

1     **Immunogenicity and Safety of 13-Valent Pneumococcal Conjugate Vaccine (PCV13)**  
2     **Formulated with 2-Phenoxyethanol in Multidose Vials Given with Routine Vaccination**  
3             **in Healthy Infants: An Open-Label Randomized Controlled Trial**

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42 **Running title:** Multidose vial presentation of PCV13

43 **Abbreviations:** \* 2-PE=2-phenoxyethanol; AE=adverse event; DMC=data monitoring  
44 committee; e-diary=electronic diary; GMC=geometric mean concentration; GMR=geometric  
45 mean ratio; GMT=geometric mean titer; IgG=immunoglobulin G; MDV=multidose vial;

46 MRC=Medical Research Council; OPA=opsonophagocytic activity; PCV=pneumococcal  
47 conjugate vaccine; PCV13=13-valent pneumococcal conjugate vaccine; SAE=serious adverse  
48 event; SDS=single-dose syringe; SDV=single-dose vial; WHO=World Health Organization.

49

50 **Abstract**

51 **Background.** This open-label randomized controlled trial in infants compared safety,  
52 tolerability, and immunogenicity of the 13-valent pneumococcal conjugate vaccine (PCV13)  
53 formulated with the preservative 2-phenoxyethanol (2-PE) in a multidose vial (MDV) to the  
54 current PCV13 without 2-PE in a single-dose syringe (SDS).

55 **Methods.** Gambian infants were randomized 1:1 to receive PCV13 as either MDV or SDS at  
56 ages 2, 3, and 4 months. Serotype-specific antipneumococcal antibody responses and  
57 opsonophagocytic activity ([OPA]; subset) were measured at age 5 months. Noninferiority  
58 was declared if the lower bound of the 97.5% CI for the difference (MDV-SDS) in  
59 proportions of subjects achieving IgG concentrations  $\geq 0.35$  ug/mL (primary endpoint) was  
60 greater than  $-10\%$ . IgG geometric mean concentrations (GMCs) were noninferior if the lower  
61 limit of the two-sided 97.5% CI of the geometric mean ratio (MDV vs SDS) was greater than  
62 0.5. Reactogenicity and other adverse events were collected.

63 **Results.** 500 participants were randomized and vaccinated; 489 (MDV: n=245; SDS: n=244)  
64 completed the trial. Noninferiority of MDV was demonstrated for all serotypes as measured  
65 by percentage of subjects achieving antibody responses above  $\geq 0.35$  ug/mL. IgG GMCs  
66 (coprimary endpoint) also demonstrated noninferiority of MDV; OPA results supported these  
67 findings. Safety and tolerability were comparable between groups.

68 **Conclusions.** PCV13 in MDV was safe and immunogenic when administered according to  
69 the routine schedule to infants. MDV was noninferior to SDS for all 13 pneumococcal  
70 serotypes. Comparable immunogenicity and safety profiles of PCV13 MDV and SDS suggest  
71 PCV13 MDV can help optimize vaccination in resource-limited settings. (ClinicalTrials.gov  
72 NCT01964716 <https://clinicaltrials.gov/ct2/show/NCT01964716>)

- 73 **Keywords:** pneumococcal conjugate vaccine; 2-phenoxyethanol; multidose vials; safety;
- 74 immunogenicity

## 75 **Introduction**

76 The prevention of pneumococcal disease remains a major international public health priority  
77 due to high disease burden and associated mortality in children, especially in developing  
78 countries [1-3]. Pneumococcal conjugate vaccines (PCVs) are proven to prevent overall  
79 pneumococcal disease by decreasing disease burden and nasopharyngeal carriage caused by  
80 serotypes included in the vaccines [4-8]. In many parts of Africa, obstacles still exist that  
81 slow the wider uptake of PCVs, including available vaccine preparations. The 13-valent PCV  
82 (PCV13), which includes the largest number of serotypes in its formulation, is currently only  
83 available in single-dose vials (SDV) and syringes (SDS). Pneumococcal conjugate vaccines,  
84 including PCV13 SDV, have been prequalified by the World Health Organization (WHO) for  
85 use in the developing world [9]. Most vaccines used routinely in Africa are formulated in  
86 multidose vials (MDV) to maximize cold-chain storage efficiency, and serve as an important  
87 resource for vaccine introduction in resource-limited countries [10,11]. In 2010 the  
88 manufacturer of PCV13 (Pfizer Inc, New York, NY) entered into an agreement with the  
89 GAVI Alliance (formerly the Global Alliance for Vaccines and Immunization) aiming to  
90 increase access to PCV13 in GAVI-eligible countries [12]. To meet the supply needs of this  
91 program, the manufacturer developed a formulation containing the preservative 2-  
92 phenoxyethanol (2-PE), a phenolic derivative currently used as a preservative in other  
93 commercially available multidose vial presentations of pediatric vaccines [13]. This 4-dose  
94 MDV presentation would help optimize vaccine delivery and facilitate sustainable coverage.

95 General concerns regarding vaccines in multidose vials include contamination risk and  
96 potential for wastage. PCV13 formulated with 2-PE as a preservative meets stringent  
97 pharmacopeia class B requirements for antimicrobial suppression that were previously  
98 accepted by the WHO. In addition, the multidose vial can be stored refrigerated and is

99 acceptable for use up to 28 days after initial dose removal. The PCV13 MDV presentation  
100 was recently prequalified by the WHO [14], licensed by the European Medicines Evaluation  
101 Agency [15], and licensed in India based on data from this clinical trial. The MDV  
102 presentation will be crucial in sustaining PCV delivery across resource-limited settings and  
103 maintaining the protection from disease that PCV13 already provides.

104 We present here an open-label randomized vaccine trial conducted in The Gambia to  
105 compare the safety, tolerability, and immunogenicity of PCV13 with 2-PE presented in a  
106 multidose vial (PCV13 MDV) to that of PCV13 without 2-PE presented in a single-dose  
107 syringe (PCV13 SDS).

## 108 **Methods**

### 109 **Trial design and population**

110 This phase 3, open-label randomized controlled trial (ClinicalTrials.gov, NCT01964716) was  
111 conducted between January and September, 2014, in The Gambia at the Medical Research  
112 Council (MRC) Unit Fajara. Participants were enrolled at the routine Expanded Programme  
113 on Immunization clinic at the Fajikunda Major Health Centre in Western Gambia. The trial  
114 was conducted per ethical principles derived from the Declaration of Helsinki and in  
115 compliance with International Conference on Harmonisation and Good Clinical Practice  
116 guidelines. The trial protocol and relevant documents were reviewed and approved by the  
117 National Regulatory Authority and the Gambian Government/MRC Joint Ethics Committee.  
118 Written informed consent was obtained from parents of each participant before enrollment  
119 and before any trial-related procedure was performed.

