Malaria Parasite Quality Assurance Programme in Hong Kong, 2002-2006

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Abstract

We report on the quality assessment of malaria laboratory diagnosis in Hong Kong from 2002 to 2006. Twenty-two laboratories, from private and public hospitals, participated in the Malaria Parasite Quality Assurance Programme. Both rechecking of routine slides and proficiency testing using unknown panels of malaria parasite blood films are valuable measures of laboratory performance. Outcome of the assessment of participants' performance showed 2003 to be the best year while 2004 the worst. We summarised the common problems that participants encountered and suggested ways by which the quality of malaria microscopy could be strengthened, including enhancing microscopist competency, and performance through constant training, supervision and quality control of malaria laboratory services.

Introduction

Malaria is one of the major causes of death from communicable diseases globally. Hong Kong was an endemic area for malaria even after the Second World War. Until 1969, local transmission was stopped and there was no indigenous case until 1976 when sporadic local cases were again reported. After that, there was an outbreak of indigenous cases in Sai Kung. In the late 1980's, Vietnamese refugees contributed a great majority of cases and thereafter a progressive decrease in incidence was observed. In the past five years from 2002- 2006, the number of malaria cases was less than 50 every year, and the majority was imported cases. Unfortunately, the mosquito vectors such as *Anopheles minimus* & *Anopheles jeyporiensis* continue to prevail in some parts of Hong Kong. Therefore, global strategies and approaches to effective malaria control is still applicable in Hong Kong [1-2]

As the burden of malaria in Hong Kong continues, the availability of high quality diagnostic services is mandatory. Laboratory diagnosis by microscopic examination of stained blood films continues to be the gold standard for confirming clinical suspicious malaria[3]. In 1983, a consultancy from the World Health Organisation (WHO) recommended the implementation of Quality Assurance Programme (QAP) for malaria microscopy in Institute of Pathology in Hong Kong. Afterwards, in 1985, Central Malaria Reference Laboratory (CMRL) was set up and with Malaria Parasite (MP) QAP went alive.

The objectives of the MP QA programmes were to (1) improve the overall performance of the malaria microscopists at each level of the laboratory services; (2) sustain the highest level of accuracy in detecting and confirming the presence of malaria parasites; (3) monitor systematically laboratory procedures, reagents and equipment; and (4) establish an information exchange laboratory network on updated malaria laboratory testing strategies.

Currently, this programme includes activities (a) cross-checking of routine positive and negative slides (b) unknown panel slides testing (c) training such as seminars and workshop. In order to gather experiences gained from this QAP, we undertook an analysis of performance for the past five years from 2002 to 2006 to look for trends in performance and lessons learned in malaria blood film diagnosis in Hong Kong.

Methods

The programme presently comprises two components: (1) blood film proficiency testing of two distributions per annum including 2 panel slides (either Giemsa stained or unstained) per distribution; (2) rechecking of slides: all malaria positives and 10% negative malaria slides were sent to CMRL for cross-checking. In 2006, twenty two laboratories participated in this QAP, with 2 (9%) from private sector laboratories and 20 (91%) from public hospital laboratories.

Malaria blood film proficiency testing: panel slides

Assessment of performance was focused on the ability of microscopist to: (1) detect malaria parasite: in terms of sensitivity and specificity; (2) differentiate different species; (3) correctly reporting parasite density within +/- 1 grade using WHO plus system; or +/-2SD using parasite count per white blood cell; or +/-2SD percentage infected red blood cell; (4) accurate *P. falciparum* reporting: for both *P. falciparum* identification and correct parasite density determination; and (5) appropriate stain slides in dispatched unstained blood film. Together these parameters assisted in assessment of abilities in parasite detection. Overall performance evaluation was based on comparison of participants' performance per year, and total score was made per laboratory.

