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Polymerization-based amplification as a tool for malaria diagnostics

Samuel Raccio

Novel diagnostic techniques are needed in the fight against tropical diseases. In the case of malaria, many infections are below the detection limit of routinely carried out tests. Our group has developed an assay to detect hemozoin, a biomarker of malaria. Under conditions of atom transfer radical polymerization, hemozoin catalyzes the formation of radicals that initiate the polymerization of N-isopropylacrylamide (NIPAAm). As the reaction is carried out above the lower critical solution temperature of the polymer, turbidity is formed. The rate of turbidity formation is proportional to the amount of biomarker present. To make the assay more rapid and sensitive, the polymerization-based amplification components were thoroughly investigated. Optimization of the reaction conditions reduced the assay time from 37 min to 3 min while maintaining a low detection limit of 1.06 ng mL-1.

Hemozoin isolation from blood samples spiked with *P. Falciparum* culture was investigated using methods such as centrifugation, size-exclusion chromatography and flow through paper-based chromatography. This method allowed to purify and analyze one sample in less than one hour. Although lacking of sensitivity, hemozoin isolation on paper was the most simple to perform, fastest and required only minimal equipment.

Isolation by centrifugation was employed during a field test in Brazil giving a sensitivity of 91.3 % and a specificity of 93.8 % for *P. vivax* infections. The purification on paper achieved a sensitivity of 30 % and a specificity of 88 %. The work carried out has therefore brought the technique closer to a field diagnostic and identified those aspects in the hemozoin extraction protocol that need further development.

Jury:
Prof. Dr. Nico Bruns (thesis supervisor)
Prof. Dr. Chris Weder(external co-examiner)
Prof. Dr. Hans-Peter Beck (external co-examiner)
Prof. Dr. Matthias Marti (internal co-examiner)
Prof. Dr. Ulrich Steiner(president of the jury)