120 The primary objective was to demonstrate noninferiority of immune responses induced by  
121 PCV13 MDV compared with PCV13 SDS as measured by serotype-specific immunoglobulin

122 G (IgG) concentrations 1 month after infant series completion. Safety profiles of each  
123 formulation were measured by rates of local reactions, systemic events, and adverse events  
124 (AEs). The secondary objective was to assess immune responses induced by PCV13 MDV  
125 relative to PCV13 SDS as measured by serotype-specific opsonophagocytic activity (OPA) in  
126 a subset of subjects.

127 Healthy infants aged 42 to 70 days weighing 3.5 kg or more at enrollment were eligible to  
128 participate. Infants with known or suspected immune deficiency or suppression, severe acute  
129 or chronic medical conditions, neurologic disorders, known major or congenital  
130 malformations, any contraindication to vaccination with pneumococcal conjugate vaccine,  
131 previous vaccination with pneumococcal vaccine, or a history of culture-proven invasive  
132 disease caused by *Streptococcus pneumoniae* were excluded from the trial.

### 133 **Randomization**

134 In this open-label trial, participants were randomly assigned 1:1 to receive 3 doses of either  
135 PCV13 MDV or SDS. Randomization was performed using sequential allocation to trial  
136 groups based on a randomization sequence prepared by the sponsor, and provided as  
137 individual randomization envelopes.

### 138 **Trial vaccines and vaccination schedule**

139 PCV13 contains saccharides from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14,  
140 18C, 19A, 19F, and 23F individually conjugated to nontoxic diphtheria toxin cross-reactive  
141 material 197. Each 0.5-mL dose contains 2.2 µg of each saccharide, except for 4.4 µg of 6B,  
142 5 mM succinate buffer, 0.02% polysorbate 80, and 0.125 mg aluminum as aluminum  
143 phosphate. PCV13 MDVs also contain 4 mg of 2-phenoxyethanol (2-PE, the concentration of  
144 which is within the range of 2-PE concentrations used in other licensed vaccines presented in  
145 MDV formulations) per 0.5-mL dose. Four doses of PCV13 (2.0 mL) are contained within



146 each MDV; for this trial, only 1 dose was used from each vial to allow for 1:1 vaccine  
147 accountability (1 vial to 1 subject) in a clinical trial conducted per Good Clinical Practice  
148 guidelines. This study compared responses to PCV13 with and without 2-PE, which has  
149 already been proven an effective preservative [16].

150 Participants received 1 dose (0.5 mL) of PCV13 MDV or SDS at approximately 2, 3, and 4  
151 months of age per the Gambian immunization schedule, into the left anterolateral thigh. A  
152 range of 42 to 70 days of age was allowed for the 2-month visit, with subsequent visits 28 to  
153 42 days following the previous visit. Other injectable vaccines routinely administered at this  
154 age (pentavalent vaccine containing diphtheria, whole-cell pertussis, tetanus, *Haemophilus*  
155 *influenzae* type b, and hepatitis B virus antigens) were given into the contralateral limb at the  
156 same time as PCV13. Children also received oral rotavirus and polio vaccines according to  
157 local guidelines.

### 158 **Safety assessments**

159 An experienced physician independent of the trial was available onsite and served as a local  
160 safety monitor to provide support for any potential safety issues. The sponsor also provided a  
161 data monitoring committee (DMC) independent of the trial site to periodically review safety  
162 data. Local reactions and systemic events were monitored for at least 30 minutes after each  
163 vaccination and from days 2 to 6 after each vaccination (day 1 being the day of vaccination);  
164 events were recorded in electronic diaries (e-diaries) by trained field workers during daily  
165 home visits. Local reactions included redness, swelling, and tenderness at the PCV13  
166 injection site. Systemic events included fever (temperature  $\geq 38.0^{\circ}\text{C}$ ), changes in appetite,  
167 sleep, and irritability; antipyretic use was also documented. If any local or systemic event  
168 persisted at day 6 and/or antipyretic use continued at day 6, field workers continued daily  
169 visits and completed the e-diary until resolution of either. Persistent fever or development of

170 other symptoms triggered a referral to the safety nurse and trial physician. A rapid diagnostic  
171 test for malaria parasitemia was conducted whenever a temperature reading  $\geq 38.0^{\circ}\text{C}$  was  
172 obtained. Any other AEs and serious AEs (SAEs) occurring between the signing of the  
173 informed consent form and the postvaccination blood draw were recorded. The relationship  
174 between AEs and the trial vaccine received was characterized as related or not related by the  
175 trial physicians.

### 176 **Immunogenicity assessments**

177 One venous blood sample (up to 5 mL) was collected 1 month after dose 3. Blood samples  
178 were transported to the MRC laboratory within 4 hours of collection, and serum was  
179 separated, barcoded, stored at  $-80^{\circ}\text{C}$  and shipped to the sponsor for analysis. For all  
180 participants, a standardized enzyme-linked immunosorbent assay measured the concentration  
181 of serotype-specific IgG to the 13 pneumococcal vaccine serotypes [17-19].

182 Functional antibodies to the 13 serotypes were assessed by OPA assays [20,21] and were  
183 performed in a randomly selected subset of samples to ensure approximately 100 valid OPA  
184 results per serotype per group. To ensure 100 valid and determinate results for each serotype  
185 per treatment group, 160 subjects were randomly chosen from each treatment group. Invalid  
186 or indeterminate results were excluded from analysis, and samples with insufficient blood  
187 volume were not tested.

### 188 **Statistical analysis**

189 The WHO has established criteria for licensing new PCV formulations, which must  
190 demonstrate noninferiority to a current formulation for mean IgG concentrations and  
191 proportion of vaccinees achieving IgG concentrations  $\geq 0.35 \mu\text{g/mL}$ , the accepted correlate of  
192 protection against invasive pneumococcal disease, all measured 1 month after primary series  
193 completion [22-24].

