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International Seroepidemiology of Adenovirus Serotypes 5, 26, 35, and 48 in Pediatric and Adult Populations

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Abstract

Recombinant adenovirus serotype 5 (rAd5) vaccine vectors for HIV-1 and other pathogens have been shown to be limited by high titers of Ad5 neutralizing antibodies (NAbs) in the developing world. Alternative serotype rAd vectors have therefore been constructed. Here we report Ad5, Ad26, Ad35, and Ad48 NAb titers in 4,381 individuals from North America, South America, sub-Saharan Africa, and Southeast Asia. As expected, Ad5 NAb titers were both frequent and high magnitude in sub-Saharan Africa and Southeast Asia. In contrast, Ad35 NAb titers proved infrequent and low in all regions studied, and Ad48 NAbs were rare in all regions except East Africa. Ad26 NAbs were moderately common in adults in sub-Saharan Africa and Southeast Asia, but Ad26 NAb titers proved markedly lower than Ad5 NAb titers in all regions, and these relatively low Ad26 NAb titers did not detectably suppress the immunogenicity of 4×10^{10} vp of a rAd26-Gag/Pol/Env/Nef vaccine in rhesus monkeys. These data inform the clinical development of alternative serotype rAd vaccine vectors in the developing world.

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1. Introduction

A limitation that has become apparent with rAd5 vaccine vectors is the high titers of Ad5 NAb in human populations, particularly in the developing world. Baseline Ad5 NAb have been shown to suppress the immunogenicity of rAd5 vector-based vaccines for HIV-1 in both preclinical studies [1–2] and clinical trials [3–5], although higher doses of rAd5 vectors can partially overcome this effect. To address this and other problems with rAd5 vectors, alternative human serotype rAd vectors [6–8], hexon-chimeric rAd vectors [2], and rAd vectors derived from other species [9–10] have been constructed.

In particular, rAd26 and rAd35 vectors are currently being evaluated in phase 1 HIV-1 vaccine clinical trials in both the United States and sub-Saharan Africa. Ad26 (subgroup D), Ad35 (subgroup B), and Ad48 (subgroup D) are derived from different Ad subgroups than Ad5 (subgroup C). Moreover, these alternative Ad serotypes differ from Ad5 in terms of their receptor usage [6, 8], tropism [11], dendritic cell stimulatory capacity [12], innate immune profile (D.H.B., unpublished data), adaptive immune phenotype [13], and capacity to protect against SIV challenge in rhesus monkeys [14].

Building on Ad seroepidemiology studies previously reported from our laboratory and others [6, 8, 15–19], we report here a large study of Ad5, Ad26, Ad35, and Ad48 NAb titers in 4,381 pediatric and adult subjects from North America, South America, sub-Saharan Africa, and Southeast Asia. We also model the impact of typical Ad26 NAb titers found in the developing world on rAd26 vaccine immunogenicity in a pilot study in rhesus monkeys.

2. Materials and Methods

2.1 Study Populations

This study involved 4,381 subjects from pediatric and adult populations in North America, South America, sub-Saharan Africa, and Southeast Asia. Subjects included both low HIV-1 risk and high HIV-1 risk adult populations from multiple geographic regions, as well as healthy infants and schoolchildren from South Africa. Random or case-controlled baseline samples were utilized in these studies to minimize selection bias. Table 1 details the specific cohorts in this study. All samples were collected with local Institutional Review Board (IRB) approvals, and adenovirus neutralization assays utilizing these samples were approved by the Beth Israel Deaconess Medical Center IRB.

