Comparison of Two Genotyping Methods for Distinguishing Recrudescence from Reinfection in Antimalarial Drug Efficacy/Effectiveness Trials

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Abstract.

Genotyping of allelic variants of *Plasmodium falciparum* merozoite surface proteins 1 and 2 (*msp-1* and *msp-2*), and the glutamate-rich protein is the gold standard for distinguishing reinfections from recrudescences in antimalarial drug trials. We compared performance of the recently developed 24-single-nucleotide polymorphism (SNP) Barcoding Assay against *msp-1* and *msp-2* genotyping in a cluster-randomized effectiveness trial of artemether–lumefantrine and dihydroartemisinin–piperaquine in Malawi. Rates of recrudescence and reinfection estimated by the two methods did not differ significantly (Fisher's exact test; $P = 0.887$ and $P = 0.768$, respectively). There was a strong agreement between the two methods in predicting treatment outcomes and resolving the genetic complexity of malaria infections in this setting. These results support the use of this SNP assay as an alternative method for correcting antimalarial efficacy/effectiveness data.

INTRODUCTION

In areas of intense malaria transmission, drug-treated malaria patients are at high risk of reinfection during long follow-up post-treatment. Without genotyping, pretreatment, and post-treatment parasites, it is difficult to resolve whether parasites persisting after therapy are due to treatment failure (recrudescence) or a new infection (reinfection) and to provide the true risk of treatment failure in the population.[1]

Genotyping of allelic variants of *Plasmodium falciparum* merozoite surface proteins 1 and 2 (*msp-1* and *msp-2*), and glutamate-rich protein is the recommended genotyping method.[1,2] However, it is labor intensive, has low discriminatory power, and produces results that are often ambiguous to interpret and reproduce between laboratories.[3] Microsatellite genotyping is an alternative approach.[4–6] However, the lack of capillary sequencers to amplify and score microsatellites has hampered its wide use. The 24-single-nucleotide polymorphism (SNP) Barcoding Assay has shown great potential[7] but requires expensive reagents and real-time PCR

instruments. We compared the performance of the 24-SNP Barcoding Assay and *msp-1* and *msp-2* genotyping in an effectiveness trial.

METHODS

This study was part of a trial exploring neuro-ototoxic adverse effects in children repeatedly treated with artemisinin-based combination therapies (NCT01038063). Ethical approvals were obtained from Liverpool School of Tropical Medicine Research Ethics Committee (Protocol 09.07), University of Malawi College of Medicine Research and Ethics Committee (Protocol P.10/08/707), and Malawi's Pharmacy, Medicines and Poisons Board (Protocol PMPB/CTRC/III/1211200904).

Children with uncomplicated malaria were randomized to receive artemether–lumefantrine (AL) or dihydroartemisinin–piperaquine (DHA-PPQ) and followed up for 42 days. A filter paper blood sample was collected before treatment and 42 days posttreatment regardless of day 42 slide positivity.

To determine if a child had recurrent parasitemia on day 42, parasite DNA was extracted from d0 and d42 samples using DNA Mini Kits (Qiagen, United Kingdom) and genotyped using the 24-SNP Barcoding Assay, and *msp-1* and *msp-2* genotyping as previously described.[2,7] Investigators genotyping samples were blinded to d42 slide positivity. Infections with ≥ 2 and ≤ 1 heterozygous SNPs were classified as multiple- and single-haplotype infections, respectively.[8] We performed a loci resampling analysis in GenClone v.2.0[9] to determine the minimum number of SNPs required to capture full haplotypic diversity amongst single-haplotype infections sampled.

Recurrent parasitemia was considered a reinfection if d0 and d42 parasites were genetically distinguishable; otherwise, it was deemed a reinfection. All proportions and their binomial exact 95% confidence intervals (CIs) were computed using Stata version 11.0 (College Station, TX).

RESULTS AND DISCUSSION

We evaluated 109 pairs of filter paper blood samples collected on days 0 and 42. Of these, 65% ($N = 71$) showed no detectable parasite DNA on d42, whereas 38 had recurrent d42 parasitemia. Detailed effectiveness data for the trial will be presented elsewhere (Terlouw et al., unpublished data). Genotype data and treatment outcomes for 38 patients with recurrent parasitemia are shown in Supplemental Tables 1 and 2, whereas genotype data for 71 patients with no detectable parasite DNA on d42 are shown in Supplemental Tables 3 and 4. A sample size of 38 recurrent infections allows us to detect a 34% difference in rates of reinfection estimated by the two methods with 80% power and 95% CI. Repeat *msp-1* and *msp-2* genotyping was performed on ∼20% of samples because of contamination in the negative control or failure to amplify some loci during the initial genotyping attempt. However, genotyping failure rate for the 24-SNP Barcoding Assay was low with > 95% of SNP assays yielding data at the first genotyping attempt and < 5% allele drop out per sample.

Rates of reinfection and treatment failure did not differ significantly between methods (Fisher's exact test; $P = 0.887$ and $P = 0.768$, respectively) (Figure 1A). There was a strong concordance between the two methods in predicting treatment responses among all the 109 patients evaluated and in 38 patients with recurrent d42 parasitemia (Figure 1B). There was also a strong agreement between the two methods in determining the clonality of parasite samples

(whether a sample is monoclonal or multiclonal) (Figure 1B). The proportion of multiclonal samples was similar between methods (Supplemental Figure 1). Relationships among 62 monoclonal samples identified using the 24-SNP Barcoding Assay are shown in the phylogenetic tree (Supplemental Figure 2). We observed a modest concordance of 56.5% (binomial exact 95% CI: 48.0–64.6) between the two methods in estimating the multiplicity of infection for individual samples (Figure 1B). This presumably reflects subtle differences in the resolution power of the two assays. Treatment failure rate was 6.4% by the 24-SNP Barcoding Assay and 4.6% by *msp-1* and *msp-2* genotyping ($P = 0.768$). The small discrepancy between recrudescence rates estimated by the two methods resulted from classifying two recurrent infections, which were otherwise considered as reinfections by *msp-1* and *msp-2* genotyping, because of treatment failures caused by using SNP genotyping (Supplemental Tables 1 and 2). Treatment failures observed may be explained by nonadherence, pharmacokinetic variations, parasite resistance, and/or drug loss through vomiting. Study participants were given a full course of AL or DHA-PPQ with only the first dose given under supervision. This may promote noncompliance but accurately represents how drugs might be used in the community. In a previous study, 79% and 88% of AL- and DHA-PPQ–treated patients complied with recommended drug dosing schedules, respectively.[13]

High rates of reinfection are of concern. Both genotyping methods showed that ∼30% of children treated for malaria are reinfected within 42 days post-treatment. This finding indicates that the intensity of transmission is very high. Compared with DHA-PPQ, AL is associated with higher risk of recurrent parasitemia[14,15] attributable to shorter elimination half-life of the partner drug, lumefantrine. However, an ACT such as DHA-PPQ, with a long elimination half-life of the partner drug, may still fail to protect against reinfections if overwhelmed by intense transmission levels.[14] To help reduce malaria transmission, new transmission reduction strategies such as mass drug administration, focal screening and treatment, or mass screening and treatment should be considered.[16]

Our findings clearly demonstrate that the 24-SNP Barcoding Assay performs *msp-1* and *msp-2* genotyping. The main advantage of *msp-1* and *msp-2* genotyping is its low cost. We estimate that genotyping costs $11.45/sample versus $3.60/sample for the 24-SNP Barcoding Assay and *msp-1* and *msp-2* genotyping, respectively. Unlike the 24-SNP Barcoding Assay that relies on expensive real-time PCR instruments, *msp-1* and *msp-2* genotyping uses relatively inexpensive and common laboratory equipment such as gel electrophoresis equipment and UV transilluminators to genotype samples. Nonetheless, inherent limitations of *msp-1* and *msp-2* genotyping outweigh its low-cost attractiveness. This method is extremely labor intensive, prone to contamination, has limited resolution power, and generates data that are often ambiguous to interpret and reproduce between different laboratories because of dependency on visual interpretation of allele migration patterns on agarose gels. In contrast, the 24-SNP Barcoding Assay is less labor intensive, has better resolution power, and generates data that are easy to score and reproduce between laboratories. The 24-SNP Barcoding Assay has better discriminatory power because it interrogates 24 highly polymorphic SNPs rather than two *msp-1* and *msp-2* loci. Because of its excellent attributes, the 24-SNP Barcoding Assay should be adopted as an alternative genotyping method. However, high cost could derail its adoption. We investigated whether an abbreviated SNP set with fewer SNPs could equally identify all parasite haplotypes as 24 SNPs. Our results indicate that 17 SNPs, irrespective of their minor allele frequencies within the 62 single-haplotype infections identified, can reliably capture all parasite haplotypes identified by 24 SNPs (Figure 2, Supplemental Table 1). Our data also indicate that if SNPs with a high minor allele frequency (≥ 0.30) are selected, only 12 of these are required to

identify all parasite haplotypes (Figure 2, Supplemental Table 1). It would cost $5.73 to genotype a single sample using the abbreviated SNP assay. Reduction in cost and availability of real-time instruments in most countries make the abbreviated SNP assay attractive and feasible to adopt.

