Pan American Health Organization

World Health Organization
REGIONAL OFFICE FOR THE Americas

TECHNICAL REPORT
FOURTH ROUND
2014-2015

EXTERNAL QUALITY ASSURANCE PROGRAM FOR MALARIA MICROSCOPIC DIAGNOSIS

REGIONAL MALARIA PROGRAM
NEGLECTED, TROPICAL AND VECTOR-BORNE DISEASES
COMMUNICABLE DISEASES AND HEALTH ANALYSIS
PAN AMERICAN HEALTH ORGANIZATION

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INTRODUCTION

One of the goals from the Pan American Health Organization Strategy and Action Plan for Malaria (2011-2015) is to ensure access to early diagnosis and prompt, effective treatment. (1)

Implementation of policies which ensure effective treatment is based on the existence of a healthcare system that offers prompt access to reliable (precise and accurate) diagnosis for better surveillance, prevention, and control of malaria in the Americas. (2)

The program for external quality evaluation has been developed because of the need for national reference laboratories to have an External Quality Assurance Program (EQAP), to contribute to the improvement of microscopic diagnosis of malaria. This effort will not only improve malaria diagnosis at the reference center level, but shall also allow the transfer of skills and the upgrading of resources at the country level.

Technical work in a laboratory should always be subject to constant supervision using quality control procedures. Such supervision is not possible without quality control which allows for evaluation of the work done by the laboratories. Success in the face of new challenges in improving the efficiency of public health response will partly depend on the quality and performance of the *LABORATORY NETWORKS.*

OBJECTIVES

GENERAL OBJECTIVES

To establish technical procedures for the organization, design, and evaluation of the microscopic diagnosis of malaria for the National Reference Laboratories of the countries in the Region, with the objective of maintaining an efficient quality management system and contributing to the strengthening of monitoring malaria diagnosis in the Region of the Americas.

SPECIFIC OBJECTIVES

1. Evaluate result concordance based on 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; Plasmodium falciparum;* and mixed slides (Pf/Pv).
- Slides with different parasite densities: low, medium and high density.
- Stages: asexual and sexual stages 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 slides (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: Refers to detection of positive and negative slides, regardless of species.
2. Species: Refers to detection of *P. vivax*, *P. falciparum*, or mixed infections.
3. Stage: Refers to detection of asexual and sexual stages (*P. vivax* and *P. falciparum* gametocytes).
4. Parasite density: Refers to quantitative detection of parasites, independent 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, a slide shall be considered concordant if the number of parasites reported by the evaluated laboratory is ±50% of the value reported 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

