TECHNICAL REPORT
THIRD ROUND
2013-2014

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

June, 2015

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INTRODUCTION

One of the goals from the Pan American Health Organization Strategy and Action Plan for Malaria (2011-2015) 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 healthcare system that offers prompt access to reliable (precise and accurate) diagnosis for better surveillance, prevention, and control of malaria in the Americas. (2)

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 program for external quality evaluation has been developed. This effort will not only improve malaria diagnosis at the reference center level, but also permit 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 *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 with regard 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; 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

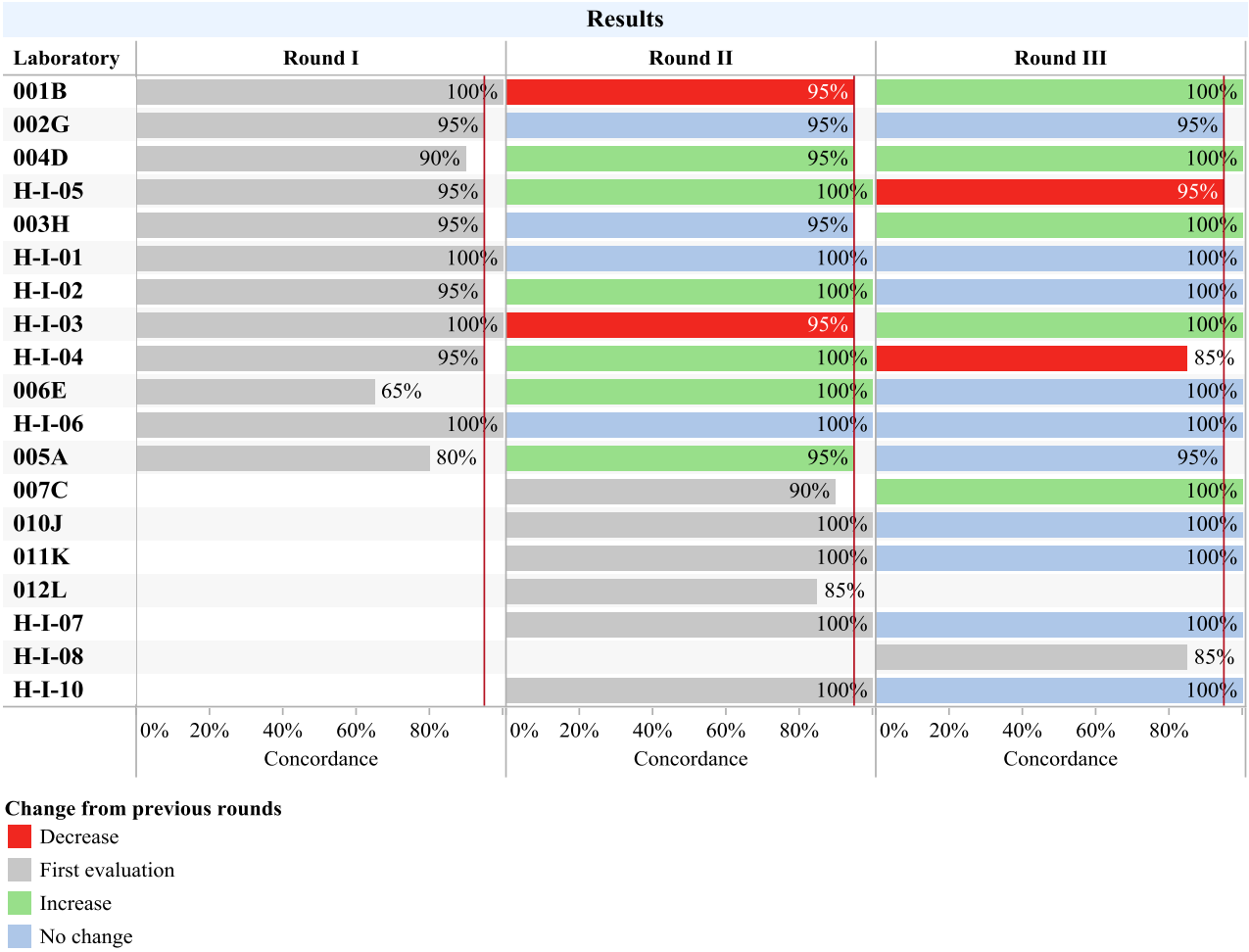
Twenty reference laboratories from the Region of the Americas participated in this third evaluation: ten from Central America and the Caribbean and ten from South America; thus including eight more than the first round. Two laboratories were not able to submit their results in time. Information from them is not included in this report and the analysis includes data from the 18 laboratories which did submit the panel results.