2.2 Adenovirus Neutralization Assay

Ad-specific NAb titers were assessed by high-throughput luciferase-based virus neutralization assays as described [20]. A549 human lung carcinoma cells were plated at a density of 1×10^4 cells per well in 96-well plates and infected with E1/E3-deleted, replication-incompetent rAd-Luc reporter constructs at a multiplicity of infection (MOI) of 500 with 2-fold serial dilutions of serum in 200 μ l reaction volumes. Following a 24-hour incubation, luciferase activity in the cells was measured using the Steady-Glo Luciferase Reagent System (Promega, Madison, WI) with a Victor 1420 Multilabel Counter (Perkin Elmer, Wellesley, MA). Neutralization titers were defined as the maximum serum dilution that neutralized 90% of luciferase activity. rAd5-Luc, rAd26-Luc, rAd35-Luc, and rAd48-Luc vectors exhibited comparable virus particle to plaque-forming unit (vp/pfu) ratios (10–30) and similar infectivity. NAb assays utilizing these four vectors demonstrated similar performance characteristics and dynamic ranges.

2.3 Animals and Immunizations

Adult Indian-origin rhesus monkeys (N=12) were housed in the bio-level 3 containment facility at New England Primate Research Center (NEPRC). Animals were inoculated twice separated by four weeks by the intranasal route with either 10^{11} viral particles (vp) replication-competent, E1-positive rAd26-Empty vectors (N=6) or saline (N=6). After four weeks, animals were vaccinated by the intramuscular route with 4×10^{10} vp replication-incompetent, E1/E3-deleted rAd26-Gag/Pol/Nef/Env vectors in the quadriceps muscles. Cellular immune responses were assessed by interferon- γ ELISPOT assays and multiparameter intracellular cytokine staining (ICS) assays essentially as described [13–14]. All animal studies were approved by the Harvard Medical School Institutional Animal Care and Use Committee (IACUC).

3. Results

3.1 Adenovirus NAb Titers in Multiple International Human Populations

We determined Ad5, Ad26, Ad35, and Ad48 NAb titers in 4,381 subjects in multiple international populations from North America, South America, sub-Saharan Africa, and Southeast Asia as detailed in Table 1. Ad-specific NAb titers were determined by luciferase-based virus neutralization assays as previously described [20]. Ad5, Ad26, and Ad35 NAb assays have been validated for use in clinical trials, whereas Ad48 NAb assays represent research assays. The vector characteristics, technical assay parameters, and assay performance and dynamic ranges were similar for the four vectors utilized in this study.

Table 2 depicts the raw seroepidemiology data for each population, and Figure 1 shows summary data. Ad5 seroprevalence was 87.9–89.5%, 90.5%, 86.4%, and 82.2% in adults in South Africa, Kenya, Uganda, and Thailand, respectively. In contrast, Ad26, Ad35, and Ad48 seroprevalence was significantly lower than Ad5 seroprevalence in all regions studied ($p < 0.0001$ for each population, chi-square test). Ad26 seroprevalence was 43.1–53.2%, 66.2%, 67.8%, and 54.6% in adults in South Africa, Kenya, Uganda, and Thailand, respectively. Ad35 seroprevalence was 10.6–17.8%, 14.8%, 5.4%, and 17.1% in these populations, and Ad48 seroprevalence was 13.3–24.6%, 51.0%, 50.0%, and 12.8% in these cohorts, respectively.

The majority of adults in these regions also exhibited high Ad5 NAb titers >200 (61.1–78.7%), and a substantial fraction had very high Ad5 NAb titers >1000 (25.1–46.8%). In contrast, markedly fewer individuals in these regions demonstrated high Ad26 NAb titers >200 (5.4–17.8%), Ad35 NAb titers >200 (0.4–5.0%), or Ad48 NAb titers >200 (0.9–10.8%), and only rare individuals had very high Ad26 NAb titers >1000 (0.0–3.8%), Ad35 NAb titers >1000 (0.0–1.7%), or Ad48 NAb titers >1000 (0.0–2.1%).

