TECHNICAL REPORT
FIFTH ROUND
2016-2017

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

November, 2017

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INTRODUCTION

One of the objectives of the Pan American Health Organization's Plan of Action for Malaria Elimination (2016-2020) is to ensure "*Universal access to good quality malaria prevention, integrated vector management interventions, **malaria diagnosis** and 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 level of reference laboratory, but shall also allow the transfer of skills and the upgrading of resources throughout the country.

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 parasitaemia) 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 fifth evaluation: ten from Central America and the Caribbean and eleven from South America. Unfortunately one of the laboratories couldn't respond, and the results of the current report represent only to 20 National Reference laboratories in de Americas Region.

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 fifth evaluation. In this report, laboratories are identified by their codes in this report to ensure anonymity of results.

The results of round V for the first parameter evaluated, concordance of results, as illustrated in Figure 1, was: of the 20 participating laboratories, all attained ≥95% concordance, deemed as acceptable. Of these, the majority with a maximum percentage of 100%, no problems were observed in relation to this first parameter.

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, with exception of one laboratory that reported 83% wherein one negative slide was reported positive (Table 1). For the positive slides, the majority of the laboratories obtained a positive predicative value (PPV) of ≥93%. A Kappa (K) index value greater than 0.8 shows good concordance among evaluators of the slides; it 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.

Result			
Laboratories	**NPV**	**PPV**	**Kappa**
006-E	100%	100%	1.00
005-A	100%	100%	1.00
001-B	100%	100%	1.00
004-D	100%	93%	0.89
002-G	100%	100%	1.00
003-H	100%	100%	1.00
H-I-02	100%	100%	1.00
H-I-01	83%	100%	0.88
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%	100%	1.00
011-K	100%	100%	1.00
010-J	100%	100%	1.00
012-L	100%	100%	1.00
007-C	100%	100%	1.00
H-I-11	100%	93%	1.00
013-M	100%	100%	1.00

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

As can be observed in Figure 2, the results for the second parameter evaluated, species concordance, in round V were: 14 of the 20 participating laboratories obtained an acceptable result (percentage greater than 95%), while the remaining six had concordance rates below the required standard.

Comparing these results with those of previous rounds, it can be observed that most of the participating laboratories improved their concordance rates for this parameter. Only three demonstrated a decline, and five maintained the same concordance.

Analyzing the data using predictive values and the Kappa index, it can be observed that only three of the 20 participating laboratories had problems in identifying positive slides for *P. falciparum* (<80% PPV) and none had problems reading negative slides 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 evaluation, high levels of sensitivity and specificity should be maintained for

diagnosis of positive cases of this species. For *P. vivax,* the 20 laboratories presented good results higher than 80% for both positive (PPV) and negative slides (NPV). It is worth mentioning that some laboratories reported *P. vivax* where it did not exist.

As seen in Table 2, the kappa index demonstrates in detail that only one laboratory had discrepancies in the identification of both species, and two had problems in the identification of *P. falciparum,* reporting rates below 0.8 but higher than 0.5 compared to the previous rounds.

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	82%	89%	0.70	100%	67%	0.69
001-B	100%	100%	1.00	100%	100%	1.00
004-D	82%	100%	0.80	91%	67%	0.59
002-G	82%	100%	0.80	100%	78%	0.79
003-H	100%	100%	1.00	91%	100%	0.90
H-I-02	100%	100%	1.00	100%	100%	1.00
H-I-01	82%	100%	0.80	100%	89%	0.90
H-I-03	100%	100%	1.00	100%	100%	1.00
H-I-04	100%	100%	1.00	91%	100%	0.90
H-I-06	100%	100%	1.00	100%	100%	1.00
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%	100%	1.00
011-K	100%	100%	1.00	100%	100%	1.00
010-J	100%	89%	0.90	100%	100%	1.00
012-L	100%	100%	1.00	100%	100%	1.00
007-C	100%	89%	0.90	100%	100%	1.00
H-I-11	100%	89%	0.90	100%	89%	0.90
013M	100%	89%	0.90	91%	100%	0.90

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

Results for the third parameter evaluated, stage concordance, show that 100% of participating laboratories obtained acceptable results (≥80% concordance, Figure 3). A noticeable improvement can be observed in this parameter in comparison to the previous rounds.

A more detailed analysis of the results by species and stage concordance shows that one of the problems is the non-identification of certain stages when they do exist, as seen in Table 3. In regard to *P. vivax,* challenges were greater in the detection of sexual stages wherein five of the 20 participating laboratories obtained Kappa indices of substantially less than 0.8, and two of them less than 0.5, indicating less than 50% concordance of slides examined with the Regional reference laboratory. For the asexual stage, all laboratories except one obtained Kappa index ≥0.8.

For *P. falciparum* challenges in the detection of both sexual and asexual stages can be observed in a single laboratory. Three laboratories had Kappa indices of substantially less than 0.8 for sexual stages or gametocytes, and four laboratories had Kappa index substantially less than 0.8 for asexual stages. Overall, the concordance obtained is much better than in previous rounds with an observed improvement.

Figure 3. Percentage concordance for stage type.

