TECHNICAL REPORT
SECOND SLIDE PANEL
2012-2013

EXTERNAL QUALITY ASSURANCE PROGRAM FOR MALARIA MICROSCOPY DIAGNOSIS

REGIONAL MALARIA PROGRAM
NEGLECTED, TROPICAL AND VECTOR-BORNE DISEASES
COMMUNICABLE DISEASES AND HEALTH ANALYSIS
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. (1)

Implementation of policies which ensure access to prompt, effective treatment is 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. (2)

Because of the necessity for national reference laboratories to have an External Quality Assurance Program (EQAP), 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 anticipation 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 OBJECTIVES

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 result concordance with regards to reproducibility of positive or negative results.
2. Evaluate species concordance in participating laboratories.
3. Evaluate stage concordance in participating laboratories.
4. Evaluate parasite density concordance in participating laboratories.

SLIDE PANEL CHARACTERISTICS

- Slides of the species present in the Region: *Plasmodium vivax; P. 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 across different laboratories and years.
- 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 *P. vivax*, *P. 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.(3-4)

$$Parasite\ Density = \frac{\text{No. of parasites}}{\text{No. of leukocytes}} \times 6000$$

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 results 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

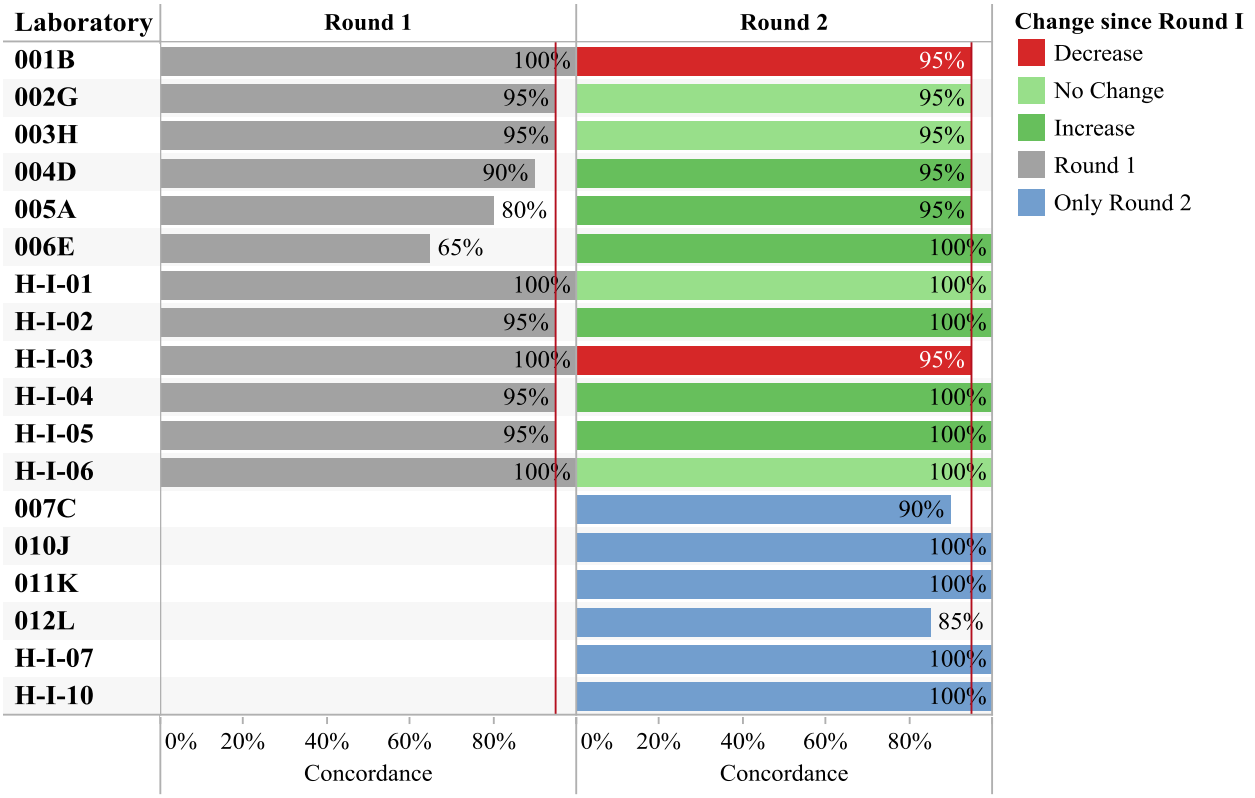
Nineteen reference laboratories in the Region of the Americas participated in this second evaluation- eight from Central America and ten from South America – seven more than the first round. Preliminary results were generated by the online NETLab system (5) for each of the participating laboratories as soon as the data was entered, making it possible to quickly obtain results for each of the parameters evaluated. One laboratory was excluded from the analysis because it could not enter the data in NETLab system. Specific support was provided to this laboratory to overcome this drawback and access the online system in the next round.

