# Statistical Analysis Plan

# A Phase 3, Randomized, Double-Blind Study to Evaluate the Safety, Tolerability, Lot-to-Lot Consistency, Immunogenicity, and Non-Interference with Concomitant Vaccinations of Serum Institute of India's 10-Valent Pneumococcal Conjugate Vaccine (PNEUMOSIL®) in Healthy Infants in The Gambia

**Protocol: VAC-056**

## STATISTICAL ANALYSIS PLAN

## APPROVAL PAGE

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## ABBREVIATIONS AND DEFINITIONS:

| | |
|---|---|
| AE | Adverse Event. In this analysis plan, "AE" refers to unsolicited events (see 'Reactogenicity Event' for solicited events) |
| CI | Confidence Interval |
| CRF | Case Report Form |
| CSR | Clinical Study Report |
| DSMB | Data Safety Monitoring Board |
| ELISA | Enzyme-linked immunosorbent assay |
| EPI | Expanded Program on Immunization |
| FIP | Full Immunogenicity Population |
| GMC | Geometric Mean Concentration (where present, subscripts designate treatment) |
| GMT | geometric mean titer (where present, subscripts designate treatment) |
| HBsAg | Hepatitis B surface antigen |
| IgA | Immunoglobulin A |
| IgG | Immunoglobulin G |
| IPP | Immunogenicity Persistence Population |
| LLOQ | Lower limit of quantification |
| NI | Non-Inferiority |
| OPA | Opsonophagocytic Assay |
| PCV | Pneumococcal Conjugate Vaccine |
| PP_IMM | Per Protocol Immunogenicity Population |
| PR | Proportion of responders (where present, subscripts designate treatment group) |
| PRC | Primary Reactogenicity Cohort |
| PRP | *Haemophilus influenzae* type b |
| PVS | PATH Vaccine Solutions |
| RCD | Reverse cumulative distribution curve |
| RE | Reactogenicity Event. In this analysis plan, "RE" refers to solicited adverse events |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SD | Standard Deviation |
| SOC | System Organ Class |
| SOP | Standard Operating Procedure |
| TEAE | Treatment-Emergent Adverse Event |
| TLFs | Tables, Listings, and Figures |
| TRS | World Health Organization Technical Report Series |
| V | Visit number (e.g. V1, V2) |
| WHO | World Health Organization |

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# 1. INTRODUCTION

Each year the bacterium *Streptococcus pneumoniae* (pneumococcus) kills hundreds of thousands of children before their fifth birthday, mostly in low-resource areas of the world. The most significant barriers to accelerated global access to pneumococcal conjugate vaccines (PCVs) are their cost and complex manufacturing process, which has underscored the importance of developing a more affordable PCV tailored to the specific serotypes causing pneumococcal disease in the developing world. SIILPCV10, the Serum Institute of India's 10-valent PCV, incorporates prevalent invasive pneumococcal disease-causing serotypes in Africa, Asia and Latin America with optimized manufacturing practices, thus offering comparable coverage to currently licensed PCVs at much lower cost.

PNEUMOSIL was shown to be well tolerated in three previously conducted clinical trials: a Phase 1 study in healthy Indian adults (PCV10-001); a Phase 1/2 study in PCV-naïve adults, PCV (Prevenar 13)-primed toddlers, and PCV-naïve infants in the Gambia (VAC-017); and a Phase 2 study in PCV-naïve Indian toddlers (PCV10-002). No safety concerns were identified in these trials. PNEUMOSIL was also shown in the VAC-017 study to be immunogenic for all 10 serotypes contained in the vaccine. The ultimate goal of PNEUMOSIL clinical development is to achieve licensure through a World Health Organization (WHO) recognized national regulatory authority, followed by prequalification by WHO to support product acquisition by Gavi and United Nations Children's Fund for its distribution to low- and middle-resource countries.

This statistical analysis plan (SAP) describes the statistical analysis to be performed for the study described in Protocol VAC-056, V5.0 (dated 01JUN2018) and titled:

*A Phase 3, Randomized, Double-Blind Study to Evaluate the Safety, Tolerability, Lot-to-Lot Consistency, Immunogenicity, and Non-Interference with Concomitant Vaccinations of Serum Institute of India's 10-Valent Pneumococcal Conjugate Vaccine (PNEUMOSIL®) in Healthy Infants in The Gambia*

The analyses identified in this SAP will support the completion of the final Clinical Study Report (CSR) and/or future manuscripts. The statistical plan for interim analysis for the Data and Safety Monitoring Board (DSMB) is described in a separate document.

The reader is encouraged to also read the clinical protocol and annotated case report forms (CRFs) for details on the planned conduct of this study. Operational aspects related to collection and timing of planned clinical assessments are not repeated in this SAP unless relevant to the planned analyses.

# 2. STUDY OBJECTIVES AND ENDPOINTS

## 2.1. Study Objectives

The purpose of this Phase 3 non-inferiority trial of PNEUMOSIL in healthy infants in The Gambia (VAC-056) is to provide the clinical safety and immunogenicity data necessary to demonstrate the critical attributes of a licensed and prequalified PCV.

### 2.1.1. Primary Objectives

**Immunogenicity:**

1. To demonstrate that the immune responses to the 10 pneumococcal serotypes in PNEUMOSIL (1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, 23F) induced by 3 different lots of PNEUMOSIL are equivalent when measured 4 weeks after a 3-dose primary series
2. To demonstrate non-inferior immune responses for at least 7 of the 10 serotypes in PNEUMOSIL in comparison to matched serotypes (for 1, 5, 6B, 7F, 9V, 14, 19F, 23F) or the lowest responder (for 6A, 19A) in Synflorix based on (a) % immunoglobulin G (IgG) response ≥ 0.35 μg/mL or (b) IgG geometric mean concentrations (GMCs) measured 4 weeks after a 3-dose primary series
3. To demonstrate that the immune responses induced by routine pediatric vaccines (pentavalent, polio and rotavirus) when co-administered with a 3-dose primary series of PNEUMOSIL are non-inferior to those induced by these vaccines when co-administered with Synflorix (subset of subjects)

**Safety, Tolerability:**

1. To demonstrate an acceptable safety and tolerability profile for PNEUMOSIL administered as a 3-dose primary series and booster dose, and when co-administered with routine pediatric vaccines through 4 weeks after a booster dose (subset of subjects for tolerability)

### 2.1.2. Secondary Objectives:

**Immunogenicity:**

1. To demonstrate that the immune responses to serotypes 6A and 19A in PNEUMOSIL are superior to the cross-reactive responses to these serotypes induced by Synflorix based on (a) % IgG response ≥ 0.35 μg/mL or (b) IgG GMCs measured 4 weeks after a 3-dose primary series
2. To evaluate the functional serotype-specific antibody responses induced by PNEUMOSIL in comparison to Synflorix, as measured by opsonophagocytic assay (OPA) at 4 weeks post 3-dose primary series (subset of subjects)
3. To evaluate the booster responses (antibody concentrations and functional responses) to PNEUMOSIL in comparison to Synflorix, from 4 weeks after a 3-dose primary series to 4 weeks after a booster dose (subsets of subjects)
4. To demonstrate that the immune responses induced by measles-rubella and yellow fever vaccines when co-administered with a booster dose of PNEUMOSIL are non-inferior to those induced by these vaccines when co-administered with a booster dose of Synflorix (subset of subjects)

### 2.1.3. Supplemental Objectives[1]:

**Immunogenicity:**

1. To evaluate the persistence of immune responses (antibody concentrations and functional responses) induced by PNEUMOSIL in comparison to Synflorix, 1 year after administration of a booster dose (subset of subjects)

**Safety:**

1. To assess the safety of a 3-dose primary series and booster dose of PNEUMOSIL co-administered with routine pediatric vaccines in regards to serious adverse events (SAEs) occurring 4 weeks after the booster dose through 12 months after the booster dose (subset of subjects)

## 2.2. Study Endpoints

### 2.2.1. Primary Endpoints:

**Immunogenicity:**

**For Primary Objective 1 (lot consistency):**

- Serotype-specific IgG GMC measured 4 weeks post dose 3

**For Primary Objective 2 (non-inferiority):**

- Percentage of subjects with serotype-specific IgG concentrations ≥ 0.35 µg/mL measured 4 weeks post dose 3
- Serotype-specific IgG GMC measured 4 weeks post dose 3