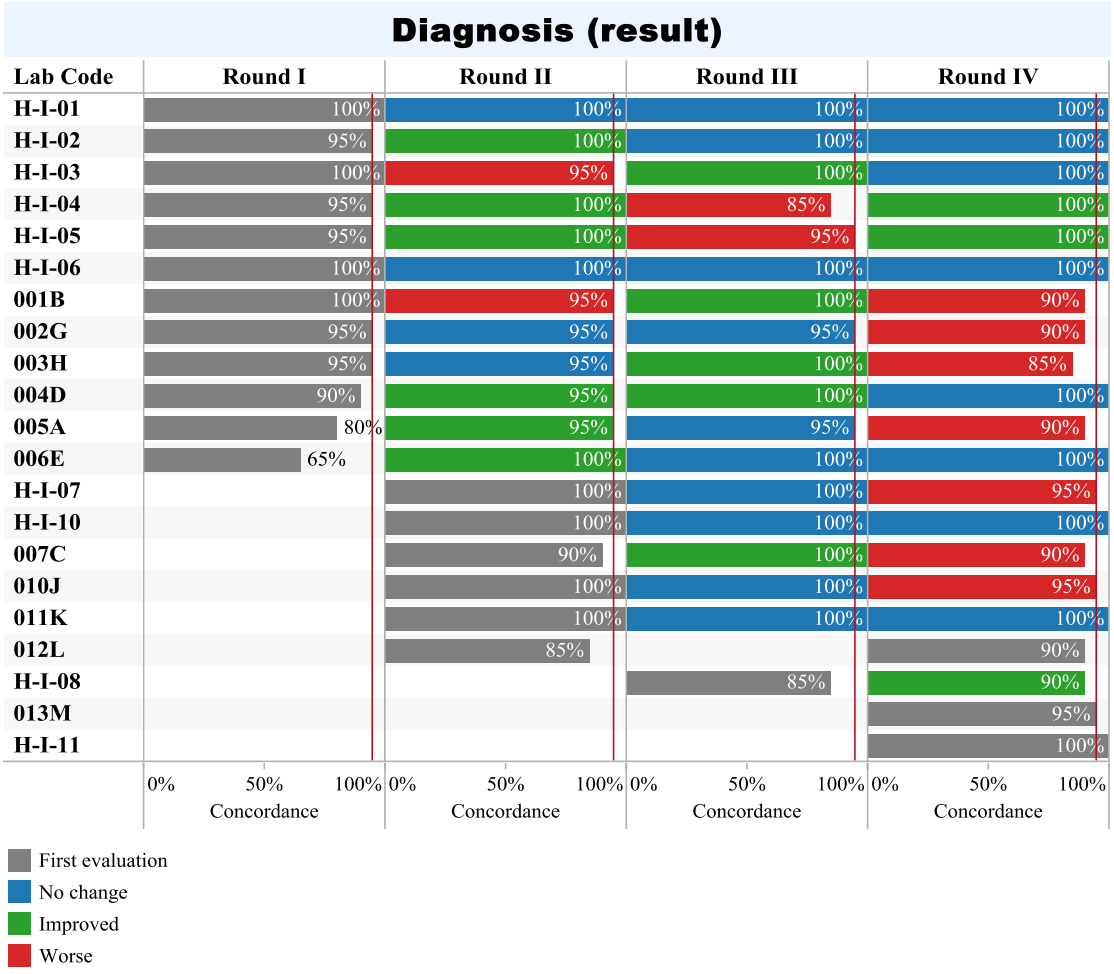
Twenty-one reference laboratories from the Region of the Americas participated in this fourth evaluation: ten from Central America and the Caribbean and eleven from South America; the total being nine more than in the first round. For the first time during the last rounds, 100% of the laboratories were able to submit their results to the electronic system, with the analysis included in this report.

Preliminary results were generated by the online NETLab system (5) for each of the participating laboratories as soon as the data was entered, and provided quick results for each of the parameters evaluated were provided.

As a second step, all participating laboratories will receive this final report compiling results from the two supranational laboratories, thus obtaining an overall result of this fourth evaluation. In this report, laboratories are identified by their codes in this report to ensure anonymity of results.

The results of round IV for the first parameter evaluated, concordance of results, as illustrated in Figure 1, was: of the 21 participating laboratories, 14 attained ≥95% concordance, deemed as acceptable, and 7 laboratories reported rates of 85%, deemed unacceptable according to pre-established criteria. One of the major problems observed for the laboratories with unacceptable concordance results for this first parameter was the reporting of positive slides with low parasite densities as negatives.

Figure 1. Percentage concordance for Results parameter.



Generally, the negative predictive value (NPV) for the laboratories evaluated was 100%, demonstrating that in general these laboratories did not have problems in reading and identifying negative slides (Table 1). For the positive slides, results varies as the positive predicative value (PPV) for all laboratories was greater than 80%, with one exception obtaining a 79%. A Kappa (K) index value greater than 0.8 shows good concordance among evaluators of the slides and demonstrates that the majority of laboratories have good concordance with the regional reference laboratories, as shown in Table 1.

Table 1. Predictive Values & Kappa for Results parameter.