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

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

The results of round III for the first parameter evaluated, concordance of results, as illustrated in Figure 1, was: of the 18 participating laboratories, 16 attained ≥95% concordance, deemed as acceptable, and 2 laboratories reported rates of 85%, deemed unacceptable according to pre-established criteria. One of the major problems observed for these two laboratories unacceptable concordance results for this first parameter was the reporting of negative slides as positive. Of these two laboratories with reported problems for this parameter, one had previously obtained acceptable rating and the other one was participating for the first time. Thus, it is important to constantly train & to improve and sustain diagnostic capacities.

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. There were two exceptions where laboratories obtained 50% (Table 1). For the positive slides, results were better as the positive predicative value (PPV) for all laboratories was greater than 90%. 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 Results parameter.

Result			
Laboratories	**NPV**	**PPV**	**Kappa**
006-E	100%	100%	1.00
005-A	83%	100%	0.88
001-B	100%	100%	1.00
004-D	100%	100%	1.00
002-G	83%	100%	0.88
003-H	100%	100%	1.00
H-I-02	100%	100%	1.00
H-I-01	100%	100%	1.00
H-I-03	100%	100%	1.00
H-I-04	50%	100%	0.58
H-I-06	100%	100%	1.00
H-I-05	100%	93%	0.89
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
007-C	100%	100%	1.00
H-I-08	50%	100%	0.58

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

As seen in Figure 2, the results for the second parameter evaluated, species concordance, in round III were: only four of the 18 participating laboratories obtained a percentage greater than 95% – deemed acceptable – while the remaining 14 had concordance rates below the required standards.

One of the major problems observed in this parameter was identification of mixed slides and their respective species. Comparing these results with those of previous rounds, it can be observed that seven of the 18 participating laboratories improved their concordance rates for this parameter, five demonstrated a decline, five maintained the same rate, and one participated for the first time. None of the laboratories participating during the three rounds have showed sustained improvement during this period.

Analyzing the data using predictive values and the Kappa index, it is observed that six of the 18 participating laboratories had problems in identifying slides positive for *P. falciparum* (<80% PPV) and only one 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 four laboratories 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, reporting index lower than 0.5. It can be observed that for one laboratory, there were problems even in identification of *P. vivax* as there were for identification of positive and negative slides, demonstrated by a kappa index of 0.05.

Figure 2. Percentage concordance for species type.

