28.8,46.8]	44 (36.4) [27.8,45.6]	136 (37.8) [32.7,43.0]	26 (24.8) [16.9,34.1]	24 (21.8) [14.5,30.7]	50 (23.3) [17.8,29.5]
Unusual crying	55 (46.2) [37.0,55.6]	59 (49.2) [39.9,58.4]	51 (42.1) [33.2,51.5]	165 (45.8) [40.6,51.1]	33 (31.4) [22.7,41.2]	29 (26.4) [18.4,35.6]	62 (28.8) [22.9,35.4]
Persistent crying	13 (10.9) [5.9,18.0]	11 (9.2) [4.7,15.8]	10 (8.3) [4.0,14.7]	34 (9.4) [6.6,12.9]	2 (1.9) [0.2,6.7]	2 (1.8) [0.2,6.4]	4 (2.1) [0.5,4.7]
Irritability	42 (35.3) [26.8,44.6]	39 (32.5) [24.2,41.7]	34 (28.1) [20.3,37.0]	115 (31.9) [27.2,37.0]	26 (24.8) [2.1,12.0]	20 (18.2) [11.5,26.7]	46 (21.4) [16.1,27.5]
Vomiting	28 (23.5) [16.2,32.2]	30 (25.0) [17.5,33.7]	26 (21.5) [14.5,29.9]	84 (23.3) [19.1,28.1]	6 (5.7) [2.1,12.0]	2 (1.8) [0.2,6.4]	8 (3.7) [1.6,7.2]
Diarrhea	20 (16.8) [10.6,24.8]	27 (22.5) [15.4,31.0]	23 (19.0) [12.4,27.1]	70 (19.4) [15.5,23.9]	6 (5.7) [2.1,12.0]	6 (5.5) [2.0,11.5]	12 (5.6) [2.9,9.5]

^aValues (%) based on number of infants experiencing an event/total number of infants receiving at least one injection (N). ^b Fever defined as body temperature ≥ 38.0°C. n = total number of infants receiving at least one injection (i.e., safety population). All local adverse events were considered vaccination-related; values for systemic adverse events represent all solicited adverse events reported, regardless of whether or not they were considered vaccination-related.

vaccination. Overall, the Quinvaxem® booster dose with either concomitant or deferred measles vaccination was well tolerated. There were no SAEs related to study vaccine during either the core study or booster phase.

Materials and Methods

Study design and population. This was a randomized, multi-center phase III study conducted at four centers in Pretoria, South Africa (Synexus Clinical Research SA Ltd, Meyerspark, (national coordinating investigator); Eersterust Clinic; East Lynne Clinic; FR Robeiro Clinic; and Mamelodi West Clinic). The study was conducted in two parts: a double-blind core study, and an open-label booster phase. Before study start, a protocol amendment occurred providing for an additional booster dose of the vaccine to be administered to infants at 18 mo of age (booster phase). The study protocols, informed consent documents, and all recruiting

material were approved by Pharma-Ethics Independent Research Committee, in South Africa and the South African Regulatory Authority (MCC). The study was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and local laws. Parents or legal guardians gave informed consent before infants were enrolled into the core study and booster phase (using a separate consent form).

In the core study, primary vaccination was performed to demonstrate clinical equivalence of three production lots of Quinvaxem®. In the booster phase, the antibody response to the booster dose of Quinvaxem® was evaluated in infants who received either a concomitant or deferred dose (one month later) of measles vaccine.

Patient disposition. Healthy infants aged 42–64 d old, born at 37 weeks of gestation or later, with a minimum birth weight of 2500 g, available for all scheduled visits and eligible for the local EPI in South Africa were invited to participate in the study.

Infants were excluded if they were participating in another clinical study, were premature or had: low birth weight < 2,500 g, previously received one dose of Hib, HBV and/or DTP vaccines, a known or suspected disease history of any of the target infections or HIV, contact or exposure within 30 d prior to start of study with a person with Hib, diphtheria, pertussis or polio disease, fever $\geq 38^{\circ}\text{C}$ within 30 d prior to start of study, suffered acute or chronic infections requiring systemic antibiotic or antiviral therapy within 7 d prior to study start, known immune system impairment, received a parenteral immunoglobulin preparation and/or blood products since birth, known chronic disease or significant acute or chronic infection, a history of anaphylaxis or vaccine related reaction or allergy to any vaccine components, any other condition hindering evaluation during study, or infants whose families were planning to leave study site area.

