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The paramagnetic properties of malaria pigment, hemozoin, yield clues to a low-cost system for its trapping and determination

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ABSTRACT

The binding of malaria pigment, hemozoin, by a gradient magnetic field has been investigated in a manual trapping column system. Two types of magnetic filling have been tested to produce field gradients: nickel-plated steel wires, wrapped around a steel core, and superparamagnetic microbeads. The latter system allows an efficient trapping (> 80%) of β-hematin (a synthetic pigment with physical and paramagnetic properties analogous to those of hemozoin). Tests with a *Plasmodium falciparum* 3D7 culture indicate that hemozoin is similarly trapped. Off-line optical spectroscopy measurements present limited sensitivity as the hemozoin we detected from *in vitro* cultured parasites would correspond to only a theoretical 0.02% parasitemia (1000 parasites/μL). Further work needs to be undertaken to reduce this threshold to a practical detectability level. Based on these data, a magneto-chromatographic on-line system with reduced dead volumes is proposed as a possible low-cost instrument to be tested as a malaria diagnosis system.

1. Introduction

Malaria, a disease caused by protozoa of the genus *Plasmodium*, transmitted by *Anopheles* mosquitoes, is endemic in 106 countries; about 50% of the world's population are exposed with a significant impact on the economic growth of developing countries [1]. In 2015, the World Health Organization (WHO) reported 212 million cases of malaria globally and 429,000 deaths from the disease; the WHO African Region accounted for most global cases of malaria (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%) [2]. Particularly vulnerable victims are children under 5 and pregnant women, mainly from the most disadvantaged populations.

The WHO-recommended selective treatment of diagnosed cases of malaria (*"Test-Treat-Track"* initiative [3]), relies on diagnostic tests both robust, sensitive, inexpensive and widely deployable in rural areas [2,4]. This strategy is catching up with the use of immunochromatographic methods, so-called *"rapid diagnostic tests"* (RDTs). In 2015, approximately 51% of children with a fever who sought care at a public health facility in 22 African countries received a malaria diagnostic test compared to 29% in 2010 [2]. Malaria diagnostic methods however present major constraints that limit their accessibility. For reliable quantitative results, the diagnosis by microscopy requires access to an operational microscope and, especially at low parasitemia, mobilization of skilled operators; obtained results are often only qualitative (positive or negative) or semi-quantitative (+++, ++, + coding systems) and the sensitivity of the test largely depends on the operator and his state of fatigue [5]. RDTs are so far not quantitative, can be unreliable for low parasitemia and require special storage conditions. Their detection is often based on *P. falciparum* histidine-rich protein 2 (HRP2) and, less frequently, on *Plasmodium* lactate dehydrogenase (pLDH). However, the median blood clearance of HRP2 (up to 4 weeks) is quite long compared to pLDH (1 week), a problem to detect recurrent malaria infections [6]; also HRP2-variants nowadays spread in some regions. Diagnosis by polymerase chain reaction (PCR) remains complex and costly, so far unsuitable for routine use in Southern countries [7]. Although the WHO has developed good practice and qualification schemes for microscopy [5] and RDTs [8], their monitoring and implementation are far from systematic.

A need clearly exists for a rapid, instrumental, undemanding and inexpensive diagnostic test, reliable at low-parasitemia (< 200 parasites/μL) [9].

Hemozoin, a large paramagnetic crystal pigment, is generated by *Plasmodium* through a detoxification pathway, the polymerization of iron III protoporphyrin-IX ring complexes released through hemoglobin digestion [10,11]; a full blood count technology incorporating analysis of depolarized laser light has been proposed to detect hemozoin in monocytes and neutrophils [12], a possible automated malaria detection system but that has not been further developed. Recently, the levels