In the second stage, this final report is being sent compiling results from the two supranational laboratories and thus obtaining an overall result for this second evaluation. Laboratories are identified by their codes in this report to ensure anonymity of the results.

The results of round II for the first parameter evaluated, concordance for result , were: of the 18 participating laboratories, 16 obtained ≥95% concordance or acceptable, and 2 laboratories reported rates of 90% or less or unacceptable according to the scale used (Figure 1). Laboratories which participated in the first round showed general improvements in this parameter..

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

Figure 1. Percentage concordance for result parameter.



Generally, the negative predictive value (NPV) in the laboratories evaluated was 100%, implying that in general these countries did not have problems with reading and identifying negative slides. There were two exceptions where laboratories scored lower than 85% (Table 1). Similarly, the positive predicative value (PPV) results for the majority of laboratories was greater than 90% - only one laboratory reported a score lower than 80%. A kappa (K) index value greater than 0.8 shows good concordance among evaluators of the slides and demonstrates that the majority of laboratories, with two exceptions, have good concordance with the regional reference laboratories, as shown in Table 1.

Table 1. Predictive Values & Kappa for Result parameter.

Result			
Laboratory	**NPV**	**PPV**	**Kappa**
006-E	100%	100%	1.00
005-A	100%	93%	0.89
001-B	100%	93%	0.89
004-D	100%	93%	0.89
002-G	100%	93%	0.89
003-H	83%	100%	0.88
H-I-02	100%	100%	1.00
H-I-01	100%	100%	1.00
H-I-03	100%	93%	0.89
H-I-04	100%	100%	1.00
H-I-06	100%	100%	1.00
H-I-05	100%	100%	1.00
H-I-10	100%	100%	1.00
H-I-07	100%	100%	1.00
011-K	100%	100%	1.00
010-J	100%	100%	1.00
012-L	100%	79%	0.69
007-C	67%	100%	0.74

*NPV- Negative Predictive Value, PPV- Positive Predictive Value

For the second parameter evaluated, species concordance, the results for second round were: only five of the 18 participating laboratories obtained a percentage >95% or acceptable while the remaining 13 had concordance rates below the required standards (Figure 2).

One of the major problems observed with this parameter was identification of mixed slides and their respective species. Comparing these results with those of round I, it is observed that 5 of the 18 participating laboratories improved their concordance rates, 7 showed decline and the remaining 6 were participating for the first time.

Analysing the data using predictive values and Kappa index, we observed that 7 of the 18 participating laboratories had problems identifying slides positive for *P. falciparum* (<80% PPV) and only one of them had problems reading negative slides (Table 2). 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 of this species. For *P. vivax,* nine laboratories had problems reading positive slides (<80% PPV), and only two laboratories had problems identifying negative slides for this species type.

The kappa index shows in detail that there exists a greater discrepancy in the identification of *P. vivax* than in *P. falciparum,* reporting indices lower than 0.5 for two of the laboratories.

Figure 2. Percentage concordance for species type.

