TECHNICAL REPORT
FIRST SLIDE PANEL
2011-2012

PROGRAM FOR EXTERNAL PERFORMANCE EVALUATION IN MICROSCOPIC DIAGNOSIS OF MALARIA

REGIONAL MALARIA PROGRAM
PREVENTION AND CONTROL OF COMMUNICABLE DISEASES
HEALTH SURVEILLANCE AND DISEASE PREVENTION AND CONTROL
PAN AMERICAN HEALTH ORGANIZATION
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INTRODUCTION

The first element in the Global Malaria Control Strategy is access to early diagnosis and prompt, effective treatment.

Implementation of policies that ensure access to prompt, effective treatment is necessarily based on the existence of a health system that offers prompt access to reliable—i.e. precise and accurate—diagnosis, for better surveillance, prevention, and control of malaria in the Americas.

Because of the necessity for national reference laboratories to have an External Performance Evaluation Program (EPEP), to contribute to improvement of the microscopic diagnosis of malaria, the Regional Malaria Program of the Pan American Health Organization (PAHO) has developed this program for external quality evaluation with the collaboration of the reference laboratories of Honduras and Peru. It is anticipated that this effort will not only improve malaria diagnosis at the reference centers, but will also permit the transfer of skills and the upgrading of resources in the countries.

Technical work in a laboratory should always be subject to constant supervision using quality control procedures. This supervision is not possible unless there is quality control that makes it possible to evaluate the work done by the laboratories. Success in the face of the new challenges to improve the efficiency of the public health response will partly depend on the quality and performance of *LABORATORY NETWORKS.*

OBJECTIVES

GENERAL OBJECTIVE

Establish technical procedures for the organization, design, and evaluation of the national reference laboratories in the countries of the Region in microscopic diagnosis of malaria, with a view to maintaining an efficient quality management system and contributing to strengthening the monitoring of malaria diagnosis in the Region of the Americas.

SPECIFIC OBJECTIVES

1. Evaluate results concordance with regard to reproducibility of positive or negative results.
2. Evaluate species concordance in participating countries.
3. Evaluate stage concordance in participating countries.
4. Evaluate parasite density concordance in participating countries.

SLIDE PANEL CHARACTERISTICS

- Slides of the species present in the Region: *Plasmodium vivax*; *Plasmodium falciparum*; and mixed slides (Pf/Pv).
- Slides with different parasite densities: low, medium, and high density.
- Stages: asexual and sexual states of *P. vivax and P. falciparum*.
- Negative slides.
- Number of slides per panel: 20.
- Groups of uniform panels with respect to the characteristics of the positive (species, stage, and parasitemia) and negative slides were used so that the evaluation can be compared among the different laboratories.
- Giemsa stain was used in the preparation of the slide panel.

PARAMETERS EVALUATED

1. Results: Detection of positive and negative slides, regardless of species.
2. Species: Detection of *Plasmodium vivax*, *Plasmodium falciparum*, or mixed infections.
3. Stage: Detection of asexual and sexual stages (*P. vivax* and *P. falciparum* gametocytes).
4. Parasite density: Independent quantitative detection of parasites for each stage of the species, calculated according to the established formula. [1][1]

$$\text{Parasite density} = \frac{\text{No. of parasites}}{\text{No. of white blood cells}} \times 6000$$

In the analysis of parasite density concordance between the evaluated laboratory and the evaluating laboratory, it will be considered concordant if the number of parasites reported is ±50% between one parasite density result and the other in the slide panel assigned by the evaluating laboratory.

RATING SCALE

Parameters Evaluated	Rating
Results concordance	Acceptable: 95-100%. Unacceptable: <95%
Species concordance	Acceptable: 95-100%. Unacceptable: <95%
Stage concordance	Acceptable: 80-100%. Unacceptable: <80%
Parasite density concordance	Acceptable: 80-100%. Unacceptable: <80%