**For Primary Objective 3 (non-interference):**

- Percentage of subjects with anti-diphtheria toxoid IgG concentration ≥ 0.1 IU/mL measured 4 weeks post dose 3
- Percentage of subjects with anti-tetanus toxoid IgG concentration ≥ 0.1 IU/mL measured 4 weeks post dose 3
- Percentage of subjects with anti-Hepatitis B surface antigen (HBsAg) IgG concentration ≥ 10 mIU/mL measured 4 weeks post dose 3
- Percentage of subjects with anti-*Haemophilus influenzae* type b (PRP) IgG concentration ≥ 0.15 µg/mL measured 4 weeks post dose 3

[1] Supplemental objectives will be reported on in an addendum to the CSR

- Anti-pertussis toxoid and fimbriae IgG GMCs measured 4 weeks post dose 3
- Percentage of subjects with anti-poliovirus types 1, 2 and 3 neutralizing antibody titers ≥ 1:8 measured 4 weeks post dose 3
- Percentage of subjects with anti-rotavirus immunoglobulin A (IgA) concentration ≥ 20 U/mL measured 4 weeks post dose 3

**Safety, Tolerability:**

- Number and severity of solicited local and systemic adverse events (reactogenicity events [REs]) through Day 6 post each vaccination
- Number, severity and relatedness of all adverse events (AEs) and SAEs during the entire study period through 4 weeks post last dose for the cohort

### 2.2.2. Secondary Endpoints:

**Immunogenicity:**

**For Secondary Objective 1 (superiority):**

- Percentage of subjects with serotype-specific IgG concentrations ≥ 0.35 µg/mL measured 4 weeks post dose 3
- Serotype-specific IgG GMC measured 4 weeks post dose 3

**Secondary Objective 2 (functional response):**

- Percentage of subjects with OPA titer ≥ 1:8 measured 4 weeks post dose 3
- OPA geometric mean titer (GMT) measured 4 weeks post dose 3

**Secondary Objective 3 (boostability):**

- Ratio of IgG GMCs measured 4 weeks post dose 4 to IgG GMCs measured 4 weeks post dose 3
- Ratio of OPA GMTs measured 4 weeks post dose 4 to OPA GMTs measured 4 weeks post dose 3

**Secondary Objective 4 (non-interference):**

- Percentage of subjects with anti-measles IgG concentration ≥ 150 mIU/mL measured 4 weeks post dose 4
- Percentage of subjects with anti-yellow fever neutralizing antibody titers ≥ 1:8 measured 4 weeks post dose 4
- Percentage of subjects with anti-rubella IgG concentration ≥ 4 IU/mL measured 4 weeks post dose 4

### 2.2.3. Supplemental Endpoints:

**Immunogenicity:**

**For Supplemental Objective 1 (immune persistence):**

- Percentage of subjects with serotype-specific IgG concentrations ≥ 0.35 μg/mL measured 1 year post dose 4
- Serotype-specific IgG GMC measured 1 year post dose 4
- Percentage of subjects with OPA titer ≥ 1:8 measured 1 year post dose 4
- OPA GMT measured 1 year post dose 4

**For Supplemental Objective 2 (Safety):**

- Number, severity and relatedness of all SAEs 4 weeks after the booster dose through 12 months after the booster dose (subset of subjects)

# 3. STUDY DESIGN

This is a prospective, single-center, randomized, active-controlled, double-blind, Phase 3 study in healthy Gambian PCV-naïve infants (6 to 8 weeks). The study will be conducted in 3 phases: an initial priming phase, in which all (n=2,250) eligible subjects will participate; second, a booster phase in which only the first 675 randomized subjects will participate; and third, a post-booster immune persistence phase, in which booster subjects whose parent consented for the additional data collection will participate. The study schema is presented in Table 1.

## 3.1. Visit Schedule and Visit Windows

During the priming phase, subjects will be randomized in a 2:2:2:3 ratio based on a pre-established randomization scheme, to receive the first dose of either PNEUMOSIL (3 groups receiving vaccine from different lots) or Synflorix (1 group) at 6-8 weeks of age at Visit 1 (V1). Subsequent primary vaccination visits will take place at 4 (+2) weeks after the previous vaccination at Visits 2 and 3 (V2 and V3), respectively. A follow-up visit (V4) will take place at 4(+2) weeks after the third vaccination visit, during which blood will be collected for immunological assessments.

**Table 1: Study Schema**

| Groups | Priming Phase | | | | | Booster Phase | | | Immune Persistence Phase |
|---|---|---|---|---|---|---|---|---|---|
| | N | Visits* | | | | N | Visits* | | Visit* |
| | | V1 | V2 | V3 | V4 | | V5 | V6 | V7 |
| | | 6-8 w | V1+4(+2)w | V2+4(+2)w | V3+4(+2)w | | 9-10 m | V5 +4(+2)w | V5+12(+1)m |
| PNEUMOSIL Lot 1 | 500 | X | X | X | B | 150[#] | X | B | B |
| PNEUMOSIL Lot 2 | 500 | X | X | X | B | 150[#] | X | B | B |
| PNEUMOSIL Lot 3 | 500 | X | X | X | B | 150[#] | X | B | B |
| Synflorix | 750 | X | X | X | B | 225[#] | X | B | B |

w = weeks; m = months; X = vaccination (+ EPI vaccines); B = blood sample for immunogenicity testing
*Age ranges indicated for V1/V5. Other vaccination/follow up visits will be at 4 weeks post prior visit + 2 week window, except for V7, which will be at 12 months post V5 + 1 month window.
[#] The total number of subjects assessed for immune persistence at V7 will depend on number of subjects whose parent provides additional informed consent.

The first 675 randomized subjects will continue on study and be asked to return to clinic at 9 (+1) months of age (V5) for a booster vaccination of study vaccine that matches the treatment received at V1. Standard Expanded Program on Immunization (EPI) vaccinations based on the Gambian EPI schedule (measles-rubella, yellow fever vaccine, oral polio vaccine) will be co-administered with the booster dose of study vaccine. A follow-up visit 4 (+2) weeks later (V6) will serve as the end-of-study visit for boosted subjects whose parent does not consent to the immune persistence phase. Blood for immunological assessments will be collected at V6. Subjects in the booster cohort whose parent consents for the immune persistence phase will return approximately one year after the booster vaccination (V7) for a blood draw and then will exit the study.

Immediate solicited reactogenicity and vital signs will be assessed at 30 (+/- 10) minutes following vaccination in all subjects. As part of the randomization process, half of the subjects assigned to each treatment group (approximately 1,125 subjects, total) are also randomly selected to be included in the Primary Reactogenicity Cohort (PRC). These subjects will be monitored daily at home by field workers for assessment of local and systemic reactogenicity during the 6 days after each primary series vaccination 1, 2, and 3. In addition, all 675 infants who receive the booster vaccination (the Booster Cohort) will be monitored daily at home by field workers during the 6 days after the booster vaccination. Serum samples will be collected 4 weeks after completion of the 3-dose primary series (V4), and 4 weeks and 12 months after the booster dose (V6 and V7; respectively). Evaluations to be performed at each study visit are provided in Table 2.