Results			
Laboratories	**NPV**	**PPV**	**Kappa**
006-E	100%	100%	1.00
005-A	100%	86%	0.78
001-B	100%	86%	0.78
004-D	100%	100%	1.00
002-G	100%	86%	0.78
003-H	100%	79%	0.69
H-I-02	100%	100%	1.00
H-I-01	100%	100%	1.00
H-I-03	100%	100%	1.00
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%	93%	0.89
011-K	100%	100%	1.00
010-J	100%	93%	0.89
012-L	100%	86%	0.78
007-C	83%	93%	0.76
H-I-08	100%	86%	0.78
H-I-11	100%	100%	1.00
013-M	100%	93%	0.89

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

As seen in Figure 2, the results for the second parameter evaluated, species concordance, in round IV were: only eight of the 21 participating laboratories obtained a percentage greater than 95% – deemed acceptable – while the remaining 13 had concordance rates below the required standards.

One of the major problems observed was identification of mixed slides and their respective species. Comparing these results with those of previous rounds, it can be observed that ten of the 21 participating laboratories improved their concordance rates for this parameter, eight demonstrated a decline, and one maintained the same rate. Two laboratories participated for the first time.

Analyzing the data using predictive values and the Kappa index, it is observed that nine of the 21 participating laboratories had problems in identifying slides positive for *P. falciparum* (<80% PPV) and only two had problems reading slides negative for this species (see Table 2). Although some of these laboratories belong to countries non-endemic for *P. falciparum*, which is also reflected in their concordance results, high levels of sensitivity and specificity should be maintained for diagnosis of positive cases of this species. For *P. vivax,* three laboratories had problems reading the positive

slides (<80% PPV), and only one laboratory had problems identifying negative slides for this species (<80% NPV).

As seen in Table 2, the kappa index demonstrates in detail that there are discrepancies in the identification of both species, but the major problem is for the identification of *P. falciparum* reporting index lower than 0.5 by two participant laboratories.

Figure 2. Percentage concordance for species type.