RESULTS

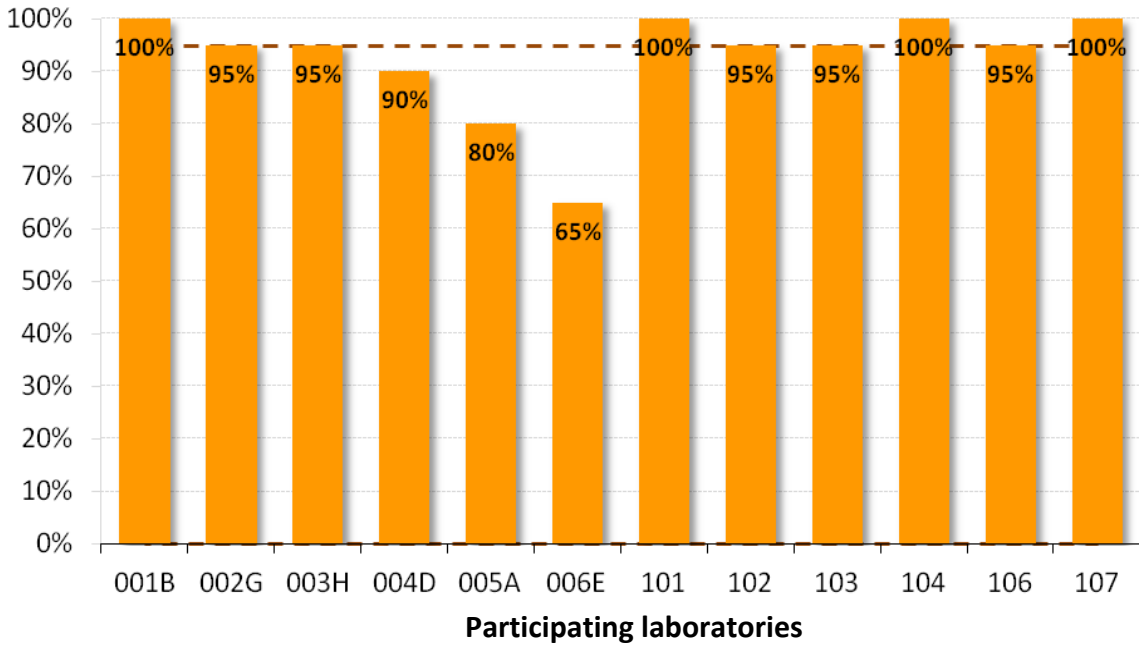
Twelve reference laboratories in the Region of the Americas participated in this first evaluation- six from Central America and six from South America, some where malaria is endemic and some where it is not endemic. Preliminary results were generated by the NETLab system in each of the participating laboratories as soon as data was entered, making it possible to quickly obtain percentages for each of the parameters evaluated.

In this second stage, we are sending this final report compiling results from the two supranational laboratories, to thus obtain an overall result for this first evaluation. Laboratories are identified by their codes in this report to ensure anonymity of the results.

Results for the first parameter evaluated—results concordance, shown in Figure 1—were: nine of the 12 participating laboratories obtained a percentage ≥95%, with a rating of acceptable, and three laboratories reported percentages <90%, receiving a rating of unacceptable according to the scale used.

One of the greatest problems observed with this first parameter was parasite detection on slides with low parasite densities.

Figure 1. Percentage for result concordance



Negative predictive value (NPV) in 10 of the 12 laboratories evaluated was above 90%, implying that in general these countries did not have problems with reading and identifying negative slides. However, positive slides were not identified with the same certainty, since the majority of the countries obtained a positive predictive value (PPV) below 90% (eight of the 12 laboratories evaluated). A kappa (K) index value greater than 0.8 shows good concordance among evaluators of the slides, and it can be seen that the majority of laboratories have good concordance with the regional reference laboratories, as shown in Table 1.

Table 1. Predictive and kappa values for results

Participating Laboratories	NPV	PPV	K
001B	100%	100%	1.00
002G	93%	89%	1.00
003H	100%	88%	0.82
004D	100%	74%	0.73
005A	71%	60%	0.82
006E	71%	20%	0.64
101	100%	100%	1.00
102	93%	89%	1.00
103	93%	89%	0.82
104	100%	100%	1.00
106	93%	89%	1.00
107	100%	100%	0.82

Results for the second parameter evaluated—species concordance, shown in Figure 2—were: only one of the 12 participating laboratories obtained a percentage >95% with a rating of acceptable; the remaining 11 had concordances below the required standards.

One of the greatest problems observed with this parameter was identification of mixed slides and their respective species.

Analyzing the data using predictive values, we observe that the laboratories overall, with one exception, have problems identifying slides positive for *P. falciparum*; some could only identify 44% of slides positive for this species, although almost none had problems reading negative slides (see Table 2). In the case of *P. vivax,* almost no laboratory had problems reading positive slides but almost all of them had problems identifying slides negative for this species. Although some of these laboratories belong to countries non-endemic for *P. falciparum*, which is also reflected in their evaluation, high levels of sensitivity and specificity should be maintained for diagnosis of positive cases, because they could have imported cases of this species.

