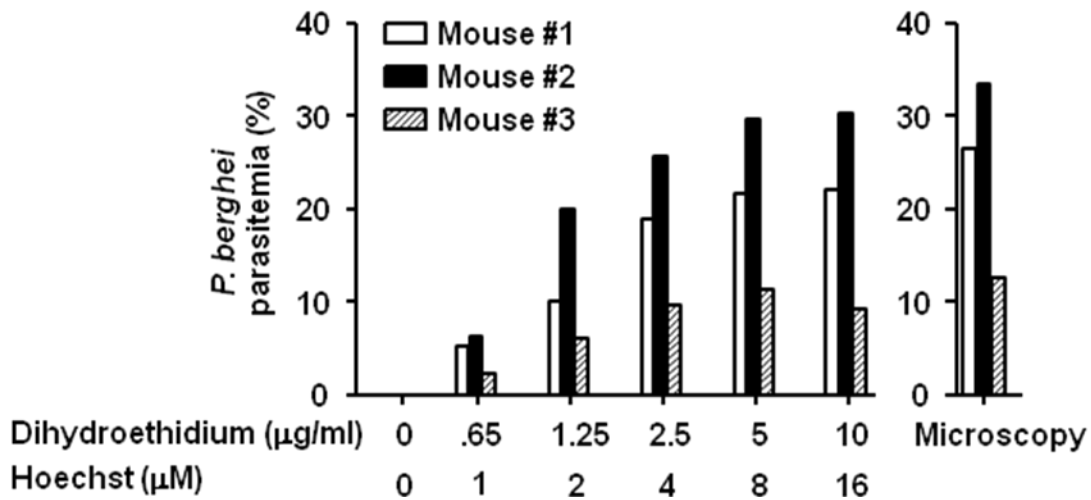


Supplementary information:

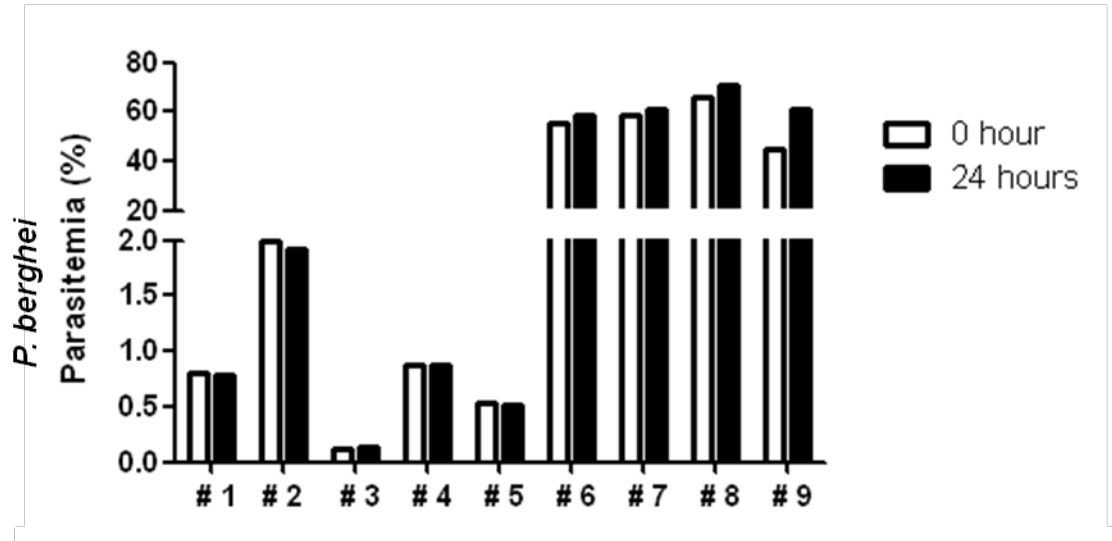
A rapid and robust tri-color flow cytometry assay for monitoring malaria parasite development