194 The primary endpoint for each vaccine group was the proportion of participants achieving a  
195 serotype-specific IgG concentration  $\geq 0.35$   $\mu\text{g/mL}$  1 month after dose 3. The coprimary  
196 endpoint was the serotype-specific IgG geometric mean concentration (GMC) one month  
197 after dose 3. The WHO [25] guidance calls for control of the type 1 error rate to be pre-  
198 specified for multiple endpoints. As there are 2 primary comparisons (GMC or % responder),  
199 a Bonferroni correction was applied (split the 5% type 1 error rate evenly for comparison of  
200 each serotype). Therefore, 97.5% CIs were used for multiple primary comparisons. For the  
201 primary endpoint, noninferiority for a serotype (MDV relative to SDS) was achieved if the  
202 lower limit of the two-sided 97.5% CI of difference in proportions (MDV–SDS) was greater  
203 than  $-10\%$ . For IgG GMCs, noninferiority for a serotype was claimed if the lower limit of the  
204 two-sided 97.5% CI of the geometric mean ratio (GMR) (MDV relative to SDS) was greater  
205 than 0.5. The overall noninferiority of immune response of the PCV13 MDV to the PCV13  
206 SDS was declared if noninferiority for either the primary or the coprimary endpoint was  
207 demonstrated for all 13 serotypes.

208 Exact, unconditional, 2-sided, 95% CIs on the proportion were computed using the F  
209 distribution. The differences in proportions for the vaccine groups were calculated with  
210 97.5% CIs for IgG. Log transformation was used for GMRs. The OPA analysis for each  
211 vaccine group calculated the serotype-specific OPA geometric mean titers (GMTs) for each  
212 pneumococcal serotype 1 month after dose 3. The evaluable immunogenicity population was  
213 the primary analysis population, and included infants who received all assigned vaccine  
214 doses, adhered to protocol requirements, had  $\geq 1$  valid and determinate assay result, and had  
215 no major protocol violations. The safety analysis included all subjects who received  $\geq 1$  dose  
216 of PCV13.

## 217 **Sample size**

218 Sample size was based on simulations for proportions of responders with IgG antibody  
219 concentrations  $\geq 0.35$   $\mu\text{g/mL}$  and for ratios of the geometric mean IgG concentrations for the  
220 MDV group relative to the SDS group. The variance-covariance input matrix and the mean  
221 vector for generating the random samples from the multiple-normal distribution were based  
222 on immunogenicity data from a previous Pfizer trial [26]. One thousand studies with 200  
223 participants per group were generated for the simulations. The sample size was chosen to  
224 meet the WHO criteria [25], with Type-I error controlled by Bonferroni's method for GMC  
225 and responder hypotheses (97.5% CIs were used for testing). Using these criteria, the trial  
226 had  $\geq 90\%$  power to achieve the criteria from the simulation. Estimating a dropout rate of  
227 20%, 500 participants (250 per group) were recommended for enrollment.

## 228 **Results**

229 Recruitment occurred between January 9 and May 19, 2014, and the last study visit was  
230 completed on September 1, 2014. Of 526 participants assessed for eligibility, 500 were  
231 enrolled, randomized, and vaccinated with either PCV13 MDV or SDS (250 per group)  
232 concomitantly with routine vaccines (**Figure 1**). Overall, 489 (97.8%) participants completed  
233 the blood draw 1 month after dose 3 and were included in the evaluable immunogenicity  
234 population (245 and 244 in the PCV13 MDV and SDS groups, respectively). Demographic  
235 characteristics of the evaluable immunogenicity population were similar between the vaccine  
236 groups (**Table 1**). Safety was assessed in all 500 participants who received  $\geq 1$  dose of trial  
237 vaccine. Characteristics of participants in the safety population were comparable to those in  
238 the evaluable immunogenicity population.

239 **Immunogenicity**

240 *Pneumococcal IgG Concentrations  $\geq 0.35 \mu\text{g/mL}$*

241 The numbers of participants in each trial arm achieving a pneumococcal IgG concentration  
242  $\geq 0.35 \mu\text{g/mL}$  are compared in **Table 2**. More than 95% of children achieved IgG  
243 concentrations  $\geq 0.35 \mu\text{g/mL}$  1 month after dose 3 for each of the 13 serotypes in both vaccine  
244 groups, and the noninferiority criterion was met for this endpoint.

245 *Pneumococcal IgG Geometric Mean Concentrations*

246 One month after dose 3, GMCs were similar in both vaccine groups for the majority of  
247 serotypes. For this coprimary endpoint, noninferiority between the PCV13 MDV group and  
248 the PCV13 SDS group was demonstrated for all 13 serotypes (**Table 3**).

249 *Pneumococcal OPA Geometric Mean Titers*

250 The OPA GMTs for all serotypes were determined for a subset of subjects in each vaccine  
251 group (MDV, 156–160 subjects; SDS, 155–160 subjects). GMTs were similar in both groups  
252 for the majority of serotypes, except serotype 3, for which titers were statistically  
253 significantly lower in the MDV group, and serotype 18C, for which titers were statistically  
254 significantly higher in the MDV group (**Table 4**). All lower limits of the 95% CIs of OPA  
255 GMRs comparing MDV to SDS were greater than 0.5 except for serotype 14, which was  
256 slightly lower (ratio 0.7; 95% CI [0.48, 1.08]) (**Table 4**). Reverse cumulative distribution  
257 curves showing OPA titers for each PCV13 serotype 1 month after dose 3 indicate that levels  
258 were similar across the full range of OPA titers for the majority of serotypes in both vaccine  
259 groups (**Supplementary Figure 1**).

260

## 261 **Safety**

262 Local reactions and systemic events after each vaccination were comparable between vaccine  
263 groups, were mainly mild, and are summarized in **Table 5**. Local reactions were reported  
264 mainly on day 2 (roughly 24 hours after vaccination) and generally lasted <72 hours.  
265 Proportions of participants reporting redness and swelling at the vaccination site were low in  
266 both groups after any dose. No participant experienced a severe local reaction. For all doses,  
267 fever rates were comparable in both groups and generally low (1.2%–3.6%), and no  
268 participant experienced a fever >40°C. Although documented fever was rare, antipyretic  
269 medication was used in up to 23.2% of participants and was similar in both vaccine groups.  
270 Other systemic events were generally mild or moderate and were reported in similar  
271 proportions in both groups.