Figure 2. Percentage for species concordance

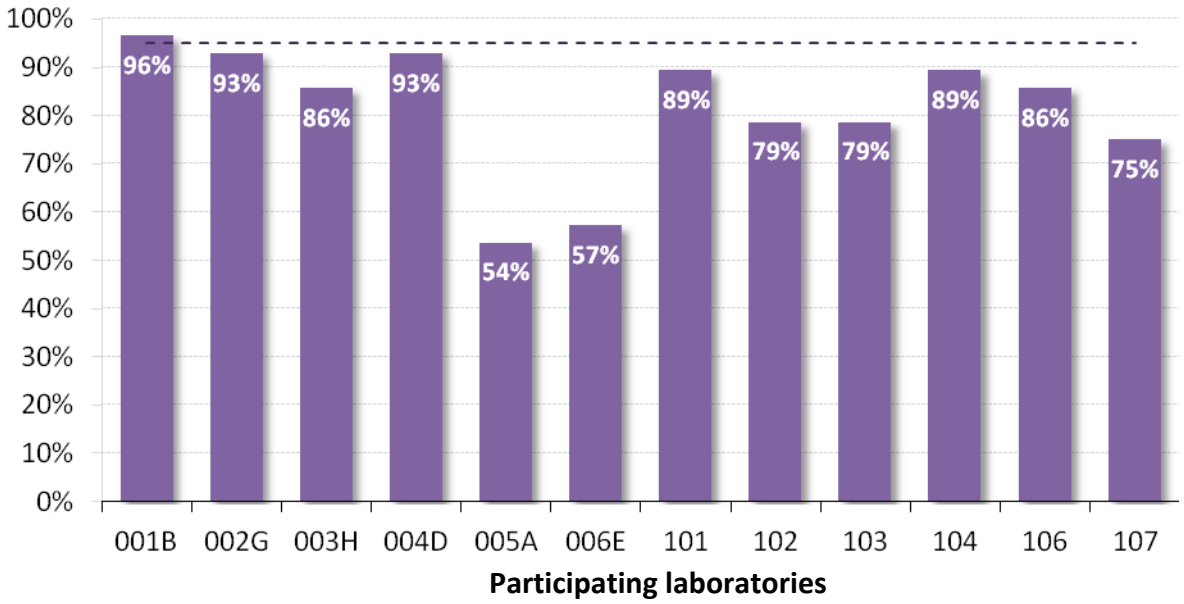


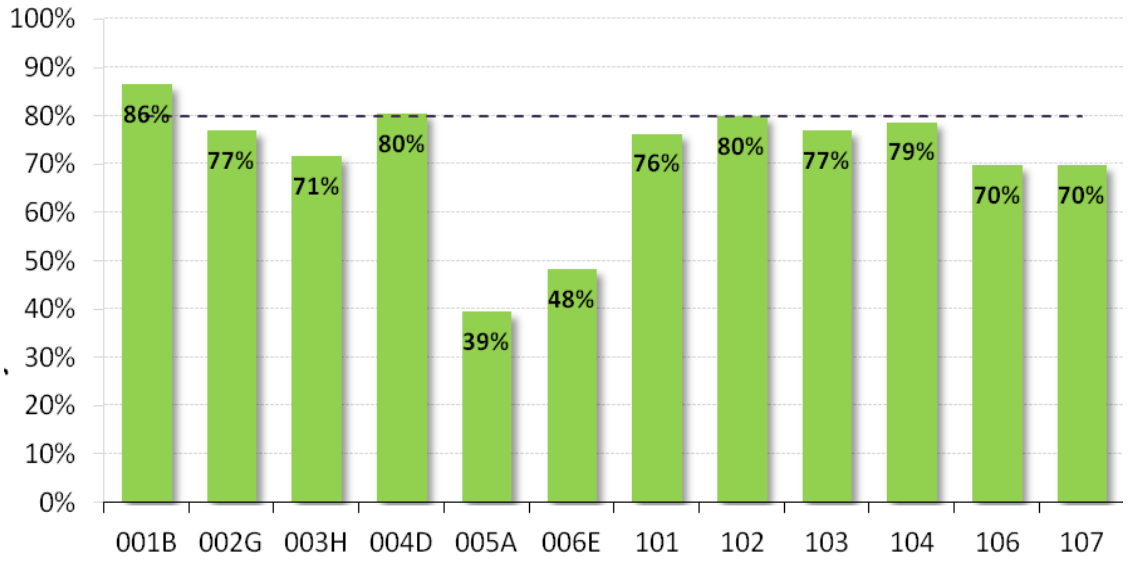
Table 2. Predictive and kappa values for species

Participating Laboratories	*P. vivax*			*P. falciparum*		
	NPV	PPV	K	NPV	PPV	K
001B	100%	89%	0.90	100%	100%	1.00
002G	100%	100%	1.00	100%	89%	0.90
003H	82%	78%	0.60	100%	89%	0.90
004D	73%	100%	0.71	100%	89%	0.90
005A	82%	78%	0.60	100%	44%	0.47
006E	64%	78%	0.41	100%	44%	0.47
101	100%	100%	1.00	91%	78%	0.69
102	100%	67%	0.69	100%	78%	0.79
103	82%	100%	0.80	100%	67%	0.69
104	100%	100%	1.00	100%	67%	0.69
106	100%	100%	1.00	100%	67%	0.69
107	82%	100%	0.80	100%	44%	0.47

Results for the third parameter evaluated—stage concordance, shown in Figure 3—were: three of the 12 participating laboratories obtained a percentage ≥80% with a rating of acceptable and the nine remaining laboratories had different percentages below the required standards.