CONCLUSION

Our results demonstrate that the 24-SNP Barcoding Assay performs *msp-1* and *msp-2* genotyping and should be adopted as an alternative method for PCR adjustment of antimalarial effectiveness/efficacy data. Resource-constrained laboratories should consider deploying an abbreviated SNP assay comprising 12 SNPs with high minor allele frequency to reduce genotyping costs while maintaining high assay resolution. Each continent must identify SNPs with high minor allele frequency to select informative SNPs.

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FIGURE 1. Comparison of two genotyping methods. (**A**) Rates of reinfection and recrudescence estimated by the 24-single-nucleotide polymorphism (SNP) Barcoding Assay and merozoite surface proteins 1 and 2 (*msp-1* and *msp-2*) genotyping. The number on top of each bar represents number of patients with a defined treatment outcome out of 109 patients evaluated. Rates of reinfection and recrudescence estimated by the two methods were similar (Fisher's exact test; $P = 0.887$ and $P = 0.768$, respectively). (**B**) Agreement between methods in determining treatment outcomes, infection clonality, and multiplicity of infection. Figures on top of each bar are percentages of concordant samples out of all samples analyzed in square brackets. Multiplicity of infection was determined from SNP data of each sample using COIL[17] and from *msp-1* and *msp-2* data as the highest number of alleles observed at the most diverse locus. In both **A** and **B**, error bars are binomial exact 95% confidence intervals.

FIGURE 2. Resolution power of the 24-single-nucleotide polymorphism (SNP) Barcoding Assay inferred from SNP resampling. The gray line shows maximum haplotype diversity captured when all 24 SNPs are used to characterize diversity, whereas the black line indicates diversity identified when only SNPs with a high minor allele frequency (≥ 0.30) are used. Error bars are 95% confidence intervals for the mean number of parasite haplotypes identified. Diversity plateaus after 17 and 12 loci if all 24 SNPs and SNPs with high minor allele frequency are used to genotype samples, respectively, indicating the assay's sufficient discriminatory power.

SUPPLEMENTAL FIGURE 1. Proportion of multiclonal samples estimated by the 24-single-nucleotide polymorphism (SNP) Barcoding Assay compared with that determined by merozoite surface proteins 1 and 2 (*msp-1* and *msp-2*) genotyping. The proportion of multiclonal samples in a combined set of pretreatment and posttreatment samples ($N = 147$) and in pretreatment samples alone ($N = 109$) did not differ significantly between the two methods (Fisher's exact test; $P = 0.186$ and $P = 0.094$, respectively). Error bars are binomial exact 95% confidence intervals for the proportion of multiclonal samples. The number on top of each bar represents the number of multiclonal samples detected by each of the two genotyping methods. For example, the proportion of pretreatment samples deemed to be multiclonal by the 24-SNP Barcoding Assay equals 61/109 = 0.560.

SUPPLEMENTAL FIGURE 2. UPGMA tree showing relationships between parasite haplotypes identified by the 24-single-nucleotide polymorphism (SNP) Molecular Barcode Assay. We computed the proportion of SNP alleles shared (ps) between all pairwise comparisons of single-haplotype infections sampled and clustered infections on the UPGMA tree based on the genetic distance metric, 1-ps, using PHYLIP.[18] Only data for single-haplotype parasite infections are shown because allele-sharing can be unambiguously computed. Pretreatment episodes of parasitemia in patients 20 and 71 (i.e., 20d0 and 71d0) have the same parasite DNA fingerprint as their respective posttreatment, episodes 20d42 and 71d42. Therefore, recurrent episodes of parasitemia in patients 20 and 71 are treatment failures. On the other hand, posttreatment episodes of parasitemia in patients 18 and 40 (i.e., 18d42 and 40d42) are genetically different from pretreatment episodes (18d0 and 40d0). These are a classical case of reinfection.

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SUPPLEMENTAL TABLE 1

SNP data for recurrent infections

Patient ID	Day	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11	SNP12	SNP13	SNP14	SNP15	SNP16	SNP17	SNP18	SNP19	SNP20	SNP21	SNP22	SNP23	SNP24	Treatment outcome	No. of heterozygous SNPs	Clonality	MOI	95% confidence interval for MOI	Probability for MOI
103d0	0	T	A	C	C	C	G	A	G	A	T	C	G	T	A	C	C	C	T	C	A	A	T	T	G	–	0	S	1	[1, 1]	1
103d42	42	–	A	C/T	C/T	C/G	G	G	G	A	T	C/T	A/G	T	C	C	C	C	A/T	C	A	A/C	C	G	G	Reinfection	7	M	2	[2, 2]	0.9954
10d0	0	T	A	C	C/T	C/G	G	A	G	A/T	C	C	G	C	A	C	C	C/T	A	A	G	C	T	T	G	–	4	M	2	[1, 2]	0.8355
10d42	42	T	A	C	C/T	C/G	G	A	G	A/T	C	C	G	C	A	C	C	C/T	A	A	G	C	T	T	G	Treatment failure	4	M	2	[1, 2]	0.8355
11d0	0	T	A	C/T	C/T	C	C/G	A/G	A/G	A/T	C	C	A/G	T	A	C	C	C/T	A/T	A	A/G	A/C	C/T	T	G	–	12	M	2	[2, 4]	0.5617
11d42	42	T	A	T	T	C	C	G	G	T	C	T	G	C	A	A	C	C	A	A	G	C	C	T	G	Reinfection	0	S	1	[1, 1]	1
12d0	0	T	A	T	T	C	G	A	G	T	C	C	G	C	A	C	C	C	A	C	G	A	C	T	G	–	0	S	1	[1, 1]	1
12d42	42	T	A	C/T	T	C	C/G	A/G	G	A/T	C/T	C/T	A/G	C/T	A	C	A/C	C/T	A	A	A/G	A/C	C/T	G/T	G	Reinfection	14	M	3	[3, 5+]	0.5103
16d0	0	T	A	C/T	T	C	C/G	A	G	A/T	T	C	A/G	C/T	A	A/C	A/C	C	A	A	A/G	C	T	T	G	–	8	M	2	[2, 3]	0.9369
16d42	42	C	A	C	T	G	C	G	G	A	T	T	G	C	A	A	C	C	T	C	G	C	C	–	G	Reinfection	0	S	1	[1, 1]	1
17d0	0	C	A	C/T	C/T	C/G	C/G	A/G	G	A	C/T	C/T	A/G	C/T	A/C	A/C	A/C	C/T	A/T	A	G	A/C	C/T	T	G	–	16	M	4	[3, 5+]	0.4335
17d42	42	C	A	C	T	G	C	A	–	A	C/T	C/T	A	T	A/C	C	C	T	A	A	G	A/C	T	T	G	Treatment failure	4	M	2	[1, 2]	0.8514
18d	0	–	–	C	–	–	C	A	G	A	T	T	–	T	C	A	–	C	–	A	A	C	C	T	G	–	0	S	1	[1, 1]	0.9998