Rechecking of routine positive and negative MP slides

Participant laboratories submitted all malaria positives and 10% negatives slides (thick & thin smear) to CMRL for cross-checking. Assessment was based on results from CMRL as the reference and compared with those from the participants. Discrepancies were classified as major or minor error. Major errors included detection error (positive- negative) and incorrect *P. falciparum* identification. Minor errors included all non- *P. falciparum* species identification reporting. Quality of the blood film preparation and staining technique were also assessed and reported for all submitted blood films. CMRL provided feedback to the participating laboratories for discussion and possible improvement measures.

Results

Comparison of participants' performance per year

Table 1 showed the trend (in percentage) of participants achieving the correct result per year. Sensitivity and specificity of detection were excellent for the past 5 years irrespective stained or unstained films. In figure 1, it can be seen that blood films with *P. malariae* showed less than 50% of accuracy in differentiation amongst participating laboratories, while in the same period 95-100% of laboratories gave correct *P. falciparum* identification. In 2004, there was clearly poorer performance with problems in both parasite quantitation and non-*P. falciparum* identification, thus giving overall correct reporting by all laboratories of less than 80%. In figure 2, more than 90% achieved correct *P. falciparum* identification but failed to give accurate parasitemia estimation. The year 2003 saw the best overall performance with greater than 90% accuracy in *P. falciparum* identification and quantitation.

Table 1. Comparison of participants' performance in the year 2002-2006 in malaria blood film proficiency testing

Percentage / Year	2002	2003	2004	2005	2006
correct_parasitemia	95	95	72	89	88
correct_id	83	86	74	92	100
P. vivax	92	82	69	86	100
P. malariae	47	NA	NA	NA	NA
P. falciparum	100	95	95	95	100
Sensitvity	100	100	100	100	100
Specificity	NA	100	100	100	NA
Pf_id + parasitemia	87	95	60	86	85
P. falciparum	100	95	95	95	100
Stained film sensitivity	100	100	100	100	100
Unstained film sensitivity	NA	100	100	100	100