One of the greatest problems with this parameter was the lack of identification of given stages, above all, asexual *P. falciparum*.

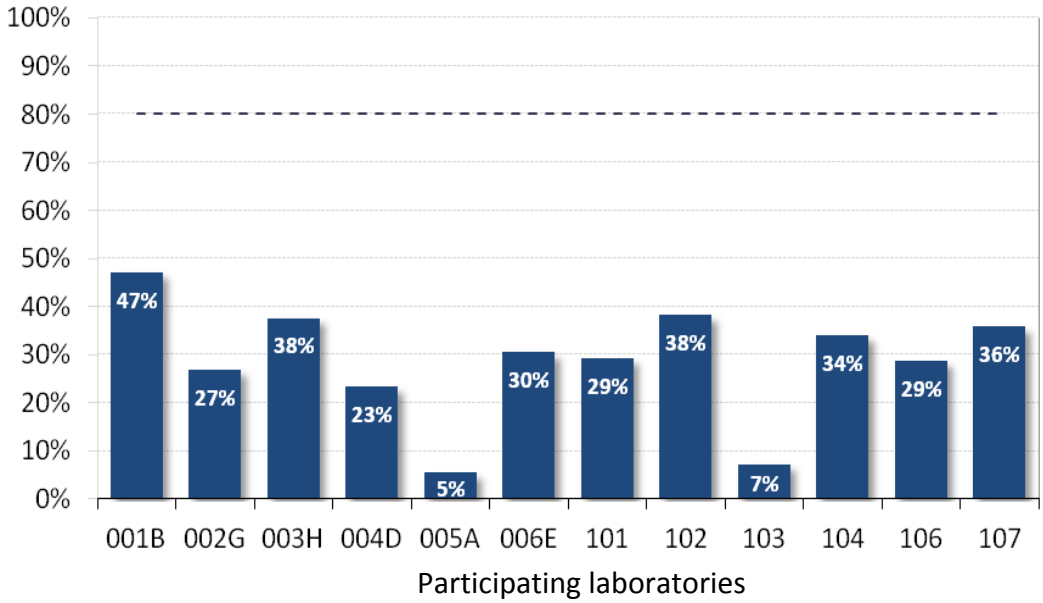
Figure 3. Percentage for stage concordance



Results for the fourth and final parameter evaluated—parasite density, shown in Figure 4—were very weak and all the laboratories evaluated obtained percentages below the required standards.

The greatest problem observed with this parameter is that counts were not done using parasites per microliter of blood (p/µl) because countries were using the 'plus' system as had been previously established. Currently, some of the countries evaluated are already performing counts of parasites per microliter of blood.

Figure 4. Percentage for parasite density concordance



CONCLUSIONS

This program has made it possible to identify certain strengths and weaknesses in reference laboratories, which will be addressed individually with each participating laboratory.

This program is also going to permit standardization of the processes for microscopic diagnosis of malaria at the regional level, since in their role as reference laboratories they should put emphasis on evaluating and supporting their laboratories in the departments and municipalities, to improve and to have high standards that assure the quality of malaria diagnosis at all levels of care in each participating country, whether endemic or non-endemic.

Recall that it is of the utmost importance for an endemic or non-endemic country to have adequate diagnostic capabilities, under a framework that guarantees their quality, to ensure rapid diagnosis and appropriate treatment for the purpose of shortening time of transmission, and not reintroducing the disease in areas where it has already been eliminated.

Laboratory personnel should be trained in the sensibleness of the formula and its use promoted in the calculation of parasite density per microliter of blood (p/µl)

RECOMMENDATIONS

With a view to overcoming the discordances obtained in the present evaluation, it is recommended that the personnel in charge of quality control for microscopic malaria diagnosis again reread the slides received, to detect errors and thus boost detection capability. Tables with the detailed results can be found at the EPEP website (http:/www.netlab.ins.gob.pe/frmloginmalaria.aspx), using the username and password you have been given for this program.

REFERENCE

[1] WHO/HTM/RBM, Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria, 2003.

ACKNOWLEDGEMENTS

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