272 The AEs reported reflected anticipated illnesses in this age group and did not differ between  
273 groups (49.2% and 50.8% for PCV13 MDV and PCV13 SDS, respectively). The most  
274 frequently reported individual AEs were upper respiratory tract infection, viral diarrhea, and  
275 dermatitis, which were reported by similar proportions in the MDV group (23.2%, 7.6%, and  
276 6.0%) and SDS group (20.0%, 12.0%, and 8.8%). There were also 3 related AEs of pyrexia  
277 (1 event in PCV13 MDV, 2 events in PCV13 SDS) that occurred on the day of vaccination  
278 (and therefore were not captured in the e-diary). One sudden infant death occurred in the  
279 PCV13 MDV group on day 20 after dose 3; this SAE was considered unrelated to trial  
280 vaccines.

## 281 **Discussion**

282 This trial compared the currently licensed PCV13 SDS presentation with a novel MDV  
283 preparation containing 2-PE in Gambian infants. Results confirmed noninferiority of the new  
284 formulation for all 13 serotypes for the primary outcome measures. Specifically, proportions

285 of participants achieving antipneumococcal IgG antibody concentrations  $\geq 0.35 \mu\text{g/mL}$  were  
286 noninferior for MDV compared to SDS, as were IgG GMCs. Additionally, functional  
287 antibody assessed using OPA was similar between groups. No safety concerns were raised  
288 with the new formulation.

289 Substituting PCV13 SDS with a preparation containing multiple doses would have  
290 considerable advantages regarding logistics associated with vaccine delivery to remote areas,  
291 but equivalent safety and immunogenicity records are paramount for WHO prequalification  
292 and introduction. More than 95% of participants achieved concentrations above the WHO-  
293 defined threshold for protection for all 13 serotypes, and no major differences were observed  
294 between vaccine groups based on predefined noninferiority criteria. Minor differences in  
295 GMCs noted for serotype 18C and serotype 3 between vaccine groups are not likely to be of  
296 clinical significance. Results from OPA assays confirmed that IgG concentrations were  
297 associated with functional antibody, and GMTs were similar between groups for the majority  
298 of serotypes.

299 These results are likely generalizable, as the current trial setting is representative of other  
300 areas where MDV would be used.

301 Solicited local and systemic reactions and AEs were generally few and mild, with no  
302 differences between vaccine arms. No new safety issues were detected, suggesting that safety  
303 profiles of MDV and SDS are at least similar. Reactogenicity was low in both groups when  
304 compared to previous studies conducted elsewhere using PCV [27]. One potential  
305 explanation for low reactogenicity is the initial day of assessment being approximately 24  
306 hours after vaccination, meaning reactions limited to the day of vaccination and resolving  
307 before day 2 could have been missed. However, this would only include reactions lasting  $< 1$   
308 day, which are likely to be mild. Related adverse events on vaccination days were also low

309 and similar between groups. Other vaccine trials in this setting using similar reporting  
310 methods and times have also reported low vaccine reactogenicity [28]. The recording of  
311 reactogenicity data in the e-diary from day 2 following vaccination may partially explain the  
312 disparity between proportions of participants reporting antipyretic use (13.8%–23.2%) and  
313 those with documented pyrexia ( $\leq 3.6\%$ ), as pyrexia may have developed after field workers  
314 departed on day 1 but before their return visit on day 2. Moreover, antipyretics may have  
315 been used for pyrexia perceived by parents rather than actual pyrexia.

316 The AEs noted were similar between trial groups and were generally consistent with  
317 conditions common in this age group. One SAE occurred in the MDV group compared with  
318 none in the SDS group, but this fatal SAE occurring 20 days after dose 3 was considered  
319 unrelated to vaccination following review by the local safety monitor and DMC. Considering  
320 infant mortality in The Gambia [29], unexplained deaths in this age group are unsurprising;  
321 overall mortality in the trial was very low.

322 In conclusion, the immune response induced by PCV13 formulated with 2-PE in multidose  
323 vials was noninferior to the immune response induced by PCV13 without 2-PE in single-dose  
324 syringes when given at approximately 2, 3, and 4 months of age with routine pediatric  
325 vaccinations in healthy infants. Therefore, PCV13 with 2-PE likely induces an immune  
326 response adequate to protect against pneumococcal disease, similar to that induced by PCV13  
327 without 2-PE. The comparable immunogenicity and safety profiles of PCV13 MDV and  
328 PCV13 SDS support use of PCV13 MDV in places where the SDS may pose unique  
329 challenges. Availability of PCV13 MDV would help ensure vaccine sustainability for infants  
330 in resource-limited settings.

331



332 **Conflict of Interest Statement**

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348 **Author Contribution Statements**

349 All authors approved and agreed to submit the final article for publication.  
350 OTI, FL, NLN, KUJ, AG, KJC, DAS, BK, and AR conceived the trial. OTI, RBM, MO, BC,  
351 BK, and AR were involved in subject recruitment and data acquisition. JZL was responsible  
352 for statistical analyses. OTI drafted the manuscript with support from AR and BK. All  
353 authors participated in data interpretation and manuscript development, including critical  
354 review of drafts and approval of the final submitted manuscript. OTI, BK, and AR are the

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362

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480

481 **Tables**482 **Table 1. Demographic Characteristics of the Population Evaluable for Immunogenicity**

<b>Characteristics</b>	<b>Vaccine Group (as Randomized)</b>		<b>Total N<sup>a</sup>=489</b>
	<b>PCV13 MDV N<sup>a</sup>=245</b>	<b>PCV13 SDS N<sup>a</sup>=244</b>	
Sex, n (%)			
Female	127 (51.8)	128 (52.5)	255 (52.1)
Male	118 (48.2)	116 (47.5)	234 (47.9)
Race, n (%)			
Black	245 (100.0)	244 (100.0)	489 (100.0)
Ethnic Group, n (%)			
Mandinka	112 (45.7)	110 (45.1)	222 (45.4)
Jola	42 (17.1)	47 (19.3)	89 (18.2)
Fula	35 (14.3)	27 (11.1)	62 (12.7)
Wolof	30 (12.2)	25 (10.2)	55 (11.2)
Sarahule	8 (3.3)	11 (4.5)	19 (3.9)
Other	18 (7.3)	24 (9.8)	42 (8.6)
Age at enrollment, days			
Mean (SD)	57.2 (8.6)	56.8 (8.7)	57.0 (8.7)
Median	58.0	56.0	57.0
Min, Max	43.0, 71.0	43.0, 71.0	43.0, 71.0
Weight, kg			
Mean (SD)	5.0 (0.7)	4.9 (0.7)	4.9 (0.7)
Median	4.9	4.8	4.8
Min, max	3.5, 7.8	3.5, 6.7	3.5, 7.8

483 PCV13 MDV=13-valent pneumococcal conjugate vaccine formulated with 2-PE in multidose  
484 vials; PCV13 SDS= 13-valent pneumococcal conjugate vaccine formulated without 2-PE in  
485 single-dose syringes.