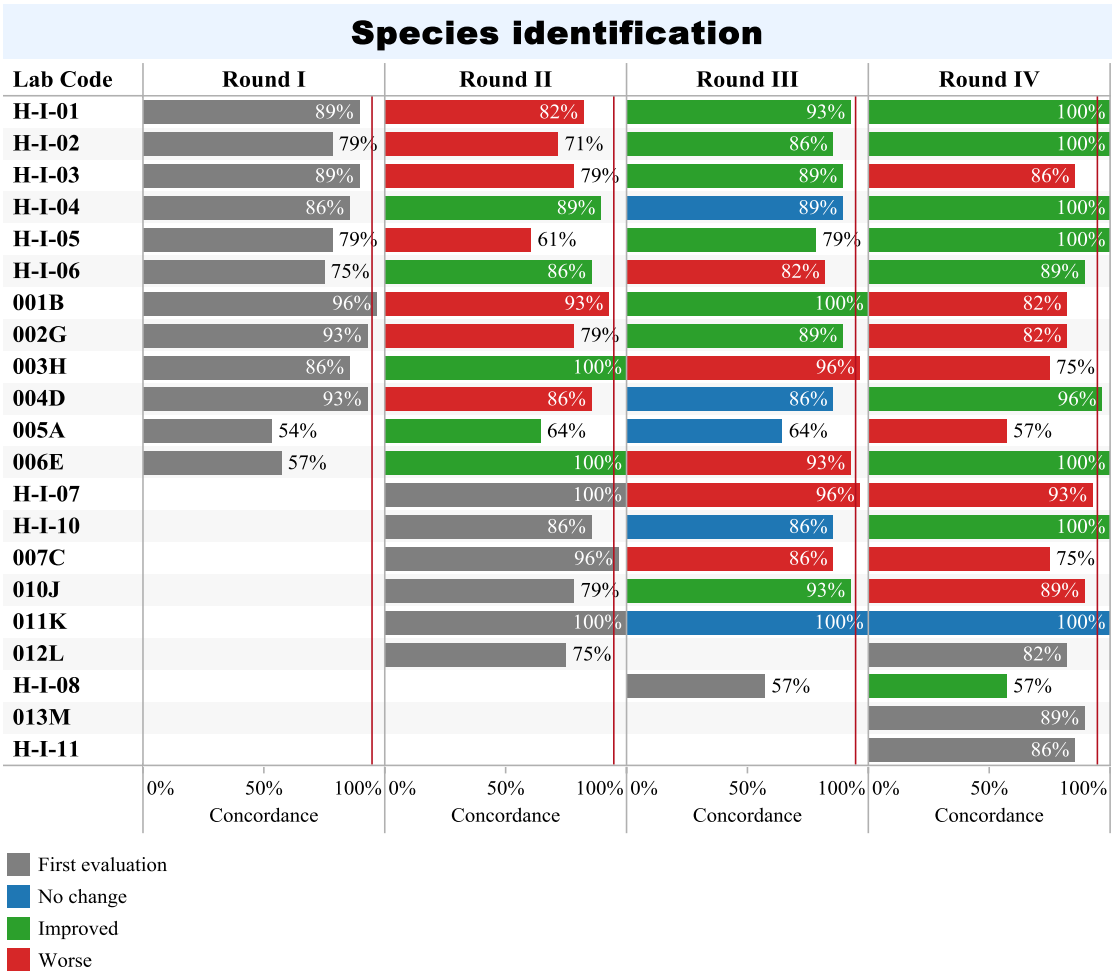


Table 2. Predictive values & Kappa for species type.

Laboratories	*P. vivax*			*P. falciparum*		
	NPV	PPV	Kappa	NPV	PPV	Kappa
006-E	100%	100%	1.00	100%	100%	1.00
005-A	73%	89%	0.60	100%	33%	0.35
001-B	100%	89%	0.90	100%	78%	0.79
004-D	100%	100%	1.00	100%	89%	0.90
002-G	100%	89%	0.90	100%	78%	0.79
003-H	100%	89%	0.90	100%	67%	0.69
H-I-02	100%	100%	1.00	100%	100%	1.00
H-I-01	100%	100%	1.00	100%	100%	1.00
H-I-03	100%	78%	0.79	82%	100%	0.80
H-I-04	100%	100%	1.00	100%	100%	1.00
H-I-06	100%	100%	1.00	100%	67%	0.69
H-I-05	100%	100%	1.00	100%	100%	1.00
H-I-10	100%	100%	1.00	100%	100%	1.00
H-I-07	100%	100%	1.00	100%	89%	0.90
011-K	100%	100%	1.00	100%	100%	1.00
010-J	100%	78%	0.79	100%	100%	1.00
012-L	100%	89%	0.90	100%	78%	0.79
007-C	91%	89%	0.80	73%	78%	0.50
H-I-08	100%	67%	0.69	73%	56%	0.29
H-I-11	100%	100%	1.00	100%	56%	0.58
013-M	100%	89%	0.90	100%	89%	0.90

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

As seen in Figure 3, results for the third parameter evaluated, stage concordance, show that 17 of the 21 participating laboratories obtained ≥80% concordance, deemed acceptable. This leaves only four laboratories with concordance rates deemed unacceptable or lower than 80%. In general, improvement has been observed in this parameter in comparison to previous rounds.

One of the major problems encountered in this parameter was the inability to identify certain stages, as seen in Table 3. In regard to *P. vivax,* challenges were greatest in the detection of sexual stages wherein 10 of the 21 participating laboratories obtained Kappa indices of less than 0.8, and one of them less than 0.5, indicating less than a 50% concordance rates with the Regional reference laboratory, and unfortunately one laboratory couldn't observe any stage for this specie in the positive slides. For the asexual stage, only four laboratories obtained Kappa index less than 0.8, but none of them reached rates lower than 0.5.

For *P. falciparum* there were greater challenges in detection of both sexual and asexual stages wherein four laboratories had Kappa indices of less than 0.5 for sexual stages or gametocytes and only eight laboratories had Kappa index greater than 0.8 or a good concordance for the asexual stages.

Figure 3. Percentage concordance for stage type.