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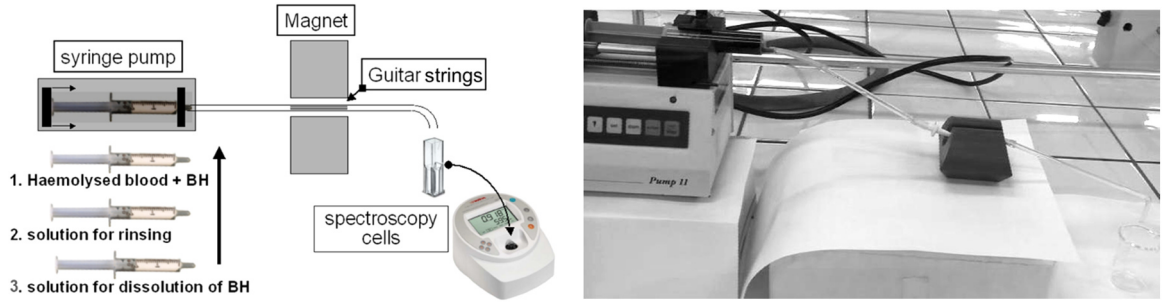

Fig. 1. Experimental device (BH = β-hematin).

of hemozoin-containing red blood cells (RBCs) [13–16] and of total blood hemozoin [17] have been proposed as a measurement of parasitemia to diagnose malaria. To this end, different microscopic and instrumental methods have been developed; in particular, a magneto-optical method achieves detectability of 50–100 parasites/μL [16] but the necessary equipment (large electro-magnet, laser, sophisticated optics…) probably limits its use in developing countries where demand is the highest.

The high field gradients generated by magnetic spheres have been used for more than ten years to produce a suspension of red blood cells enriched by erythrocytes infected with the parasite [15,18]. Indeed, the magnetization of large crystals of hemozoin is sufficient to trap infected erythrocytes in a magnetic field gradient [13,19,20]; these studies mostly aim at increasing the sensitivity of microscopy gametocytes detection, useful in the latent phase of the disease [20–22], or to separate infected RBCs and hemozoin for further studies [18,23]. However, the use of this separation method has not yet been envisaged for the routine quantitative measurement of whole blood hemozoin levels.

The present work proposes a simple and cost-effective approach for the determination of hemozoin, based on haemolysis of erythrocytes, trapping of paramagnetic crystals in a magnetic field gradient, elution by dissolution in NaOH and measurement of absorbance.

2. Material and method

Experiments were performed with β-hematin; this synthetic analogue of hemozoin has comparable structural properties although its immunogenicity may differ because of different crystal sizes and/or shapes [24–27]. Both crystals are paramagnetic because they contain Fe^{3+} ions in high-spin configuration [28–31]. Values of the magnetic susceptibility of hemozoin and β-hematin are comparable [14,28,29,32,33].

2.1. Synthesis of β-hematin and preparation of spiked blood samples

β-hematin (MW, 634 g mol^{-1}) was prepared from a protocol described by Slater et al. [24]. Briefly, a solution of 0.151 g of hemin (Sigma Life Science, > 98% pure) in 5 ml of N_2-degassed 0.66 M NaOH was acidified to pH 4.22 with 2% propionic acid, diluted to a final volume of 100 ml and heated overnight at 70 °C. The precipitate was washed in distilled water, 0.1 M sodium bicarbonate (pH 9.1) and distilled water, recovered by centrifugation and dried overnight at 50 °C. A stock suspension was prepared by suspending the precipitate in 100 ml of distilled water and adding 2 drops of Tween 80.

A red blood cells concentrate for transfusion was hemolyzed by 1/10 dilution in distilled water and spiked at different levels with dilutions of the β-hematin stock suspension (1 ml of β-hematin dilution + 9 ml of hemolyzed RBC).

2.2. Plasmodium falciparum cultures

Plasmodium falciparum (chloroquine-sensitive strain 3D7, originally isolated from a patient living near Schipol airport, The Netherlands) asexual erythrocytic stages was cultivated *in vitro* according to the procedure described by Trager and Jensen [34] at 37 °C and under an atmosphere of 5% CO_2, 5% O_2 and 90% N_2. The host cells were human red blood cells (A or O Rh+). The culture medium was RPMI 1640 (Gibco) containing 32 mM $NaHCO_3$, 25 mM HEPES and L-glutamine. The medium was supplemented with 1.76 g/L glucose (Sigma–Aldrich), 44 mg/mL hypoxanthin (Sigma–Aldrich), 100 mg/L gentamycin (Gibco) and 10% human pooled serum (A or O Rh+). Parasites were subcultured every 3–4 days with initial conditions of 0.5% parasitemia and 1% haematocrit.