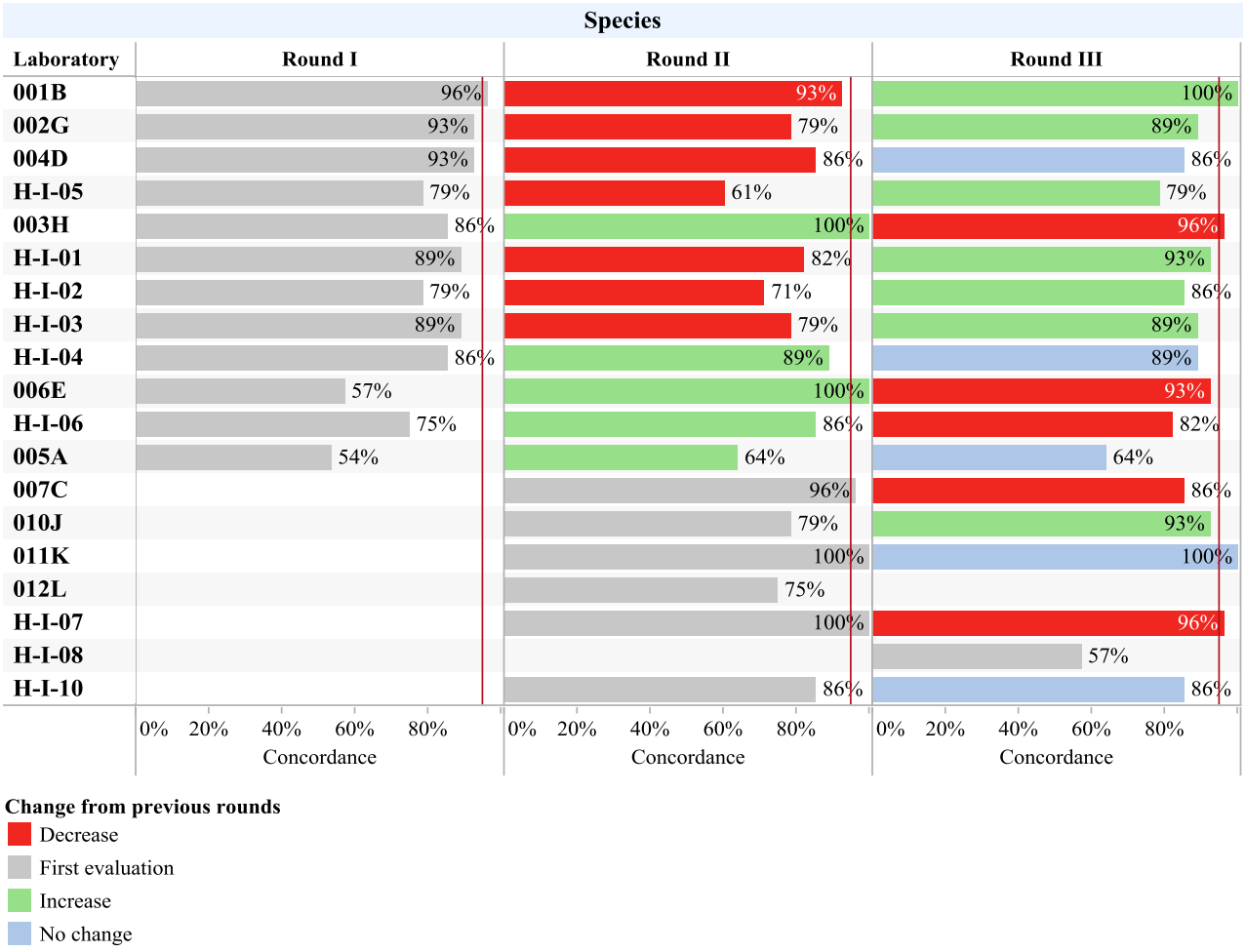


Table 2. Predictive values & Kappa for species type.

	P. vivax			*P. falciparum*		
Laboratories	**NPV**	**PPV**	**Kappa**	**NPV**	**PPV**	**Kappa**
006-E	91%	100%	0.90	100%	89%	0.90
005-A	55%	89%	0.42	100%	44%	0.47
001-B	100%	100%	1.00	100%	100%	1.00
004-D	73%	100%	0.71	100%	89%	0.90
002-G	82%	100%	0.80	91%	89%	0.80
003-H	100%	100%	1.00	100%	89%	0.90
H-I-02	91%	89%	0.80	100%	78%	0.79
H-I-01	100%	90%	0.90	92%	100%	0.90
H-I-03	82%	100%	0.80	100%	89%	0.90
H-I-04	91%	100%	0.90	64%	89%	0.51
H-I-06	73%	100%	0.71	100%	78%	0.79
H-I-05	91%	78%	0.69	100%	78%	0.79
H-I-10	82%	100%	0.80	100%	78%	0.79
H-I-07	91%	100%	0.90	100%	100%	1.00
011-K	100%	100%	1.00	100%	100%	1.00
010-J	100%	89%	0.90	100%	89%	0.90
007-C	100%	67%	0.69	91%	100%	0.90
H-I-08	27%	78%	0.05	100%	44%	0.47