PF = *Plasmodium falciparum*; ID = identification

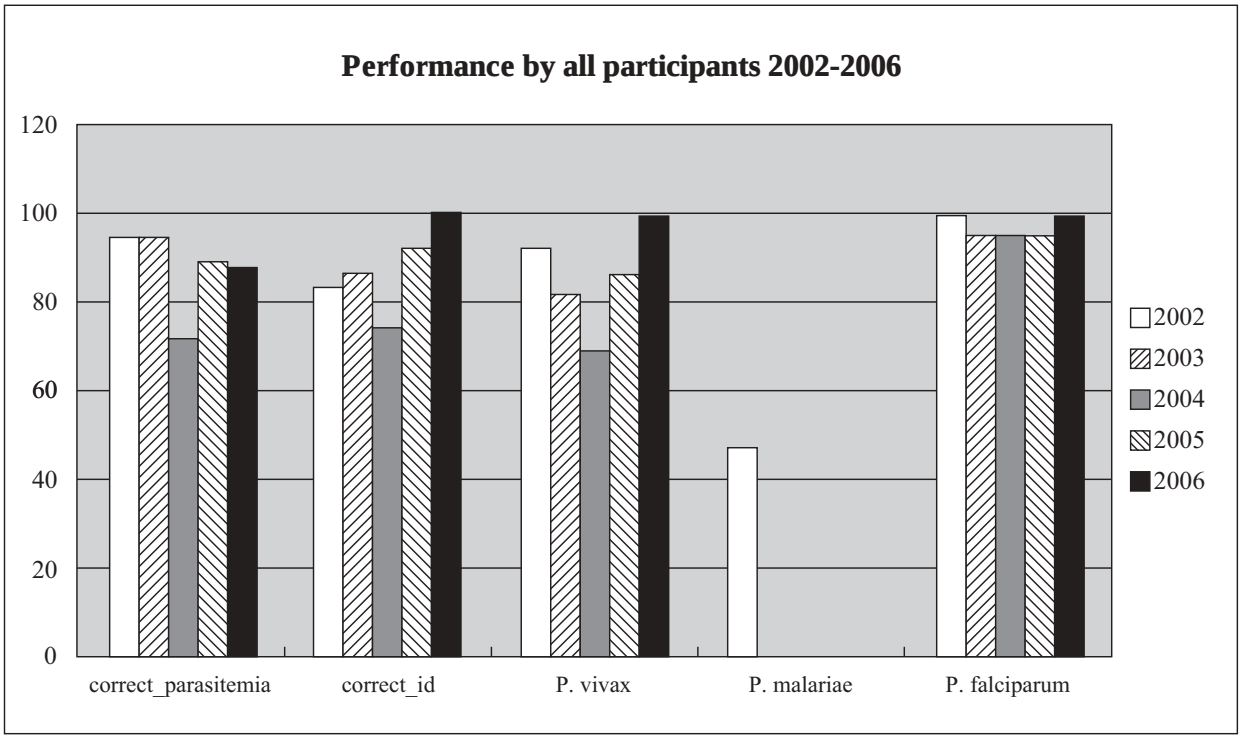

Figure 1. Comparison of participants' performance 2002-2006 in malaria parasite blood film examination

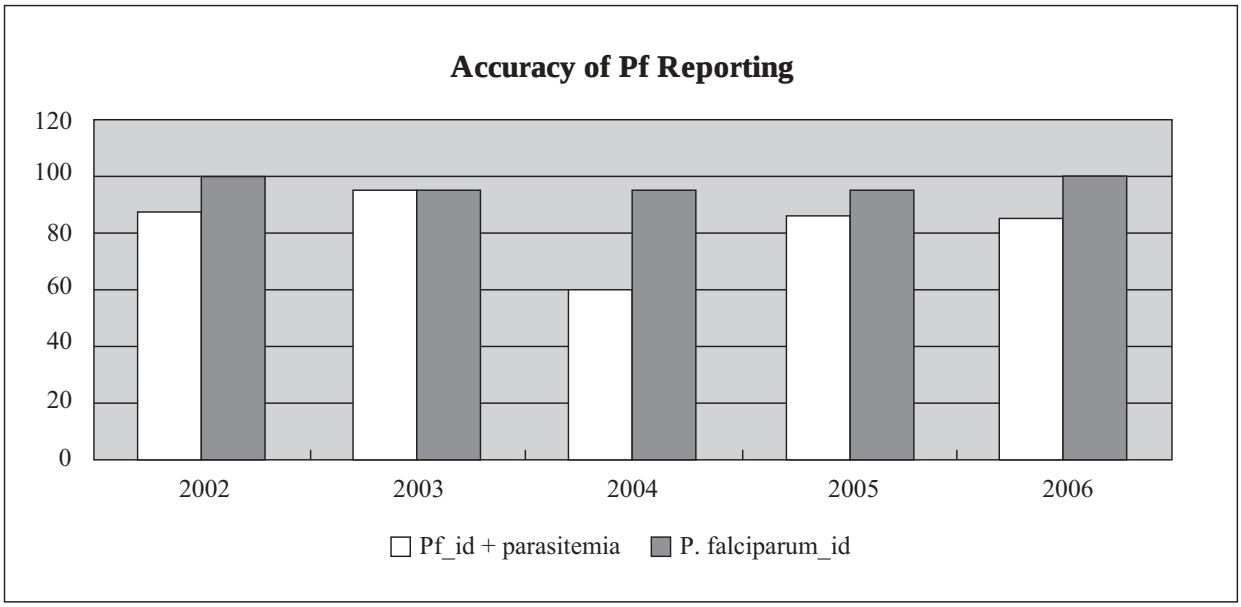

Figure 2. Comparison of participants' performance in *P. falciparum* (Pf) identification (id) and density (parasitemia) reporting

Comparison of total score between laboratories

A total of 20 unknown panel slides, including 4 negatives and 16 positives, were issued to 22 participating laboratories in 2002 to 2006. Each laboratory was assessed with a target full score of 600 including score of 100 for each of sensitivity, specificity, correct identification, correct parasite density, *P. falciparum* identification, combination of correctly identifying *P. falciparum* and quantifying parasitemia (Table 2). The mean score was 566 with the highest score of 596 (from 5 laboratories) and the lowest score was 499.