Plasmodium falciparum culture flasks, a generous gift from Prof. Michel Frédérich, Laboratoire de Pharmacognosie, Phytochimie et Phytothérapie, Université de Liège, Belgium, were obtained at a parasitemia level of 1.75% and stored at 5 °C between 24 and 48 h before testing. Such a storing has a severe killing effect on all life stages of the parasite, except rings [35]. This should not impact our measurements as crystallized hemozoin is insoluble in water and chemically quite stable.

2.3. Device for magnetic separation of β-hematin

A macroscopic trapping system was formed by a tubing column, filled either with a bunch of guitar strings (nickel-plated steel wire wrapped around a steel core; diameter, 1.32 mm; D'Addario USA, type EXL140 10–52) or with micro beads extracted from MACS column (Miltenyi Biotec, Bergisch Gladbach, Germany), fitted in the gap of a 0.45 T magnet (MidiMACS, Miltenyi Biotec) and connected to a syringe pump (Pump-II, Harvard Apparatus, USA) set at 0.5 ml/min (Fig. 1). The sample, rinsing solution (1% Triton X-100% and 0.9% NaCl in deionized water) and 1 M NaOH were sequentially injected in the system. The evaluates were collected in semi-micro disposable cuvettes (Plastibrand®) and their UV–visible spectra were recorded from 330 to 800 nm (Spectrawave S800, Biochrom, UK).

3. Results and discussion

3.1. Magnetized wires effectively trap β-hematin crystals

In a first experiment, the trapping of β-hematin was visually tested by infusing 8 ml of spiked hemolyzed RBCs in a magnetized wires column at a rate of 0.5 ml/min; the concentration of β-hematin (225 μM) was relatively high for these preliminary tests. The trapping appears effective as the solution eluted through the bunch is visually free from β-hematin particles; rinsing the column with 4 ml of water containing Triton X-100 and NaCl yields a perfectly clear evaluate. Upon removing the magnet, β-hematin is released as a coloured trail eluting from the column (Fig. 2).

β-hematin is immobilized by the magnetic field gradient because each crystal is made of an aggregate of several hundred million of paramagnetic atoms; the overall magnetization provided by this multitude of ferric ions constitutes a high resultant magnetic moment, yielding a strong overall interaction with the gradient created by the

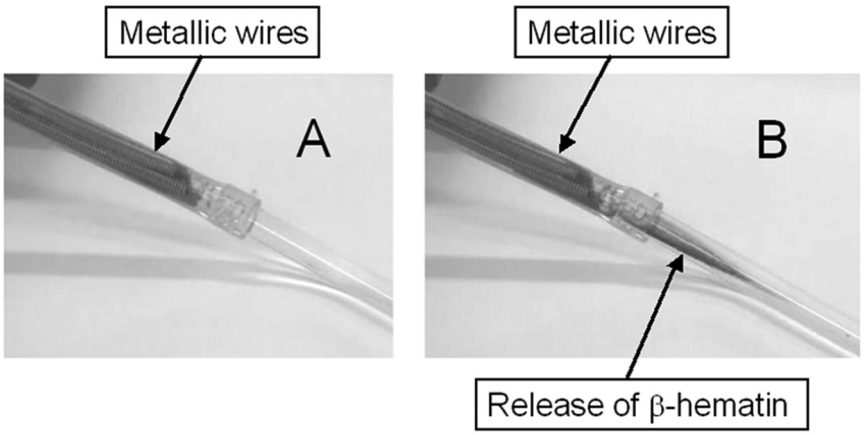

Fig. 2. Trapping and elution of β-hematin (A) β-hematin trapped on metallic wires (magnet removed; flow off) (B) Elution of β-hematin (magnet removed; flow on).

steel wires. Indeed, the energy **E** of a particle in a magnetic field $\mathbf{B_0}$ is given by

$$\mathbf{E} = \frac{\mathbf{N_p}\cdot\mathbf{u_p^2}}{\mathbf{k}\cdot\mathbf{T}}\cdot\mathbf{B_0^2} \quad (1)$$

where $\mathbf{N_p}$ is the number of paramagnetic ions in the crystal or aggregate, $\mathbf{u_p}$ the paramagnetic moment of the ion, **k** the Boltzmann constant and **T** the absolute temperature (K). The force exerted on the particle is given by the gradient of change of magnetic energy in the three dimensions of space. It therefore increases with Np, the number of paramagnetic ions per crystal or per aggregate.