486 <sup>a</sup>Number of participants in the vaccine group, or total sample. These values are used as  
487 denominators for percentages.

488



489 **Table 2. Participants Achieving a Pneumococcal IgG Antibody Concentration  $\geq 0.35$   $\mu\text{g/mL}$  1 Month After Dose 3 (Evaluable**  
 490 **Immunogenicity Population)**

Serotype	Vaccine Group (as Randomized)								Difference <sup>d</sup> (97.5% CI <sup>e</sup> )	
	PCV13 MDV				PCV13 SDS					
	N <sup>a</sup>	n <sup>b</sup>	%	(95% CI <sup>c</sup> )	N <sup>a</sup>	n <sup>b</sup>	%	(95% CI <sup>c</sup> )		
1	245	243	99.2	(97.1, 99.9)	244	244	100.0	(98.5, 100.0)	-0.8	(-3.4, 1.2)
3	245	242	98.8	(96.5, 99.7)	243	242	99.6	(97.7, 100.0)	-0.8	(-3.7, 1.6)
4	245	244	99.6	(97.7, 100.0)	244	243	99.6	(97.7, 100.0)	0.0	(-2.3, 2.4)
5	245	235	95.9	(92.6, 98.0)	244	237	97.1	(94.2, 98.8)	-1.2	(-5.4, 2.8)
6A	243	234	96.3	(93.1, 98.3)	244	238	97.5	(94.7, 99.1)	-1.2	(-5.3, 2.6)
6B	245	233	95.1	(91.6, 97.4)	244	232	95.1	(91.6, 97.4)	0.0	(-4.7, 4.7)
7F	245	244	99.6	(97.7, 100.0)	244	244	100.0	(98.5, 100.0)	-0.4	(-2.7, 1.6)
9V	245	240	98.0	(95.3, 99.3)	244	240	98.4	(95.9, 99.6)	-0.4	(-3.8, 2.8)
14	245	239	97.6	(94.7, 99.1)	244	240	98.4	(95.9, 99.6)	-0.8	(-4.3, 2.5)
18C	245	243	99.2	(97.1, 99.9)	244	239	98.0	(95.3, 99.3)	1.2	(-1.6, 4.5)
19A	245	244	99.6	(97.7, 100.0)	244	241	98.8	(96.4, 99.7)	0.8	(-1.6, 3.6)
19F	245	237	96.7	(93.7, 98.6)	244	237	97.1	(94.2, 98.8)	-0.4	(-4.4, 3.5)
23F	245	235	95.9	(92.6, 98.0)	244	234	95.9	(92.6, 98.0)	0.0	(-4.3, 4.4)

491 IgG=immunoglobulin G; PCV13 MDV=13-valent pneumococcal conjugate vaccine formulated with 2-PE in multidose vials;  
 492 PCV13 SDS= 13-valent pneumococcal conjugate vaccine formulated without 2-PE in single-dose syringes.

493 <sup>a</sup>Number of participants with a valid and determinate IgG concentration to the given serotype.

494 <sup>b</sup>Number of participants with an antibody concentration  $\geq 0.35$   $\mu\text{g/mL}$  for the given serotype.

495 <sup>c</sup>Exact 2-sided confidence interval (Clopper and Pearson) based on the observed proportion of participants.

496 <sup>d</sup>Difference in proportions, PCV13 MDV–PCV13 SDS, expressed as a percentage.

497 <sup>e</sup>Exact 2-sided CI (based on Chan and Zhang [30]) for the difference in proportions, PCV13 MDV–PCV13 SDS, expressed as a  
 498 percentage.

499 **Table 3. Comparison of Pneumococcal IgG GMCs (µg/mL) 1 Month After Dose 3 (Evaluable Immunogenicity Population)**

Serotype	Vaccine Group (as Randomized)						Ratio <sup>d</sup>	(97.5% CI <sup>e</sup> )
	PCV13 MDV			PCV13 SDS				
	n <sup>a</sup>	GMC <sup>b</sup>	(95% CI <sup>c</sup> )	n <sup>a</sup>	GMC <sup>b</sup>	(95% CI <sup>c</sup> )		
1	245	4.59	(4.11, 5.12)	244	4.45	(4.01, 4.93)	1.03	(0.87, 1.22)
3	245	1.38	(1.29, 1.49)	243	1.74	(1.62, 1.87)	0.79	(0.71, 0.90)
4	245	5.30	(4.84, 5.81)	244	5.28	(4.76, 5.85)	1.00	(0.86, 1.18)
5	245	2.00	(1.79, 2.22)	244	1.98	(1.79, 2.20)	1.01	(0.85, 1.19)
6A	243	2.25	(2.02, 2.50)	244	2.19	(1.96, 2.44)	1.03	(0.86, 1.22)
6B	245	3.42	(2.91, 4.02)	244	3.24	(2.77, 3.78)	1.06	(0.82, 1.36)
7F	245	3.92	(3.59, 4.27)	244	4.18	(3.83, 4.55)	0.94	(0.82, 1.08)
9V	245	2.83	(2.56, 3.13)	244	2.75	(2.49, 3.04)	1.03	(0.87, 1.21)
14	245	4.78	(4.06, 5.63)	244	4.96	(4.27, 5.77)	0.96	(0.75, 1.24)
18C	245	3.47	(3.17, 3.79)	244	2.72	(2.46, 3.00)	1.28	(1.09, 1.49)
19A	245	6.49	(5.70, 7.38)	244	6.44	(5.66, 7.32)	1.01	(0.82, 1.24)
19F	245	5.19	(4.59, 5.86)	244	5.00	(4.43, 5.63)	1.04	(0.85, 1.26)
23F	245	2.61	(2.30, 2.97)	244	2.17	(1.92, 2.46)	1.20	(0.98, 1.48)

500 GMC=geometric mean concentration; IgG=immunoglobulin G; PCV13 MDV=13-valent pneumococcal conjugate vaccine formulated  
501 with 2-PE in multidose vials; PCV13 SDS= 13-valent pneumococcal conjugate vaccine formulated without 2-PE in single-dose  
502 syringes.