**Table 2. Study Visits**

| Step No. | Evaluation | V1* | V2 | V3 | V4 | V5[#] | V6[#] | V7[#] |
|---|---|---|---|---|---|---|---|---|
| | | 6-8 weeks | V1+4 (+2) | V2+4 (+2) | V3+4 (+2) | 9-10 mos | V5+4 (+2) | V5+12 (+1) |
| 1 | Signing of ICF and confirmation of ongoing informed consent (+) | ✓ | + | + | + | + | +✓[$] | + |
| 2 | Assign screening ID and confirm (+) | ✓ | + | + | + | + | + | + |
| 3 | Demographics | ✓ | | | | | | |
| 4 | Record contact information – address and telephone number(s) – and confirm (+) | ✓ | + | + | + | + | + | + |
| 5 | Full medical history (including concomitant medications) and vaccination history. | ✓ | ~ | ~ | ~ | ~ | ~ | |
| 6 | Vital signs and PE (targeted after screening)* | ✓^✓ | ✓^✓ | ✓^✓ | ✓ | ✓^✓ | ✓ | |
| 7 | Blood sample for immunogenicity testing | | | | ✓ | | ✓ | ✓ |
| 8 | Rapid malaria diagnostic test* | ✓ | ✓ | ✓ | | ✓ | | |
| 9 | Eligibility check* | ✓ | ✓ | ✓ | | ✓ | | |
| 10 | Assign randomization ID | ✓ | | | | | | |
| 11 | Study vaccination | ✓ | ✓ | ✓ | | ✓ | | |
| 12 | EPI vaccination | ✓ | ✓ | ✓ | | ✓ | | |
| 13 | Record local/systemic solicited reactions | ✓ | ✓ | ✓ | | ✓ | | |
| 14 | Record adverse events (including SAE)* | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ (only SAEs) |
| 15 | Record concomitant medications* | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓[%] |
| 16 | Schedule/confirm next visit | ✓ | ✓ | ✓ | ✓[#] | ✓ | ✓ | |
| 17 | Exit study | | | | ✓[‡] | | ✓[‡] | ✓ |

Age ranges indicated for V1/V5. Other vaccination/follow up visits to be scheduled at 4 weeks post prior visit +2 week window, or at 12 months post booster dose + 1 month window in the case of V7.

* If screening extends beyond 1 clinic visit assessments (*) need to be repeated on the day of randomization/1st vaccination.

[#] Visits 5 and 6 will only be completed by subjects in the booster cohort. Visit 7 will only be completed by subjects in the booster cohort whose parent provides additional consent for this assessment.

(~) Confirmation of medical history

^ Evaluations will be conducted twice – before and after vaccination

[‡] V4 is the end of study visit for subjects who do not participate in the booster phase, and V6 is the end of study visit for subjects in the booster phase whose parent does not additionally consent for assessment at V7.

[$] Informed consent for subjects in the booster cohort to continue in study in order to be assessed at V7 will be obtained at or after V6.

[%]- if relevant to immune persistence objectives

## 3.2. Sample Size and Power Calculations[2]

The study is designed to have at least 90% power to meet all 3 primary objectives. The sample size was chosen in an iterative, trial-and-error fashion to give the desired power. We plan to enroll (i.e., assign a treatment by a randomization process) 2250 subjects: 1500 subjects to receive PNEUMOSIL (500 in each of 3 lots) and 750 to receive Synflorix. The sample size for the primary non-interference study will be lower: 675 total (450 recipients of PNEUMOSIL and 225 recipients of Synflorix). Power calculations were done using PASS 13 (Number Cruncher Statistical Systems, Kaysville, Utah).

### 3.2.1. Primary objective 1: Lot-to-lot comparisons

Assuming a 10% loss of data from withdrawals, loss to follow-up, etc., the numbers of subjects with analyzable data on immune response will be approximately 450 per lot of PNEUMOSIL and 675 for Synflorix. With these numbers, as shown below the power will be ~94% for showing equivalence of lots for all serotypes, under somewhat conservative assumptions about variability of antibody levels and differences between lots. The standard deviations (SDs) of $\log_{10}$ (antibody concentration) assumed for the power calculations were chosen by the following procedure: The observed SDs for PNEUMOSIL serotypes from study VAC-017 were arranged in descending order. For each of the 5 pairs of ranked SDs, beginning with the largest SD, the SD for power calculations was assumed to be the higher of the 2 SDs in the pair. Thus for 5 of the serotypes, the assumed SD was the same as observed in VAC-017, and for the other 5 the assumed SD was higher than the observed SD in VAC-017. We estimated the overall power by assuming the values for different serotypes were independent, so that the power to show equivalence for multiple serotypes is estimated by multiplying the powers for the individual serotypes. For each serotype there are 3 possible comparisons of two lots; for the two serotypes with the highest SDs, we assumed a true ratio of GMCs of 1.4 for 2 of the comparisons of lots, which implies a ratio of 1 for the 3rd comparison. (This is a slightly more conservative assumption – i.e., gives a lower power – than assuming equal spacing on the multiplicative scale of the GMCs of the three lots). For the other serotypes, we assumed a ratio of 1.3 for 2 of the comparisons and 1 for the 3rd comparison.

The assumed values of the SDs by serotype and the resulting power estimates are given in Table 3, below. The power to show equivalence of lots for all serotypes is approximately 94.2%, which is calculated by taking the product of the power estimates and calculating the square of that product.

[2] From protocol

**Table 3. Power of lot-to-lot comparisons for 3 lots to show equivalence of IgG GMCs after the 3-dose primary series, for sample sizes of 450 per lot and equivalence margins of 0.5 and 2 for the GMC ratio**

| Serotype | SD of $\log_{10}$ (concentration), VAC-017 | Assumed SD for power calculations | Assumed true GMC ratio* | Power** |
|---|---|---|---|---|
| 6A | 0.56 | 0.56 | 1.4 | 0.9856 |
| 14 | 0.55 | 0.56 | 1.4 | 0.9856 |
| 6B | 0.53 | 0.53 | 1.3 | 0.9996 |
| 19A | 0.45 | 0.53 | 1.3 | 0.9996 |
| 23F | 0.43 | 0.43 | 1.3 | 1.0000 |
| 19F | 0.38 | 0.43 | 1.3 | 1.0000 |
| 7F | 0.38 | 0.38 | 1.3 | 1.0000 |
| 9V | 0.37 | 0.38 | 1.3 | 1.0000 |
| 1 | 0.36 | 0.36 | 1.3 | 1.0000 |
| 5 | 0.34 | 0.36 | 1.3 | 1.0000 |

*Ratio for 2 of the 3 between-lot comparisons; ratio is 1 for the 3rd comparison.

**Power for the 2 comparisons with the indicated ratio; power for the 3rd is 1.0000.

### 3.2.2. Primary objective 2: Comparison of responses to PNEUMOSIL and Synflorix

Assuming 1350 subjects who received PNEUMOSIL and 675 recipients of Synflorix are included in the analysis, the power will be approximately 99.8% to show non-inferiority (NI) of PNEUMOSIL to Synflorix for at least 7 of 10 serotypes in PNEUMOSIL, under the assumption that the true underlying proportions responding with IgG antibody $\geq 0.35$ µg/mL after the 3-dose primary series are 0.02 lower after PNEUMOSIL vaccination than after receipt of Synflorix for each serotype. This assumption is made to introduce some conservativeness into the calculations. For each serotype, NI will be shown if either a two-sided 97.5% CI for the absolute difference in response proportions (proportion of responders with Synflorix minus proportion with PNEUMOSIL) has upper limit $< 0.10$, or a two-sided 97.5% CI for the GMC ratio (Synflorix GMC divided by PNEUMOSIL GMC) has upper limit $< 2$. For serotypes 6A and 19A, the response rate with Synflorix will be assumed to be the lowest observed rate among the 8 serotypes in common with PNEUMOSIL, and the GMC will be assumed to be the GMC of the serotype with the lowest response rate. Table 4 shows the assumed proportions and resulting power for each serotype. The approximate power for meeting primary objective 2, obtained by multiplying the 7 highest powers in Table 4, is 99.8%.

**Table 4. Power of comparisons between PNEUMOSIL and Synflorix to show non-inferiority of proportion of subjects with IgG antibody concentration ≥ 0.35 µg/mL after the 3-dose primary series, for sample sizes of 1350 for PNEUMOSIL and 675 for Synflorix and NI inferiority margin of 0.10 for the difference between the proportion responding for PNEUMOSIL and the proportion responding for Synflorix**

| Serotype | Assumed true proportion responding after Synflorix* | Assumed true proportion responding with PNEUMOSIL | Power |
|---|---|---|---|
| 6A | 0.89 | 0.87 | 0.9993 |
| 14 | 0.98 | 0.96 | 1.0000 |
| 6B | 0.89 | 0.87 | 0.9993 |
| 19A | 0.89 | 0.87 | 0.9993 |
| 23F | 0.91 | 0.89 | 0.9999 |
| 19F | 0.92 | 0.90 | 1.0000 |
| 7F | 0.97 | 0.95 | 1.0000 |
| 9V | 0.94 | 0.92 | 1.0000 |
| 1 | 0.99 | 0.97 | 1.0000 |
| 5 | 0.999 | 0.979 | 1.0000 |

*Assuming response proportions are as for PNEUMOSIL in VAC-017 for the 8 common serotypes and the lowest of these observed proportions for 6A and 19A.