In blood samples, hemoglobin could be partly converted to deoxyhemoglobin, a structure that also presents paramagnetic properties; however, in deoxyhemoglobin, the number of iron ions per molecule is only four and the overall magnetic moment is too tiny to significantly interact with magnetic field gradients; even considering a large aggregate of deoxyhemoglobin proteins, the very low amount of iron in the agglomerate (mass ratio = 0.0033) predicts a very low magnetization of the whole entities. Also, nano-sized particles such as ferritin are too small to create an overall magnetic moment that could interact with the field gradient at the surface of steel wires.

This preliminary experiment clearly indicates that the magnetic field gradient created on the surface of steel wires is sufficient to immobilize the crystals of β-hematin; upon removing of the magnet, the residual magnetization and the magnetic field gradient amplitude at their surface become sufficiently low to allow elution of β-hematin.

3.2. Quantitative aspects of β-hematin crystals trapping

Progressive dilutions of β-hematin were mixed with hemolyzed RBCs down to the μM range, which corresponds to a rate of *Plasmodium* infection of about 0.1% [32], and 8 ml were injected into the magnetic trapping system. The β-hematin fixed in the bunch of steel wires was rinsed to remove residual blood, dissolved in 1 M NaOH and collected in a spectroscopy cell (0.5–1 ml were collected; the exact volume was measured by weighing the cell and absorbances were adjusted to 1.0 ml). The obtained UV–visible spectra (Fig. 3-A) are characteristic of solubilized hematin, clearly showing the strong intensity Soret band around 400 nm and the tiny Q-bands in the region between 450 and 750 nm [14]. A calibration curve (2.0–22.5 μM; Fig. 3-B) was established with synthetic β-hematin dissolved in 1 M NaOH, quantitative measurements being based on the absorbance at 390 nm. From this calibration curve, the molar absorption coefficient of β-hematin dissolved in NaOH was estimated at 4.37 10^4 Mcm^{-1}.

The trapping of β-hematin was investigated in the 2–6 μM concentrations range both for steel wires (Fig. 4-A) and micro-beads (Fig. 4-B) columns. In these macro-systems, β-hematin was detectable below 2 μM (or 1.26 μg/ml) in 8-mL blood solutions. The obtained linear trend corresponds to that obtained by Newman et al. (1–5 μg/mL) using a magneto-optic device [16]. The measured β-hematin binding is only 23% on steel wires, compared to 80% binding on microbeads. This can be explained by widely differing and less efficient magnetic field lines for rod-shaped compared to spherical magnets, poles being largely separated in the case of rods [36]; magnetic field gradients also increase for decreasing sizes of the ferromagnetic objects that fill the separation column.

3.3. Trapping of Plasmodium falciparum hemozoin

Different volumes of a *Plasmodium falciparum* culture (haematocrit, 1%; parasitemia, 1.75%, corresponding to ~ 87.5 parasites/μL; dilution 1:2) were injected into the microbeads magnetic column; after washing and elution, the measured absorbances indicate a linear relationship with injected volumes (Fig. 5). Regarding the comparison of tested cultures with real samples, it should be considered that (i) the development of *P. falciparum* in *in vitro* cultures is usually asynchronous with all asexual stages of the parasite present at any given time [35]; and (ii) transcriptional patterns in *ex vivo* culture display little variation across patients with diverse clinical profiles and closely resemble transcriptional profiles that occur *in vitro* [37]. The *in vitro* culture is however quite different from natural infection samples, notably with regard to haematocrit (1% vs 20–53%, depending on age, sex and severity of malaria [38–40]; note that the measurement of haematocrit in malaria patients is subject to artifacts, i.e. slow-centrifuging trains of cells and wall shear stress [41]).