0																														
18d 42	42	T	A	C	T	G	C	G	G	A	C	C	G	C	A	A	C	T	A	C	G	C	C	T	G	Reinfection	0	S	1	[1, 1]	1
20d 0	0	T	A	T	T	C	G	A	G	A	T	T	G	C	A	A	C	C	A	A	G	C	T	T	G	–	0	S	1	[1, 1]	0.9999
20d 42	42	T	A	T	T	C	G	A	G	A	T	T	G	C	A	A	C	C	A	A	G	C	T	T	G	Treatment failure	0	S	1	[1, 1]	0.9999
2d0	0	T	A	C/T	T	C	C	A	G	A	C/T	C/T	A/G	C	A	C	C	C	A	A	G	A	T	G	G	–	4	M	2	[2, 2]	0.9719
2d42	42	T	A	C	T	C	C	A	G	A	T	C	A	C	A	C	A/C	T	T	A	G	A	T	T	G	Reinfection	1	S	1	[1, 1]	0.9996
30d 0	0	T	A	C	C/T	C	C	A	A/G	A	T	C/T	G	C/T	A	C	C	C	A/T	A	A	A/C	C/T	G/T	G	–	8	M	2	[2, 3]	0.9416
30d 42	42	C	A	C	T	C	C	A	G	A	T	C	G	C	A	C	C	T	A	A	G	A	C	G	G	Reinfection	0	S	1	[1, 1]	1
31d 0	0	T	A	C	C	C	C	G	G	A	T	–	A	C	A	C	C	C	A	A	G	C	C	T	G	–	0	S	1	[1, 1]	1
31d 42	42	T	A	T	T	C	C	A	G	A	T	C	G	C	A/C	C	C	C	A/T	A	A/G	A	T	T	G	Reinfection	3	M	1	[1, 2]	0.6299
32d 0	0	C	A	C/T	T	C	C	A/G	G	A	C/T	C	G	C	A	A	C	T	A	A	A/G	C	C/T	T	G	–	5	M	2	[2, 2]	0.993
32d 42	42	T	A	C	T	C/G	G	A/G	G	T	T	C/T	A	C/T	A/C	C	A	C/T	A	A	G	A/C	C/T	T	G	Reinfection	8	M	2	[2, 2]	0.9686
37d 0	0	C	A	C/T	C/T	C	C/G	A/G	G	A	T	C	G	C	A	A/C	C	C/T	A/T	A	A	C	C/T	T	G	–	8	M	2	[2, 3]	0.9497
37d 42	42	C/T	A	C/T	T	C	C/G	A/G	G	A/T	C/T	C/T	A/G	C	A	A/C	C	C	A	A	G	A/C	C/T	G/T	G	Reinfection	12	M	3	[2, 4]	0.6139
40d 0	0	T	A	C	T	C	C	G	G	A	C	C	A	C	A	C	C	C	T	A	A	A	C	G	G	–	0	S	1	[1, 1]	1
40d 42	42	T	A	T	T	C	G	A	G	A	T	T	G	C	A	C	A	C	T	A	G	C	T	G	G	Reinfection	0	S	1	[1, 1]	1
47d 0	0	C	A	C	T	C	G	G	G	A	T	C	A	C	A	C	C	C	A	A	G	A/C	C/T	G/T	G	–	3	M	1	[1, 2]	0.6478
47d 42	42	T	A	C	C	G	G	G	G	T	C	T	G	C	A	A	C	C	A	C	G	C	T	T	G	Reinfection	0	S	1	[1, 1]	1

48d0	0	T	A	C	C	G	C	G	G	A	C/T	T	A	T	A	A	C	C	A	A	A	C	C	T	G	–	1	S	1	[1, 1]	0.9999
48d42	42	T	A	C	T	C	C/G	A	G	A	C/T	T	A	C	–	–	–	–	–	–	–	–	–	–	G	Reinfection	2	M	2	[1, 3]	0.8519
49d0	0	T	A	C	C/T	C	G	A/G	G	A	C/T	C/T	A	C	A	C	C	C/T	A	A	A/G	C	C	T	G	–	6	M	2	[2, 2]	0.9889
49d42	42	T	A	C/T	T	C/G	C/G	G	G	A	C	T	A	C	A	A	A/C	C/T	A	A	A/G	C	C	T	G	Treatment failure	6	M	2	[2, 2]	0.992
4d0	0	T	A	C	C	C	C	A/G	G	A	T	T	G	C	C	C	C	C	T	A	A/G	C	T	T	G	–	2	M	1	[1, 1]	0.9857
4d42	42	T	A	C	T	C	C	A/G	G	A	C	T	G	C	A	A	C	C/T	T	C	A/G	A	C/T	G/T	G	Reinfection	5	M	2	[2, 2]	0.9915
53d0	0	C	A	C/T	T	C	C/G	A	G	A/T	C/T	C/T	A/G	T	A	C	C	C	A/T	A	A/G	C	T	T	G	–	8	M	2	[2, 3]	0.9473
53d42	42	C/T	A	T	C/T	C/G	G	A	G	A	T	C	G	C	C	A	A/C	C	A	C	G	A/C	C	T	G	Reinfection	5	M	2	[2, 2]	0.976
55d0	0	–	A	C	–	C	C	A	G	A	C	–	–	C	A	C	A	C/T	–	A	G	C	T	T	G	–	1	S	1	[1, 1]	0.9906
55d42	42	T	A	C	T	C	C/G	A/G	G	T	C/T	T	G	T	A	A	A	C	T	A	G	A/C	C/T	T	G	Reinfection	5	M	2	[2, 2]	0.9909
56d0	0	T	A/G	C/T	T	C	C/G	A/G	G	A/T	C/T	C/T	A/G	C/T	A	C	A/C	C	A/T	A	A/G	A/C	C/T	G/T	G	–	15	M	3	[3, 5+]	0.4084
56d42	42	C	A	C/T	C/T	C	C/G	A/G	G	A/T	T	C/T	G	C/T	A	C	C	C/T	A	A	A/G	A/C	C/T	T	G	Reinfection	11	M	2	[2, 3]	0.5907
57d0	0	T	A	C	C	C	G	A	G	A	C	T	A	C	A	C	C	C	A	A	G	A	C	T	G	–	0	S	1	[1, 1]	1
57d42	42	T	A	C	T	C	G	A	G	A	C	T	G	C	A	A	C	C	T	C	G	A	T	G/T	G	Reinfection	1	S	1	[1, 1]	0.9983
59d0	0	C	A/G	C/T	T	C/G	C/G	A/G	G	A/T	C/T	C	A/G	C/T	A/C	C	A/C	C/T	A/T	A	A/G	A/C	C/T	T	G	–	16	M	4	[3, 5+]	0.3749
59d42	42	C	A/G	C	T	–	G	A/G	A/G	A	C/T	C/T	A/G	C/T	C	C	A/C	C	A/T	A	G	A/C	C/T	G/T	G	Treatment failure	12	M	2	[2, 4]	0.4954
5d0	0	C	A	C/T	C/T	C	C/G	A	A/G	A	C/T	C/T	A/G	C	A/C	A/C	A/C	C/T	A	A	G	A/C	C/T	G/T	G	–	14	M	3	[2, 5+]	0.5362

5d42	42	T	A	C	T	C	C	G	G	T	T	C/T	A	C/T	A	A	C	T	T	C	G	C	T	T	G	Reinfection	2	M	1	[1, 1]	0.994
60d0	0	C	A	C	C/T	C	C	A/G	G	A	C/T	T	A/G	C	A	C	A/C	C/T	A	A	G	A/C	C/T	G/T	G	–	9	M	2	[2, 3]	0.8293
60d42	42	C/T	A	C/T	T	C/G	C/G	A/G	G	A	C/T	C/T	A/G	C/T	A/C	C	A/C	C/T	A/T	A	A/G	A/C	C/T	G/T	G	Treatment failure	17	M	5	[3, 5+]	0.4997
61d0	0	T	A	T	T	C/G	C	G	G	A	C/T	T	G	C	A	C	A	C	A/T	A	G	A/C	T	T	G	–	4	M	2	[1, 2]	0.9096
61d42	42	C	A	C	T	C	C	A	G	A	T	T	G	T	A	A	C	T	T	A	A	A	C	G	G	Reinfection	0	S	1	[1, 1]	1
62d0	0	T	A	T	C	G	C/G	G	G	T	T	C	A	T	A	C	C	T	A	A	G	C	C/T	T	G	–	2	M	1	[1, 1]	0.9998
62d42	42	C/T	A	C	C/T	C/G	C	A	G	A	C/T	C/T	A	C	A	C	C	T	A	C	A/G	A/C	C/T	G/T	G	Reinfection	9	M	2	[2, 3]	0.9053
63d0	0	C	A	C	T	G	C	G	G	A	T	T	A	T	A	A	C	C	A	A/C	G	C	T	T	G	–	1	S	1	[1, 1]	0.9996
63d42	42	C	A	C	T	C/G	C/G	A	G	A/T	C/T	T	A	T	C	C	C	T	A/T	C	G	C	T	G/T	G	Reinfection	6	M	2	[2, 2]	0.9965
64d0	0	T	A	T	T	C	C	G	G	T	C/T	T	G	T	A	A/C	C	C	A	C	G	A	C/T	T	G	–	3	M	1	[1, 2]	0.8251
64d42	42	T	A	C	T	C/G	C	A	G	A	C/T	T	G	C	C	C	A/C	T	A	A	G	C	C/T	T	G	Reinfection	4	M	2	[1, 2]	0.926
71d0	0	C	A	C	T	G	G	A	G	A	T	T	A	T	C	A	A	C	T	C	G	A	T	T	G	–	0	S	1	[1, 1]	1
71d42	42	C	A	C	T	G	G	A	G	A	T	T	A	T	C	A	A	C	T	C	G	A	T	T	G	Treatment failure	0	S	1	[1, 1]	1
72d0	0	T	A	C	T	C	C/G	G	G	A	T	C/T	G	C	A	A/C	C	T	A/T	A/C	G	A/C	T	T	G	–	6	M	2	[2, 2]	0.975
72d42	42	T	A	C	T	C	C	A	G	A	T	C	G	C	A/C	A	C	T	A/T	A	A/G	C	T	T	G	Reinfection	3	M	2	[1, 2]	0.5512
81d0	0	T	A	C/T	C/T	C/G	C/G	A	A/G	A	C/T	C/T	G	C/T	A/C	C	A/C	C/T	A	A	A/G	A/C	C	G/T	G	–	14	M	3	[2, 4]	0.5971
81d42	42	C	A	C	C	C	C	A	G	T	T	T	G	C	C	A	C	C	A	C	G	A	T	T	G	Reinfection	0	S	1	[1, 1]	1