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

As seen in Figure 3, results for the third parameter evaluated, stage concordance, show that 15 of the 18 participating laboratories obtained ≥80% concordance, deemed acceptable. This leaves only three laboratories with concordance rates deemed unacceptable or lower than 80%. In general, improvement has been observed in this parameter in comparison to previous rounds, and two laboratories showed a sustained improvement during the three 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 13 of the 18 participating laboratories obtained Kappa indices of less than 0.8, and three less than 0.5, indicating less than a 50% concordance rates with the Regional

reference laboratory. For the asexual stage, seven laboratories obtained Kappa index less than 0.8, and of these seven, two reached rates lower than 0.5.

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

Figure 3. Percentage concordance for stage type.

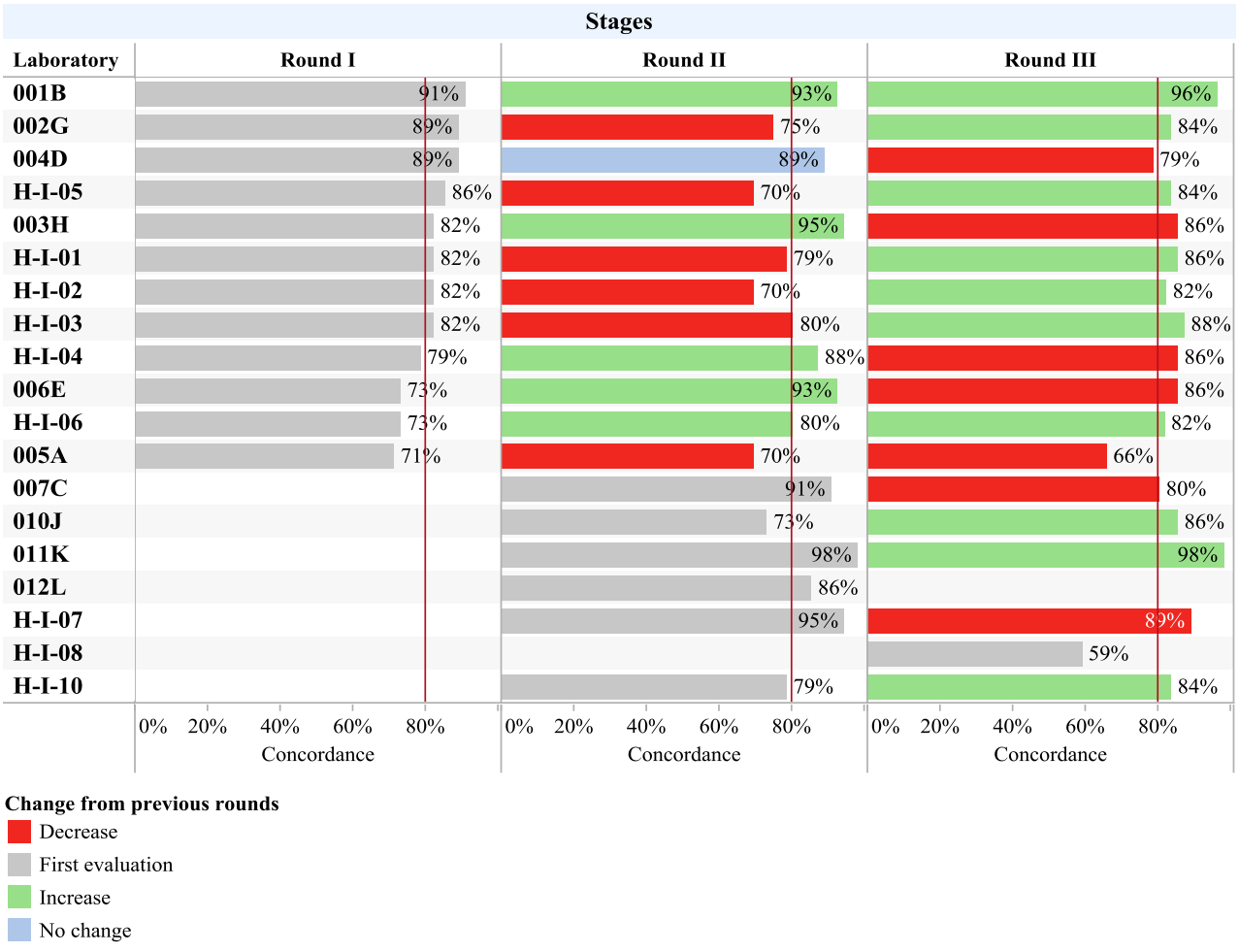


Table 3. Predictive Values & Kappa for stage type.