These data demonstrate that Ad35 seroprevalence and NAb titers were low in all regions studied. Ad48 seroprevalence was low in all regions except for East Africa. Ad26 seroprevalence was moderate in adults in the developing world, but Ad26 NAb titers proved consistently and substantially lower than Ad5 NAb titers in all regions studied. Moreover, Ad26 and Ad35 seroprevalence and NAb titers were negligible in infants (3–9 months old) and were low in children (6–12 years old) in South Africa, which represent potential ultimate target populations for an HIV-1 vaccine. NAb titers to each of these Ad serotypes appeared to be independent (data not shown).

Table 3 and Figure 2 depict the median and geometric mean Ad NAb titers in each geographic region. Median titers with interquartile ranges (IQRs) were calculated for each population, and geometric mean titers were determined among seropositives in each population. These data confirm that Ad26, Ad35, and Ad48 NAb titers were significantly

lower than Ad5 NAb titers in all regions studied ($p < 0.001$ for each region, ANOVA with post-hoc Dunnett correction). In particular, median (IQR) Ad5 NAb titers were 391 (94–1,069), 877 (259–2,369), 505 (77–1,501), and 521 (88–1,328), and geometric mean Ad5 NAb titers were 461, 967, 598, and 669 in adults in South Africa, Kenya, Uganda, and Thailand, respectively. In contrast, median (IQR) Ad26 NAb titers were 22 (18–65), 37 (18–115), 38 (18–132), and 30 (18–134), and geometric mean Ad26 NAb titers were 67, 97, 86, and 127 in these populations, respectively. Geometric mean Ad35 NAb titers were 75, 103, 50, and 86 in these regions, and geometric mean Ad48 NAb titers were 38, 90, 87, and 54 in these populations, respectively. These data show that median Ad26, Ad35, and Ad48 NAb titers were approximately 10-fold lower than median Ad5 NAb titers in these regions in the developing world. Median Ad26, Ad35 and Ad48 NAb titers were also substantially lower than median Ad5 NAb titers in the developed world.

3.2 Impact of Low Ad26 NAb Titers on rAd26 Vaccine Immunogenicity in Rhesus Monkeys

Given the moderately common but low titer Ad26 NABs in the developing world, we evaluated the potential impact of such Ad26 NAb titers on rAd26 vaccine vector immunogenicity in a pilot study in nonhuman primates. 12 adult rhesus monkeys were pre-immunized twice by the intranasal route with either 10^{11} vp replication-competent rAd26-Empty (N=6) or saline (N=6). Animals that received rAd26-Empty developed serum Ad26 NAb titers that were similar in magnitude to those found in humans in sub-Saharan Africa and Southeast Asia (median titer 102; geometric mean titer 85). Four weeks later, all animals were immunized once by the intramuscular route with 4×10^{10} vp of the rAd26-Gag/Pol/Env/Nef vaccine.

Env-specific antibody responses were assessed by ELISA, and Gag-, Pol-, Env-, and Nef-specific cellular immune responses were assessed by interferon- γ ELISPOT assays at week 2 following vaccination. As shown in Figure 3A, Env-specific binding antibody responses were comparable in monkeys with and without low baseline Ad26 NAb titers ($p = \text{NS}$, Mann-Whitney test). Similarly, as shown in Figure 3B, Gag-, Pol-, Env-, and Nef-specific cellular immune responses were comparable in monkeys with and without low baseline Ad26 NAb titers ($p = \text{NS}$, Mann-Whitney test). Humoral and cellular immune responses also proved comparable between groups at week 4 and week 12 following vaccination (data not shown).

We also evaluated Ad26 NAb titers in these monkeys both before and after rAd26 vaccination. As shown in Figure 3C, Ad26 NAb titers proved high (range 1,157 – 16,384) in the Ad26 pre-immunized monkeys following rAd26-Gag/Pol/Env/Nef vaccination. These titers were significantly higher than those observed in naïve monkeys following vaccination ($p = 0.002$, Mann-Whitney test) but did not detectably suppress rAd26-elicited humoral or cellular immune responses and did not alter the ratio of CD4/CD8 cellular immune responses (Figure 3D). These data demonstrate the dynamic range of the Ad26 NAb assay and confirm that the relatively low Ad26 NAb titers observed in human populations (Figures 1–2) did not simply reflect technical characteristics of the Ad26 NAb assay.