87d0	0	C	A	C	T	C	C	A	A	A	T	T	A	C	C	C	C	C	T	C	G	C	T	G	G	–	0	S	1	[1, 1]	1
87d42	42	T	A	T	C	C	G	G	G	A/T	C/T	C/T	A	T	A	C	A	T	A	A	G	A/C	C/T	T	G	Reinfection	5	M	2	[1, 2]	0.9003
88d0	0	C	A	T	T	C	C	A	G	A/T	T	C	–	T	C	A	C	C	A	A	A/G	A/C	T	T	G	–	3	M	1	[1, 2]	0.8727
88d42	42	T	A	C	T	C/G	C	A	G	T	C	T	A/G	C/T	A/C	A/C	C	C	T	A	A/G	A/C	T	T	G	Reinfection	7	M	2	[2, 2]	0.9667
89d0	0	T	A	C	T	C	C	A	G	A	T	T	G	C	A	C	C	C	T	C	G	C	C	G	G	–	0	S	1	[1, 1]	1
89d42	42	T	A	T	T	G	C	A	G	A	C	T	A	C	A	A	C	T	A	C	G	C	T	G	G	Reinfection	0	S	1	[1, 1]	1
8d0	0	T	A	C	T	G	G	A	G	A	T	T	G	C	A	A	C	C	A	A	G	A	T	T	G	–	0	S	1	[1, 1]	0.9999
8d42	42	T	A	C	C	G	C	A	G	T	T	T	G	C	A	C	C	C	A/T	A	A/G	C	C	T	G	Reinfection	2	M	1	[1, 1]	0.9953
94d0	0	C/T	A/G	C/T	C/T	C/G	C/G	A/G	G	A/T	C/T	C/T	A/G	C/T	A	C	C	C/T	A/T	A	A/G	A/C	C/T	T	G	–	17	M	4	[3, 5+]	0.4262
94d42	42	T	A	C	T	C	C	A/G	G	A	T	T	G	C	A	A	A	C	T	A	G	A	T	G	G	Reinfection	1	S	1	[1, 1]	1
98d0	0	–	–	C/T	–	C/G	C/G	–	–	T	C	T	A/G	T	A	A	A/C	C/T	A/T	A	A/G	A/C	T	G	G	–	9	M	2	[2, 3]	0.79
98d42	42	–	A	–	–	C	G	–	G	T	C	T	G	C	A/C	C	C	T	A/T	C	G	A	C	G	G	Reinfection	2	M	1	[1, 1]	0.9872
DD2	N/A	T	A	T	C	C	G	G	A	T	T	T	A	T	C	A	A	T	A	C	A	A	C	G	T	N/A	0	S	1	[1, 1]	1
HB3	N/A	T	G	C	C	C	C	A	G	A	T	C	A	C	A	A	C	T	A	A	G	T	T	T	T	N/A	0	S	1	[1, 1]	1
K1	N/A	C	G	C	T	C	G	G	A	T	T	T	A	T	C	C	C	T	A	C	G	C	C	G	T	N/A	0	S	1	[1, 1]	1
R033	N/A	C	A	T	T	G	C	A	G	A	C	T	–	C	A	C	C	T	T	A	G	A	T	T	G	N/A	0	S	1	[1, 1]	1
W2	N/A	T	A	T	C	C	G	G	A	T	T	T	A	T	C	A	A	T	A	C	A	A	C	G	T	N/A	0	S	1	[1, 1]	1
3D7	N/A	C	G	C	T	C	C	G	G	A	C	T	G	C	A	C	C	C	A	A	G	A	T	T	G	N/A	0	S	1	[1, 1]	1

N/A = not applicable; SNP = single-nucleotide polymorphism. For each patient, both the d0 and d42 filter paper samples were genotyped using the 24-SNP Barcoding Assay (Daniels et al.).[7] Treatment outcomes inferred from genotyping the d0 and d42 samples are coded as "Reinfection" and "Treatment failure." Only data for 38 recurrent infections are shown. "–" denotes that an allele was not detected. No. of heterozygous SNPs is the number of loci out of the 24 genotyped that carry both alternate SNP alleles. Clonality denotes the genetic complexity of an infection, that is, whether the infection contains multiple-parasite haplotypes (M) or a single-parasite haplotype (S). MOI = multiplicity of infection as determined by the maximum likelihood method called COIL (Galinsky et al.).[17] Twelve SNPs highlighted in blue are proposed for the abbreviated SNP assay. Highlighted in green at the bottom of the table are SNP data for laboratory control parasites. Highlighted in red are alleles that allowed to conclude that the outcome for the paired samples was a "Reinfection."

SUPPLEMENTAL TABLE 2

Msp-1 and *msp-2* genotype data for recurrent infections

Patient ID	Day	MAD 20	MAD 20 fragment size	K1	K1 fragment size	RO33	R033 fragment size	3D7/IC	3D7/IC fragment size	FC27	FC27 fragment size	Treatment outcome	Clonality	MOI
103d0	0	–	–	†	200	–	–	††	500; 600	†	300	–	M	2
103d42	42	†	200	†	250	–	–	†	500	†	400	Reinfection	M	2
10d0	0	††	200; 300	†	150	–	–	†	500	†	300	–	M	3
10d42	42	††	200; 300	†	150	–	–	†	500	†	300	Treatment failure	M	3
11d0	0	†	300	†	200	–	–	††	300; 500	†	450	–	M	2
11d42	42	–	–	†	200	–	–	†	600	–	–	Reinfection	S	1
12d0	0	†	200	–	–	–	–	–	–	†	500	–	S	1
12d42	42	–	–	†	250	†	160	†††	300; 500; 700	†††	400; 300; 500	Reinfection	M	3
16d0	0	–	–	†	200	–	–	†	350	†	400	–	M	2
16d42	42	†	200	–	–	–	–	†	500	–	–	Reinfection	S	1
17d0	0	†	250	††	200; 300	†	160	††	350; 400	–	–	–	M	3
17d42	42	†	200	†	250	†	160	†	500	–	–	Reinfection	M	3
18d0	0	–	–	–	–	†	160	†	550	–	–	–	S	1
18d42	42	†	200	–	–	–	–	†	300	–	–	Reinfection	S	1
20d0	0	†	200	–	–	–	–	–	–	†	300	–	S	1
20d42	42	†	200	–	–	–	–	–	–	†	300	Treatment failure	S	1
2d0	0	†	200	†	200	–	–	†	500	–	–	–	M	2
2d42	42	–	–	–	–	†	160	–	–	†	300	Reinfection	S	1
30d0	0	†	200	†	200	–	–	†	500	††	200; 300	–	M	2
30d42	42	†	200	–	–	–	–	†	500	–	–	Treatment failure	S	1
31d0	0	–	–	†	200	–	–	–	–	†	350	–	S	1
31d42	42	–	–	††	200; 300	–	–	††	300; 500	††	200; 300	Reinfection	M	2
32d0	0	†	200	†	300	†	160	†††	300; 500; 600	†††	300; 400; 450	–	M	3
32d42	42	–	–	†	250	–	–	–	–	†	400	Reinfection	S	1
37d0	0	–	–	†	150	–	–	†	500	†	250	–	M	2
37d42	42	†	150	†	250	–	–	†	400	†	350	Reinfection	M	2
40d0	0	–	–	–	–	†	160	–	–	†	350	–	S	1
40d42	42	–	–	†	200	–	–	–	–	†	300	Reinfection	S	1
47d0	0	†	200	–	–	–	–	†	250	†	250	–	M	2
47d42	42	–	–	†	200	–	–	†	500	–	–	Reinfection	S	1
48d0	0	–	–	†	200	–	–	–	–	†	220	–	S	1
48d42	42	†	200	–	–	–	–	†	500	†	350	Reinfection	M	2
49d0	0	–	–	†	200	–	–	†	500	†	400	–	M	2
49d42	42	–	–	†	250	–	–	†	600	†	250	Reinfection	M	2
4d0	0	–	–	–	–	†	160	†	600	†	400	–	M	2
4d42	42	–	–	–	–	†	160	†	350	†	300	Reinfection	M	2