Rechecking of routine positive and negative MP slides

Table 3 and figure 3 show the highest case load (311 cases) of re-checking blood films was in 2005, while the highest number of positive films was occurred in 2002 (89 cases). This corresponded with the actual occurrence of malaria cases in the territory. Figure 4 shows the breakdown of positive cases to species level with species prevalence in the order of *P. vivax* > *P. falciparum*>*P. malariae* > *Mixed infection* >*P. ovale*.

The breakdown of laboratory discrepancies is shown in Table 4, with the lowest in 2003 when no major error occurred. Table 5 shows the sensitivity, specificity and agreement of species differentiation in the past 5 years. In both 2003 and 2006, there was excellent performance in malaria detection. On the contrary, there was least agreement in species identification in 2004. Overall, there was no agreement in *P. ovale* identification for the past 5 years, although the number of involved cases was not too many.

Table 2. Comparison of participants' performance by laboratory 1 to 22

% / Laboratory	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Sensitivity	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Specificity	100	100	100	100	100	100	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100
Correct_ID	87	91	100	96	96	96	96	91	100	96	91	100	83	91	94	87	91	65	100	100	84	89
Correct_density	78	91	96	100	100	91	91	91	96	100	91	71	91	91	82	100	91	100	91	67	100	83
Pf_ ID	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	67	100	100	100	83
Pf_ID + density	60	80	100	100	100	80	100	90	100	100	90	66	80	90	78	100	100	67	80	83	100	67
Total Score	**525**	**562**	**596**	**596**	**596**	**567**	**587**	**572**	**596**	**596**	**572**	**537**	**537**	**572**	**554**	**587**	**582**	**499**	**571**	**550**	**584**	**522**

PF = *Plasmodium falciparum*; ID = identification

Table 3. Distribution of number of re-checked cases and discrepancies 2002-2006

Year	Neg	Pos	Total	PV	PF	PM	PO	Mixed	Discrepancy	Major error	Minor error
2002	202	89	291	60	26	1	0	2	4	3	1
2003	238	40	278	17	20	2	1	0	1	0	1
2004	230	55	285	30	18	4	0	3	4	3	1
2005	256	55	311	24	28	1	1	1	4	3	1
2006	197	66	263	38	25	2	1	0	5	1	4

PV = *Plasmodium vivax*; PF = *Plasmodium falciparum*; PM = *Plasmodium malariae*; PO = *Plasmodium ovale*