### 3.2.3. Primary objective 3: Non-interference with responses to EPI vaccines

For each EPI vaccine antibody tested (except antibodies to pertussis antigens), NI will be shown if a two-sided 95% CI for the absolute difference in response proportions (proportion of responders with Synflorix minus proportion with PNEUMOSIL) has an upper limit of < 0.10. For pertussis, NI is defined as observing a two-sided 95% CI for the GMC ratio (GMC with Synflorix co-administration divided by GMC with PNEUMOSIL co-administration) with an upper limit of < 2 for each of two separate antigens (pertussis toxin and fimbriae). Based on prior studies, we assume response proportions of at least 96% for each antibody level tested (other than pertussis antibodies) and a standard deviation of 0.82 for $\log_{10}$ (pertussis antibodies). We assume there is no interference, i.e., the underlying response probabilities are equal for co-administration with PNEUMOSIL and with Synflorix. For 450 recipients of PNEUMOSIL and 225 recipients of Synflorix, each comparison will have >99% power, and the power will also be >93% to show non-interference of PNEUMOSIL with routine vaccinations for all the comparisons simultaneously, i.e. to show NI of PNEUMOSIL to Synflorix for all responses to co-administered pentavalent, polio and rotavirus vaccines that are tested.

The overall power to show lot-to-lot consistency, NI of PNEUMOSIL to Synflorix for responses to antigens in PNEUMOSIL, and non-interference of PNEUMOSIL with responses to pentavalent, polio and rotavirus vaccines, is obtained by multiplying the powers of the study to meet primary objectives 1, 2, and 3 – approximately 93%.

### 3.2.4. Secondary objective 1: Superiority of responses to 6A and 19A

We assume the true GMCs and proportions of responders 4 weeks after the 3-dose primary series are as in VAC-017 for PNEUMOSIL and as in the COMPAS study for Synflorix cross-reactivity; in the COMPAS study the response rate was 64.4% for 6A and 61.1% for 19A, and the GMC was 0.32 (95% CI 0.27-0.37) for 6A and 0.29 (95% CI 0.25-0.33) for 19A (Palmu et al., 2013). For tests at the two-sided 0.025 significance level on data from 1350 recipients of PNEUMOSIL and 675 recipients of Synflorix, the power of the study is virtually 100% to show that the GMC for PNEUMOSIL is significantly higher than the GMC for Synflorix for both serotype 6A and serotype 19A. The power is > 99% to show that the GMC ratio (GMC for PNEUMOSIL divided by GMC for Synflorix) is greater than 2 for both serotypes. The power is virtually 100% to show the response rates for both serotypes are significantly higher for PNEUMOSIL. For showing the response rate after PNEUMOSIL vaccination is higher by at least 0.10 than the response rate after Synflorix vaccination, the power is approximately 69% for serotype 6A and 100% for serotype 19A.

### 3.2.5. Safety

The sample size for evaluation of solicited AEs (local and systemic reactions to vaccine, i.e., REs) will be approximately 1125. For 750 recipients of PNEUMOSIL and 375 recipients of Synflorix, the power to find a significant increase in the rate of severe local or systemic reactions after PNEUMOSIL vaccination compared to Synflorix, using a z-test at the one-sided 2.5% significance level, will be about 84% if the rate of severe reactions or AEs is 5% after Synflorix vaccination and 10% after PNEUMOSIL vaccination. The power will be approximately 99% if the respective rates are 10% and 20%.

In a sample size of 1500 infants vaccinated with PNEUMOSIL, the probability of observing at least one occurrence of an unsolicited AE that occurs with frequency 1% will be virtually 100%. For an event that occurs with frequency 0.1%, the probability will be ~78%. If there are no events of a specific AE in 1500 PNEUMOSIL recipients, the upper limit of a two-sided 95% CI for the probability of the event's occurrence will be 0.25%.

## 3.3. Randomization and blinding

After a subject is confirmed to have met all study eligibility requirements, the subject will be randomized in a 2:2:2:3 ratio based on a pre-established randomization scheme, and receive the first dose of either PNEUMOSIL (3 groups reflecting the 3 vaccine lots) or Synflorix (1 group) at 6-8 weeks of age (V1). Treatment assignment will be stratified by field site.

Half of the subjects assigned to each field-site-by-treatment subgroup (approximately 1,125 subjects total) will be randomly selected as part of the randomization scheme for the Primary

Reactogenicity Cohort. These subjects will be monitored daily at home by field workers for assessment of local and systemic reactogenicity during the 6 days after each primary series vaccination.

The eligible subject will be assigned the next numeric randomization ID for the given field site. Treatment and cohort assignments will be communicated to the unblinded dosing nurse via special sealed, opaque, numerically numbered envelopes identified by a unique randomization ID. Once the envelope is open, the cohort assignment will not be blinded.

The PI, clinic staff, the Sponsor, and the contract research organization will remain blinded to subject treatment assignment until after database closure following completion of V6 by the last subject, unless unblinding is warranted due to subject safety concerns or because the staff person is unblinded by virtue of their role in the study, e.g. the unblinded dosing nurse who prepares study vaccine. Details regarding maintenance of blinding for DSMB safety analyses are addressed in the DSMB Charter and separate DSMB analysis plan. See below for a description of blinding status after scheduled study unblinding.

## 3.4. Blinded data review

Prior to scheduled study unblinding, the data will be reviewed by the PATH Study Director or designee for analysis decisions. Based on analysis needs, the lead statistician will develop specific listings for this review and will document the PATH Study Director's decisions prior to unblinding. These reviews will include (but not necessarily be restricted to) protocol deviations for inclusion/exclusion of subjects and/or data points, particularly with respect to the Per Protocol Immunogenicity (PP_IMM) Populations; and distribution of time points of data collection that are out of window per protocol. Protocol deviations will include those documented on the protocol deviation CRF as well as selected deviations that can be detected directly from the data base. Blinded data review may also include clinical evaluation of safety data for reasonableness or to issue clinical queries to the site if needed. Any decisions affecting analysis will be described in internal documentation and the CSR.

## 3.5. Scheduled Study Unblinding

The requirements for scheduled unblinding for this study are:

1. Statistical Analysis Plan has been finalized and approved.
2. All CRF data through V6 have been collected, double-entered, and all discrepancies resolved.[3]
3. Blinded data review for primary objectives 1 and 2 is completed and corresponding analysis decisions documented.

[3] This step constitutes first data base closure as described in Protocol v5.0.

4. Key analysis data sets based on CRF data have been independently validated. At a minimum, variables defining inclusion/exclusion into key analysis populations must be independently validated.[4]
5. Medical, concomitant medication and other text coding has been completed.

Unblinded statisticians will not have access to PCV immunogenicity data needed to assess primary objectives 1 and 2 until [redacted] data management staff (who will be blind to treatment assignment) complete record-level quality control checks against the clinical data base and resolve any discrepancies. Immunogenicity data for certain EPI vaccines (including those required to assess components of primary objective 3) may be received from external laboratories after unblinding. The timing of unblinding with respect to availability of data from each source will be documented in the CSR.

Before receiving immunogenicity data, statisticians will use blinded V4 ELISA IgG data and dummy treatment assignments to validate analysis programs assessing (1) lot-to-lot consistency (key primary objective) and (2) non-inferiority of immune responses comparing PNEUMOSIL to Synflorix. [redacted] data management staff will create blinded immunogenicity data sets for statisticians by dropping subject ID, visit date, and any other variables that might reveal subject ID. The statisticians will create dummy IDs and arbitrarily assign them to treatment group in a 2:2:2:3 (Lot1:Lot2:Lot3:Synflorix) allocation ratio for program validation.

Analysis of supplementary objectives for the one-year post-booster immune persistence phase will take place following closure of V7 data; i.e., after all CRF data are double-entered, discrepancies are closed, and V7 immunogenicity record-level checks completed.

## 3.6. General Issues

General descriptive statistics for numeric variables include the number of observed values, the mean, SD, median, minimum, and maximum values. For categorical variables, the number and percentage of subjects with a specific level of the variable will be presented. Percentages will be calculated based on subjects with reported values (i.e., subjects with missing data will not contribute to denominators of percentages). Descriptive statistics and tabular summaries will be presented by treatment group and, as relevant, by vaccination number, visit or other subdivision.

