Field application of in vitro assays *for the* sensitivity of human malaria parasites *to* antimalarial drugs

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World Health Organization

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Abbreviations used

ADP	adenosine diphosphate
cpm	counts per minute
DELI	double-site enzyme-linked lactate dehydrogenase immunodetection
DHFR	dihydrofolate reductase
DHPS	dihydropteroate synthase
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FACS	fluorescence-activated cell sorter
FW	formula weight
HEPES	*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)
HPLC	high-performance liquid chromatography
HRP II	histidine-rich protein II
IC_{50}	50% inhibitory concentration
LDH	lactate dehydrogenase
MIC	minimal inhibitory concentration
NAD	nicotinamide adenine dinucleotide
OD	optical density
PABA	*para*-aminobenzoic acid
PCR	polymerase chain reaction
pfcrt	*P. falciparum* chloroquine resistance transporter gene
*pfmdr*1	*P. falciparum* multidrug resistance gene 1
pvcrt	*P. vivax* chloroquine resistance transporter
RPMI	Roswell Park Memorial Institute
TDR	Special Programme for Research and Training in Tropical Diseases
Tween 80	polyoxyethylenesorbitan monooleate
vs	versus
WHO	World Health Organization

Executive summary

In vitro assays for the sensitivity of human malaria parasites to antimalarial drugs provide information complementary to that derived from the epidemiology of drug-resistant malaria. Its principles were initially based on a suboptimal short-term culture method reported in 1912, applied for the World Health Organization (WHO) macrotest system in the 1960s and used until the 1980s. The principles of in vitro culture underwent a major modification in 1976, when a new method for continuous culture of *Plasmodium falciparum* was reported by Trager & Jensen (1976). The essential components of the complete blood-medium mixture are Roswell Park Memorial Institute (RPMI) 1640 medium buffered with *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) (HEPES) and sodium bicarbonate, human serum (or serum substitutes, including animal sera and lipid-enriched bovine albumin) and *P. falciparum*-infected human erythrocytes. This technical improvement led to the elaboration of several in vitro assay systems in the late 1970s and in the 1980s, including the WHO microtest system (morphological assay), the 48-h test (morphological assay) and the radioisotope microtest. Over the past few years, two novel in vitro drug sensitivity assays have been evaluated: an enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies directed against either plasmodial lactate dehydrogenase (LDH) or histidine-rich protein II (HRP II) and a fluorometric assay with DNA-binding fluorescent dyes. These two assays are non-morphological and non-radioactive and are also based on Trager & Jensen's (1976) culture method.

Continuous culture of *P. vivax* requires a modified or alternative culture medium and regular addition of reticulocytes. The minimal requirement for performing in vitro drug sensitivity assays is schizont maturation during the first intraerythrocytic cycle, without necessarily attaining the ring stage of

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