53d0	0	†	200	†	250	–	–	†	500	†	400	–	M	2
53d42	42	†	200	††	200; 300	–	–	†	500	–	–	Reinfection	M	2
55d0	0	††	200; 300	–	–	–	–	†	700	†	350	–	M	2
55d42	42	†	200	†	300	–	–	†	600	†	300	Reinfection	M	2
56d0	0	†	200	†	200	–	–	††	300; 400	††	350; 450	–	M	2
56d42	42	†	200	†	250	–	–	†	400	–	–	Reinfection	M	2
57d0	0	–	–	–	–	†	160	†	500	††	300; 500	–	M	2
57d42	42	–	–	–	–	†	160	–	–	†	300	Treatment failure	S	1
59d0	0	†	200	–	–	–	–	†	600	†	400	–	M	2
59d42	42	–	–	†	250	–	–	†	500	†	300	Reinfection	M	2
5d0	0	–	–	††	200; 250	–	–	–	–	†	350	–	M	2
5d42	42	†	200	†	300	–	–	†	500	†	400	Reinfection	M	2
60d0	0	†	200	†	300	–	–	†	600	–	–	–	M	2
60d42	42	–	–	†	200	–	–	–	–	†	300	Reinfection	S	1
61d0	0	†	200	–	–	–	–	†	600	†††	300; 350; 400	–	M	3
61d42	42	–	–	†	200	–	–	–	–	†	350	Reinfection	S	1
62d0	0	††	200; 300	†	300	–	–	–	–	†	400	–	M	2
62d42	42	†	250	–	–	–	–	†	500	†	350	Reinfection	M	2
63d0	0	–	–	†	250	–	–	†	700	†	350	–	M	2
63d42	42	††	200; 300	†	200	–	–	†	500	–	–	Reinfection	M	2
64d0	0	–	–	–	–	†	160	†	500	†	600	–	M	2
64d42	42	–	–	–	–	†	160	†	600	–	–	Reinfection	S	1
71d0	0	†	200	–	–	–	–	†	500	†	300	–	M	2
71d42	42	†	200	–	–	–	–	†	500	†	300	Treatment failure	M	2
72d0	0	–	–	†	200	–	–	†	700	†	450	–	M	2
72d42	42	†	200	†	250	–	–	†	500	†	400	Reinfection	M	2
81d0	0	††	200; 250	†	250	–	–	–	–	†	400	–	M	2
81d42	42	–	–	–	–	†	160	–	–	†	300	Reinfection	S	1
87d0	0	†	200	–	–	–	–	†	500	–	–	–	S	1
87d42	42	–	–	†	250	–	–	†††	400; 450; 500	†	350	Reinfection	M	3
88d0	0	†	200	†	300	–	–	††	400; 450	†	250	–	M	2
88d42	42	–	–	†	200	–	–	†	700	†	200	Reinfection	M	2
89d0	0	†	200	–	–	–	–	†	450	††	200; 250	–	M	2
89d42	42	†	300	†	300	–	–	†	350	††	200; 300	Reinfection	M	2
8d0	0	–	–	†	150	–	–	†	500	–	–	–	S	1
8d42	42	†	250	–	–	–	–	†	700	†	350	Reinfection	M	2
94d0	0	†	200	††	200; 250	–	–	†	300	††	350; 450	–	M	2
94d42	42	–	–	†	300	–	–	–	–	†	400	Reinfection	S	1
98d0	0	†	250	†	200	–	–	††	400; 500	†	300	–	M	2
98d42	42	†	200	–	–	–	–	†	600	†	450	Reinfection	M	2
DD2	N/A	†	220	–	–	–	–	–	–	†	400	N/A	S	1
HB3	N/A	†	180	–	–	–	–	–	–	†	300	N/A	S	1

K1	N/A	–	–	†	180	–	–	–	–	†	380	N/A	S	1
R033	N/A	–	–	–	–	†	160	†	480	–	–	N/A	S	1
W2	N/A	†	220	–	–	–	–	–	–	†	400	N/A	S	1
3D7	N/A	–	–	†	250	–	–	†	500	–	–	N/A	S	1

For each patient, both the d0 and d42 filter paper samples were genotyped at *msp-1* and *msp-2* loci (Snounou et al.).[2] Only data for 38 recurrent infections are shown. "†" denotes that one allele is present at a locus, whereas "–" shows that it is absent. If two alleles are present, the data are shown as shown as "††" and "†††" if three are present, etc. Allele size is the approximate molecular size in bp of an *msp-1* or *msp-2* fragment detected. Treatment outcomes inferred from genotyping the d0 and d42 samples are coded as "Reinfection" and "Treatment failure." Clonality denotes the genetic complexity of an infection, that is, whether the infection contains multiple-parasite haplotypes (M) or a single-parasite haplotype (S). N/A = not applicable. Multiplicity of infection (MOI) is an estimate of the minimum number of parasite haplotypes present within an infection and was determined as the highest number of alleles observed at the most diverse locus. Highlighted in green at the bottom of the table are *msp-1* and *msp-2* genotype data for laboratory control parasites. Highlighted in red are alleles that allowed to conclude that the outcome for the paired samples was a "Reinfection."

SUPPLEMENTAL TABLE 3

SNP data for patients with no detectable d42 parasitemia

Patient ID	Day	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11	SNP12	SNP13	SNP14	SNP15	SNP16	SNP17	SNP18	SNP19	SNP20	SNP21	SNP22	SNP23	SNP24	Treatment outcome	No. of heterozygous SNPs	Clonality	MOI	95% confidence interval for MOI	Probability for MOI
100 d0	0	T	A/G	C/T	C/T	C	C/G	A/G	G	A	C/T	C/T	G	C	A	A/C	A/C	C/T	A	A	G	A/C	C/T	G/T	G	–	13	M	3	[2, 4]	0.6102
100 d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
101 d0	0	T	A	C	C	C	C	A	G	A	C	T	A	T	A	A	A	C	A	A	A	A	C	G	G	–	0	S	1	[1, 1]	1.0000
101 d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
102 d0	0	T	A	C	T	C	C	A/G	G	A	T	C	G	T	A	A	C	T	A	A	G	A/C	C/T	T	G	–	3	M	2	[1, 2]	0.5327
102 d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
104 d0	0	T	A	C/T	T	C/G	C	A/G	A/G	A/T	C/T	C	G	C	A	C	A/C	C/T	A/T	A/C	A/G	A/C	C/T	G/T	G	–	14	M	3	[2, 5+]	0.5642
104 d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
105 d0	0	T	A	C	T	G	G	A	G	A	T	C	A	T	C	C	C	T	T	A	A	C	T	G	G	–	0	S	1	[1, 1]	1.0000
105 d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitolog	–	–	–	–	–

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106 d0	0	T	A	C	T	C	C	G	G	A	T	T	G	C	A	A	A	C	T	A	G	A	T	T	G	–	0	S	1	[1, 1]	1.0000
106 d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
13d 0	0	T	A	T	T	C	C	A	G	A	C	T	G	C	C	A	C	C	T	C	A	A	C	T	G	–	0	S	1	[1, 1]	1.0000
13d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
14d 0	0	T	A	T	C	G	C	A	A	A	T	C	G	T	A	A	C	C	T	C	A	A	C	G	G	–	0	S	1	[1, 1]	1.0000
14d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
15d 0	0	C	A	C	C/T	C	C	A/G	G	A	C/T	T	A/G	C/T	A	C	A/C	C	T	A	A/G	C	T	T	G	–	7	M	2	[2, 2]	0.9757
15d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
19d 0	0	C	A	C/T	T	C/G	C	G	G	A	C	C	A	C	A	C	C	C	T	A	G	A/C	T	G/T	G	–	4	M	2	[1, 2]	0.8341
19d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
1Ad 0	0	T	A	C	T	C/G	C/G	G	G	A/T	C/T	C	G	C/T	A	A	A/C	C/T	A	A	G	A/C	C/T	G/T	G	–	10	M	2	[2, 3]	0.7645
1Ad 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological	–	–	–	–	–

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1d0	0	T	A	C	T	C	C	A	G	A	T	T	G	C/T	A	A	C	C	A	A	G	A/C	T	G/T	G	–	3	M	2	[1, 2]	0.8189
1d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
21d0	0	C	A	C	T	C	C	A	G	T	C	T	A	C	A	A	C	T	A	A	G	C	C	T	G	–	0	S	1	[1, 1]	1.0000
21d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
22d0	0	T	G	C	C	C	C	G	G	T	C	C	A	C	A	A	C	C	A	A	A	C	C	T	G	–	0	S	1	[1, 1]	1.0000
22d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
23d0	0	T	A	C	T	C	C	A	A	A	C	C	A	C	A	A	A/C	C	A	A	G	C	C	G/T	G	–	2	M	1	[1, 1]	0.9920
23d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
24d0	0	T	A	T	T	C	C	A	G	A	C	T	G	T	C	A	C	C	T	C	G	C	T	T	G	–	0	S	1	[1, 1]	1.0000
24d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
25d0	0	C	A	C	T	C	C	A	G	A	T	T	G	C	A	A	C	T	A	C	G	A	C	T	G	–	0	S	1	[1, 1]	1.0000
25d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
26d	0	C	A	C	T	C	G	A	A	A	T	T	A	C	C	C	A	T	T	A	G	C	T	T	G	–	0	S	1	[1, 1]	1.0000