General reporting conventions will include the following:

- The baseline values for any measurement is the last value obtained prior to receiving the first vaccination at V1.
- Unless decided otherwise during blinded data review, nominal time points will be used for analysis; actual assessment times will not be used to reclassify the time point at which a measure was taken.

[4] Data regarding protocol deviations that may affect inclusion into the Immune Persistence Population will not have been obtained at the time of scheduled unblinding.

- Study day will be calculated relative to the date of randomization/1st vaccination as the following: Date of event – Date of randomization/1st vaccination administration (Day 0). This formula will be used when calculating days to a specific event (i.e., concomitant medication and/or AE start date). Event durations will be calculated as date of event resolution minus date of event onset +1.
- Other than log-transformations as described below for the immunogenicity data, no data transformations are planned.
- Unless otherwise noted, all percentages will be presented to one decimal place; standard deviations to one decimal more than that used for the mean; and p-value will be rounded to four decimal places.
- All data listings will be sorted by treatment group and subject screening ID.
- Except for AEs and REs (for which the highest grade may occur at an unscheduled visit), post-randomization data from unscheduled visits will be excluded from the summary tables but included in listings.

## 3.7. Analysis Populations

The following analysis populations will be used in the study:

- **Enrolled Population** includes all screened subjects who provide informed consent, regardless of whether the subject is randomized to receive a study treatment. This population will be used to account fully for subject disposition, starting with the informed consent. The enrolled population will not be analyzed as such but will be available in the clinical database.
- **Safety Analysis Set (Safety Set)** includes all subjects who were randomized, received a study vaccination, and provided at least some post-vaccination safety data. Treatment groups for safety analyses will be based on actual treatment received at V1. This population will serve as the primary analysis population for demographics and study disposition as well as safety, and is the basic population for all analyses except immunogenicity.
- **The Full Immunogenicity Population (FIP)** includes subjects in the enrolled population who were randomized, received a study vaccination, and have post-vaccination immunogenicity measurement(s). Analysis will be according to the treatment received at enrollment, regardless of whether the subject received all study vaccines or received a study vaccine different from that to which they were randomized. The FIP will be analyzed only if more than 10% of the FIP are excluded from the PP_IMM (defined below), and only to serve as supportive results.
- **The Per Protocol Immunogenicity Population (PP_IMM)** includes all subjects in FIP who received all study vaccines and have post-dose immunogenicity measurement(s) with no major protocol deviations (such as not receiving all doses of the same vaccine) that were determined to potentially interfere with immune response to the study vaccine.

Analyses based on this population will be performed according to treatment received. This population will serve as the primary analysis population for the immunogenicity-related primary and secondary objectives.

- **The Immunogenicity Persistence Population (IPP)** is a subset of PP_IMM consisting of subjects in the booster cohort who provide evaluable data at V7, excluding subjects with protocol deviations that could affect this analysis (e.g. use of prohibited medication or treatment).

Primary analysis populations and conditions for supportive analysis are summarized in Table 5.

**Table 5. Summary of Planned Analyses, by Population**

| ANALYSIS | ENROLLED | SAFETY | FIP | PP_IMM | IPP |
|---|---|---|---|---|---|
| **Disposition** | √[5] | √ | | | |
| **Baseline** | | √ | | | |
| **Safety** | | √[6] | | | |
| **Immunogenicity: Primary & Secondary Objectives** | | | √[7] | √ | |
| **Immune Persistence: Supplemental Objectives** | | √[8] | | | √ |

## 3.8. Covariates

No covariates will be utilized in any planned statistical analyses of treatment effects, safety, or tolerability. Exploration of covariates associated with level of immune response may be conducted as post-hoc analysis.

## 3.9. Pooling of Sites and Evaluation of Site Differences

Information from the population flow chart, demographics and final disposition tables will be reported by the field sites to describe potential site differences. No statistical tests for differences in characteristics across field sites or between treatment groups within a field site will be conducted.

[5] The Enrolled Population will contribute only to population flow charts.

[6] Reactogenicity assessments through Day 6 post-vaccination are conducted on subsets within the Safety Set.

[7] Only if more than 10% of FIP are excluded from PP_IMM.

[8] Safety evaluation for the Immune Persistence Population will consist of a separate listing of SAEs with onset after V6 (4-week post booster) and before V7 (1-year post booster).

Except for tests of treatment-group differences in safety outcomes, which will be stratified on field site, analyses will be performed on data pooled across field sites. However, potential site-level effects will be assessed in exploratory analyses of treatment-by-site interactions for selected safety endpoints, specifically: site effects on (a) treatment-group difference in proportions of subjects with TEAE; and (b) treatment-group differences in the distribution of highest grade of reactogenicity observed within Day 6 of any vaccination (pooling doses 1, 2, 3, and 4). Interactions significant at $\alpha = 0.05$ will be described. Site-specific tables summarizing treatment-group differences in key TEAE categories (e.g. SAE, vaccine-related AE) may be produced but formal statistical testing of treatment-by-site interactions within such categories will not be conducted.

## 3.10. Multiple Comparisons

Two-sided 97.5% confidence intervals (CIs) (rather than the standard 95%) will be used when a claim of NI with respect to a particular outcome can be made based either on the upper bound of the difference in seroresponse proportions or the GMC ratio between treatment groups. Likewise, two-sided 0.025-level tests (rather than the standard 0.05 level) will be used when a claim of superiority can be made based on either the difference in seroresponse proportions between groups or the GMC ratio. For secondary and supplemental objectives to evaluate treatment effect on immunogenicity measures (e.g. functional antibody response, boostability) without a pre-specified directional hypothesis, two-sided 95% CIs will be calculated as descriptive measures. Statistical comparisons of safety data will not be adjusted for multiple comparisons, so as not to miss a potentially important safety signal. It is acknowledged that the chance of falsely concluding that one or more differences in safety outcomes exist will be greater than the nominal two-sided 0.05 level used for each individual comparison.

## 3.11. Interim Analyses

No formal interim analyses are planned for this study.

## 3.12. Data Review Meeting

Per protocol, one formal DSMB meeting will be conducted during the trial when approximately one-fourth of the infants in the Primary Reactogenicity Cohort have received their first vaccination. The DSMB will conduct an unblinded review of the available safety data, as well as quality of data generated and protocol deviations to make recommendations for study continuation and design. Details regarding the operation of and planned analyses for the DSMB are described in the DSMB Charter[9] and DSMB SAP.[10]

[9] V1.0 approved 9 May 2017; updated V2.0 approved 9 Nov 2017.

[10] V1.0 approved 11 May 2017.

## 3.13. Handling missing and incomplete data

### 3.13.1. Premature Discontinuation and Missing Data

For any subject who withdraws prematurely from the study, all relevant available data up to the time of discontinuation will be included in analyses unless otherwise excluded during blind review. Per protocol, subjects who are discontinued from the study after vaccination (regardless of reason) will not be replaced, but a subject discontinued after randomization but prior to 1st vaccination will be replaced using a new randomization assignment for the replacing subject.

Missing data are assumed to be missing at random and ignorable. Except where otherwise noted (see section 3.13.2), missing data will not be estimated or imputed. Denominators for percentages will be based only on the number of subjects with non-missing values.

### 3.13.2. Imputed Data

All immunogenicity assays reported as being below the limit of quantification or below a specified threshold (e.g. "<0.15") will be assigned a value of one-half the lower limit of quantification (LLOQ) or threshold value (e.g. 0.075 for the given example), using values of LLOQ or other information provided by the responsible laboratory.

In the event missing event dates or times are needed to compute durations of outcomes, the following rules will be applied:

[redacted]

Otherwise, no imputation for missing values is planned.

## 3.14. Evaluation of Normality Assumption

The assumption of $\log_{10}$ normality of immunogenicity data will be assessed by visual inspection of quantile-to-quantile plots and tests of departures from normality. The lead statistician will use these results to determine whether the normality assumption is substantively violated; that is, if the deviation from normality is to a degree that alternative analysis methods should be employed.