From these data, the concentration of hemozoin in the *Plasmodium* culture amounts to 0.92 μmol L^{-1}, corresponding to 0.59 μg/mL^{-1}. Based on the hypothesis that 1 μg whole blood hemozoin correlates to a parasitemia of 0.033% [16], this level corresponds to an infection of 0.02%.

Supposing *(i)* no influence of blood components and blood concentration; *(ii)* an eventual relationship between hemozoin and parasitemia; and *(iii)* and that the haematocrit of our sample is 1% *versus* about 45% for whole blood, it could be speculated that the determination of this amount of hemozoin from the blood of a patient would correspond to a parasitemia of 1.75%/45 = 0.04% ~ 2000 parasites/μL.

4. Conclusions

This work indicates the possibility of developing a quite simple instrument to determine the amount of hemozoin, the malaria pigment, in haemolyzed red blood cells and in *Plasmodium* cultures. The tested macroscopic trapping system achieved a detection limit comparable to

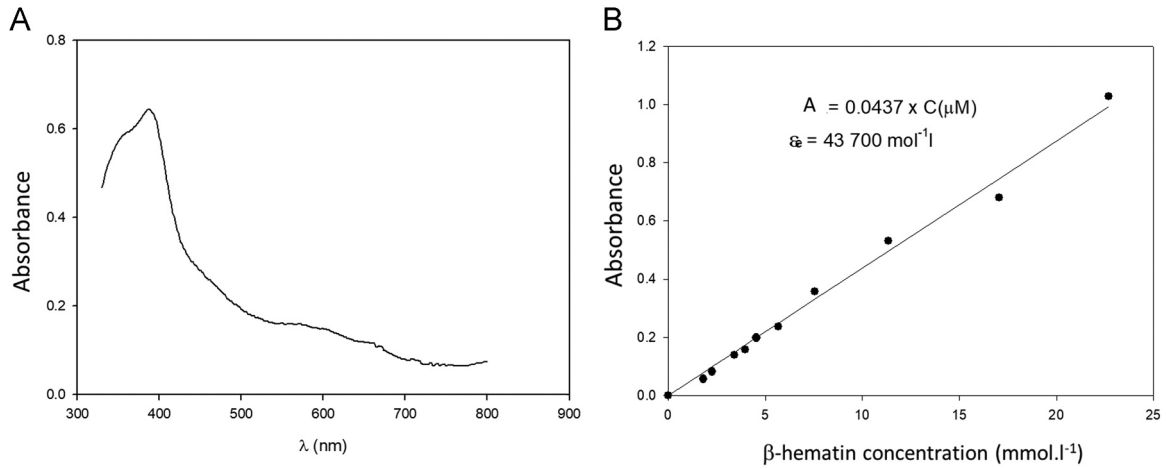

Fig. 3. (A) Optical spectrum of β-hematin eluted in 1 M NaOH (17 μM) (B) Calibration curve for the spectrophotometric determination of β-hematin.