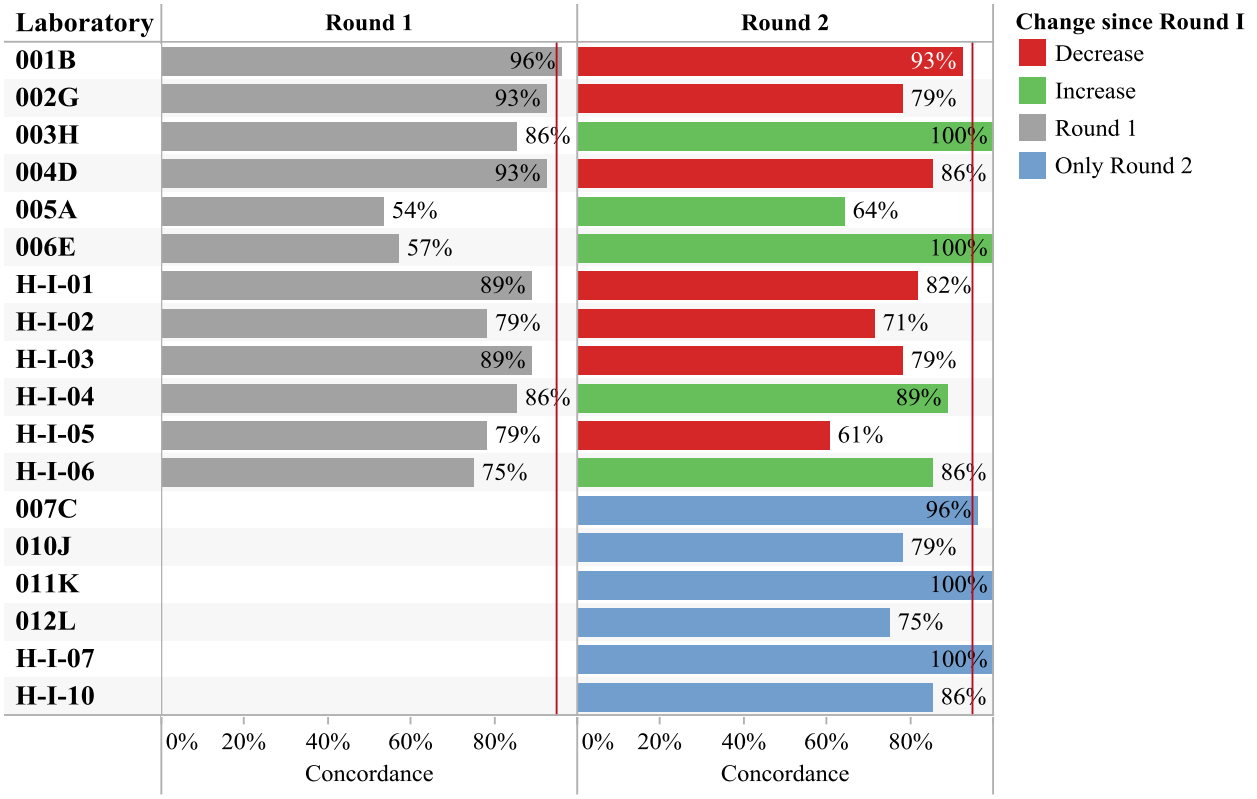


Table 2. Predictive Values & Kappa for species type.