Core study. Allocation of the three lots of Quinvaxem® was double blinded. A computer generated randomization list was prepared and participants were sequentially randomized and allocated in a 1:1:1 ratio to receive one of three lots of Quinvaxem®. All vaccines were administered intramuscularly in the anterolateral region of the thigh at 6, 10 and 14 weeks of age. Blood samples (approximately 3 mL blood taken by venepuncture) were obtained from all infants immediately prior to the first and one month after the third vaccination. Concomitant administration of oral polio vaccine was permitted with each dose of investigational vaccine in accordance with the local EPI schedule. Quinvaxem® lots, supplied by Crucell, Berna Biotech Korea Corporation (formerly GreenCross Vaccine Corporation), each contained ≥ 30 IU diphtheria toxoid, ≥ 60 IU tetanus toxoid, ≥ 4 IU (lower limit of 95% CI ≥ 2 IU) inactivated *B. pertussis*, 10 μg HBsAg, and 10 μg Hib oligosaccharide conjugated to CRM197 per 0.5 mL dose. Vaccinations were given at least 28 d (including the day of vaccination), but not more than 35 d after the preceding vaccination using a 6–10–14 week schedule.

Booster phase. For the open-label booster phase, the parents or legal guardians of participants of the core study were asked if their child could participate. After informed consent, infants aged 18 ± 3 mo were randomly reallocated in a 1:1 ratio to receive a Quinvaxem® booster dose. The booster dose was either given concomitantly with or followed by a monovalent measles vaccination one month later. The booster dose was administered intramuscularly in the right deltoid muscle, whereas the measles vaccination was administered subcutaneously in the left deltoid muscle. Blood samples (approximately 3 mL blood taken by venepuncture) were obtained from all infants immediately prior to and one month after booster vaccination. The measles vaccine (Rouvax, Sanofi Pasteur MSD) contained $\geq 1,000$ TCID₅₀ live attenuated measles virus per 0.5 mL dose.

Serology. Antibodies against HBsAg were determined using a commercial kit (Enzygnost® Anti-HBs II, Dade Behring), with seroprotection defined as a titer ≥ 10 mIU/mL.²³ Anti-diphtheria and anti-tetanus antibodies were determined using an indirect ELISA, with seroprotection defined as a titer ≥ 0.1 IU/mL.^{24,25} A whole-cell ELISA was used to detect IgG antibodies to *B. pertussis*.²⁶ No correlate of protection exists for pertussis; the clinically relevant response was chosen to be

seroconversion, defined by percentages of infants with either a titer of ≥ 20 EIU/mL or a 4-fold increase from pre- to post-vaccination levels. The Hib ELISA specifically detected antibodies against type b capsular polysaccharide (PRP) of *H. influenzae* and was set up as a competitive ELISA.²⁷ Hib seroprotection rates were determined for both levels indicative of short-term (≥ 0.15 $\mu\text{g/mL}$) and long-term (≥ 1.0 $\mu\text{g/mL}$) seroprotection. Anti-diphtheria, anti-tetanus, anti-PRP and anti-HBs assays were performed and validated at the Novartis Clinical Serology Laboratory, Marburg, Germany. The *B. pertussis* antibody ELISA was performed and validated at the University of Turku Department of Medical Microbiology, Finland.

Safety assessment. For both the core study and booster phase, study personnel monitored infants for 30 min immediately following each vaccination. Parents or legal guardians recorded solicited local and systemic AEs in a subject diary for five days following each vaccination. The local AE that was solicited was induration. Systemic AEs solicited were fever ($\geq 38^{\circ}\text{C}$, axillary temperature), vomiting, diarrhea, irritability, change in eating habits, sleepiness, unusual crying, persistent crying (> 3 h) and rash. SAEs and unsolicited AEs (collected by neutral questioning on each visit) were recorded throughout the study period. All SAEs were reported on a case report form.