4. Discussion

Our data show that Ad26, Ad35, and Ad48 NAb titers were substantially lower than Ad5 NAb titers in multiple large international human populations (N=4,381). Ad35 NABs were rare in all populations studied, and Ad48 NABs were uncommon in all cohorts except for East Africa. In contrast, Ad26 NABs were moderately common in sub-Saharan Africa and Southeast Asia, but Ad26 NAb titers proved markedly lower than Ad5 NAb titers in all regions studied. To the best of our knowledge, this is the largest and most comprehensive international Ad seroepidemiology study to date.

Our findings confirm and extend prior studies showing that Ad35 seroprevalence and NAb titers were low in the developing world [6, 17]. Ad26 seroprevalence in adults in the present study proved somewhat higher than our initial estimate from a different population [8] and comparable with more recent estimates [16, 18–19]. However, this study shows that Ad26 NAb titers were markedly and consistently lower than Ad5 NAb titers across all geographic populations, risk groups, and age ranges studied. Our data also address several discrepancies between two recent studies that have compared Ad5 and Ad26 NAb titers in the developing world [16, 19]. For example, we find substantially higher magnitude Ad5 NAb titers in sub-Saharan Africa and higher Ad26 seroprevalence in Southeast Asia than reported by one of these groups [19]. We suspect that these differences may relate to differences in the sample sizes and specific assays utilized. Additional novel features of the present study that are not addressed in these previous reports [16, 19] include direct comparisons of Ad NAb titers in infants, schoolchildren, and adults in South Africa; NAb titers to Ad35 and Ad48 in addition to Ad5 and Ad26; and Ad NAb titers in low and high HIV-1 risk cohorts in both the United States and Africa.

The low Ad26 and Ad35 seroprevalence and NAb titers in pediatric populations suggest that seroconversion to these Ads is an age-dependent process. We previously reported that Ad5 seroconversion was also age-dependent, with maternal antibodies disappearing by approximately 6 months of age and high Ad5 NAb titers often developing by age 2 in sub-Saharan Africa [15]. Our current data from infants, schoolchildren, and adults from South Africa suggest that Ad26 and Ad35 NAb titers increase at a slower rate than do Ad5 NAb titers, likely reflecting a lower rate of natural infection with these serotypes. These data may be relevant for HIV-1 vaccine development, since infants and pre-adolescents represent potential ultimate vaccine target populations.

We also observed that relatively low baseline Ad26 NAb titers did not detectably suppress humoral or cellular immune responses elicited by a rAd26-Gag/Pol/Env/Nef vaccine in rhesus monkeys. Baseline Ad26-specific T cell responses were also induced in these animals (data not shown), although the impact of Ad-specific cellular immune responses on rAd vector immunogenicity remains unclear. Although these data were derived utilizing a preclinical model, analogous rhesus monkey immunogenicity studies [1–2] accurately predicted the suppressive impact of high titers but not low titers of Ad5 NAb titers on rAd5 vaccine immunogenicity in humans [3–5, 21], thus suggesting the potential clinical relevance of these data. However, the nonhuman primate model also has several important differences from the natural human setting, including a substantially shorter time interval between pre-exposure and vaccination and poor replication of the rAd26-Empty vector in this host species. Clinical trials are therefore required to determine the utility of these rAd vectors as vaccine candidates in humans.

The phase 2b Step study (HVTN 502/Merck 023) demonstrated that rAd5 vectors expressing clade B HIV-1 Gag/Pol/Nef antigens failed to afford protection in high-risk individuals in North and South America, the Caribbean, and Australia and may have increased HIV-1 acquisition risk in certain subgroups [3]. Alternative human serotype rAd vectors, such as rAd26 and rAd35, are therefore being explored as alternatives and are currently in phase 1 clinical trials. Our studies show that baseline NAb titers to these serotypes are substantially lower than baseline Ad5 NAb titers in multiple populations worldwide. These data suggest that further clinical studies are warranted with these and other rAd vectors in both the developing world and in the developed world.