503 <sup>a</sup>Number of participants with a valid and determinate antibody concentration for the specified serotype.

504 <sup>b</sup>GMCs were calculated using all participants with available data for the specified blood draw.

505 <sup>c</sup>CI<sub>s</sub> are back transformations of a CI based on the Student *t* distribution for the mean logarithm of the concentrations.

506 <sup>d</sup>Ratio of GMCs, PCV13 MDV to PCV13 SDS, was calculated by back transforming the mean difference between the vaccine groups  
507 on the logarithmic scale.

508 <sup>e</sup>CI<sub>s</sub> for the ratio are back transformations of a CI based on the Student *t* distribution for the mean difference of the logarithms of the  
509 measures (PCV13 MDV–PCV13 SDS).

510 **Table 4. Comparison of Pneumococcal OPA GMTs 1 Month After Dose 3 (Evaluable Immunogenicity Population)<sup>a</sup>**

Serotype	Vaccine Group (as Randomized)						Ratio <sup>e</sup>	(95% CI <sup>f</sup> )
	PCV13 MDV			PCV13 SDS				
	n <sup>b</sup>	GMT <sup>c</sup>	(95% CI <sup>d</sup> )	n <sup>b</sup>	GMT <sup>c</sup>	(95% CI <sup>d</sup> )		
1	159	48	(39.0, 58.0)	160	52	(43.2, 62.6)	0.9	(0.70, 1.20)
3	160	97	(87.3, 108.8)	160	122	(110.1, 135.7)	0.8	(0.69, 0.93)
4	159	1666	(1412.3, 1966.3)	159	1492	(1285.2, 1732.3)	1.1	(0.89, 1.39)
5	160	79	(67.7, 92.4)	159	80	(69.1, 92.6)	1.0	(0.80, 1.22)
6A	160	1690	(1460.5, 1955.9)	160	1968	(1698.5, 2279.3)	0.9	(0.70, 1.06)
6B	156	1990	(1611.7, 2456.9)	155	2014	(1639.1, 2475.4)	1.0	(0.74, 1.33)
7F	159	2891	(2565.4, 3258.5)	160	3450	(3014.7, 3947.9)	0.8	(0.70, 1.00)
9V	158	709	(600.5, 836.2)	160	706	(597.0, 835.3)	1.0	(0.79, 1.27)
14	157	567	(415.4, 773.5)	160	786	(607.8, 1015.3)	0.7	(0.48, 1.08)
18C	159	2792	(2387.5, 3264.8)	160	1605	(1352.0, 1904.4)	1.7	(1.38, 2.19)
19A	160	305	(256.2, 362.9)	160	329	(284.8, 379.8)	0.9	(0.74, 1.16)
19F	158	430	(357.6, 517.1)	159	470	(391.4, 565.3)	0.9	(0.71, 1.18)
23F	159	918	(729.0, 1156.4)	160	998	(810.6, 1229.6)	0.9	(0.67, 1.25)

511 GMT=geometric mean titer; OPA=opsonophagocytic activity; PCV13 MDV=13-valent pneumococcal conjugate vaccine formulated  
 512 with 2-PE in multidose vials; PCV13 SDS=13-valent pneumococcal conjugate vaccine formulated without 2-PE in single-dose  
 513 syringes.

514 <sup>a</sup>OPA assays were performed on the blood sample taken 1 month after dose 3 in a subset of randomly selected  
 515 participants from each group.

516 <sup>b</sup>Number of participants with a valid and determinate antibody titer for the specified serotype.

517 <sup>c</sup>GMTs were calculated using all participants with available data for the specified blood draw.

518 <sup>d</sup>CI<sub>s</sub> are back transformations of a CI based on the Student *t* distribution for the mean logarithm of the titers.

519 <sup>e</sup>Ratio of GMTs, PCV13 MDV to PCV13 SDS, was calculated by back transforming the mean difference between the vaccine groups  
 520 on the logarithmic scale.

521 <sup>f</sup>CI<sub>s</sub> for the ratio are back transformations of a CI based on the Student *t* distribution for the mean difference of the logarithms of the  
 522 measures (PCV13 MDV–PCV13 SDS).