Statistical methods. *Core study.* The sample size was calculated using the normal approximation for large samples method. Assuming a reference seroprotection of 95% for anti-HBs and an equivalence limit of $\pm 10\%$, then a sample size of 100 evaluable subjects per group would provide a power of 81% to demonstrate equivalence between the three lots using a two-sided 90% CI for all pairwise comparisons between Lots A, B and C. The sample size was increased by a suitable percentage to give 360 subjects to account for potential dropouts.

The primary study objective was to demonstrate immunological equivalence of three consecutive production lots of Quinvaxem® given to infants at 6, 10 and 14 weeks of age with regard to seroprotection rates for hepatitis B surface antigen antibodies (anti-HBs) one month after the third vaccination. Quinvaxem was developed by co-formulating hepatitis B vaccine with a licensed vaccine Quattvaxem® (liquid DTwP-Hib vaccine). As lot-to-lot consistency has been previously established for Quattvaxem®, the antigenic responses to HBV were viewed to be the highest priority of the five antigens. As a result, this was selected as the primary antigen for analysis of lot-to-lot consistency.^{18,28} For this anti-HBs antibody, the two-sided 90% CI of the difference in seroprotection rates (percentage of infants with titer ≥ 10 mIU/mL) between the two lots at endpoint was calculated using the normal approximation for large samples method. The calculated 90% CI was compared with the pre-specified equivalence margin $\pm 10\%$. If all 90% CIs for all pairwise differences between the three production lots were included in this pre-specified equivalence margin, then equivalence was demonstrated. Multiple comparisons made the analysis more conservative than an analysis based on a single comparison.

Exact two-sided 95% CIs (Pearson-Clopper) were provided for the seroprotection rates for each lot and by timepoint, and for pooled data across lots. Calculation of the geometric means of

antibody concentrations (GMCs) were performed by taking the anti-log₁₀ of the mean of the log₁₀ titer transformation. The primary immunogenicity variable (also the primary endpoint) was the percentage of children with anti-HBs titer ≥ 10 IU/L at one month after the third vaccination.

The secondary objectives were to evaluate the anti-PRP, anti-*Bordetella pertussis*, anti-diphtheria and anti-tetanus antibody response at one month after the third vaccination in infants who received one of the three production lots at 6, 10 and 14 weeks of age; and to evaluate the safety of the three production lots. The secondary immunogenicity variables included the percentage of children one month after the third vaccination with: an anti-PRP titer ≥ 0.15 $\mu\text{g/mL}$ and ≥ 1.0 $\mu\text{g/mL}$, antibody levels against diphtheria toxoid ≥ 0.1 IU/mL, antibody levels against tetanus toxoid ≥ 0.1 IU/mL, seroconversion for anti-*B. pertussis* antibodies (≥ 20 EIU or 4-fold increase), and the evaluation of GMT for anti-HBs, anti-diphtheria, anti-tetanus, anti-*B. pertussis* and anti-PRP antibodies at one month after the third vaccination.

Booster phase. The objectives of the booster phase were: to evaluate the anti-HBs, anti-PRP, anti-*B. pertussis*, anti-diphtheria and anti-tetanus antibody responses one month after the booster vaccination in infants receiving a Quinvaxem® booster one month prior to or concomitantly with a measles vaccine; and to evaluate the tolerability and safety of a booster dose of Quinvaxem® administered alone or concomitantly with measles vaccine. Exact two-sided 95% CIs (Pearson-Clopper) were provided for the seroprotection and seroconversion rates for each antigen by study group and timepoint, and calculations for GMCs were performed as described above for the core study.

Descriptive statistics were generated for the safety data for the core study and booster phases individually.

Conclusions

Quinvaxem® was immunogenic and well tolerated when administered according to a 6–10–14 week vaccination schedule. The three production lots met the protocol primary objective of demonstrating equivalent immunogenicity and the lots demonstrated similar reactogenicity profiles. The booster dose of Quinvaxem® was also immunogenic and safe, regardless of whether a monovalent measles vaccine was administered concomitantly or one month later. Furthermore, reactogenicity of the primary and booster doses appeared to be similar. This study further confirms the excellent efficacy and safety profile of Quinvaxem®.

Conflicts of Interest

K.H. is an employee of Crucell Switzerland AG; P Bedford is a consultant to Crucell Switzerland AG; S Aspinall was the coordinating investigator for this study, but otherwise has no conflicts to declare; D.T. assisted with the operational management and has no conflicts to declare.

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