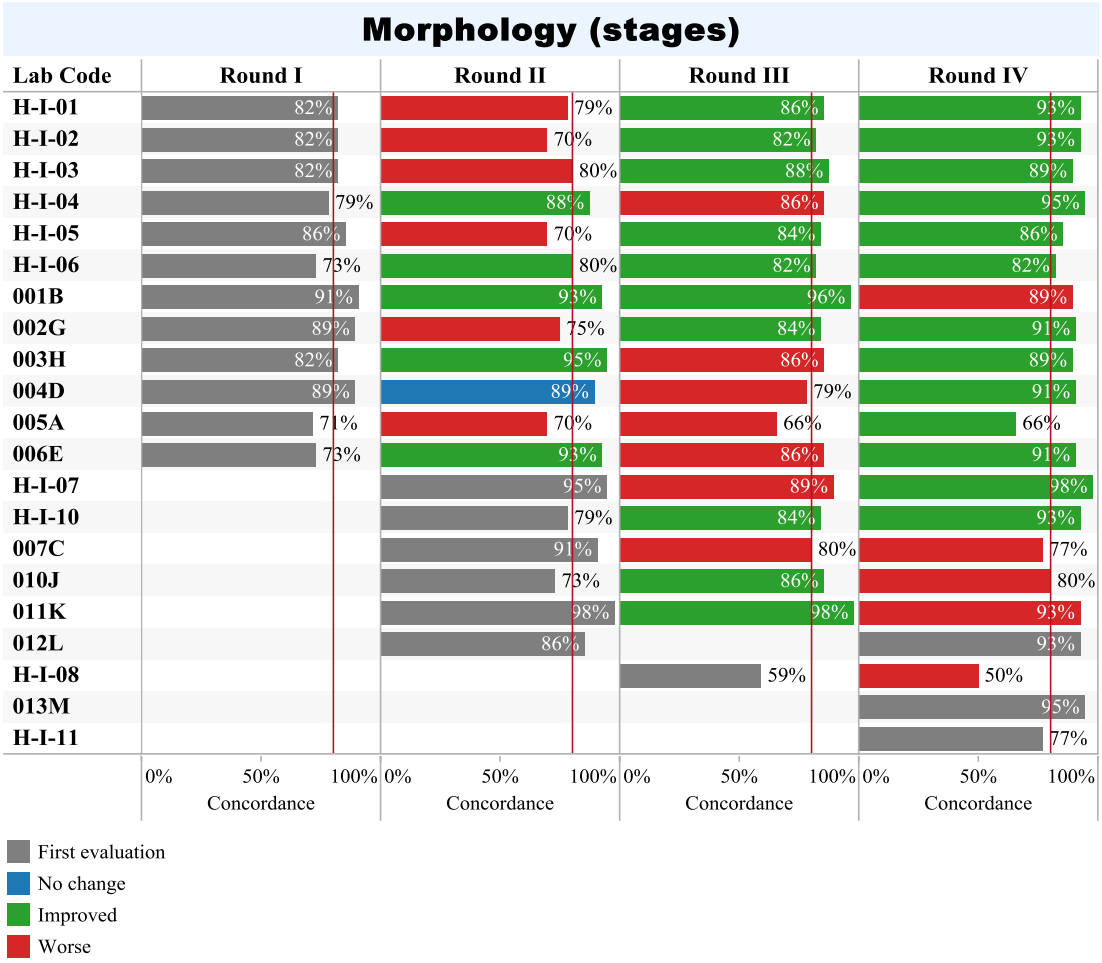


Table 3. Predictive Values & Kappa for stage type.