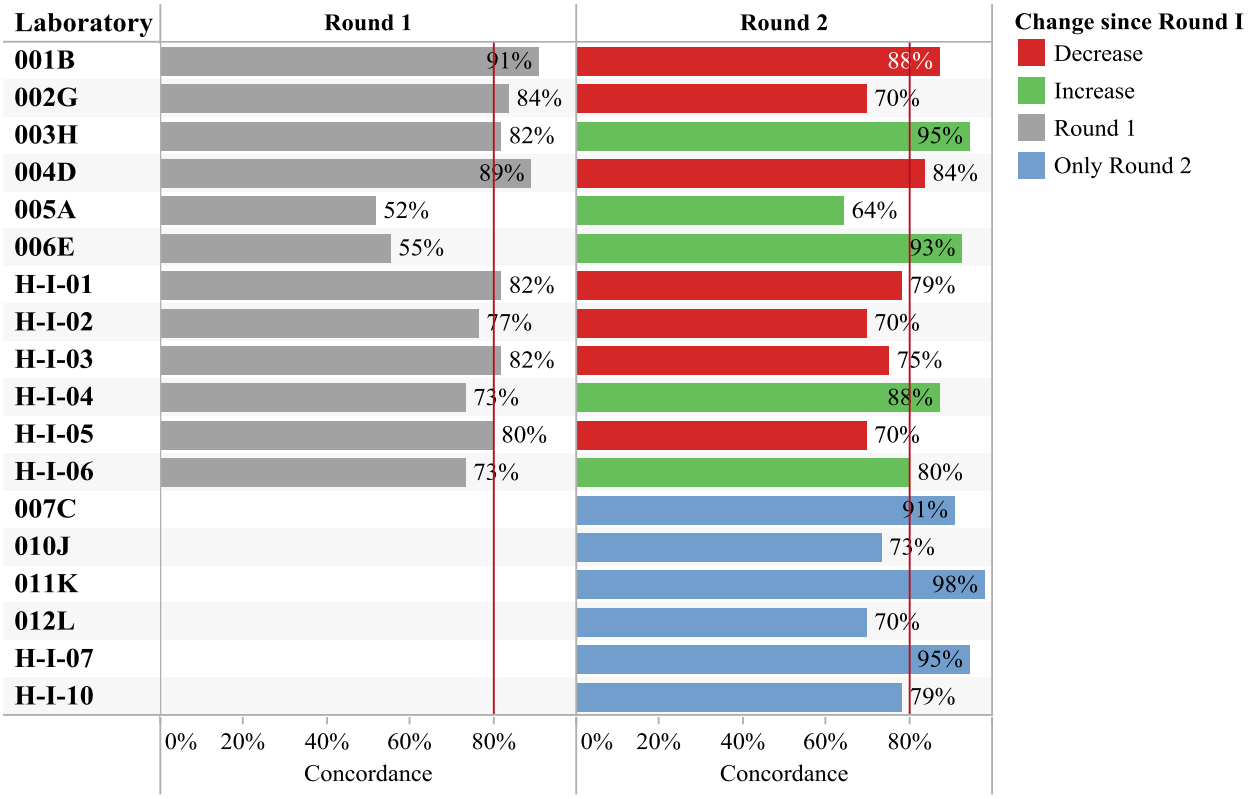
	P. vivax		*P. falciparum*		*P. falciparum*	*P. vivax*
Laboratories	**NPV**	**PPV**	**NPV**	**PPV**	**Kappa**	
006-E	100%	100%	100%	100%	1.00	1.00
005-A	73%	78%	100%	56%	0.58	0.50
001-B	100%	100%	100%	89%	0.90	1.00
004-D	91%	100%	100%	78%	0.79	0.90
002-G	91%	100%	100%	56%	0.58	0.90
003-H	91%	100%	100%	100%	1.00	0.90
H-I-02	100%	44%	73%	100%	0.71	0.47
H-I-01	91%	78%	91%	89%	0.80	0.69
H-I-03	100%	67%	100%	78%	0.79	0.69
H-I-04	100%	89%	82%	100%	0.80	0.90
H-I-06	100%	78%	100%	78%	0.79	0.79
H-I-05	73%	56%	82%	78%	0.60	0.29
H-I-10	100%	67%	91%	100%	0.90	0.69
H-I-07	100%	100%	100%	100%	1.00	1.00
011-K	100%	100%	100%	100%	1.00	1.00
010-J	100%	63%	91%	100%	0.89	0.66
012-L	100%	78%	100%	78%	0.79	0.79
007-C	100%	89%	82%	100%	0.80	0.90

*NPV- Negative Predictive Value, PPV- Positive Predictive Value

Results for the third parameter evaluated, stage concordance, show that 9 of the 18 participating laboratories obtained ≥80% concordance or acceptable (Figure 3). Also, 5 laboratories showed an improvement over the previous evaluation round, but 7 showed the opposite, obtaining lower concordance rates than in the previous round.

One of the major problems encountered in this parameter was the inability to identify certain stages, especially the detection of *P. vivax* sexual stage where 5 laboratories obtained Kappa indices lower than 0.5, indicating a concordance of less than 50% with the supranational laboratory (Table 3). For *P. falciparum* there were some challenges in detection of sexual and asexual stages where 3 laboratories showed Kappa index values less than 0.5 for sexual stages or gametocytes. Only 9 laboratories had acceptable concordance rates of 0.8 for the asexual stages.

Figure 3. Percentage concordance for stage type.



The concordance results for Round 1 were modified according to the formula used by NETLAB

Table 3. Predictive Values & Kappa for stage type.

