Flow Cytometry for Antimalarial Drug Testing: More than Meets the Eye

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Resistance to antimalarial drugs has been increasing, with resistance to artemisinins in Southeast Asia being of particular concern. Monitoring resistance requires useful and feasible *in vitro* tests, and Woodrow et al. should be applauded for their report on a two-color flow cytometry assay (1), a right step in the direction of establishing a practical, objective, alternative testing strategy which addresses the shortcomings of other existing assays. Despite the impressive performance of the assay, it should be noted that this assay requires two nucleic acid stains (dihydroethidium and SYBR green), whereas another recent report using this assay format to assess drug sensitivity used only a single stain, which is certainly a desirable improvement (2). Importantly, the authors mention incorrectly that all flow cytometry assays are based on nucleic acid stains (1). A review by Grimberg on this issue already showed that viability stains are a useful alternative (3). Furthermore, our group has shown that the flow cytometric detection of hemozoin (Hz) is a possible reagent-free assay alternative, both *in vitro* for laboratory strains and *ex vivo* in the field (4, 5).

Notably, the use of nucleic acid stains is prone to interference by other cells. The method described requires the removal of white blood cells, since staining DNA/RNA may interfere with measurements. Although this may not require expensive materials, it certainly makes the assay much more complex and labor-intensive.

However, the most relevant question is whether the described flow cytometric assay has the potential to detect artemisinin resistance. In fact, only recently, a very time-consuming and complex assay was described as being able to achieve this, the ring-stage assay (RSA). This method requires a complicated and meticulous synchronization protocol and is based on microscopic readouts (6).

In contrast, flow cytometric detection of Hz allows tracking of parasite maturation in real time and detection of drug effects much earlier than other phenotypic assays. This is very relevant in the case of artemisinin resistance, as the very young parasite forms are responsible for the observed resistance to this drug. Therefore, this approach may be an attractive alternative to consider and investigate in the context of artemisinin resistance testing.

In conclusion, more flow cytometric assays are available than the one described by Woodrow et al. Some need fewer or no detection reagents, such as the Hz assay, which may have the additional advantage of detecting artemisinin resistance. A prudent

approach would be considering all novel approaches, including cytometric detection of Hz, rather than contemplating only flow cytometry solutions with multicolor staining of nucleic acids.

FUNDING INFORMATION

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sector.

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Citation Rebelo M, Hänscheid T. 2016. Flow cytometry for antimalarial drug testing: more than meets the eye. *J Clin Microbiol* 54:817. [doi:10.1128/JCM.03017-15](http://dx.doi.org/10.1128/JCM.03017-15).

Editor: A. J. McAdam

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For the author reply, see [doi:10.1128/JCM.03158-15](http://dx.doi.org/10.1128/JCM.03158-15).

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