External Quality Assurance In Malarial Parasite Density Counts

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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 efficacy of treatment. Density counts are highly variable and the method used influences the accuracy of results[1, 2]. Standardisation of methods may assist in reducing variability[1]. The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) conducts a biannual malarial parasite 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. falciparum* 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 100 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 thin films compared to thick films (figure 2). The majority of thick film results were obtained by counting 100 WBC to estimate parasite density and the majority of thin film results were obtained using a miller ocular square (table 1). 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).

Figure 1. Film type used to perform malarial parasite density count at a range of parasite densities (Mean ± SEM).

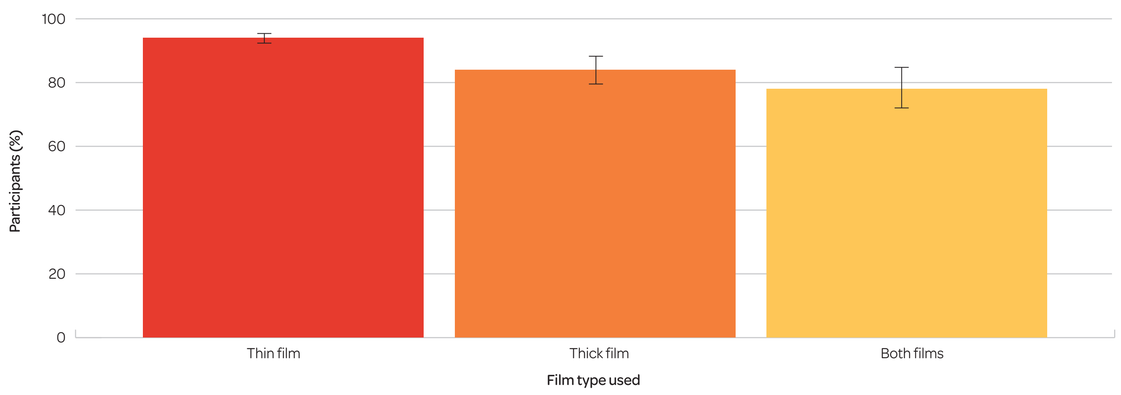


Figure 2. Coefficients of variation (CV) of thin and thick film malarial parasite density counts at a range of parasite densities.

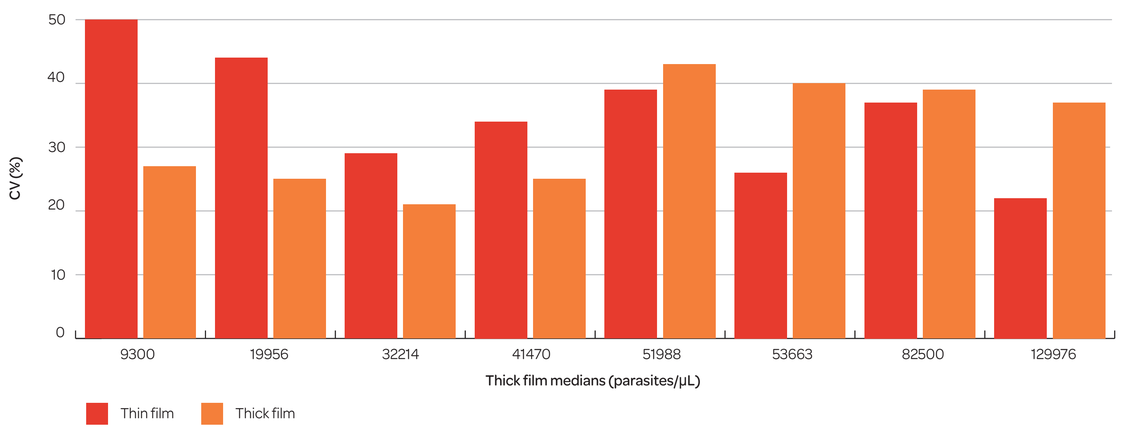
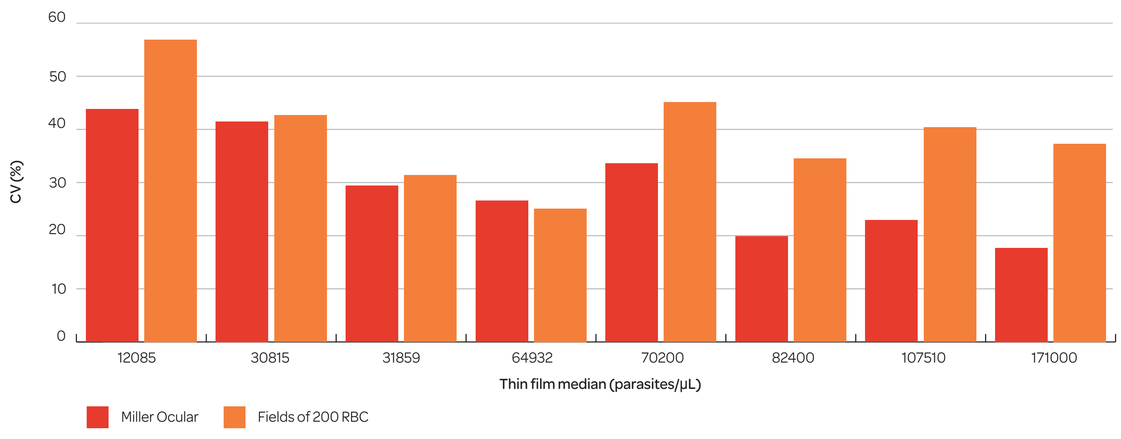


Figure 3. Coefficients of variation (CV) of thin film malarial parasite density counts using miller ocular square or counting fields of 200 red blood cells (RBC) at a range of parasite densities.



Discussion

The World Health Organisation (WHO) standard operating procedure (SOP) stipulates that, when available, thick film counts should be performed[3]. If parasite count is >100 parasites in each field, this equates to >80 000 parasites/µL and a thin film count should follow[3]. Microscopists are required to count 200 WBC on thick films or 500 WBC if parasite count is ≤100 parasites in 200 WBC[3]. Approximately 5000 RBC should be counted on thin films[3].

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[4], and were also found to be more accurate at lower parasite densities (figure 2). 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 ≥77% 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 >2000RBC 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[3]. 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[3]. 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

Thick film median (parasites/µL)	Thin film median (parasites/µL)	WBC counted (% Thick film users)		Counting method (% Thin film users)		RBC counted (% Thin film users)		
		100 WBC	200 WBC	Miller Ocular	Fields of 200 RBC	<2000 RBC	2000 RBC	>2000 RBC
9300	12085	79	21	55	45	37	47	15
19956	30815	80	20	60	40	44	43	12
32214	31859	86	14	58	42	41	47	12
41470	64932	80	20	61	39	44	42	14
51988	70200	87	13	56	44	39	45	15
53663	82400	77	23	63	37	47	42	11
82500	107510	87	13	52	48	36	49	15
129976	171000	87	13	58	42	41	45	13
Number of users (%) (Mean ± SEM)		83 ± 1.5	17 ± 1.5	58 ± 1.2	42 ± 1.2	41 ± 1.3	45 ± 0.9	14 ± 0.6
CV (%) of density count (Mean ± SEM)		30 ± 3.3	34.5 ± 4.8	29.4 ± 3.2	39.2 ± 3.2	33.5 ± 3.5	35.8 ± 2.7	30.4 ± 2.7

References

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