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Figure 1. Ad5, Ad26, Ad35, and Ad48 NAb Titers

Ad5, Ad26, Ad35, and Ad48 Nab titers are shown for each population (Table 1) and are stratified in the following titers: <18 (negative), 18–200, 200–1000, and >1000. HR=high HIV-1 risk, LR=low HIV-1 risk.

Figure 2. Median and Geometric Mean Ad NAb Titers

Median and interquartile ranges are shown as box and whisker plots for each population (Table 1). Titers of 18, 200, and 1000 are indicated by dashed lines. Raw data and geometric mean titers (red bar) are also shown in vector seropositives. Data are summarized from

populations in (A) South Africa, (B) additional African and Asian regions, and (C) North and South America. HR=high HIV-1 risk, LR=low HIV-1 risk.

Figure 3. Immunogenicity of rAd26-Gag/Pol/Env/Nef in Rhesus Monkeys

Monkeys either with (N=6) or without (N=6) low baseline Ad26 NAb titers (18–200) were immunized once with 4×10^{10} vp rAd26-Gag/Pol/Env/Nef. (A) Env ELISA responses and (B) Gag/Pol/Env/Nef IFN- γ ELISPOT responses are shown at week 2 following immunization. Monkey numbers and baseline Ad26 NAb titers are shown for each animal. (C) Ad26 NAb titers and median titers (bar) are shown before (Pre) and at week 2 after (Post) immunization in animals with and without baseline Ad26 NAb titers. (D) CD4/CD8 ratios of Gag- and Env-specific IFN- γ ELISPOT responses and median responses (bar) are depicted in animals with and without baseline Ad26 NAb titers.

Table 1
Study populations for adenovirus seroepidemiology studies

Age groups include infants (3–9 months), schoolchildren (6–12 years), and adults (18–40 years). Geographic region, HIV-1 risk group, study population, and number of subjects (N) are also depicted for each population.

Age Group	Region	Risk Group	Study Population	N
Infants	South Africa	Low Risk	Community	149
Children	South Africa	Low Risk	Community	185
Adults	South Africa-1	High Risk	IAVI B	477
Adults	South Africa-2	High Risk	HSV-2 PIP	171
Adults	South Africa-3	High Risk	MIRA	903
Adults	Africa (Various)	Low Risk	Community	199
Adults	Kenya	High Risk	IAVI B	263
Adults	Uganda	Low Risk	RV 172	242
Adults	Thailand	High Risk	VAX 003 [22]	438
Adults	United States	Low Risk	IPCAVD 001	222
Adults	United States	High Risk	VAX 004 [23]	727
Adults	North/South America	High Risk	HVTN 502 / Merck 023 [3]	405
Total				4,381

Table 2

Neutralizing antibodies against Ad5, Ad26, Ad35 and Ad48

Numbers (and percentages) of individuals for each population are shown stratified by NAb titers of <18, 18–200, 200–1000, and >1000.