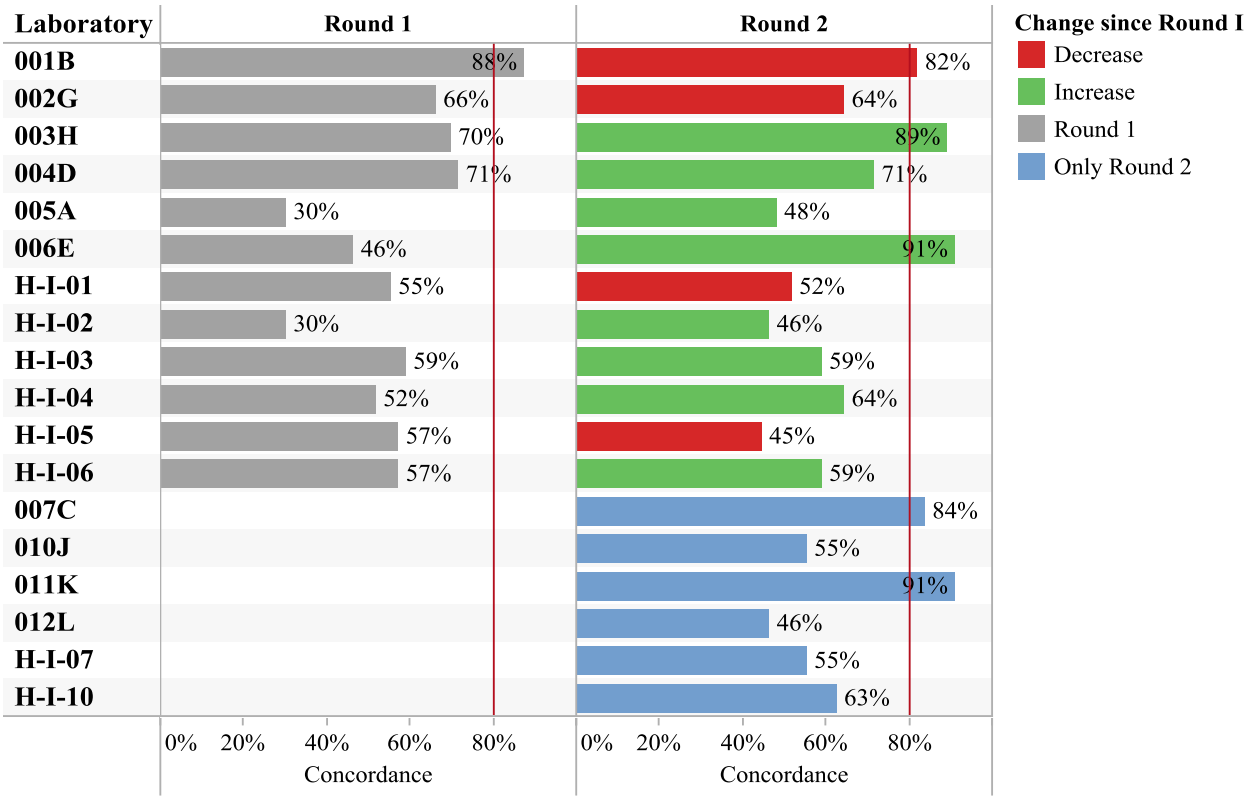
	P. vivax asexual		*P. vivax* sexual		*P. falciparum* asexual		*P. falciparum* sexual		Kappa			
Laboratories	**NPV**	**PPV**	**NPV**	**PPV**	**NPV**	**PPV**	**NPV**	**PPV**	***P. vivax* asexual**	***P. vivax* sexual**	***P. falciparum* asexual**	***P. falciparum* sexual**
006-E	100%	100%	92%	63%	100%	100%	100%	100%	1.00	0.57	1.00	1.00
005-A	73%	78%	92%	38%	100%	56%	100%	50%	0.50	0.32	0.58	0.62
001-B	100%	100%	100%	88%	100%	67%	100%	100%	1.00	0.89	0.69	1.00
004-D	91%	100%	83%	100%	100%	78%	100%	75%	0.90	0.80	0.79	0.83
002-G	91%	89%	92%	63%	100%	44%	100%	25%	0.80	0.57	0.47	0.35
003-H	91%	100%	100%	86%	100%	78%	100%	100%	0.90	0.89	0.79	1.00
H-I-02	100%	44%	85%	29%	73%	100%	87%	100%	0.47	0.15	0.71	0.76
H-I-01	91%	78%	100%	56%	91%	78%	92%	86%	0.69	0.58	0.69	0.78
H-I-03	100%	67%	100%	75%	100%	78%	85%	71%	0.69	0.78	0.79	0.56
H-I-04	100%	89%	92%	88%	82%	100%	87%	100%	0.90	0.79	0.80	0.76
H-I-06	100%	78%	100%	63%	100%	78%	93%	40%	0.79	0.67	0.79	0.38
H-I-05	73%	56%	86%	50%	82%	78%	100%	86%	0.29	0.38	0.60	0.89
H-I-10	100%	67%	92%	50%	91%	78%	92%	100%	0.69	0.44	0.69	0.90
H-I-07	100%	100%	85%	100%	100%	100%	100%	86%	1.00	0.79	1.00	0.89
011-K	100%	100%	100%	88%	100%	100%	100%	100%	1.00	0.89	1.00	1.00
010-J	100%	56%	92%	50%	91%	89%	88%	50%	0.58	0.44	0.80	0.38
012-L	100%	78%	100%	63%	100%	67%	100%	100%	0.79	0.67	0.69	1.00
007-C	100%	89%	92%	88%	82%	78%	100%	100%	0.90	0.79	0.60	1.00

