Evaluation of a Direct Counting Technique for Malaria in Thailand

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OBJECTIVE: To evaluate a direct quantitative technique for the determination of parasite densities in malaria in rural hospitals in Thailand.

BACKGROUND: Various methods have been used for the direct quantification of malaria parasites but none has been widely used in tropical countries. The technique discussed in this report was introduced by Earle and Perez in 1932.

DESCRIPTION: A measured volume of blood (e.g. 0.005 ml) is spread evenly on a measured rectangle (e.g. 3 x 15 mm) on a slide. Thus the thickness of the film is known (0.11 mm). A grid is placed in one eyepiece of a binocular microscope and calibrated with a stage micrometer. Thus the volume of blood seen under one grid is known. For example, in the SEATO studies, about 1800 grids correspond to 1 cu. mm. of blood. Two types of staining are used. For outpatient screening 0.002 ml of blood are placed in the rectangle and stained with concentrated Giemsa (1:5 dilution) for five minutes. In patients admitted to the ward, 0.005 ml of blood are used and the thick film is stained with dilute Giemsa (1:50 dilution) for 30 minutes. Two rectangular thick films are prepared on inpatient slides. A circular thick film and a thin film are also prepared on all patients.

In this study the technicians were observed performing the parasite counts. There were five technicians on the study team. A stop watch was used to time the duration of the count. The following data were recorded for each determination of the parasite density. The name of the technician, the microscope used, the number of fields counted, the actual count obtained, the count per cu.mm. and the time elapsed in performing the count. A hand calculator was used for determining the parasite densities.

PROGRESS: The first factor assessed was the variability among technicians. Numerous duplicate counts on coded slides were performed by all the technicians. One technician counted with an accuracy of 3.4 per cent (Table 1) as determined on nine slides. The microscopes used were recalibrated frequently. No consistent difference in the two microscopes was observed.

The number of fields that need to be counted varies with the parasite count. For example, if the parasite count is 500,000 per cu.mm. there are about 250 parasites under each grid. The slide is scanned to find typical concentrations of parasites and with such a high count, a minimum of two fields have to be counted. At the other end of the scale a count of 500 requires the counting of at least 90 fields. Usually the number of fields counted is 2, 20, 90 or a larger number. Except for very low counts, about 200–500 parasites are counted. The accuracy of the technique is shown in Table 1. In this experiment the most accurate count was achieved in the patient with a parasite density of 10,000 (0.4 percent precision). The technique was less accurate with both high (4.1% precision), and low (11.1% precision) parasite densities. However the accuracy of the technique was satisfactory through all ranges of the parasite count. This technician read the nine slides twice in an average of 3.8 minutes per slide (range 2.1 to 6.3 minutes). Numerous other experiments have shown that only about four minutes is needed to make an accurate count.

SUMMARY: In rural hospitals in Thailand, a direct quantification technique has proved useful and accurate for the determination of parasite densities in malaria.

Table 1. Duplicate Counts on 9 Falciparum Malaria Slides by One Observer Using The Direct Quantitative Technique

Slide	1st (a) Reading	2nd (b) Reading	Mean (c)	c—a	Precision $(\frac{c-a\%}{c})$
1	220220	202748	211484	8736	4.1
2	188188	182364	185276	2912	1.6
3	46592	47593	47092	501	1.1
4	29757	29302	29529	228	0.8
5	10920	10829	10874	46	0.4
6	2020	2220	2120	100	4.7
7	1660	1900	1780	120	6.7
8	400	500	450	50	11.1
9	10	20	—	—	—
	Mean 55529	53052	—	—	3.4*

* This figure is the reproducibility from the mean or "PRECISION" and is an index of the accuracy of the technique.