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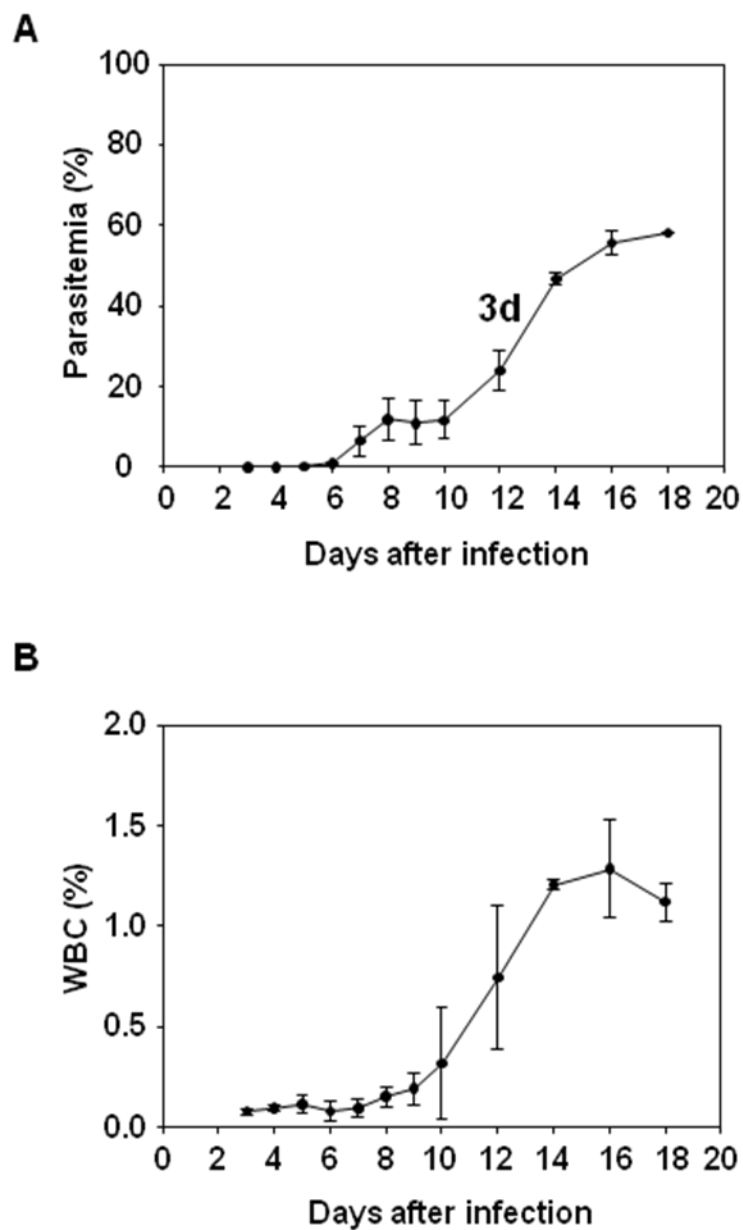
Supplemental Figure 1. Optimization of Hoechst and dihydroethidium doses used.

Mouse blood infected with *P. berghei* ANKA whole blood was exposed to different levels of dihydroethidium (0.65 to 10 μg/mL) and Hoechst (1 to 16 μM) to evaluate the appropriate doses of dyes. The parasitemia obtained by flow cytometry is compared with that determined from a Giemsa-stained blood smear.

