Global **Malaria** Programme

Informal consultation on methodology to distinguish reinfection from recrudescence in high malaria transmission areas

Report of a virtual meeting, 17–18 May 2021

World Health Organization

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Contents

ABBREVIATIONS

Africa CDC	Africa Centres for Disease Control and Prevention
AL	artemether-lumefantrine
AmpSeq	amplicon sequencing
AS	artesunate
ASAQ	artesunate-amodiaquine
ASSP	artesunate+sulfadoxine-pyrimethamine
bp	base pair
CE	capillary electrophoresis
COI	complexity of infection
DP	dihydroartemisinin-piperaquine
glurp	gene of glutamate-rich protein
GMP	Global Malaria Programme
HeOME	heterozygome
IBC	image barcode
SNP	single nucleotide polymorphism
TEG DER	Technical Expert Group on Drug Efficacy and Response
TES	therapeutic efficacy study
MIP	molecular inversion probe
MMV	Medicines for Malaria Venture
MOI	multiplicity of infection
msp1	gene of merozoite surface protein 1
msp2	gene of merozoite surface protein 2
PCR	polymerase chain reaction
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
US-CDC	United States Centers for Disease Control and Prevention
WGS	whole genome sequencing
WHO	World Health Organization

EXECUTIVE SUMMARY

This data-driven meeting assessed the advantages and disadvantages of changing the way in which recurrences are differentiated as reinfection or recrudescence following the treatment of uncomplicated *Plasmodium falciparum* malaria. This has implications for the evaluation of antimalarial efficacy in therapeutic efficacy studies (TESs), as well as in regulatory trials for the development of new antimalarial drugs.

Guidance for discriminating *P. falciparum* recrudescence from reinfection was published by the World Health Organization (WHO) in 2008 *(1)*. Blood samples collected pre-treatment (day 0) and on the day of treatment failure (day X) are compared using three markers: genes for merozoite surface protein 1 (*msp1*), merozoite surface protein 2 (*msp2*) and glutamate-rich protein (*glurp*). A standard genotyping methodology is recommended, including the use of capillary electrophoresis (CE). The decision algorithm (referred to as the WHO/MMV algorithm) requires the three markers to be genotyped and analysed in sequence, starting with *msp1*, followed by *msp2* and then *glurp*, and stopping once a marker has classified the paired samples as a reinfection. In this case, if any marker indicates reinfection, the recurrence is deemed a reinfection. An alternative approach has been suggested (termed the 2/3 algorithm) whereby *msp1/msp2* are evaluated, and only in cases where these two markers are discordant, *glurp* is used as the deciding factor. In this case, even if *msp1* indicates a reinfection, if *msp2* and *glurp* indicate a recrudescence, the recurrence is deemed a recrudescence.

The consultation examined evidence around changes in the genetic markers used to determine the relatedness of initial and recurrent parasites, as well as the algorithms used to analyse these markers to classify recurrences as either a recrudescence or reinfection. In particular, the panel examined the applicability of recent advances in genotyping and analysis. The meeting focused on areas of high transmission in Africa because the high multiplicity of infection (MOI; i.e., the number of concurrent clones in an infection) and high reinfection rates in such areas complicate the discrimination of recrudescence from reinfection.

Summary conclusions

The different methodologies for genotyping and analysis used to differentiate recrudescence from reinfection all have advantages and limitations, and clearly give different results. This has important consequences because, in some cases, the difference in the number of recurrences classified as recrudescence drives the efficacy rate below 90%, which is the currently recommended threshold requiring a change in treatment policy. In addition, this may affect decisions during drug development and adoption of new treatments, where a 95% efficacy threshold is recommended.

The panel considered which methods are most likely to be closest to the 'true' values for reinfection and recrudescence. The most robust and reliable genotyping method is amplicon sequencing (AmpSeq). However, the capacity to apply this technology in Africa needs to be strengthened before this method can be adopted as a standard. This could be achieved by capacity building in African countries and/or offering deep sequencing at core facilities(s) in Africa or elsewhere, which could process samples and return data to countries for analysis – the dual aim being to ensure that drug efficacy is accurately measured and that countries retain ownership of their data. The examined data clearly indicate that *glurp* is not an ideal marker. Therefore, until AmpSeq implementation is feasible, as an interim solution, *glurp* should be replaced with alternative markers. Microsatellites with a diversity relevant to the study site location appear to be the most

feasible and reliable option. For the transition period, data from the new methods and the current *msp1/msp2/glurp* markers should be reported to enable historical comparison.

In terms of analysis, the 2/3 approach is comparable to the WHO/MMV algorithm in low to moderate transmission settings, but may overestimate recrudescence rates for artemether-lumefantrine (AL) in high transmission settings. The 2/3 method ignores data from the 'third' discordant marker, which is a reasonable strategy when *msp1* and *msp2* agree; however, ignoring the other markers is not necessary if microsatellites are used in place of *glurp*. Match-counting is simple to use and does not disregard information from any markers; however, this method may underestimate recrudescence. The panel also considered Bayesian analysis, which has been applied to TESs conducted by the United States Centers for Disease Control and Prevention (US-CDC). The main advantage of this approach is that it provides a measure of uncertainty around the results. However, validation of the model used for Bayesian analysis is needed, including when AmpSeq data are used to distinguish between reinfection and recrudescence. Furthermore, the feasibility of using Bayesian analysis at the country level needs to be carefully assessed.

It is unclear what impact a change in methodology would have on the drug efficacy thresholds that are used to establish antimalarial treatment policy in countries and to support new drug approvals. Therefore, it is anticipated that a transition period will be required to generate comparative data for the different uses of this information. This should be considered in the context of expanding expertise and capacity in Africa for next generation sequencing. Furthermore, the broader trend towards genetic analysis of infectious diseases suggests that such approaches are likely to become more widely accepted and better understood over the next few years.

Recommendations

1 As an interim solution, *msp1* and *msp2* should continue to be used, but *glurp* should be replaced with one microsatellite from the following: *Poly-α*, *Pfpk2* and *TA1*. For simplicity and reasons of practical implementation, WHO/MMV match-counting (3/3) should be maintained as the primary analysis methodology for reporting and policy change. Bayesian and 2/3 algorithms may be applied for evaluation and comparison, but not for primary reporting. These methods should be applied in both low to moderate and high transmission settings in Africa. Outside Africa, the current method (*msp1/msp2/glurp*) should still be applied.

2 For a transition period, data should be analysed and reported using both the current (*msp1/msp2/glurp*) and new (*msp1/msp2/*microsatellites) methods to enable historical comparison and to understand the implications of the new methods in terms of thresholds for treatment policy change and introduction of new antimalarial drugs. Countries are not required to use *glurp* if they have already switched microsatellite markers or *msp1/msp2/*microsatellites. Data transparency will be critical for comparative analysis and to provide a database for analytical methodology development.