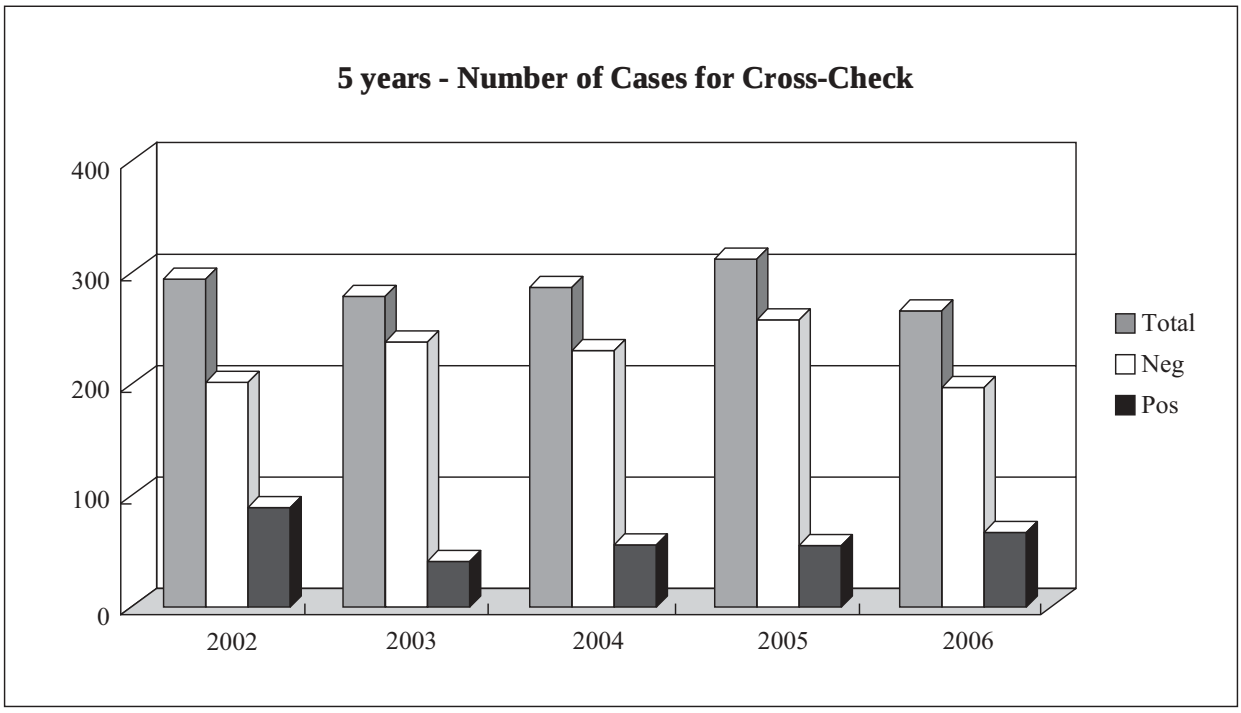

Figure 3. Distribution of annual case load 2002-2006 Neg = Negative; Pos = Positive