If the $\log_{10}$ normality assumption is not met, an appropriate non-parametric method will be used. If sample sizes are relatively small (e.g. <=50), bootstrap resampling may be considered. If bootstrap resampling is implemented, point estimates will be calculated directly from the data but confidence intervals will use the bias-corrected and accelerated method (Efron and

Tibshairani [1993]; Barker [2005]), which addresses discrepancies (bias) between point estimates obtained from bootstrap samples and the original calculation from the raw data as well as possible variation in the standard error as a function of the value of the estimate. Each bootstrap estimate will be based on 10,000 randomly selected replicates.

## 3.15. Software Package

Version 9.4 or higher of the SAS/STAT® software will be used for all analysis.

# 4. EVALUATION OF DEMOGRAPHIC AND BASELINE CHARACTERISTICS

## 4.1. Subject Enrollment and Disposition

A population flowchart ("population tree") will summarize the numbers of subjects screened, randomized, and in each of the analysis populations and cohorts (Safety Analysis Set, PRC, Booster Cohort, FIP[11], PP_IMM, and IPP), by treatment group. As noted above, the population flowchart will be repeated separately by field site.

Subject disposition will be summarized for the Safety Set pooling across field sites and by field site. Highest visit completed, number of subjects discontinued and reason for early discontinuation will be summarized as well. Subjects who discontinued early, together with the reason for withdrawal/termination, will be listed, as will subjects lost to follow-up, with amount of observation time contributed to the analysis.

## 4.2. Protocol Deviations and Measures of Study Conduct

As described above, protocol deviations will be reviewed during blinded data review and analysis decisions, if any, made as a consequence of these deviations documented prior to unblinding. Protocol deviations will be listed. Information captured on the UNBLIND CRF regarding individual subject unblinding, whether for safety reasons or by accident, will be listed.

As a measure of the quality of study conduct, the number of vaccination visits or home visits that were missed and follow-up visits missed or outside of protocol window will be summarized.

## 4.3. Treatment Compliance

Treatment compliance for the priming phase is defined as having received all three doses of study vaccine whereas compliance for the booster phase is defined as having received the three

[11] If analysis in the FIP is conducted.

doses plus the booster vaccination. Subjects with less than full compliance and reasons why (including early discontinuation or loss to follow-up) will be listed.

## 4.4. Demographics and Baseline Characteristics

Demographic characteristics (including age, sex, race, and ethnicity), and socioeconomic status will be summarized. No statistical comparisons of treatment-group differences in demographics or baseline data will be performed. As noted above (Sec 3.9), demographics data will also be reported by field site.

## 4.5. Medical History and Baseline Assessment

Medical history and baseline anthropometrics, vital signs, etc. will be summarized.

## 4.6. Concomitant Medications

A summary of all concomitant medications taken during the study will be presented in listings. Information of drug name, dose, route, frequency, indication, and start and end dates will be presented.

# 5. EVALUATION OF IMMUNOGENICITY AND NON-INTERFERENCE

**Analysis Populations:** Primary and secondary immunogenicity will be evaluated within the Per Protocol Immunogenicity Population (PP_IMM); the supplemental immunogenicity objective will be evaluated within the Immunogenicity Persistence Population (IPP).

**Data Sources:** Primary immunogenicity objectives will be based on serotype-specific IgG antibody concentrations and IgG antibody concentrations induced by co-administration of EPI vaccines (or neutralizing antibody titers for polio) measured 4 weeks after the primary vaccination series (V4). Secondary objectives will be based on the serotype-specific IgG concentration and OPA titer 4 weeks after the 3-dose primary series (V4) and 4 weeks after the booster dose (V6), and IgG antibody concentrations induced by co-administration of EPI vaccines (or neutralizing antibody titers for yellow fever) 4 weeks after the booster dose. The supplemental immunogenicity objective will be based on the serotype-specific IgG concentration and OPA titer 1 year after the booster dose (V7).

**Endpoint Definitions:**

1. **GMC [or GMT]:** The geometric mean concentration (or titer) = antilog (mean [$\log_{10}$ x]), where x is the assay result
2. **Response to study vaccine:**
   - IgG concentration ≥ 0.35 μg/mL
   - OPA titer ≥ 1:8
3. **Response to EPI vaccines**:

   **a. vaccines co-administered with primary series (measurements obtained at V4)**

   - anti-diphtheria toxoid ≥ 0.1 IU/mL
   - anti-tetanus toxoid ≥ 0.1 IU/mL
   - anti-HBs concentration ≥ 10 mIU/mL
   - anti-PRP concentration ≥ 0.15 μg/mL
   - Anti-pertussis IgG GMCs
   - anti-polio types 1, 2 or 3 titer ≥ 1:8
   - anti-rotavirus concentration ≥ 20 U/mL

   **b. vaccines co-administered with booster (measurements obtained at V6)**

   - anti-measles IgG concentration ≥ 150 mIU/mL
   - anti-yellow fever neutralizing titers ≥ 1:8
   - anti-rubella IgG concentration ≥ 4 IU/mL

## 5.1. General Comments Regarding Analysis of Immunogenicity Objectives:

### 5.1.1. Statistical Methods for Determining Non-Inferiority:

1. Difference in proportion of responders (proportion of responders associated with Synflorix use/co-administration [$PR_S$] minus proportion associated with PNEUMOSIL use/co-administration [$PR_P$]): upper limit of the CI around the difference is < 0.10. Confidence intervals will be calculated using the Miettinen-Nurminin (1985) likelihood score method.
2. GMC ratio (Synflorix GMC [$GMC_S$] divided by PNEUMOSIL GMC [$GMC_P$]): upper limit of the CI is < 2, where the ratio and CI are calculated assuming a normal distribution for $\log_{10}$ (concentration) (Gart and Nam, 1990). The ratio is the antilog (base 10) of the difference in mean $\log_{10}$ (concentration), i.e. $\log_{10}(GMC_S)$ minus $\log_{10}(GMC_P)$; thus, confidence limits for the ratio are calculated as the antilogs (base 10) of the limits of a CI for the difference in mean $\log_{10}$ (concentration).

### 5.1.2. Reporting of Immunogenicity Data

Responders: Number of observations contributing to analysis; treatment-group PR and their 95% CI; treatment-group difference in proportions ($PR_S$ minus $PR_P$) and CI around the difference.

GMC[GMT]: Number of observations contributing to analysis; treatment-group-specific GMC[GMT] and its 95% CI; treatment-group ratios (e.g., $GMC_S$ divided by $GMC_P$) and CI around the GMC[GMT] ratio.

Coverage levels for two-sided confidence intervals around the measure of treatment group effect (ratio or difference in proportions) will be 97.5% when a conclusion of NI or superiority can be made based on either a difference in proportions or a GMC ratio. Otherwise, two-sided 95% confidence intervals will be reported.

### 5.1.3. Measure of Synflorix Serotypes 6A and 19A

Serotypes 6A and 19A are not contained in Synflorix. Per the WHO Technical Report Series (TRS) guidance (No. 927, Annex 3), the evaluation of PNEUMOSIL non-inferiority for serotypes 6A and 19A will use a surrogate measure for seroresponse rates and IgG concentrations induced by Synflorix against serotypes 6A and 19A; see 5.2.2. Measured concentrations and proportions responding from Synflorix cross-reactive response will be used to evaluate PNEUMOSIL superiority for these two serotypes (secondary objective 1) as well as all other secondary and supplemental objectives.

## 5.2. Analysis of primary immunogenicity endpoints

### 5.2.1. Primary Objective 1: Equivalence of PNEUMOSIL lots

**Primary objective 1** is to show equivalence of 3 lots of PNEUMOSIL 4 weeks after the primary vaccination series. For each of the 10 serotypes in PNEUMOSIL, IgG concentration will be summarized separately by lot and overall. These descriptive statistics will be accompanied by reverse cumulative distribution (RCD) curves displaying each product lot as a separate curve.

For each serotype and each pair of lots, two-sided 95% CIs for the ratio of the geometric mean concentration (GMC) of IgG antibody in one lot to the GMC in the other lot will be calculated, assuming a normal distribution for $\log_{10}$ (concentration), unadjusted for multiple analyses. The 3 lots will be considered equivalent if, for each serotype, all 3 CIs for GMC ratios lie within the interval (0.5, 2). Since the equivalence criterion must be met for all 3 comparisons, no α-adjustment is necessary. If equivalence of lots is shown, data for the three lots will be combined for all further immunogenicity analyses.