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26d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
27d 0	0	C	A	C	T	C	C	A	G	A	T	C	G	T	A	C	C	T	A	A	G	C	T	T	G	–	0	S	1	[1, 1]	1.0000
27d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
28d 0	0	–	G	C/T	C	C	C	A/G	G	A/T	T	C	A	T	C	C	A/C	C	T	A	A/G	C	C	T	G	–	5	M	2	[1, 2]	0.8329
28d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
29d 0	0	T	A	C	T	C	C	A	G	A	T	C	A	C	C	C	C	C	A	A	G	A	T	G	G	–	0	S	1	[1, 1]	1.0000
29d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
2Ad 0	0	T	A	C	C/T	C	C	A	G	A	T	C	A	C	C	C	C	C	A	A	A/G	A	T	T	G	–	2	M	1	[1, 1]	0.9903
2Ad 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
33d 0	0	T	A	T	T	C	G	A	G	A	T	C	A	C	A	A	A	C	T	A	G	A/C	C	T	G	–	1	S	1	[1, 1]	0.9998
33d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
34d 0	0	T	A	C	C/T	C	C/G	A	G	A/T	C/T	C/T	A/G	C/T	A/C	A/C	A/C	C/T	A	A/C	G	A/C	C/T	T	G	–	14	M	3	[3, 5+]	0.4759

34d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
35d0	0	T	A	C	C/T	C/G	C/G	A/G	G	A	T	C/T	A/G	C/T	A	C	A/C	T	A/T	A	A/G	A/C	T	T	G	–	11	M	2	[2, 3]	0.5896
35d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
36d0	0	C/T	A	C	T	C	G	A	A	A	T	T	A	C	A	A/C	C	C/T	A	C	G	A	T	T	G	–	3	M	1	[1, 2]	0.6725
36d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
38d0	0	T	A	T	T	C	G	A	G	A	C	T	A	T	A	A	C	C	A	A	G	A	T	G	G	–	0	S	1	[1, 1]	1.0000
38d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
39d0	0	T	A	C	T	C	C	A/G	G	T	C/T	C	A	C	A	C	C	C	T	A	G	A/C	T	T	G	–	3	M	1	[1, 2]	0.6289
39d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
3Ad0	0	T	A	C	T	C	C/G	A	G	A/T	T	C	G	C	A	C	C	C	A	A	A/G	C	C/T	T	G	–	4	M	2	[2, 2]	0.9651
3Ad42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
3d0	0	C	A	C	C/T	C	C	A/G	G	A	C/T	T	A	C/T	A	A/C	A/C	C	T	A	G	A/C	C/T	T	G	–	8	M	2	[2, 3]	0.9287
3d4	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate	–	–	–	–	–

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41d 0	0	T	A	C	T	C	G	G	G	A	T	T	G	T	C	A	A	C	A/T	A/C	G	A/C	C	T	G	–	3	M	1	[1, 2]	0.8034
41d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
42d 0	0	C	G	C	T	C	C	A	G	A	C	T	A	T	C	C	C	C	A	A	A	A	T	G	G	–	0	S	1	[1, 1]	1.0000
42d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
43d 0	0	T	A	C/T	T	C/G	G	A/G	G	T	T	C	A	T	A	C	C	C/T	T	A	G	A/C	C/T	T	G	–	6	M	2	[2, 2]	0.9957
43d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
44d 0	0	C	A/G	C	C/T	C/G	C	A/G	G	T	C/T	C/T	A	C	A	A/C	A	C/T	A/T	A	A/G	A/C	C	G/T	G	–	12	M	2	[2, 4]	0.6187
44d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
45d 0	0	C	A	C	C	C	C/G	A/G	G	A/T	C/T	T	A/G	C/T	A	C	C	T	A	A	G	C	C/T	T	G	–	7	M	2	[2, 2]	0.9799
45d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
46d 0	0	T	A	T	T	G	G	A	G	A	C	T	A	C	A	A	C	C	T	A	G	A	T	T	G	–	0	S	1	[1, 1]	1.0000
46d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitolog	–	–	–	–	–

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50d 0	0	T	A	C/T	T	C	C/G	A/G	G	A	T	C/T	G	T	A	C	C	C	A/T	A	G	C	T	G/T	G	–	6	M	2	[2, 2]	0.9830
50d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
51d 0	0	C	A	C	T	G	C	G	G	T	T	T	A	T	A	A	C	C	A	A	A	C	C	T	G	–	0	S	1	[1, 1]	1.0000
51d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
52d 0	0	C	A	C	T	C	C	G	G	A	T	T	G	C	C	A	C	T	T	A	A	A/C	T	G	G	–	1	S	1	[1, 1]	0.9998
52d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
54d 0	0	T	A	C	C/T	C/G	C	A	G	A/T	C/T	C	G	C/T	A/C	C	C	C	A/T	C	A/G	A/C	C/T	G/T	G	–	11	M	2	[2, 3]	0.6454
54d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
58d 0	0	T	A	C	T	C/G	C	A/G	G	A	T	C/T	G	C/T	A	A	A/C	C	A	A	A/G	A/C	T	G/T	G	–	8	M	2	[2, 3]	0.8987
58d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
65d 0	0	C	A	C	T	C	G	G	G	A	C	T	G	T	A	A	C	C	A	A	A	C	T	T	G	–	0	S	1	[1, 1]	1.0000
65d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological	–	–	–	–	–

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66d0	0	T	A	C	T	C	C	A/G	A/G	A/T	C/T	C	A/G	C	A/C	C	A	C/T	A/T	A	G	A	C/T	G/T	G	–	10	M	2	[2, 3]	0.8352
66d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
67d0	0	T	A/G	C/T	T	C/G	C/G	A/G	G	A	C/T	T	A	C/T	A/C	A	A/C	C/T	A/T	A	A/G	A/C	C/T	T	G	–	14	M	3	[2, 5+]	0.5520
67d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
68d0	0	T	A	C	C	C	C	A	G	A	C	C	A	T	A	C	C	C	A/T	A/C	G	A/C	C	G	G	–	3	M	1	[1, 2]	0.8726
68d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
69d0	0	T	A/G	C	C/T	C/G	C/G	A	G	A	C/T	C	A/G	C/T	A/C	C	C	C	A/T	A	A/G	C	T	G/T	G	–	11	M	2	[2, 3]	0.6565
69d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
6d0	0	T	A	C	T	C	C	G	G	A	C	T	G	C	A	A	A	T	T	C	G	C	T	T	G	–	0	S	1	[1, 1]	1.0000
6d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
70d0	0	C	A	C	T	C	C	G	G	A	C	T	G	C	A	C	C	C	A	C	G	C	T	T	G	–	0	S	1	[1, 1]	1.0000
70d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
73d	0	T	A	C	T	C	C	G	G	A	C	T	A	C	A	A	A	T	A/T	C	A	A	C/T	G	G	–	2	M	1	[1, 1]	0.9973

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73d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
74d 0	0	T	A	C	C	C	G	A	G	A	T	–	G	C	A	A	C	C	A	A	G	C	T	G	G	–	0	S	1	[1, 1]	1.0000
74d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
75d 0	0	T	A	C	T	C	C	A	G	A	C	C	G	C	A	A	C	T	A	A	G	A	C	G	G	–	0	S	1	[1, 1]	1.0000
75d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
76d 0	0	T	A	C	T	C	C/ G	A/ G	G	A	C/T	C/T	A/G	C	A/C	C	C	C/T	A/T	A	A/G	A/C	C/T	T	G	–	11	M	3	[2, 4]	0.5806
76d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
77d 0	0	T	A	C	T	C	G	G	G	A	T	C	A	C	A	A	C	C	A	C	G	A	T	T	G	–	0	S	1	[1, 1]	1.0000
77d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
78d 0	0	C	A	C	T	C	C	G	G	A	T	T	G	C	C	A	A	T	T	A	A	C	C	T	G	–	0	S	1	[1, 1]	1.0000
78d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
79d 0	0	T	A	C	T	C	G	G	G	A	C	T	G	C	A	C	C	C	T	A	G	C	C	T	G	–	0	S	1	[1, 1]	1.0000