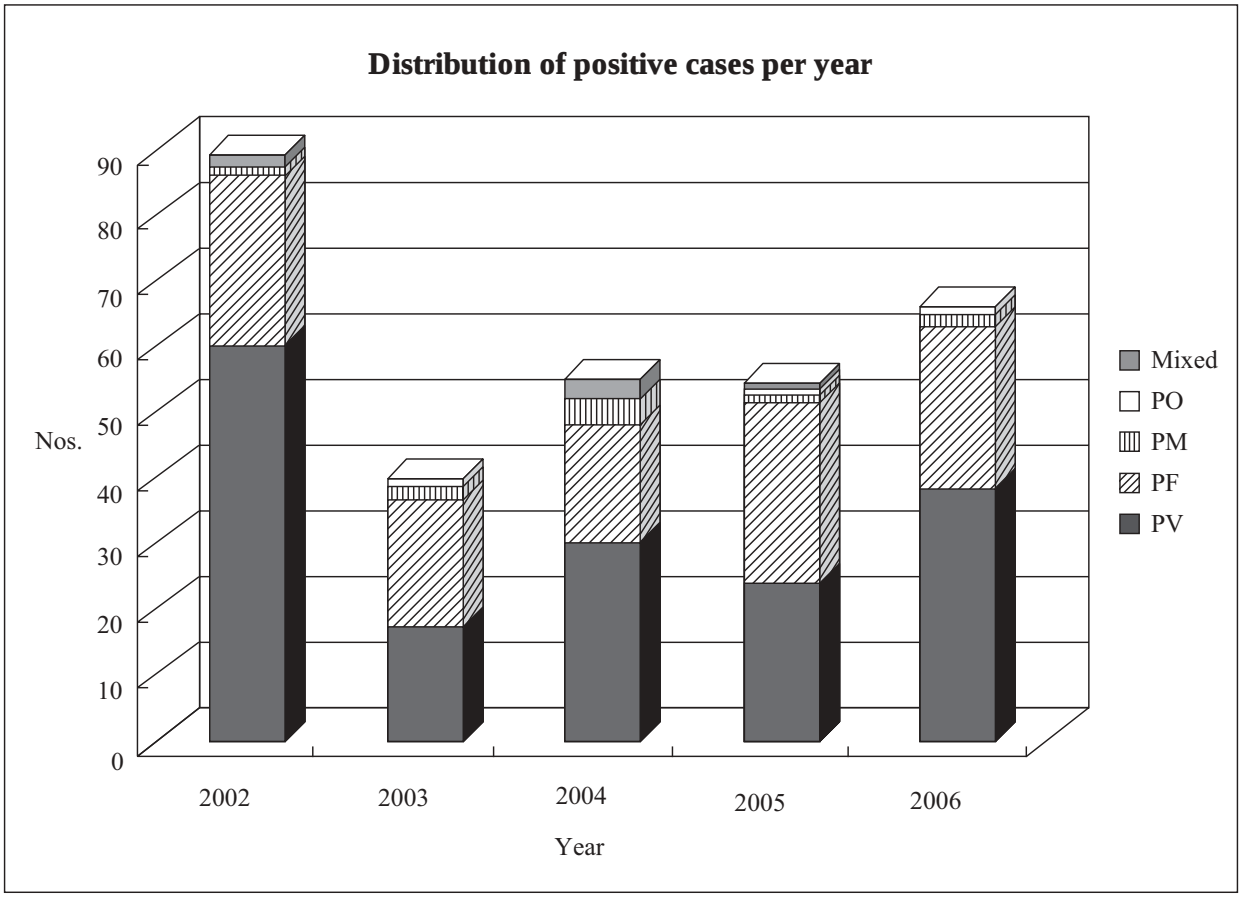

Figure 4. Breakdown of positive cases by species per year 2002 - 2006
PO = *Plasmodium ovale*; PM = *Plasmodium malariae*; PF = *Plasmodium falciparum*;
PV = *Plasmodium vivax*

Table 4. Breakdown of discrepancies 2002-2006

Year	No. of Discrepancies	Major error (false neg; false pos; Pf unidentified)	Minor error (non Pf unidentified)
2002	4	3 (1 false pos, 2 Pf)	1 (1 Pv)
2003	1	0	1 (1 Po)
2004	4	3 (2 false neg, 1 Pf)	1 (mixed infection)
2005	4	3 (1 Pf, 2 false pos)	1 (1 Po)
2006	5	1 (1 Pf)	4 (3 Pv, 1 Po)

pos = positive; neg = negative; Pv = *Plasmodium vivax*; Pf = *Plasmodium falciparum*;
Pm = *Plasmodium malariae*; Po = *Plasmodium ovale*

Table 5. Agreement of re-checking cases 2002 - 2006

Year	% Sensitivity	% Specificity	% agreement Pv	% agreement Pf	% agreement Pm	% agreement Po	% agreement mixed species
2002	100	99.5	98	92	100	NA	100
2003	100	100	100	100	100	0	NA
2004	96.3	100	100	83	100	NA	67
2005	100	99.2	100	96	100	0	100
2006	100	100	92	96	100	0	NA

Pv = *Plasmodium vivax*; Pf = *Plasmodium falciparum*; Pm = *Plasmodium malariae*;
Po = *Plasmodium ovale*; NA = *Not applicable*

Discussion

Light microscopy remains the main stay for detecting presence of malaria parasite, and for confirming clinical diagnosis.[3] It has the many advantages of (1) low cost (2) sensitive (3) can be used to differentiate species (4) can determine parasite density (5) can be used to diagnosis many other conditions. However, experience over the world, even in malaria endemic areas, has shown that it can be difficult to maintain good microscopy in all the health services providers where most malaria patients are detected and treated.

