External Quality Assurance In Malarial Parasite Density Counts

N.H Ali Pour, F Estepa, L Wienholt, E Dales

The Royal College of Pathologists of Australasia Quality Assurance Programs Pty Ltd, St Leonards, NSW, Australia

Background
Microscopy is the gold standard for determining malarial parasite density. Density counts by microscopic visualisation of malarial parasites on thick and/or thin blood smears, provide information on the severity of infection and of treatment. Density counts are highly variable and the method used influences the accuracy of results. Standardisation of methods may assist in reducing variability. The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) conducts a biannual malarial external quality assurance program, using digital images of Plasmodium falciparum (P. falciparum) infected peripheral blood films. Density count results from the 2014–2017 programs were analysed to assess the methods used and the accuracy of reported values.

Method
Survey samples were digital images of P. falcoparum-infected peripheral blood films at parasite densities ranging from 9300 parasites/µL – 171000 parasites/µL. Participants received digital images and were asked to perform density counts using either the thick and/or the thin film image and report their results and methods used. Method options for thick film counts were 150 white blood cells (WBC) or 200 WBC counted. Method options for thin film counts were use of a miller ocular square or counting fields of 200 red blood cells (RBC). Thin film users also reported the number of RBC counted; <2000, 2000 or >2000. Results received over four years (8 samples) were used to assess the influence of methodology on the accuracy of reported values.

Results
The majority of participants reported both thick and thin film results for all surveys (Figure 1). At lower parasite densities, coefficients of variation (CV) were lower in thick films compared to thin films (Figure 2). At higher parasite densities CVs were lower in thick films compared to thick films (Figure 2). The majority of thick film results were obtained by counting 100 WBC on a thick film or >2000 RBC on a thin film appeared to reduce CV (table 1). For thin films, CVs were lower when a miller ocular square was used (mean CV 29.4%) compared to counting fields of 200 RBC (mean CV 39.2%) (figure 3).

Discussion
The World Health Organisation (WHO) standard operating procedure (SOP) stipulates that, when available, thick film counts should be performed. If parasite count is >100 parasites in each field, this equates to >80 000 parasites/µL and a thin film count should follow. Microscopists are required to count 200 WBC on thick films or 500 WBC if parasite count is >100 parasites in 200 WBC. Approximately 5000 RBC should be counted on thin films.

For RCPAQAP surveys, the majority of participants reported on both thick and thin films (figure 1). There was a slight preference for thin films (figure 1) that was observed for all surveys, including those with >80 000 parasites/µL. Thick films are known to be more sensitive to low parasite densities, and were also found to be more accurate at lower parasite densities (table 1). As parasite load increased, thin film CV reduced to less than that of thick films, corresponding to the increased accuracy of thin films at higher parasite load (figure 2). The number of WBC counted on thick films was not correlated to parasite density, with 97% of thick film users consistently counting 100 WBC at all parasite densities (table 1). Reduction of CV was evident for counting >2000 RBC as opposed to <2000 or 2000, as expected (table 1). However, only 17% of participants counted 200 WBC and 14% counted >2000 RBC on thick and thin films respectively, and it is possible that the bias in user numbers skewed the CVs (table 1). This also highlighted that the number of cells counted was, in most cases, well below that recommended by WHO (table 1). When thin films were used, most participants used a miller ocular square (table 1). Use of a miller ocular square was shown to improve accuracy (figure 3).

Conclusion
The suitability of film type was directly related to parasite density; thick films at lower densities and thin films at higher densities. This reflects the WHO SOP that stipulates thin films should be used when parasite load is >80 000 parasites/µL. The number of cells counted on both thick and thin films was lower than recommended and the number of WBC counted on thick films was not correlated to parasite density, as would be expected. When thin films were used, use of a miller ocular square consistently improved accuracy and use is recommended where possible. Variability in reported density counts remains high. Use of appropriate methodologies as per the WHO SOP and use of a miller ocular square on thin films may assist in reducing variability.

Table 1. Malarial parasite density count methods – Proportion of users at varying densities