79d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
7d0	0	T	A	C	T	C	C	A	G	A	C	C	G	C	C	C	C	C	A	A	G	A	T	G	G	–	0	S	1	[1, 1]	1.0000
7d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
80d0	0	T	A	C	T	G	C	A	G	A/T	C/T	T	G	T	A	C	C	T	T	C	G	C	C/T	T	G	–	3	M	1	[1, 2]	0.8769
80d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
82d0	0	T	A	C	T	C	C	A	G	A	T	C	A	C	C	C	C	C	A	A	G	C	T	T	G	–	0	S	1	[1, 1]	1.0000
82d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
83d0	0	T	A	C	T	C	C	G	G	A	C	C	G	C	A	C	C	T	A	C	G	A	T	T	G	–	0	S	1	[1, 1]	1.0000
83d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
84d0	0	C	A	T	T	C	G	A	G	A	T	–	A	C	A	A	C	T	A	C	G	A	T	T	G	–	0	S	1	[1, 1]	1.0000
84d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
85d0	0	C	A	C	T	C	G	G	G	T	C	C	G	C	A	C	C	C	T	A	A	C	C	T	G	–	0	S	1	[1, 1]	1.0000
85d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitolog	–	–	–	–	–

																										ical response					
86d 0	0	T	A	C	T	C	G	G	A	A	C	C	G	C	A	C	C	T	T	C	A	C	C	T	G	–	0	S	1	[1, 1]	1.0000
86d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
90d 0	0	T	A	C	C	C	C/G	A	A/G	A	C/T	C	G	C	A/C	C	C	C/T	A/T	A	G	C	T	T	G	–	6	M	2	[2, 2]	0.9892
90d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
91d 0	0	T	A/G	C	T	C/G	C/G	A/G	A/G	A/T	C/T	C/T	A/G	C	A/C	A/C	C	T	A	A	A/G	A/C	T	G/T	G	–	14	M	3	[2, 5+]	0.5631
91d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
92d 0	0	C	A	C	C/T	C	G	A/G	A/G	A	C	T	G	C	C	C	A/C	C/T	A	A/C	A/G	A/C	C/T	T	G	–	9	M	2	[2, 3]	0.9487
92d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
93d 0	0	T	A	T	T	C	C	A/G	A	A	T	T	A	T	A	A	A	C	T	A	G	C	C	T	G	–	1	S	1	[1, 1]	0.9999
93d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	0	–	–	–	–
95d 0	0	C/T	A	C/T	C/T	C	C/G	A/G	G	A	C/T	C/T	G	C/T	A/C	C	C	C	A/T	A/C	A/G	A/C	C/T	G/T	G	–	15	M	4	[3, 5+]	0.4350
95d 42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological	–	–	–	–	–

																										response					
96d0	0	T	A	C	T	C	C	A	G	A/T	T	C	A	C	C	C	A	T	A/T	C	A/G	A/C	C	T	G	–	4	M	2	[2, 1]	–
96d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
97d0	0	–	–	C	–	C	–	G	–	A	C/T	T	G	C	A	–	A	C	T	A/C	G	C	T	G/T	G	–	3	M	2	[1, 2]	0.8524
97d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
99d0	0	T	A	C/T	T	C	C	A/G	G	A	T	T	G	C	C	C	C	C	A	A	G	C	C	T	G	–	2	M	1	[1, 1]	1.0000
99d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
9d0	0	C	A	C	T	C	C	G	G	A	T	T	G	T	C	A	A	T	T	A	G	C	T	T	G	–	0	S	1	[1, 1]	1.0000
9d42	42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–	–	–	–
DD2	N/A	T	A	T	C	C	G	G	A	T	T	T	A	T	C	A	A	T	A	C	A	A	C	G	T	N/A	0	S	1	[1, 1]	1.0000
HB3	N/A	T	G	C	C	C	C	A	G	A	T	C	A	C	A	A	C	T	A	A	G	T	T	T	T	N/A	0	S	1	[1, 1]	1.0000
K1	N/A	C	G	C	T	C	G	G	A	T	T	T	A	T	C	C	C	T	A	C	G	C	C	G	T	N/A	0	S	1	[1, 1]	1.0000
R033	N/A	C	A	T	T	G	C	A	G	A	C	T	–	C	A	C	C	T	T	A	G	A	T	T	G	N/A	0	S	1	[1, 1]	1.0000
W2	N/A	T	A	T	C	C	G	G	A	T	T	T	A	T	C	A	A	T	A	C	A	A	C	G	T	N/A	0	S	1	[1, 1]	1.0000
3D7	N/A	C	G	C	T	C	C	G	G	A	C	T	G	C	A	C	C	C	A	A	G	A	T	T	G	N/A	0	S	1	[1, 1]	1.0000

msp-1 = merozoite surface protein 1; *msp-2* = merozoite surface protein 2; N/A = not applicable; SNP = single-nucleotide polymorphism. For each patient, both the d0 and d42 filter paper samples were genotyped using the 24-SNP Barcoding Assay (Daniels et al.).[7] Because d42 samples for these patients did not have detectable parasite DNA, these patients were deemed to have achieved "adequate parasitological response." Only data for 71 patients with no detectable d42 parasitemia are shown. "–" denotes that an allele was not detected. Number of heterozygous SNPs is the number of loci out of the 24 genotyped that carry both alternate SNP alleles. Clonality denotes the genetic complexity of an infection, that is, whether the infection contains multiple-parasite haplotypes (M) or a single-parasite haplotype (S). MOI = multiplicity of infection as determined by the maximum likelihood method called COIL (Galinsky et al.).[17] Twelve SNPs highlighted in blue are proposed for the abbreviated SNP assay. Highlighted in green at the bottom of the table are SNP data for laboratory control parasites

SUPPLEMENTAL TABLE 4

Msp-1 and *msp-2* genotype data for patients with no detectable d42 parasitaemia

Patient ID	Day	MAD 20	MAD 20 fragment size	K1	K1 fragment size	RO33	R033 fragment size	3D7/IC	3D7/IC fragment size	FC27	FC27 fragment size	Treatment outcome	Clonality	MOI
100d0	0	†	200	††	200; 300	–	–	†	600	††	300; 350	–	M	2
100d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
101d0	0	†	300	–	–	–	–	†	500	–	–	–	S	1
101d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
102d0	0	†	200	†	300	–	–	†	500	†	400	–	M	2
102d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
104d0	0	†	250	†	300	–	–	††	400; 500	††	300; 350	–	M	2
104d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
105d0	0	†	300	–	–	–	–	†	600	–	–	–	M	2
105d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
106d0	0	†	200	–	–	–	–	†	500	–	–	–	S	1
106d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
13d0	0	–	–	–	–	–	–	††	550; 650	†	300	–	M	2
13d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
14d0	0	–	–	–	–	†	160	†	550	–	–	–	S	1
14d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
15d0	0	–	–	–	–	†	150	†	500	†	350	–	M	2
15d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
19d0	0	†	300	–	–	–	–	†	500	†	450	–	M	2
19d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
1Ad0	0	–	–	††	200; 300	–	–	†	500	††	250; 350	–	M	2
1Ad42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological	–	–

												l response		
1d0	0	†	200	–	–	–	–	†	500	†	300	–	M	2
1d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
21d0	0	–	–	–	–	†	160	†	600	–	–	–	S	1
21d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
22d0	0	–	–	†	200	–	–	–	–	†	450	–	S	1
22d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
23d0	0	–	–	†	150	–	–	†	600	†	250	–	M	2
23d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
24d0	0	†	200	–	–	–	–	–	–	†	400	–	S	1
24d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
25d0	0	†	300	†	200	–	–	–	–	†	250	–	M	2
25d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
26d0	0	–	–	–	–	†	160	†	600	–	–	–	S	1
26d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
27d0	0	–	–	–	–	†	160	–	–	†	250	–	S	1
27d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
28d0	0	–	–	††	200; 250	–	–	††	400; 500	–	–	–	M	2
28d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
29d0	0	–	–	†	200	–	–	–	–	††	250; 300	–	M	S
29d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
2Ad0	0	†	200	†	250	–	–	†	500	†	350	–	M	2
2Ad42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
33d0	0	†	200	–	–	–	–	†	500	–	–	–	S	1
33d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
34d0	0	–	–	–	–	†	180	†	600	†	400	–	M	2