	SA infants	SA children	SA adults-1 high-risk	SA adults-2 high-risk	SA adults-3 high-risk	Africa low-risk	Kenya high-risk	Uganda low-risk	Thailand high-risk	US low-risk	US high-risk	N/S America high-risk
Ad5, total n (%)	ND	185 (100)	477 (100)	171 (100)	903 (100)	199 (100)	263 (100)	242 (100)	438 (100)	192 (100)	727 (100)	405 (100)
<18		63 (34.1)	54 (11.3)	18 (10.5)	109 (12.1)	33 (16.6)	25 (9.5)	33 (13.6)	78 (17.8)	118 (61.5)	451 (62.0)	195 (48.1)
18–200		17 (9.2)	120 (25.2)	46 (26.9)	242 (26.8)	43 (21.6)	31 (11.8)	54 (22.3)	66 (15.1)	22 (11.5)	60 (8.3)	44 (10.9)
200–1000		25 (13.5)	170 (35.6)	64 (37.4)	312 (34.6)	54 (27.1)	84 (31.9)	66 (27.3)	154 (35.2)	13 (6.8)	113 (15.5)	82 (20.2)
>1000		80 (43.2)	133 (27.9)	43 (25.1)	240 (26.5)	69 (34.7)	123 (46.8)	89 (36.8)	140 (32.0)	39 (20.3)	103 (14.2)	84 (20.7)
Ad26, total n (%)	149 (100)	185 (100)	477 (100)	171 (100)	903 (100)	199 (100)	263 (100)	242 (100)	438 (100)	222 (100)	727 (100)	405 (100)
<18	148 (99.3)	136 (73.5)	233 (48.8)	80 (46.8)	514 (56.9)	114 (57.3)	89 (33.8)	78 (32.2)	199 (45.4)	203 (91.4)	617 (84.9)	302 (74.6)
18–200	1 (0.7)	33 (17.8)	216 (45.3)	78 (45.6)	340 (37.7)	77 (38.7)	132 (50.2)	129 (53.3)	161 (36.8)	12 (5.4)	84 (11.6)	83 (20.5)
200–1000	0 (0)	15 (8.1)	25 (5.2)	13 (7.6)	41 (4.5)	8 (4.0)	32 (12.2)	31 (12.8)	67 (15.3)	7 (3.2)	22 (3.0)	16 (4.0)
>1000	0 (0)	1 (0.5)	3 (0.6)	0 (0)	8 (0.9)	0 (0)	10 (3.8)	4 (1.7)	11 (2.5)	0 (0)	4 (0.6)	4 (1.0)
Ad35, total n (%)	149 (100)	180 (100)	477 (100)	171 (100)	903 (100)	199 (100)	263 (100)	242 (100)	438 (100)	192 (100)	727 (100)	401 (100)
<18	149 (100)	154 (85.5)	392 (82.2)	149 (87.1)	807 (89.4)	156 (78.4)	224 (85.2)	229 (94.6)	363 (82.9)	181 (94.3)	672 (92.4)	370 (92.3)
18–200	0 (0)	23 (12.8)	64 (13.4)	18 (10.5)	66 (7.3)	37 (18.6)	26 (9.9)	12 (5.0)	53 (12.1)	11 (5.7)	35 (4.8)	23 (5.7)
200–1000	0 (0)	3 (1.7)	13 (2.7)	4 (2.3)	24 (2.7)	6 (3.0)	13 (4.9)	1 (0.4)	18 (4.1)	0 (0)	13 (1.8)	6 (1.5)
>1000	0 (0)	0 (0)	8 (1.7)	0 (0)	6 (0.7)	0 (0)	0 (0)	0 (0)	4 (0.9)	0 (0)	7 (1.0)	2 (0.5)
Ad48, total n (%)	ND	178 (100)	477 (100)	171 (100)	903 (100)	199 (100)	263 (100)	242 (100)	438 (100)	192 (100)	726 (100)	405 (100)
<18		166 (93.3)	362 (75.9)	129 (75.4)	783 (86.7)	184 (92.5)	129 (49.0)	121 (50.0)	382 (87.2)	187 (97.4)	637 (87.7)	342 (84.4)
18–200		10 (5.6)	105 (22.0)	39 (22.8)	112 (12.4)	13 (6.5)	108 (41.1)	95 (39.3)	51 (11.6)	5 (2.6)	72 (9.9)	54 (13.3)
200–1000		2 (1.1)	8 (1.7)	3 (1.8)	7 (0.8)	1 (0.5)	21 (8.0)	21 (8.7)	3 (0.7)	0 (0)	12 (1.7)	8 (2.0)
>1000		0 (0)	2 (0.4)	0 (0)	1 (0.1)	1 (0.5)	5 (1.9)	5 (2.1)	2 (0.5)	0 (0)	5 (0.7)	1 (0.2)

Table 3
Descriptive statistics on neutralizing antibodies against Ad5, Ad26, Ad35 and Ad48

Median neutralizing antibody titers and interquartile ranges (IQR) as well as percentages of subjects with titers above the level of quantification (>18) for each population are shown. Geometric mean titers (GMT) and ranges are calculated among those subjects with quantifiable titers >18.