We have implemented the quality assurance programme for some time. The review of the proficiency tests in malaria parasite detection in 2002-2006 showed excellent results as reflected in 100% sensitivity and specificity per year by all participants in Table 1. However, in the rechecking programme, only in 2003 and 2006 could participants attain 100% detection (Table 5). This shows the pros and cons in both components of a quality assurance programme. Proficiency testing by unknown panels is measuring the best possible work of a microscopist or a laboratory rather than its daily standard because all participants are well aware that they are examining an EQA sample. The benefit of proficiency test is to allow a rapid assessment of gross deficiencies and identify factors contributing errors such as competency and training need of new microscopists. Re-checking programme has the advantage of reflecting the reality of routine laboratory performance as well as for monitoring laboratory performance[4]. The prime objective of implementing this

element is to detect unacceptable level of errors in their routine laboratory work with continuous motivation for better performance.

In the assessment process by proficiency testing with unknown panels (Table 1, Figs. 1-2), accuracy in *P. falciparum* identification with estimation of the percentage of parasitemia definitely require improvement; with the lowest being 60% (2004), 85% (2006), 86% (2005), 87% (2002) and highest was 95% (2003). Taking this as an overall assessment, participants' performance level was at the lowest in 2004 (less than 80% correct) and highest in 2003 and 2006. When comparing performance across laboratories (Table 2), five consistently gave excellent output in the past 5 years. For the laboratory with the lowest score (499), we found that all the incorrect results occurred before 2005. This implied that this laboratory made a lot of effort in improving performance since 2005. In the assessment process by rechecking routine slides (Table 3- 5), the best overall performance with no major error occurring was seen in 2003, when there was also 100% agreement in species identification. The lowest level of performance is again in 2004 with least agreement in mixed infection (67%), *P. falciparum* (83%) and 3 major errors occurred.

It is apparent that two separate QA processes can simultaneously detect similar outcomes in assessment of participants' performance levels in the past 5 years, with 2003 being the best year and 2004 the worst. These results indicated quality of microscopists was the critical determining factor of laboratory performance in Hong Kong.

Overall, participant laboratories had problems with calculating the parasitemia of *P. falciparum,* confusing *P. vivax and P. ovale*, confusing yeasts as merozoites, confusing other plasmodia-like parasites as malaria, and difficulties in identifying mixed species infection. Such observations have also been made in other part of the world[5].

Most of the problems encountered by our participants in achieving a correct result are summarized as below.

Firstly, some participants failed to produce a set of quality stained blood film, both thick and thin film, which is mandatory in accurate detection and identification of malaria. Secondly, some laboratories received very few requests for malaria resulting in lack of awareness and confidence in picking up the parasite and especially in species differentiation of malaria even when present. Third, there were too rapid turnover of laboratory staff posting, especially in some private sector laboratories that staff competency and performance in malaria microscopy could not be maintained. Fourth, some participants did not spend sufficient time to examine the slides and thus not aware the presence of more than one species of malaria in a mixed infection. Fifth, some participants were not aware of the importance of parasitemia of *P. falciparum* and have a tendency to over estimate the parasitemia.

Figure 4 shows *P. vivax* being the commonest species of malaria encountered, whereas *P. ovale* is very rare in Hong Kong. Therefore majority of participants have experience in making correct diagnosis of *P. vivax* but very often made no attempt in speciation of *P. ovale*.

From the problems the participants encountered, there is an urgent need to strengthen the quality of malaria microscopy by (1) timely blood film collection, staining and reporting to clinicians for initiating effective treatment; (2) enhancing microscopist competency and performance through (3) constant training, supervision and quality control of laboratory services[3].

Conclusion

From the past five years experience of performance evaluation described in this review, we confirm that rechecking and proficiency testing in malaria parasite blood film detection are both very valuable measures of laboratory performance. The malaria quality assurance programme in Hong Kong now has increasing awareness in terms of maintenance and continuous improvement in standard of malaria laboratory diagnostic capacity by all participants. By overcoming practical problems encountered and with continuous training and motivation, we expect that our participants' performance level will be not less than 90% in microscopic accuracy[6] in the

proficiency tests; and the number of discrepancies detected in the rechecking programme reduced to very low or, better still, zero level with a hope that all suspect patients will benefit from having an appropriate diagnosis and treatment of malaria.

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