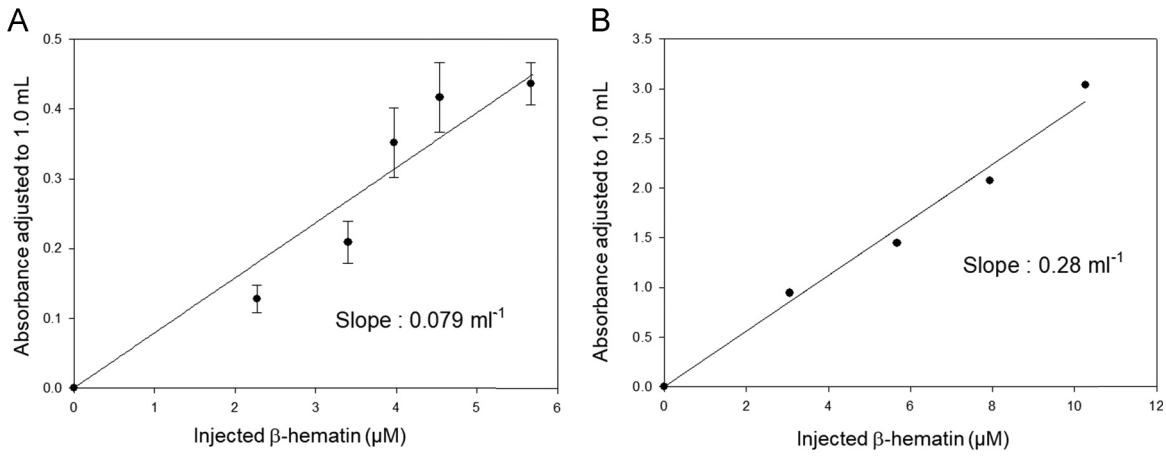

Fig. 4. (A) Correlation between the absorbance of β-hematin trapped/eluted on a magnetic steel wires column and the injected β-hematin blood concentration (n = 3) (B) Correlation between the absorbance of β-hematin trapped/eluted on a magnetic beads column and the injected β-hematin blood concentration (data from a single experiment).

that of the first prototype of magneto-optical detection described by Newman et al. [16].

For use in malaria diagnosis, both the amount of injected blood and achieved sensitivity are obviously unpractical. The injection volume should be reduced in the range 50–300 μL (50 μL for finger prick collection) and the detectability should be reduced by a factor of about 200 to reach about 10 parasites/μL [26,42].

Further work will aim at implementing a flow-system with on-line injection and detection combined to a miniaturized trapping system, an obvious way to achieve magneto-chromatography of hemozoin, to reduce dead volumes and to increase sensitivity. Reducing the diameters of microbeads should increase the intensity of the magnetic field gradient [36] to improve the proportion of trapped hemozoin. Based on a paper from the malERA Consultative Group on Diagnoses and Diagnostics [43], and on the cost of subsidized treatments, a final target cost of about 1 $/diagnostic test would be desirable. This could probably be achieved with a microchips-type flow device; the prices strongly depend on production volumes, but projected costs of polymer, manufacturing and microbeads packing were found compatible with this target (data not shown).

More than 80% of the iron-porphyrin complexes released during the meal of the parasite would be converted into hemozoin [15,44]; upon erythrocytes lysis, hemozoin is rapidly captured by white blood cells and accumulates in organs (liver, spleen, bone marrow) but its fate and role in malaria infection remain hypothetical [45]. As no reliable measurement is available so far, the relevance of hemozoin levels for malaria diagnosis and prognostic is still unknown [26]. Many questions indeed remain open. On one hand, the relationship between whole-blood hemozoin level and parasitemia is uncertain; since immature forms of the parasite contain only a very small amount of hemozoin,

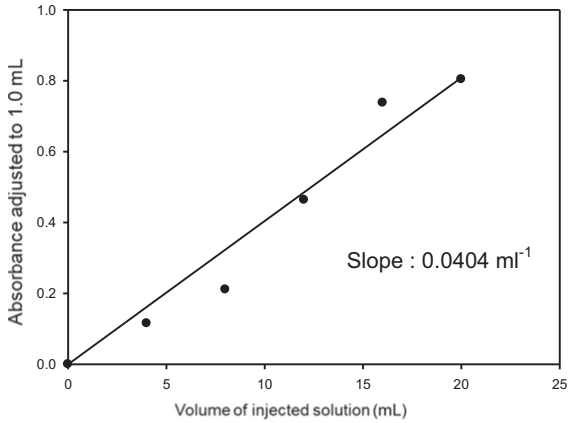

Fig. 5. Correlation between the absorbance of hemozoin trapped/eluted on a magnetic beads column and the volume of *Plasmodium falciparum* culture injected.

detected levels depend on both *Plasmodium* cycle phase, parasitemia and hemozoin levels in white blood cells and serum. On the other hand, the clearance of hemozoin from blood after infection cure is not known and other parasitic diseases, prevalent in endemic areas, such as schistosomiasis, also produce hemozoin [46].

An accurate and sensitive determination method for the determination of whole blood hemozoin is needed to answer these questions. The macroscopic system we tested gives an interesting clue for the development of such a method based on magnetic trapping for the concentration of hemozoin and its determination.