523

**Table 5. Subjects Reporting Local Reactions, Systemic Events, and Antipyretic Medication Use on Days 2–6**

Condition Reported	Dose 1		Dose 2		Dose 3	
	PCV13 MDV	PCV13 SDS	PCV13 MDV	PCV13 SDS	PCV13 MDV	PCV13 SDS
	% (n <sup>a</sup> /N <sup>b</sup> )	% (n <sup>a</sup> /N <sup>b</sup> )	% (n <sup>a</sup> /N <sup>b</sup> )	% (n <sup>a</sup> /N <sup>b</sup> )	% (n <sup>a</sup> /N <sup>b</sup> )	% (n <sup>a</sup> /N <sup>b</sup> )
<b>Local Reaction</b>						
Tenderness <sup>c</sup>						
Any	16.9 (42/248)	18.8 (47/250)	13.7 (34/248)	13.0 (32/247)	15.0 (37/246)	14.5 (35/241)
Mild	12.9 (32/248)	14.8 (37/250)	10.9 (27/248)	11.3 (28/247)	12.2 (30/246)	13.3 (32/241)
Moderate	5.2 (13/248)	5.6 (14/250)	2.8 (7/247)	2.0 (5/247)	3.7 (9/246)	1.7 (2/241)
Severe	0.0 (0/248)	0.0 (0/250)	0.0 (0/247)	0.0 (0/247)	0.0 (0/246)	0.0 (0/241)
Swelling <sup>d</sup>						
Any	0.4 (1/248)	0.0 (0/250)	0.8 (2/247)	0.0 (0/247)	0.0 (0/246)	0.0 (0/241)
Mild	0.4 (1/248)	0.0 (0/250)	0.8 (2/247)	0.0 (0/247)	0.0 (0/246)	0.0 (0/241)
Moderate	0.0 (0/248)	0.0 (0/250)	0.4 (1/247)	0.0 (0/247)	0.0 (0/246)	0.0 (0/241)
Severe	0.0 (0/248)	0.0 (0/250)	0.0 (0/247)	0.0 (0/247)	0.0 (0/246)	0.0 (0/241)
Redness <sup>d</sup>						
Any	0.4 (1/248)	0.0 (0/250)	0.8 (2/247)	0.0 (0/247)	0.0 (0/246)	0.0 (0/241)
Mild	0.4 (1/248)	0.0 (0/250)	0.8 (2/247)	0.0 (0/247)	0.0 (0/246)	0.0 (0/241)
Moderate	0.0 (0/248)	0.0 (0/250)	0.0 (0/247)	0.0 (0/247)	0.0 (0/246)	0.0 (0/241)
Severe	0.0 (0/248)	0.0 (0/250)	0.0 (0/247)	0.0 (0/247)	0.0 (0/246)	0.0 (0/241)
<b>Systemic Event</b>						
Fever <sup>e</sup>						
≥38°C	3.6 (9/248)	2.8 (7/250)	2.8 (7/247)	2.8 (7/247)	1.2 (3/246)	3.3 (8/241)
≥38°C but ≤39°C	3.6 (9/248)	2.8 (7/250)	2.8 (7/247)	2.8 (7/247)	1.2 (3/246)	2.9 (7/241)
>39°C but ≤40°C	0.0 (0/248)	0.0 (0/250)	0.4 (1/247)	0.0 (0/247)	0.0 (0/246)	0.4 (1/241)
>40°C	0.0 (0/248)	0.0 (0/250)	0.0 (0/247)	0.0 (0/247)	0.0 (0/246)	0.0 (0/241)
Decreased appetite <sup>f</sup>						
Any	6.9 (17/248)	10.4 (26/250)	11.3 (28/247)	7.3 (18/247)	9.8 (24/246)	8.3 (20/242)
Moderate	6.9 (17/248)	10.4 (26/250)	11.3 (28/247)	7.3 (18/247)	9.8 (24/246)	7.9 (19/242)
Severe	0.0 (0/248)	0.0 (0/250)	0.0 (0/247)	0.0 (0/247)	0.0 (0/246)	0.4 (1/241)
Irritability <sup>g</sup>						
Any	41.4 (103/249)	37.2 (93/250)	37.5 (93/248)	33.6 (83/247)	33.7 (83/246)	37.9 (92/243)
Mild	34.5 (86/249)	30.8 (77/250)	30.2 (75/248)	27.1 (67/247)	28.9 (71/246)	30.6 (74/242)

Moderate	7.7 (19/248)	6.8 (17/250)	9.3 (23/247)	6.9 (17/247)	6.1 (15/246)	7.4 (18/242)
Severe	0.0 (0/248)	0.0 (0/250)	0.0 (0/247)	1.2 (3/247)	0.4 (1/246)	2.1 (5/241)
Increased sleep <sup>h</sup>						
Any	6.5 (16/248)	5.6 (14/250)	9.7 (24/247)	5.7 (14/247)	4.9 (12/246)	5.0 (12/241)
Mild	4.4 (11/248)	4.0 (10/250)	8.1 (20/247)	4.9 (12/247)	4.1 (10/246)	4.6 (11/241)
Moderate	2.4 (6/248)	1.6 (4/250)	1.6 (4/247)	0.4 (1/247)	0.8 (2/246)	0.4 (1/241)
Severe	0.0 (0/248)	0.0 (0/250)	0.0 (0/247)	0.4 (1/247)	0.4 (1/246)	0.0 (0/241)
Use of antipyretic medication	22.2 (55/248)	23.2 (58/250)	18.5 (46/248)	17.4 (43/247)	13.8 (34/246)	14.9 (36/241)

525 PCV13 MDV=13-valent pneumococcal conjugate vaccine formulated with 2-PE in multidose vials; PCV13 SDS=13-valent pneumococcal  
526 conjugate vaccine formulated without 2-PE in single-dose syringes.

527 <sup>a</sup>Number of subjects reporting the reaction.

528 <sup>b</sup>Number of subjects reporting yes for at least 1 day or no for all days.

529 <sup>c</sup>Tenderness was categorized as mild (hurts if gently touched), moderate (hurts if gently touched with crying), or severe (causes  
530 limitation of limb movement).

531 <sup>d</sup>Swelling and redness were measured by caliper (each caliper unit representing 0.5 cm) and categorized as mild (0.5–2.0 cm), moderate  
532 (2.1–7.0 cm) or severe (>7.0 cm).

533 <sup>e</sup>Fever severity was categorized as: 38°C–39°C, 39.1°C–40°C, and >40°C.

534 <sup>f</sup>Loss of or decreased appetite defined as moderate (decreased oral intake), or severe (refusal to feed).

535 <sup>g</sup>Irritability defined as mild (easily consolable), moderate (requiring increased attention) or severe (inconsolable, crying that cannot be  
536 comforted).

537 <sup>h</sup>Increased sleep defined as drowsiness, mild (increased or prolonged sleeping bouts), moderate (slightly subdued, interfering with daily  
538 activity) or severe (disabling, not interested in usual daily activity).