	SA infants	SA children	SA adults-1 high-risk	SA adults-2 high-risk	SA adults-3 high-risk	Africa low-risk	Kenya high-risk	Uganda low-risk	Thailand high-risk	US low-risk	US high-risk	N/S America high-risk
Ad5, n	ND	185	477	171	903	199	263	242	438	192	727	405
Median, IQR		493 (18-2528)	410 (102-1169)	391 (94-1069)	366 (75-1106)	506 (73-1726)	877 (259-2369)	505 (77-1501)	521 (88-1328)	18 (18-346)	18 (18-388)	25 (18-723)
Positive (>18), n (%)		122 (65.9)	423 (88.7)	153 (89.5)	794 (87.9)	166 (83.4)	238 (90.5)	209 (86.4)	360 (82.2)	74 (38.5)	276 (38.0)	210 (51.9)
GMT (range) amongst positives		1457 (21-16384)	509 (19-16384)	461 (20-16384)	458 (19-16384)	620 (20-16384)	967 (19-16384)	598 (19-16386)	669 (20-16384)	643 (20-16384)	537 (19-16384)	636 (19-16384)
Ad26, n	149	185	477	171	903	199	263	242	438	222	727	405
Median, IQR	18 (18-18)	18 (18-21)	20 (18-55)	22 (18-65)	18 (18-49)	18 (18-40)	37 (18-115)	38 (18-132)	30 (18-134)	18 (18-18)	18 (18-18)	18 (18-19)
Positive (>18), n (%)	1 (0.7)	49 (26.5)	244 (51.2)	91 (53.2)	389 (43.1)	85 (42.7)	174 (66.2)	164 (67.8)	239 (54.6)	19 (8.6)	110 (15.1)	103 (25.4)
GMT (range) amongst positives	32 (3-32)	96 (19-1044)	63 (19-1709)	67 (19-682)	69 (19-16384)	61 (19-851)	97 (19-4241)	86 (19-1568)	127 (19-3691)	134 (21-768)	94 (20-1190)	85 (19-5527)
Ad35, n	149	180	477	171	903	199	263	242	438	192	727	401
Median, IQR	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)
Positive (>18), n (%)	0 (0)	26 (14.4)	85 (17.8)	22 (12.9)	96 (10.6)	43 (21.6)	39 (14.8)	13 (5.4)	75 (17.1)	11 (5.7)	55 (7.6)	31 (7.7)
GMT (range) amongst positives	-	51 (19-512)	109 (19-16384)	75 (21-783)	111 (19-5440)	55 (20-790)	103 (26-315)	50 (20-579)	86 (19-4308)	40 (19-78)	166 (21-4798)	76 (19-1099)
Ad48, n	ND	178	477	171	903	199	263	242	438	192	726	405
Median, IQR		18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)	19 (18-75)	19 (18-69)	18 (18-18)	18 (18-18)	18 (18-18)	18 (18-18)
Positive (>18), n (%)		12 (6.7)	115 (24.1)	42 (24.6)	120 (13.3)	15 (7.5)	134 (51.0)	121 (50.0)	56 (12.8)	5 (2.6)	89 (12.3)	63 (15.6)
GMT (range) amongst positives		62 (22-455)	49 (19-16384)	38 (19-647)	47 (19-1484)	75 (22-3506)	90 (19-9116)	87 (19-16384)	54 (19-3940)	47 (26-116)	86 (19-11279)	67 (20-16384)