*NPV- Negative Predictive Value, PPV- Positive Predictive Value

Results for the fourth parameter evaluated, parasite density, show substantial improvements for the majority of the participant laboratories (Figure 4). From the 18 participating laboratories, 5 obtained ≥80% concordance or acceptable rating. Concordance for this parameter is calculated such that it tolerates a variance of ±50% of reference laboratory value of parasitemia on each slide.

The biggest problem observed with this parameter is that parasite counts were not done using parasites per microliter of blood parasites (p/µl) as well as the error in application of the formula. The latter is due to the fact that countries were still utilizing the 'plus' system which had been established in previous years. Currently, some of the countries evaluated are now implementing the counts of parasites per microliter (p/µl) and an improvement from the first round can be seen.

Figure 4. Percentage of Density Concordance.



The concordance results for Round 1 were modified according to the formula used by NETLAB

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. As national reference laboratories, they should put emphasis on evaluating and supporting laboratories in the departments and municipalities, to improve and maintain high standards that assure the quality of malaria diagnosis at all levels of care in each participating country, whether endemic or non-endemic.

It is of utmost importance for an endemic or non-endemic country to have adequate diagnostic capabilities, using a framework that guarantees their quality. This is to ensure rapid diagnosis and appropriate treatment for the purpose of shortening time of transmission, thereby decreasing malaria incidence and also preventing reintroduction of the disease in areas where it has already been eliminated.

RECOMMENDATIONS

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

The previous report (6) as well as this report can be downloaded from the following link, under *'Relevant documents'*:

English:
http://www.paho.org/hq/index.php?option=com_content&view=article&id=2453&Itemid=3624

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ANNEX

I. Formulas used by the NetLab system to calculate the percent agreement.

1. Concordance in result

The software awards 1 point for every laboratory-tested slide consistent with the reference panel of evaluation laboratory. Both positive and negative slides are counted.

The total score obtained by the evaluated laboratory is divided by 20 (total number of slides) and is expressed as a percentage.

2. Concordance in species

The software awards 1 point for every slide, for each individual species : *P. vivax* or *P. falciparum*; or in the case of mixed slides (containing *P. vivax* and *P. falciparum*), the software awards 0.50 points for each specie per slide, identified by the evaluated laboratory and consistent with the reference panel of the evaluation laboratory

Only positive slides that match the reference panel will be counted (concordance in result). The total score obtained by the evaluated laboratory is divided by the total number of positive slides from the reference panel.

3. Concordance in stages

The software awards 0.25 points for each slide that the evaluated laboratory has identified one of the four stages (the sexual stages for *P. falciparum and for* P*. vivax* and the asexual stages for *P. falciparum* and *P.* vivax) and matches the reference panel from the evaluating laboratory. The software also awards 0.25 points when the slide does not have parasites in any of these stages and the evaluated laboratory correctly identifies the slide as such.

Up to 1, 0.25, 0.5, and 0.75 points can be awarded for each slide.

Only positive slides that match the reference panel will be counted (concordance in result).

The total score for the evaluated laboratory is divided by the total number of positive slides from the reference panel.

4. Concordance in parasitemia

The software awards 0.25 points when the number of parasites per microliter for each of the four stages (the sexual and asexual stages for *P. vivax* and *P. falciparum respectively*) for each slide identified by the evaluated laboratory matches (with a variation of up to 50% above or below) the parasite density from the evaluating laboratory's reference panels. The software awards 0.25 points when a slide from the reference panel does not contain a parasite in any of its stages, and the evaluated laboratory indicates this by not entering an amount.

The software awards 0.25 points when there the reference panel has fewer than 50 parasites (in any stage) and the evaluated laboratory enters any amount.

Up to 1, 0.25, 0.5, and 0.75 points can be awarded for each slide.

Only positive slides that match the reference panel will be counted (concordance in result).

The total score for the evaluated laboratory is divided by the total number of positive slides from the reference panel.