34d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
35d0	0	††	200; 250	††	200; 300	–	–	†	500	††	350; 400	–	M	2
35d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
36d0	0	†	300	–	–	–	–	†	500	††	300; 400	–	M	2
36d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
38d0	0	–	–	†	150	–	–	–	–	†	300	–	S	1
38d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
39d0	0	†	200	–	–	–	–	†	550	†	400	–	M	2
39d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
3Ad0	0	†	200	†	300	–	–	†	500	–	–	–	M	2
3Ad42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
3d0	0	†	200	††	200; 300	–	–	†	550	†	350	–	M	2
3d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
41d0	0	–	–	†	200	–	–	†	500	†	400	–	M	2
41d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
42d0	0	†	200	–	–	–	–	–	–	†	400	–	S	1
42d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
43d0	0	†	200	†	250	–	–	†	550	†	350	–	M	2
43d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
44d0	0	†	250	†	200	–	–	–	–	†	400	–	M	2
44d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
45d0	0	†	200	–	–	–	–	†	500	–	–	–	S	1
45d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
46d0	0	†	200	–	–	–	–	†	500	–	–	–	S	1
46d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological	–	–

												l response		
50d0	0	†	200	†	300	–	–	†	500	–	–	–	M	2
50d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
51d0	0	–	–	†	250	–	–	†	500	–	–	–	S	1
51d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
52d0	0	†	200	–	–	–	–	–	–	†	300	–	S	1
52d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
54d0	0	††	200; 250	†	200	–	–	†	600	†	300	–	M	2
54d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
58d0	0	†	200	†	300	–	–	†	500	††	300; 400	–	M	2
58d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
65d0	0	††	200; 300	†	300	–	–	†	500	†	–	–	M	2
65d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
66d0	0	–	–	†	200	–	–	†	500	†	300	–	M	2
66d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
67d0	0	††	200; 300	†	200	–	–	†	500	†	350	–	M	2
67d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
68d0	0	–	–	†	250	–	–	†	500	†	700	–	M	2
68d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
69d0	0	†	200	–	–	–	–	–	–	†	350	–	S	1
69d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
6d0	0	†	200	–	–	–	–	††	400; 500	–	–	–	M	2
6d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
70d0	0	–	–	†	200	–	–	†	500	–	–	–	S	1
70d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
73d0	0	†	250	†	300	–	–	†	500	†	600	–	M	2

73d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
74d0	0	–	–	†	200	–	–	†	500	–	–	–	M	2
74d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
75d0	0	–	–	†	300	–	–	–	–	†	400	–	S	1
75d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
76d0	0	†	200	†	300	–	–	†	500	–	–	–	M	2
76d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
77d0	0	†	300	†	300	–	–	–	–	†	400	–	M	2
77d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
78d0	0	–	–	–	–	†	160	††	500; 600	–	–	–	M	2
78d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
79d0	0	–	–	†	200	–	–	†	500	–	–	–	S	1
79d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
7d0	0	–	–	†	200	–	–	††	500	–	–	–	M	2
7d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
80d0	0	†	200	–	–	–	–	†	500	†	600	–	M	2
80d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
82d0	0	†	300	†	250	–	–	†	500	†	350	–	M	2
82d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
83d0	0	†	200	–	–	–	–	†	500	–	–	–	S	1
83d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
84d0	0	–	–	†	200	–	–	†	500	–	–	–	S	1
84d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
85d0	0	†	250	–	–	–	–	†	500	–	–	–	S	1
85d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitologica	–	–

												l response		
86d0	0	–	–	†	300	–	–	–	–	†	400	–	S	1
86d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
90d0	0	–	–	†	200	–	–	–	–	†	300	–	S	1
90d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
91d0	0	–	–	††	180; 240	–	–	††	500; 550	†	300	–	M	2
91d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
92d0	0	–	–	†	200	–	–	††	400; 600	†	350	–	M	2
92d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
93d0	0	–	–	–	–	†	160	†	400	–	–	–	S	1
93d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
95d0	0	†	200	†	180	–	–	†	500	–	–	–	M	2
95d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
96d0	0	†	300	–	–	–	–	†	500	–	–	–	S	1
96d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
97d0	0	–	–	†	200	–	–	††	500; 600	–	–	–	M	2
97d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
99d0	0	–	–	†	250	–	–	†	500	†	300	–	M	2
99d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
9d0	0	–	–	–	–	†	160	††	500	–	–	–	S	1
9d42	42	–	–	–	–	–	–	–	–	–	–	Adequate parasitological response	–	–
DD2	N/A	†	220	–	–	–	–	–	–	†	400	N/A	S	1
HB3	N/A	†	180	–	–	–	–	–	–	†	300	N/A	S	1
K1	N/A	–	–	†	180	–	–	–	–	†	380	N/A	S	1
R033	N/A	–	–	–	–	†	160	†	480	–	–	N/A	S	1
W2	N/	†	220	–	–	–	–	–	–	†	400	N/A	S	1

	A													
3D7	N/A	–	–	†	250	–	–	†	500	–	–	N/A	S	1

msp-1 = merozoite surface protein 1; *msp-2* = merozoite surface protein 2. For each patient, both the d0 and d42 filter paper samples were genotyped at *msp-1* and *msp-2* loci (Snounou et al.).[2] Only data for 71 patients with no detectable d42 parasitaemia are shown. "†" denotes that one allele is present at a locus, whereas "–" shows that it is absent. If two alleles are present, the data are shown as "††," "†††" if three are present, etc. Allele size is the approximate molecular size in bp of an *msp-1* or *msp-2* fragment detected. Because d42 samples for these patients did not have detectable parasite DNA, these patients were deemed to have achieved "adequate parasitological response." Clonality denotes the genetic complexity of an infection, that is, whether the infection contains multiple-parasite haplotypes (M) or a single-parasite haplotype (S). N/A = not applicable. Multiplicity of infection (MOI) is an estimate of the minimum number of parasite haplotypes present within an infection and was determined as the highest number of alleles observed at the most diverse locus. Highlighted in green at the bottom of the table are *msp-1* and *msp-2* genotype data for laboratory control parasites.

Figure 1

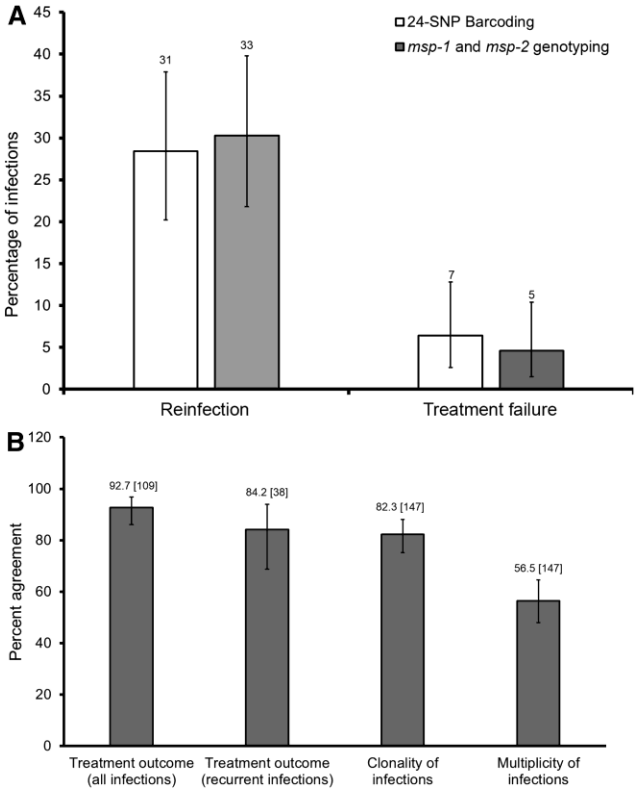
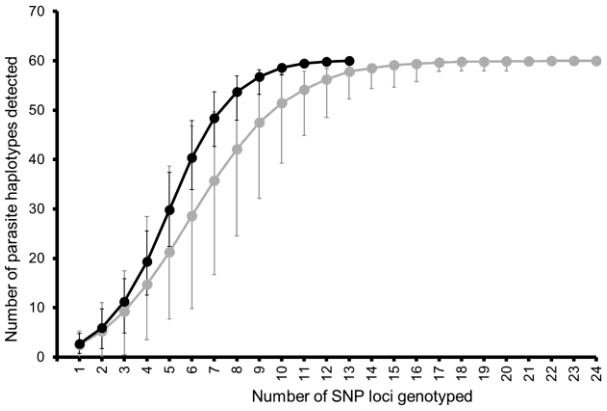
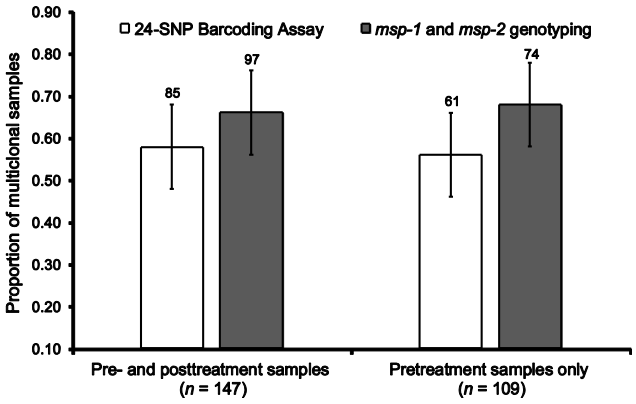

A
24-SNP Barcoding
msp-1 and msp-2 genotyping
Percentage of infections
31
33
7
5
Reinfection
Treatment failure
B
Percent agreement
92.7 [109]
84.2 [38]
82.3 [147]
56.5 [147]
Treatment outcome (all infections)
Treatment outcome (recurrent infections)
Clonality of infections
Multiplicity of infections

Figure 2


Number of parasite haplotypes detected
0
10
20
30
40
50
60
70
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Number of SNP loci genotyped

Supplemental Figure 1



Supplemental Figure 2