ABSTRACT

Malaria rapid diagnostic tests (RDTs) are a great achievement to the implementation of parasite based diagnosis before treatment. They use a nitrocellulose strip coated with antibodies that target the *Plasmodium* antigens; histidine rich protein II (HRP2), lactose dehydrogenase (LDH), and aldolase. A major drawback of the method is that the majority of RDTs do not have positive control materials to test different batches of RDTs at the point of care. Dried *Plasmodium*-infected samples with the RDT target antigens has been suggested as a possible positive control, but their utility is hampered by rapid loss of activity over time and variability of performance over different ranges of temperatures. This study hypothesized that the temporal and thermal stability HRP2, LDH and Aldolase can be improved by addition of chemical additives. The objectives of the study were to determine the effect of chemical additives on temporal stability of HRP2, LDH and aldolase proteins as well as to investigate the effect of chemical additives on thermal stability of HRP2, LDH and aldolase proteins, and finally, to compare the effect of chemical additives on stability of HRP2, LDH and aldolase obtained from wild versus cultured parasites present in dried *Plasmodium* infected samples. This was a retrospective cross-sectional study utilizing forty archived whole blood patient samples from Kombewa Sub-County Hospital. *In vitro* cultivated *Plasmodium falciparum* parasites were utilized; prepared at Walter Reed Project. Three RDTs (SD Bioline, First Response and Binax NOW) were selected based on round two of the World Health Organization's performance evaluations and their ability to detect at least two of the target antigens. Blood samples were tested for baseline activity of the antigens then treated with chemical additives. Repetitive RDT testing of the stabilized specimens was done for a period of eight months. To determine temporal and thermal stability of the proteins, the Z-test of proportions was used to compare the proportionate stability time difference between control and stabilized samples. Logistic regression model for repeated measures was used to determine the differences in responses between cultured samples and patient samples. HRP2 was shown to loses stability in <21 weeks (63% stability) while aldolase and LDH lost stability in <12 week (33% stability) during the eight months of storage. Temporal stability of HRP2, LDH, and aldolase increased significantly (>18%, $P<0.001$) in the presence sucrose, trehalose, sucrose/trehalose, biostab/trehalose, and LDH stabilizer/trehalose. Thermal stability of HRP2 was shown to be lost in <21weeks at temperatures of 37°C and 45°C while LDH and aldolase lost stability in <12 weeks at similar conditions. There was significant increase in stability (>24%, $P<0.05$) of HRP2, LDH, and Aldolase in the presence of trehalose, sucrose/trehalose, and LDH stabilizer/trehalose at both 37°C and 45°C across all three parasite densities. Cultured samples at 2000p/µL can be used to develop the positive controls in the same capacity as patient samples ($P>0.05$). In conclusion, trehalose and its combination with biostab enzyme stabilizer, sucrose and LDH stabilizer improved the temporal stability of HRP2, LDH and Aldolase for eight months of storage. Thermal stability of HRP2, LDH and Aldolase was increased at 25°C, 37°C and 45°C in the presence of trehalose, sucrose/trehalose, and LDH stabilizer/trehalose. We recommend that trehalose or in combination with sucrose, biostab or LDH stabilizers can be used in the development of stable field deployable positive controls for validation of malaria rapid tests. Routine validation of malaria RDTs would improve reliability and confidence of the results thereby ensuring accurate treatment of malaria.