### 5.2.2. Primary Objective 2: Non-Inferiority of IgG Antibody Response

**Primary objective 2** is to show non-inferiority of the IgG antibody response after PNEUMOSIL vaccination to the response after Synflorix for at least 7 of the 10 serotypes in PNEUMOSIL at V4. For each serotype, NI will be shown if (a) the two-sided 97.5% CI for the difference in

proportions of responders, as defined above, has an upper limit of < 0.10, or (b) the two-sided 97.5% CI for the GMC ratio, as defined above, has an upper limit of < 2. Per the requirements of the WHO TRS, for serotypes 6A and 19A the response rate for Synflorix will be assumed to be the lowest observed rate among the 8 serotypes in common with PNEUMOSIL, and the GMC will be assumed to be the GMC of the serotype with the lowest response rate among the 8 common serotypes.

#### 5.2.3. Primary Objective 3: Non-Inferiority of EPI Vaccine Response Co-Administered with PNEUMOSIL following Primary Vaccination Series

**Primary objective 3** is to show NI of responses, 4 weeks after the primary series, to pentavalent, polio and rotavirus vaccines when co-administered with PNEUMOSIL, to those responses when co-administered with Synflorix. Except for pertussis, for each antigen in the pentavalent, polio and rotavirus vaccines, NI will be shown if a two-sided 95% CI for the difference in response proportions (proportion with Synflorix co-administration minus proportion with PNEUMOSIL co-administration) has an upper limit of < 0.10. For pertussis, NI will be shown if the two-sided 95% CIs for the GMC ratios for both anti-pertussis toxin and fimbriae antigens (GMC with Synflorix co-administration to GMC with PNEUMOSIL co-administration) have an upper limit of < 2. Analysis will be conducted on 450 PNEUMOSIL recipients and 225 Synflorix recipients.[12]

### 5.3. Analysis of Secondary Immunogenicity Endpoints

#### 5.3.1. Secondary Objective 1: Superiority of Measured 6A and 19A Serotypes

**Secondary objective 1** is to demonstrate that the immune responses to serotypes 6A and 19A in PNEUMOSIL recipients 4 weeks after the 3-dose primary series are superior to the cross-reactive responses to these serotypes induced by Synflorix after a 3-dose primary series. For each of the two serotypes, proportions with IgG concentration ≥ 0.35 μg/mL will be compared using a z-test for proportions and GMCs will be compared by a two-sample t-test on the difference between means of $\log_{10}$ (antibody). Both tests will be done at the two-sided 2.5% significance level to adjust for the two superiority tests. 95% CIs around treatment-group-specific responses, and 97.5% CIs for treatment-group differences in response, will also be reported.

#### 5.3.2. Secondary Objective 2: Evaluate Functional Antibody Response

**Secondary objective 2** is to evaluate, in a subset of subjects (250 PNEUMOSIL recipients and 250 Synflorix recipients), the serotype-specific functional antibody responses, measured by OPA, to PNEUMOSIL in comparison with Synflorix for each of the 10 serotypes in

[12] Precise treatment-group sample sizes are cited when samples will be selected for lab assay by an unblinded statistician per pre-specified quota. Only vials identified on the lab CRF as having sufficient volume will be eligible for selection; thus, no loss is anticipated.

PNEUMOSIL, when measured 4 weeks after a 3-dose primary series. The percentage of subjects with OPA titers ≥ 1:8 and OPA GMTs will be reported, as well as treatment-group comparisons of differences in percentages and ratios of GMTs and the corresponding 95% CIs. An exploratory analysis will assess the Spearman correlation between IgG concentration and OPA titer for each serotype.

### 5.3.3. Secondary Objective 3: Evaluate Boostability

**Secondary objective 3** is to evaluate, in a subset of subjects, serotype-specific booster responses (antibody concentrations and functional responses) to PNEUMOSIL in comparison to Synflorix, from 4 weeks after a 3-dose primary series to 4 weeks after a booster dose. The comparisons will be based on the ratio of IgG GMC post-booster (V6) to the IgG GMC post-primary series (V4), and on a similar ratio of OPA GMTs. Analyses will be performed on the Booster Cohort subgroup of PP_IMM, restricted to subjects to contribute relevant data at both time points. The vaccines will be compared using the ratios of these ratios for the two treatment groups, i.e., the PNEUMOSIL ratio divided by the Synflorix ratio, and the corresponding 95% CIs based on log-linear random effects models. Supporting material will include the treatment group ratios of IgG GMCs and OPA GMTs at V6 (with corresponding 95% CIs) among all subjects in in the Booster Cohort subgroup of PP_IMM; RCDs for IgG concentration and OPA titer, by treatment group, at V6; descriptive statistics, e.g. GMCs [GMTs] by treatment group and time point; and GMCs [GMTs] plotted by treatment group and time point.

### 5.3.4. Secondary Objective 4: Non-Interference with EPI Vaccines Co-Administered at 9-10 Months of Age

**Secondary objective 4** is to demonstrate non-inferior immune responses to routine pediatric vaccines (measles, rubella, yellow fever) co-administered with a booster dose at 9-10 months of age (as measured at V6, 4-weeks after booster). Non-inferiority will be shown if a two-sided 95% CI for the treatment-group difference in response proportions (proportion with Synflorix co-administration minus proportion with PNEUMOSIL co-administration) has an upper limit of < 0.10.

### 5.3.5. Supplemental Objective 1: Evaluate Immune Persistence 1 Year Post Booster

**Supplemental objective 1** is to evaluate, in subsets of subjects, the persistence of serotype-specific immune responses (antibody concentrations and functional responses) to PNEUMOSIL in comparison to Synflorix for each of the 10 serotypes in PNEUMOSIL, when measured 1 year after a booster dose. To evaluate the persistence of serotype-specific antibody concentrations, V7 treatment-group-specific IgG GMCs and percentage of IgG responders, with 95% CI, will be reported. PNEUMOSIL will be compared to Synflorix by the ratio of IgG GMCs (and its 95% CI) and the difference in percentage of responders (and the 95% CI around the difference). The analysis population is the IPP. The same approach will be used to evaluate the persistence of serotype-specific functional responses based on treatment-group GMTs and percentage of

responders (OPA titer ≥ 1:8). To explore the rate of decay in immune response, the ratios of IgG GMCs at V7 to the corresponding measures at V6 will be reported by treatment group (among subjects contributing data at both timepoints), together with 95% CIs for the GMC ratio. Analyses will be supported by summary descriptive tables for V4, V6, and V7, by treatment group; RCD curves at V7, by treatment group; and graphs of GMCs/GMTs and 95% confidence intervals over time: 4 weeks post primary series, 4 weeks post booster, and 1 year post booster.

## 5.4. Evaluation of Safety and Tolerability

Note: In this document solicited adverse events are called reactogenicity events; the term "adverse event (AE)" refers to unsolicited events only. This reflects the fact that while both are adverse situations, data collection and analysis for the two types of events are handled quite differently.

### 5.4.1. Safety Analysis: General Issues

Safety and tolerability of study vaccines will be evaluated using the following endpoints:

- Number and severity of solicited local and REs through Day 6 after each vaccination
- Number, severity and relatedness of all AEs and SAEs during the entire study period through 4 weeks post dose 4
- Number, severity and relatedness of all AEs and SAEs during the 2-week period post each vaccination
- Number, severity and relatedness of all SAEs from 4 weeks post dose 4 through 12 months post dose 4

**Analysis Population**: All analysis of safety and tolerability will be performed using the Safety Set, with analysis of REs conducted within subsets of the Safety Set as described below.

Generally, safety evaluations will be descriptive in nature, and observed differences will be evaluated for medical relevance. Any statistical tests of treatment-group differences in safety endpoints, including calculation of 95% confidence intervals around treatment-group differences, are not adjusted for multiple testing and are provided solely as a guide to clinical and scientific judgment.

Clinical assessments (e.g. vital signs) will be summarized separately by baseline and scheduled measurement time point pre-vaccination, post-vaccination and at follow-up visits. Results from unscheduled visits will be included in listings. For ease of review, baseline results may be reported on the same table as post-randomization results.