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Declaration of interest

A. Roch and P. Duez are inventors for a patent based on the research described in the present manuscript *("Malaria detection", British Patent Office, 29 October 2014)*

References

[1] WHO, World malaria report, World Health Organization Geneva, 2015.
[2] WHO, World Malaria Report 2016, World Health Organization, Geneva, 2016.
[3] WHO, Test. Treat. Track. Scaling up Diagnostic Testing, Treatment and Surveillance For Malaria, WHO, Geneva, 2012.
[4] WHO, Management of Severe Malaria, WHO, Geneva, 2012.
[5] WHO, Malaria Microscopy Quality Assurance Manual, WHO, Geneva, 2009.
[6] B. Aydin-Schmidt, M. Mubi, U. Morris, M. Petzold, B.E. Ngasala, Z. Premji, A. Bjorkman, A. Martensson, Malar. J. 12 (2013) 349.
[7] T. Hanscheid, M.P. Grobusch, Trends Parasitol. 18 (2002) 395–398.
[8] WHO, Good Practices for Selecting and Procuring Rapid Diagnostic Tests of Malaria, (2011).
[9] G. Hassanpour, M. Mohebali, A. Raeisi, H. Abolghasemi, H. Zeraati, M. Alipour, E. Azizi, H. Keshavarz, Parasitol. Res. 108 (2011) 1519–1523.
[10] T.J. Egan, J. Inorg. Biochem. 102 (2008) 1288–1299.
[11] T.J. Egan, J.M. Combrinck, J. Egan, G.R. Hearne, H.M. Marques, S. Ntenteni, B.T. Sewell, P.J. Smith, D. Taylor, D.A. van Schalkwyk, J.C. Walden, Biochem. J. 365 (2002) 343–347.
[12] B.V. Mendelow, C. Lyons, P. Nhlangothi, M. Tana, M. Munster, E. Wypkema, L. Liebowitz, L. Marshall, S. Scott, T.L. Coetzer, Br. J. Haematol. 104 (1999) 499–503.
[13] F. Paul, S. Roath, D. Melville, D.C. Warhurst, J.O. Osisanya, Lancet 2 (1981) 70–71.
[14] A. Butykai, A. Orban, V. Kocsis, D. Szaller, S. Bordacs, E. Tatrai-Szekeres, L.F. Kiss, A. Bota, B.G. Vertessy, T. Zelles, I. Kezsmarki, Sci. Rep. 3 (2013) 1431.
[15] S. Hackett, J. Hamzah, T.M.E. Davis, T.G. St Pierre, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1792 (2009) 93–99.
[16] D.M. Newman, J. Heptinstall, R.J. Matelon, L. Savage, M.L. Wears, J. Beddow, M. Cox, H.D. Schallig, P.F. Mens, Biophys. J. 95 (2008) 994–1000.
[17] I. Silva, R. Lima, G. Minas, S.O. Catarino, in: Proceedings of the 5th Portuguese Meeting on Bioengineering (ENBENG), 2017, pp. 1–4.
[18] C.C. Kim, E.B. Wilson, J.L. DeRisi, Malar. J. 9 (2010) 17.
[19] S.Y. Ahn, M.Y. Shin, Y.A. Kim, J.A. Yoo, D.H. Kwak, Y.J. Jung, G. Jun, S.H. Ryu, J.S. Yeom, J.Y. Ahn, J.Y. Chai, J.W. Park, Parasitol. Res. 102 (2008) 1195–1200.
[20] A.H. Bates, J. Mu, H. Jiang, R.M. Fairhurst, X.Z. Su, Exp. Parasitol. 126 (2010) 278–280.
[21] S. Karl, T.M. Davis, P.T.G. St, Malar. J. 9 (2010) 116.
[22] S. Vyas, V. Genis, G. Friedman, J. Magn. Magn. Mater. 423 (2017) 301–305.
[23] A.H. Fairlamb, F. Paul, D.C. Warhurst, Mol. Biochem. Parasitol. 12 (1984) 307–312.