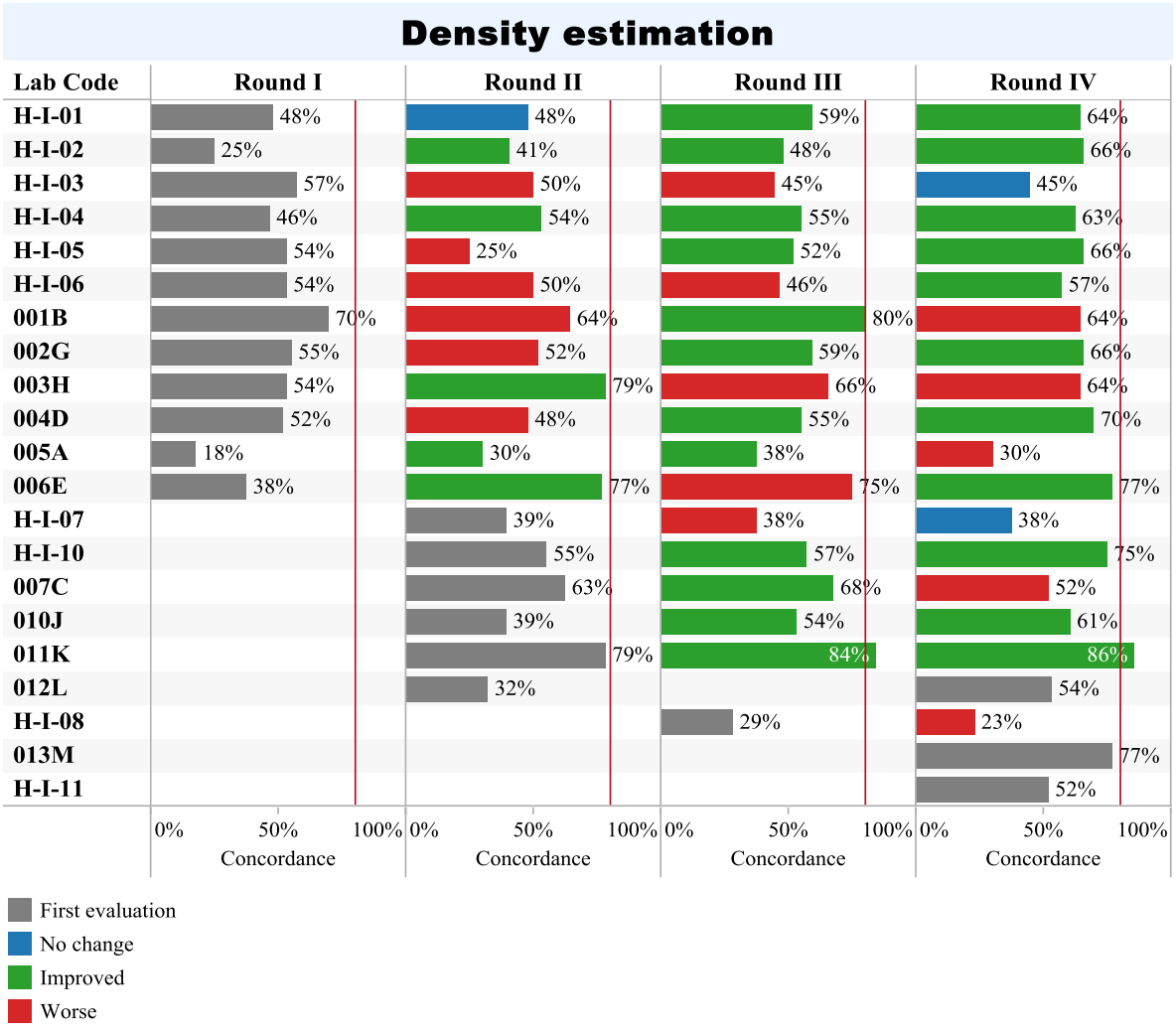
Laboratories	*P. vivax* asexual		*P. vivax* sexual		*P. falciparum* asexual		*P. falciparum* sexual		Kappa			
	NPV	PPV	NPV	PPV	NPV	PPV	NPV	PPV	*P. vivax* asexual	*P. vivax* sexual	*P. falciparum* asexual	*P. falciparum* sexual
006-E	100%	100%	100%	78%	79%	100%	100%	100%	1.00	0.79	0.69	1.00
005-A	73%	89%	92%	100%	93%	17%	100%	50%	0.60	0.90	0.12	0.62
001-B	100%	89%	100%	100%	100%	67%	88%	75%	0.90	1.00	0.74	0.57
004-D	100%	100%	100%	100%	86%	100%	94%	50%	1.00	1.00	0.78	0.48
002-G	100%	89%	100%	88%	93%	83%	100%	75%	0.90	0.89	0.76	0.83
003-H	100%	89%	100%	100%	86%	67%	100%	75%	0.90	1.00	0.52	0.83
H-I-02	100%	100%	80%	100%	100%	100%	93%	100%	1.00	0.67	1.00	0.89
H-I-01	100%	100%	79%	100%	100%	100%	93%	100%	1.00	0.69	1.00	0.89
H-I-03	100%	78%	93%	100%	82%	100%	92%	100%	0.79	0.89	0.80	0.88
H-I-04	100%	100%	92%	100%	100%	100%	87%	100%	1.00	0.90	1.00	0.76
H-I-06	100%	100%	86%	67%	100%	67%	100%	50%	1.00	0.52	0.69	0.58
H-I-05	100%	100%	80%	80%	100%	89%	100%	50%	1.00	0.53	0.90	0.58
H-I-10	100%	100%	79%	100%	100%	100%	93%	100%	1.00	0.69	1.00	0.90
H-I-07	100%	100%	100%	100%	100%	89%	100%	100%	1.00	1.00	0.90	1.00
011-K	100%	100%	92%	100%	79%	100%	100%	100%	1.00	0.90	0.69	1.00
010-J	100%	78%	100%	50%	86%	83%	94%	75%	0.79	0.55	0.66	0.69
012-L	100%	89%	100%	100%	93%	83%	100%	75%	0.90	1.00	0.76	0.83
007-C	91%	89%	100%	50%	86%	67%	81%	75%	0.80	0.55	0.52	0.47
H-I-08	100%	67%	100%	0%	100%	44%	73%	40%	0.69	0.00	0.47	0.13
H-I-11	100%	100%	69%	100%	100%	56%	100%	33%	1.00	0.47	0.58	0.41
013-M	100%	89%	100%	88%	100%	100%	100%	75%	0.90	0.89	1.00	0.83