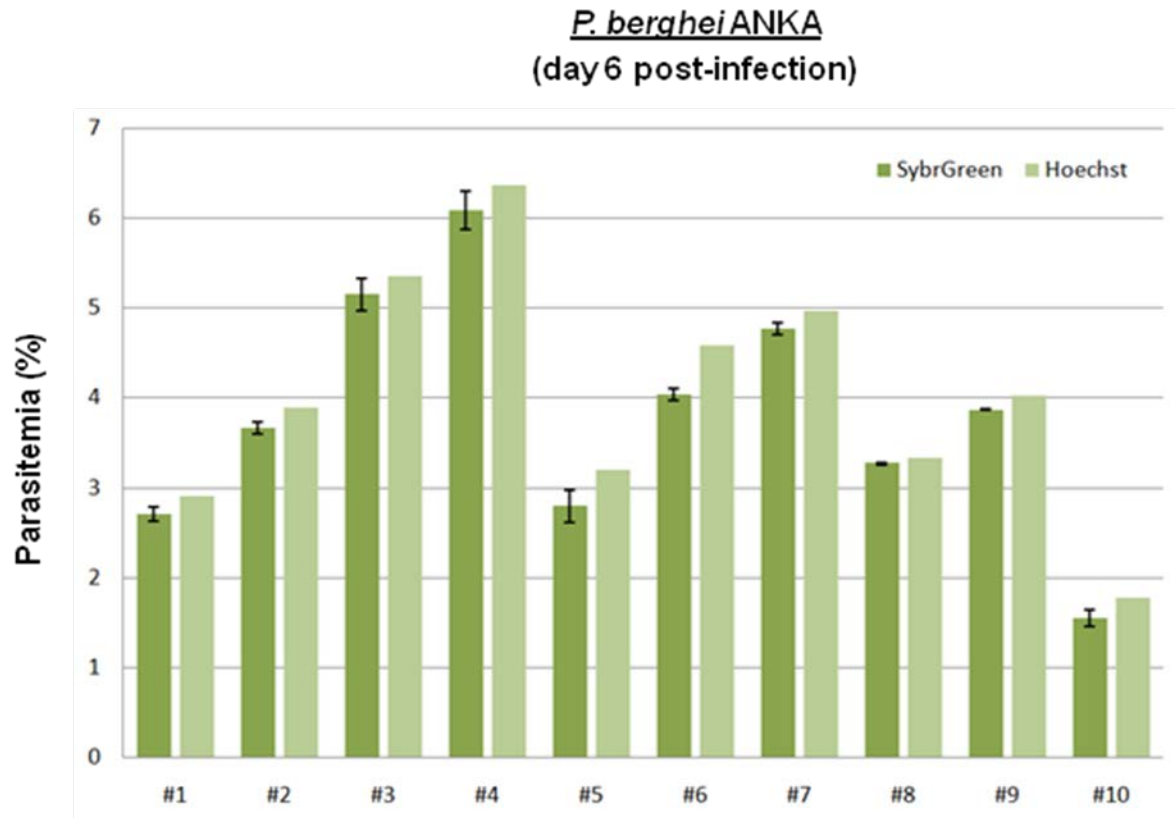


Supplemental Figure 2. Stability of flow cytometry staining. The parasitemia of nine mice infected with *P. berghei* ANKA was evaluated immediately after staining and stored during 24 hours at 4 °C in the dark.

P. berghei ANKA GFP



Supplemental Figure 3. Correlation between *P. berghei* ANKA GFP parasitemia monitored with GFP expression and with TCM. Spearman rank correlation analysis of the parasitemia determined from GFP expression and the TCM in blood samples from 27 infected mice (slope = 1.044, $R^2=0.999$).



Supplemental Figure 4. Comparison between mouse parasitemia monitored with Hoechst or SYBR Green staining. The parasitemia of 10 mice infected by *P. berghei* ANKA were monitored at day 6 post-infection with the TCM using either SYBR Green (duplicate) or Hoechst (monoplicate).

Marker	uninfected Normocyte	infected Normocyte	uninfected Reticulocyte	infected Reticulocyte	Leukocyte
Hoechst	-	++	-	++	+++
SYBR Green	-	++	+	++	+++
Ethidium	-	++	+	++	+++
CD45	-	-	-	-	++

Supplemental Table 1. Flow cytometry gating parameters for circulating blood cells.
 (–) negative, (+) low, (++) medium and (+++) high signal.

Technique	Reagent cost per test	Processing Time	Acquisition Time	Interest element number per test	Required equipment
Microscopy Thin film	~15 US\$	60 min	1,000 min	1000 RBC	microscope
TCM	~8 US\$	25 min	60 min	300,000 RBC	flow cytometer

Supplemental Table 2. Comparison between microscopy and TCM in term of cost, processing and acquisition time, number of interest events and required equipment.

The different categories were evaluated for 100 tests.