[24] A.F. Slater, W.J. Swiggard, B.R. Orton, W.D. Flitter, D.E. Goldberg, A. Cerami, G.B. Henderson, Proc. Natl. Acad. Sci. USA 88 (1991) 325–329.
[25] M. Jaramillo, M.J. Bellemare, C. Martel, M.T. Shio, A.P. Contreras, M. Godbout, M. Roger, E. Gaudreault, J. Gosselin, D.S. Bohle, M. Olivier, PLoS One 4 (2009) e6957.
[26] L.M. Coronado, C.T. Nadovich, C. Spadafora, Biochim. Biophys. Acta 1840 (2014) 2032–2041.
[27] C. Coban, M. Yagi, K. Ohata, Y. Igari, T. Tsukui, T. Horii, K.J. Ishii, S. Akira, Allergol. Int. 59 (2010) 115–124.
[28] C. Brémard, J.J. Girerd, J.C. Merlin, S. Moreau, J. Mol. Struct. 267 (1992) 117–122.
[29] D.S. Bohle, B.J. Conklin, D. Cox, S.K. Madsen, S. Paulson, P.W. Stephens, G.T. Yee, Structural and Spectroscopic Studies of β-Hematin (the Heme Coordination Polymer in Malaria Pigment), in: Inorganic and Organometallic Polymers II, American Chemical Society, Washington DC, 1994, pp. 497–515.
[30] D.S. Bohle, P. Debrunner, P.A. Jordan, S.K. Madsen, C.E. Schulz, J. Am. Chem. Soc. 120 (1998) 8255–8256.
[31] A. Sienkiewicz, J. Krzystek, B. Vileno, G. Chatain, A.J. Kosar, D.S. Bohle, L. Forró, J. Am. Chem. Soc. 128 (2006) 4534–4535.
[32] S. Hackett, J. Hamzah, T.M. Davis, P.T.G. St, Biochim. Biophys. Acta 1792 (2009) 93–99.
[33] Y. Gossuin, P. Okusa Ndjolo, Q.L. Vuong, P. Duez, Sci. Rep. 7 (2017) 14557.
[34] W. Trager, J.B. Jensen, Science 193 (1976) 673–675.
[35] L. Yuan, M. Hao, L. Wu, Z. Zhao, B.M. Rosenthal, X. Li, Y. He, L. Sun, G. Feng, Z. Xiang, L. Cui, Z. Yang, Exp. Parasitol. 140 (2014) 18–23.
[36] G.P. Hatch, R.E. Stelter, J. Magn. Magn. Mater. 225 (2001) 262–276.
[37] J.E. Lemieux, N. Gomez-Escobar, A. Feller, C. Carret, A. Amambua-Ngwa, R. Pinches, F. Day, S.A. Kyes, D.J. Conway, C.C. Holmes, C.I. Newbold, Proc. Natl. Acad. Sci. USA 106 (2009) 7559.
[38] I.A. Carneiro, C.J. Drakeley, S. Owusu-Agyei, B. Mmbando, D. Chandramohan, Malar. J. 6 (2007) 67.
[39] L. Quintó, J.J. Aponte, J. Sacarlal, M. Espasa, P. Aide, I. Mandomando, C. Guinovart, E. Macete, M.M. Navia, R. Thompson, C. Menéndez, P.L. Alonso, Trop. Med. Int. Health 11 (2006) 1741–1748.
[40] N.N. Odunukwe, L.A. Salako, C. Okany, M.M. Ibrahim, Trop. Med. Int. Health 5 (2000) 582–586.
[41] T. Wang, Z. Xing, Biomed. Res. Int. 2018 (2018) 14.
[42] WHO, New Perspectives: Malaria Diagnosis. Report of a joint WHO/USAID Informal Consultation, World Health Organization, Geneva, 2000.
[43] The malERA consultative group on diagnoses and diagnostics, PLoS Med. 8 (2011) e1000396.
[44] T.J. Egan, Targets 2 (2003) 115–124.
[45] M. Boura, R. Frita, A. Gois, T. Carvalho, T. Hanscheid, Trends Parasitol. 29 (2013) 469–476.
[46] S.Q. Toh, A. Glanfield, G.N. Gobert, M.K. Jones, Parasit. Vectors 3 (2010) 108.