### 5.4.2. Adverse events

The onset date will be compared to the date of 1$^{st}$ vaccination (Day 0) to determine if it is a treatment emergent adverse event (TEAE). Adverse events are considered treatment emergent (TEAEs) if (a) onset occurs on or after the date of first vaccination, or (b) an event with onset

prior to the first vaccination but increases in severity after administration of the vaccination. Only TEAEs will be included in the analysis. Adverse events in the data base for subjects in the Safety Set that are not TEAEs will be listed. SAEs for subjects not in the Safety Set, if any, will also be listed.

TEAEs will be summarized through 4 weeks post vaccination 4, i.e. through V6. Since only SAEs are collected during the 1-year post booster immune persistence phase, these events will be listed separately (and included in an addendum to the CSR) but not included in TEAE summaries or listings.

A high-level summary of TEAEs will be presented in a table with numbers of events, and number and percent of subjects, with (a) any TEAE, (b) any serious TEAE, (c) any vaccine-related TEAE, (d) any vaccine-related SAE, (e) a TEAE leading to discontinuation of the vaccination series in a subject, or (f) a TEAE leading to death. Treatment-group differences in percent of subjects will be statistically compared separately for each TEAE category (a)-(f) using the Cochran-Mantel-Haenszel test stratifying on field site and using exact methods if the number of events is low (i.e. number of events is < 5 for at least one treatment group). The 95% CI around the treatment-group difference will also be reported. Treatment-group differences in percentages of subjects with at least one TEAE in a category of special clinical interest (identified in blind review) may also be statistically compared.

The process for medical coding will be conducted according to the [redacted] SOP 06006 "Medical Coding of Clinical Study Data using MedDRA". TEAEs will be summarized and grouped by MedDRA System Organ Class (SOC) and AE preferred term using MedDRA™ v 19.1 or higher coding terminology. Results will be displayed in order of decreasing frequency, both across SOC and within each SOC term. In addition, summaries will be provided by severity (mild, moderate, severe, or life threatening/death if any), and by relationship to study vaccine (Related, Unrelated).

A listing of all TEAEs through V6 will be presented by treatment group and will include subject identifier, AE verbatim description, preferred term, SOC, duration, relatedness to product, seriousness, severity, outcome, and action taken with respect to the investigational product. Because of the number of variables to be reported, TEAE listings will be produced in two parts, sorted in the same order and with the same key identifiers to allow easy cross-reference.

TEAE listings will be repeated for (a) those that were serious, severe (Grade 3 or 4), considered related to vaccine, or leading to discontinuation of the vaccination series, and (b) events of special clinical interest (identified in blind review).

The concomitant medication/drug/treatment verbatim text will be coded using a combination of Lexi-comp and the on-line Physicians' Desk Reference. Concomitant medications will be listed.

### 5.4.3. Reactogenicity (Solicited Adverse Events)

**Reactogenicity Assessment Subgroups:** REs are assessed at 30 (+/- 10) minutes following each vaccination for all subjects in the Safety Set. Those in the Primary Reactogenicity Cohort

will be monitored daily at home by field workers for assessment of local and systemic reactogenicity during the 6 days after each vaccination 1, 2, and 3. Vaccination 4, the booster vaccination, is administered only to the Booster Cohort, defined as the first 675 randomized infants. All subjects in the Booster Cohort will be monitored daily at home by field workers during the 6 days after the booster vaccination.

**Populations for Analysis of Reactogenicity Assessments**

| Observation Period | Population |
|---|---|
| 30-minutes post vaccination: Vaccinations 1, 2, 3 | Safety Set |
| Daily through Day 6 post vaccination: Vaccinations 1, 2, 3 | Primary Reactogenicity Cohort (subset of Safety Population) |
| 30-minutes post vaccination: Vaccination 4 (Booster) | Booster Cohort (subset of Safety Population) |
| Daily through Day 6 post vaccination: Vaccination 4 (Booster) | Booster Cohort (subset of Safety Population) |

If more than one measurement was obtained on a given day (e.g. from unscheduled clinic visits for RE follow-up) the maximum level observed that day will be used in analysis. Note that by protocol, an RE with onset on or after Day 7 is an unsolicited AE reported on the AE CRF.

To provide a picture of REs at the subject level, we will determine for each subject and type of reaction the highest level that subject experienced through Day 6 post-vaccination, separately for each vaccination. This will be done for the Primary Reactogenicity Cohort for post-vaccination 1, 2, and 3, and for the Booster Cohort post-vaccination 4.

Treatment groups will be compared on the distribution of highest reactogenicity grades (0,1,2,3,4) (a) post any vaccination and (b) post each vaccination, using the Cochran-Mantel-Haenszel test stratifying on field site. The modified ridit method will be implemented to take advantage of the ordinality of grades. To avoid analysis issues due to sparse data, categories may be pooled (e.g. 0, 1, 2+) based on the statistician's blinded review of distributions.

Any subjects with at least one Grade 3+ reactogenicity event post vaccination will be listed. For clinical context, the list will include all observations through Day 6 for that subject, reactogenicity endpoint, and vaccination.

### 5.4.4. Vital signs

Vital signs (temperature, resting respiration rate and resting pulse rate) will be summarized via standard descriptive statistics as well as by protocol severity (toxicity) grades for each treatment group and measurement time point (pre-vaccination, 30 minutes post vaccination, scheduled follow-up visit). Data from subjects with Grade 2+ vital signs at any time, including unscheduled visits, will be listed.

# 6. TABLES, LISTINGS, AND FIGURES

Planned Tables, Listing and Figures (TLFs) intended to capture analyses described in the SAP are reviewed by the Sponsor before going into production. Since numbering and other formatting of the TLFs will change during the analysis, review, and CSR writing process, and because additional TLFs may be created for exploratory analyses not anticipated prior to un-blinding, the TLF shells are maintained as a 'living' document separate from the approved version of the SAP.

## 6.1. Programs and Tables Quality Control

Report production and review will be conducted in accordance with HI 360 BIOS Work Instructions (WI) 03003 (Verification of Analyses and Reports) and 03006 (Preparation and Review of Statistical Reports). In brief, a primary statistician-programmer for a given output will carefully review the program and output, verifying that no error message is highlighted in the "LOG" file and that titles, footers, footnotes, text body, etc. are correct. A second statistician-programmer will independently validate the output by checking the results against separately created SAS programs and checking textual material. Prior to delivery of any statistical output to Sponsor, the lead statistician will review the statistical package for internal inconsistencies or any items where clarifying notes will be helpful to the reviewer. The package is then thoroughly reviewed by the Biostatistics Director or designee before it is distributed.

## 6.2. Programming Conventions

Reporting conventions will adhere, when possible, to the International Conference on Harmonization Guidance document E3, "Structure and Content of Clinical Study Reports".

All tables and listings will be in landscape format unless otherwise requested.

Each table/figure/listing will have at least three titles:

- The 1st title will have the study/report name;
- The 2nd and as needed 3rd titles will be the TLF number and description, and will identify the objective being addressed
- The last title will identify the study population

Each table/figure/listing will also identify:

- Date of data freeze
- Data source (analysis data set)
- Listing source (associated listing, if any, for further details)
- File reference (name of output file)
- Run date

All SAS output for tables and listings will be distributed in PDF files, with RTF files created for inclusion into the CSR or sponsor presentations. PDFs may be generated as part of the output program or later. It is recommended that RTF (or MS Word) should be created as part of the output program so that file date matches run date.

# 7. LITERATURE AND REFERENCES

Barker N. (2005) A Practical Introduction to the Bootstrap Using the SAS System. Paper PK02, PhUSE conference.

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Gart JJ, Nam J. (1990). Approximate interval estimation of the difference in binomial parameters: correction for skewness and extension to multiple tables. *Biometrics* 46: 637-643

Miettinen OS, Nurminen M. (1985). Comparative analysis of two rates. *Statistics in Medicine*; 4:213–226.

Palmu AA1, Jokinen J, Borys D, Nieminen H, Ruokokoski E, et al. (2013). Effectiveness of the ten-valent pneumococcal Haemophilus influenzae protein D conjugate vaccine (PHiD-CV10) against invasive pneumococcal disease: a cluster randomised trial. *Lancet*; 381(9862):214-22.