

Laboratory methods

Sickling Testing

Sickling test was conducted among patients suspected of having a sickle cell syndrome. The sickling tests were conducted using Sodium metabisulphite. The Sodium metabisulphite reduces the oxygen tension inducing the typical sickle-shape of red blood cells. This test uses fresh blood in any anticoagulant. To prepare reagents for this method, 0.2 g of sodium metabisulphite in 10 ml of distilled water. Stir until dissolved. Prepare fresh each time. You normally, Mix 1 drop of blood with 1 drop of 2% sodium metabisulphite solution on a microscope slide. Then you cover with a cover slip and seal the edge with wax/vaseline mixture or with nail varnish. Allow to stand at room temperature for 1 to 4 hours. Thereafter, examine under a microscope with the dry objective. In positive samples, the typical sickle-shaped red blood cells will appear Occasionally the preparation may need to stand for up to 24 hours. In this case put the slides in a moist Petri dish. False negative results may be obtained if the metabisulphite has deteriorated or if the cover slip is not sealed properly(23).

Malaria testing

Whole blood was used to do Malaria Rapid Diagnostic Test (MRDT) using The Alere SD BIOLINE Malaria Ag P.f/Pan test which is a rapid, qualitative and differential test for the detection of histidine-rich protein II (HRP-II) antigen of *Plasmodium falciparum* and common *Plasmodium* lactate dehydrogenase (pLDH) of *Plasmodium* species in human whole blood. It detects HRP2 Ag specific to *P. falciparum* and pLDH specific to *Plasmodium* species with a fast test result (15-30 minutes) and not beyond. This is a lateral flow technology with a very high sensitivity and specificity of 99.7% (P.f), 95.5% (non-P.f) 99.5% (251,252) respectively. Malawi has a high prevalence of *Plasmodium falciparum* and hence a need to use such a diagnostic tool(24).

FBC Analysis

FBC analysis was done by Sysmex Haematology analyser (Sysmex XP-300™ Automated Haematology Analyzer) which uses 50µL on whole blood. Using this analyser, WBCs, RBCs and PLTs were counted using the direct current detection method with coincidence correction. Automatic discriminators separate the cell populations based on complex algorithms. The intensity of the electronic pulse from each analysed cell was proportional to the cell volume. The haematocrit (HCT) was directly determined based on the red cell count and volume detection of each individual RBC. Even with samples at extremely low or

unusually high concentrations, the Sysmex cell counters analyse WBCs, RBCs and PLTs with uncompromised precision and accuracy(25).