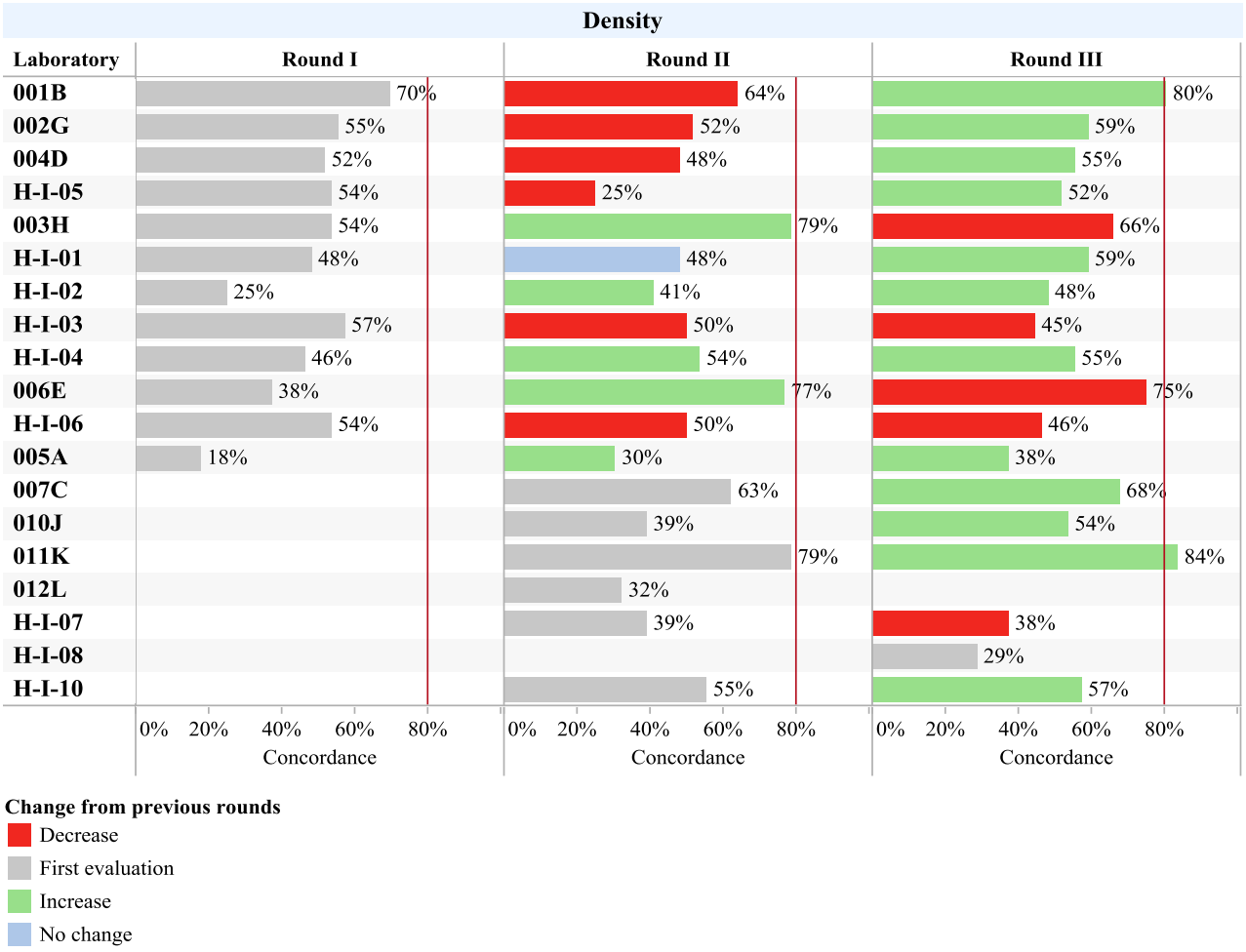
Laboratories	*P. vivax* asexual		*P. vivax* sexual		*P. falciparum* asexual		*P. falciparum* sexual		Kappa			
	VPN	VPP	VPN	VPP	VPN	VPP	VPN	VPP	*P. vivax* asexual	*P. vivax* sexual	*P. falciparum* asexual	*P. falciparum* sexual
006-E	91%	100%	92%	75%	100%	89%	92%	71%	0.90	0.68	0.90	0.66
005-A	55%	89%	100%	38%	100%	44%	100%	43%	0.42	0.42	0.47	0.49
001-B	100%	100%	92%	100%	100%	100%	100%	86%	1.00	0.90	1.00	0.89
004-D	73%	100%	67%	100%	100%	78%	100%	57%	0.71	0.62	0.79	0.63
002-G	82%	100%	83%	100%	100%	67%	92%	71%	0.80	0.80	0.69	0.66
003-H	100%	100%	100%	88%	100%	56%	100%	57%	1.00	0.89	0.58	0.63
H-I-02	91%	89%	86%	83%	100%	78%	92%	71%	0.80	0.66	0.79	0.66
H-I-01	100%	90%	79%	83%	92%	100%	87%	100%	0.90	0.57	0.90	0.76
H-I-03	82%	89%	79%	100%	100%	89%	100%	100%	0.70	0.69	0.90	1.00
H-I-04	91%	100%	86%	83%	64%	89%	80%	100%	0.90	0.66	0.51	0.67
H-I-06	73%	100%	85%	57%	100%	78%	100%	100%	0.71	0.43	0.79	1.00
H-I-05	91%	78%	100%	83%	100%	78%	100%	57%	0.69	0.88	0.79	0.63
H-I-10	82%	100%	79%	100%	100%	78%	100%	67%	0.80	0.69	0.79	0.74
H-I-07	91%	100%	77%	100%	100%	100%	86%	100%	0.90	0.70	1.00	0.78
011-K	100%	100%	92%	100%	100%	100%	100%	100%	1.00	0.90	1.00	1.00
010-J	100%	89%	92%	88%	100%	89%	85%	71%	0.90	0.79	0.90	0.56
007-C	100%	67%	100%	50%	91%	100%	92%	71%	0.69	0.55	0.90	0.66
H-I-08	27%	78%	43%	83%	100%	44%	100%	44%	0.05	0.20	0.47	0.47

*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. Two of the 18 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 measuring 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.

Due to problems found within the NETlab system and the corresponding changes made to the system for the current analysis of this third round, this report updates results obtained in previous rounds.

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 *'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 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.