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541 **Figure Legend**

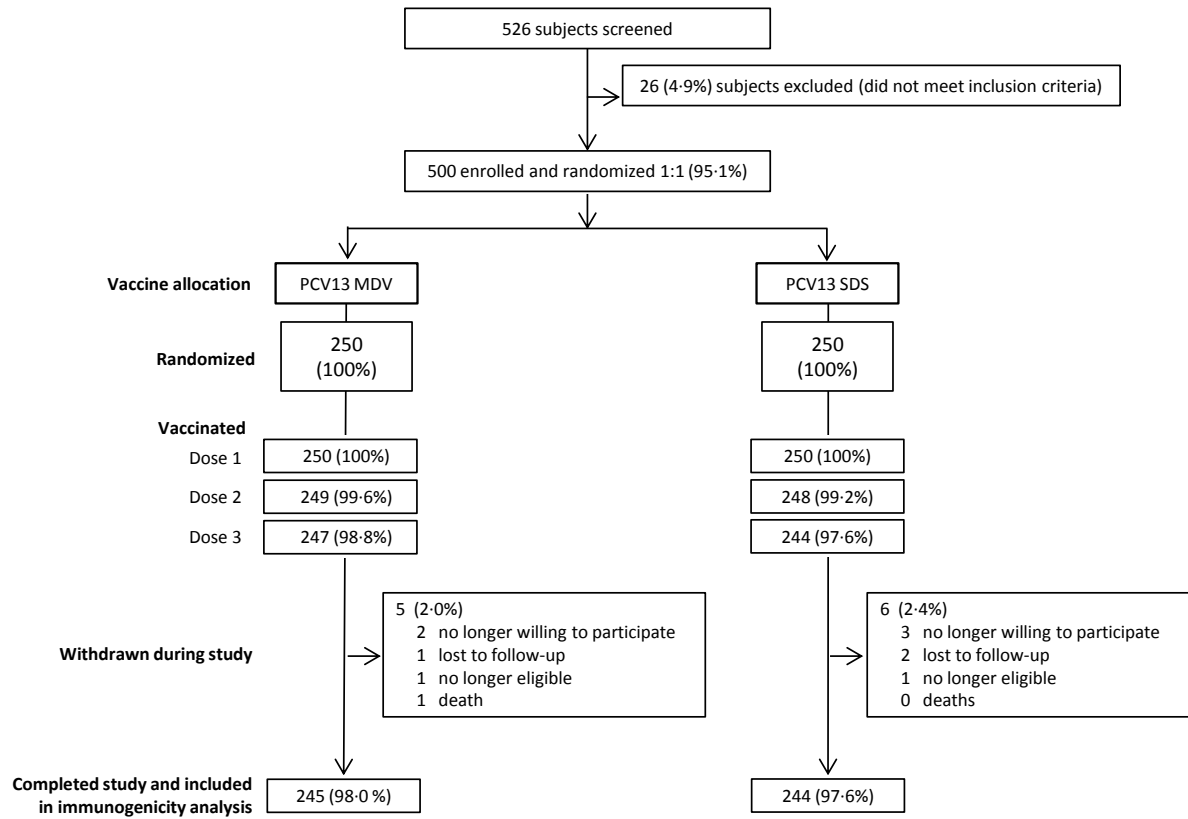
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543 Figure 1. CONSORT diagram. All 26 ineligible participants were excluded for failure to meet  
544 inclusion criteria or for meeting exclusion criteria: 14 (54%) weighed less than 3.5 kg, 5 (20%)  
545 suffered from an intercurrent illness and would have been too old for enrollment after the  
546 allowable window of 5 days after completing antibiotic therapy, 3 (11%) had underlying  
547 congenital anomalies, and 2 (7.5%) each were not available for the entire trial period, or had  
548 received previous pneumococcal vaccination.

549 MDV=multidose vials; PCV13=13-valent pneumococcal conjugate vaccine; SDS=single-dose  
550 syringe.

551 **Figure 1.**

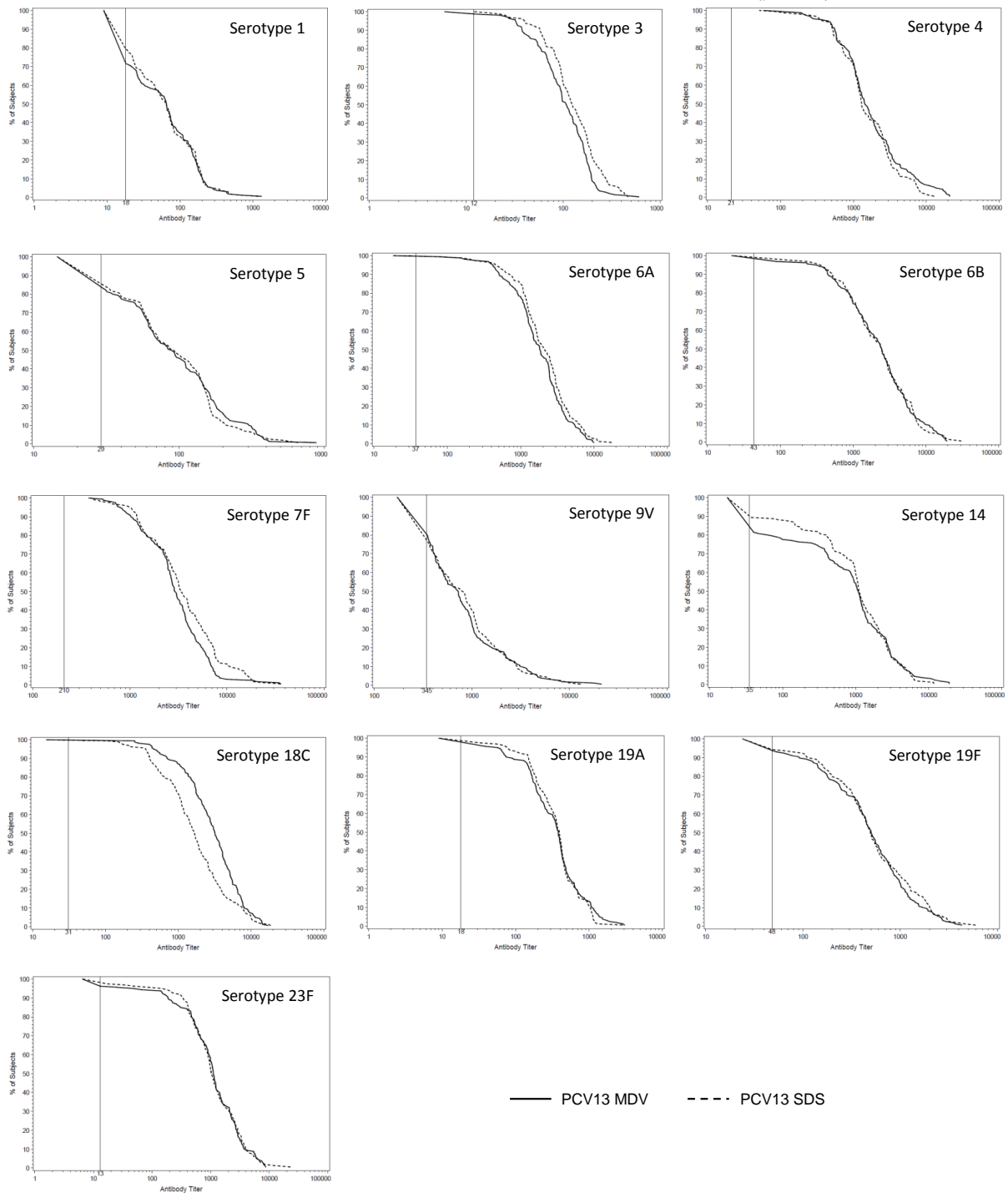
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555 **Supplementary Figure 1. Reverse Cumulative Distribution Curve of Pneumococcal**  
 556 **OPA Antibody Titers 1 Month After PCV13 Dose 3, Evaluable Immunogenicity**  
 557 **Population.**



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