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

As seen in Figure 4, results for the fourth parameter evaluated, parasite density, show substantial improvement for the majority of participating laboratories. One of the 21 laboratories reached an acceptable concordance rate of ≥80%. Although measurement of parasite density needs strengthening, in the last round the concordance rates obtained by almost all laboratories have been higher than those of the previous rounds. Concordance for this parameter is calculated such that it allows on each slide for a variance of ±50% from the parasite density reported by the Regional reference laboratory. See Annex 1 for the details of the formulas used in the NETLab system for the calculation of concordance rates.

The biggest problem observed with this parameter was the correct application of the formula for calculation of parasite density by parasites per microliter of blood (p/µl). This is due to the fact that laboratories were still utilizing the 'plus' system which had been previously established for estimating parasite density. Currently, several of the countries evaluated are now implementing the counting of parasites per microliter (p/µl) and improvement since the first round has been observed.

Figure 4. Percentage of parasite density concordance.



CONCLUSIONS

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

This program will also permit standardization of the processes for microscopic diagnosis of malaria at the regional level. Participating laboratories, being national reference laboratories, should place emphasis on evaluating and supporting laboratories at the department and municipal level in order to improve and maintain high standards that assure the quality of malaria diagnosis at all levels of care in each participating country, be it endemic or non-endemic.

It is of utmost importance that an endemic or non-endemic country be able to rely on adequate diagnostic capabilities, under a framework that guarantees their quality. This ensures rapid diagnosis and appropriate treatment with the purpose of shortening time of transmission and preventing reintroduction of the disease in areas where it has already been eliminated.

RECOMMENDATIONS

Looking 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 read the slides received again in order to detect errors and thus improve detection capability. Tables with the detailed results can be found at the EQAP website using the username and password provided for this program (http:/www.netlab.ins.gob.pe/frmloginmalaria.aspx).

The previous report (6) as well as the current one can be downloaded from the following link, under *'Technical reports*:'

English:
http://www.paho.org/hq/index.php?option=com_topics&view=readall&cid=5524&Itemid=40757&lang=en

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ANNEX

I. Formulas used by the NETLab system to calculate concordance rates.

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 identified: *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 species 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 stage

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 are counted (concordance of species).

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 between 01 and 75.

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 are counted (concordance of species).

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