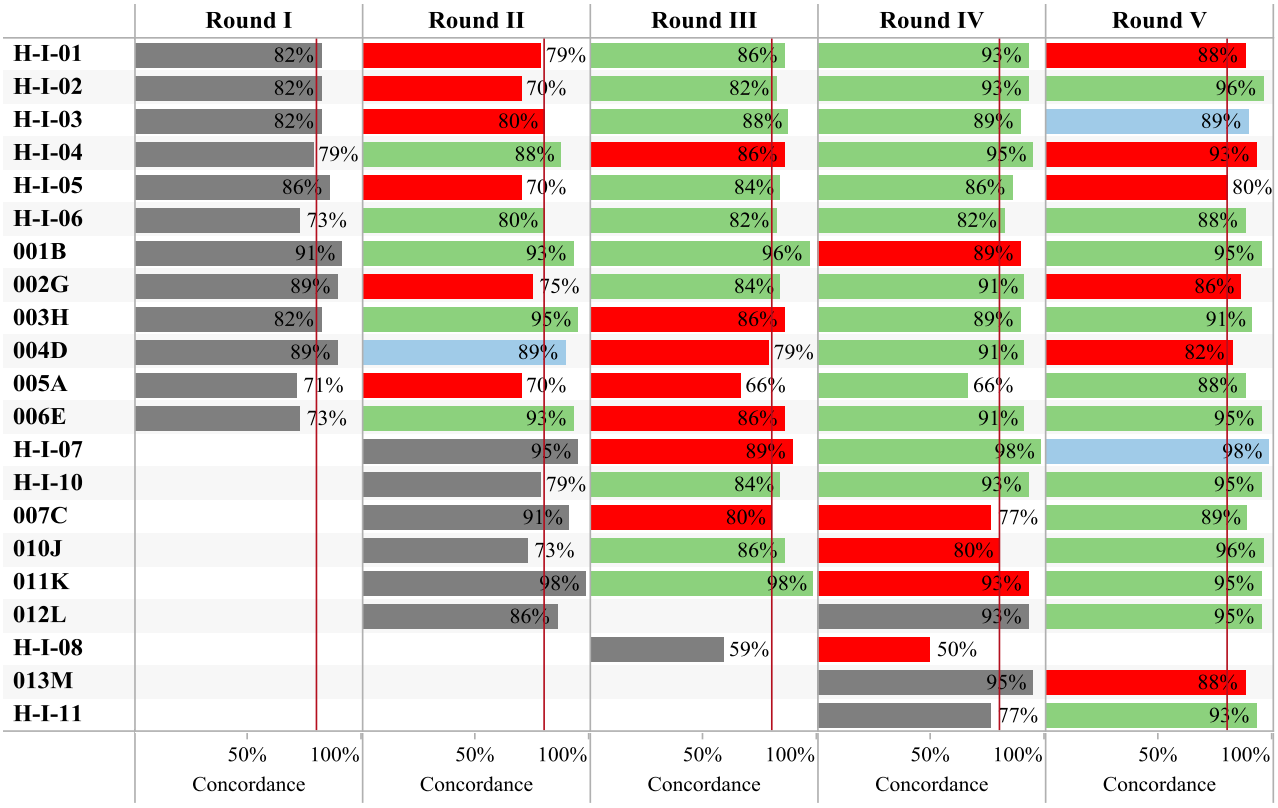


Table 3. Predictive Values & Kappa for stage type.

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%	67%	100%	100%	100%	100%	1.00	0.69	1.00	1.00
005-A	82%	89%	100%	89%	100%	75%	100%	75%	0.70	0.90	0.78	0.83
001-B	100%	100%	85%	100%	100%	88%	100%	100%	1.00	0.79	0.89	1.00
004-D	82%	100%	91%	100%	83%	63%	93%	80%	0.80	0.90	0.47	0.73
002-G	82%	100%	100%	78%	100%	63%	100%	83%	0.80	0.79	0.67	0.88
003-H	100%	89%	100%	100%	86%	67%	100%	75%	0.90	1.00	0.52	0.83
H-I-02	100%	100%	100%	100%	100%	100%	100%	75%	1.00	1.00	1.00	0.78
H-I-01	82%	100%	91%	100%	100%	67%	100%	75%	0.80	0.90	0.69	0.78
H-I-03	100%	100%	100%	50%	100%	100%	92%	86%	1.00	0.55	1.00	0.78
H-I-04	100%	100%	100%	100%	91%	100%	85%	86%	1.00	1.00	0.90	0.68
H-I-06	100%	100%	100%	33%	100%	100%	100%	86%	1.00	0.35	1.00	0.89
H-I-05	100%	100%	100%	22%	100%	100%	100%	50%	1.00	0.24	1.00	0.55
H-I-10	100%	100%	100%	78%	100%	100%	100%	88%	1.00	0.79	1.00	0.89
H-I-07	100%	100%	92%	100%	100%	100%	100%	100%	1.00	0.90	1.00	1.00
011-K	100%	100%	92%	86%	92%	100%	100%	100%	1.00	0.78	0.90	1.00

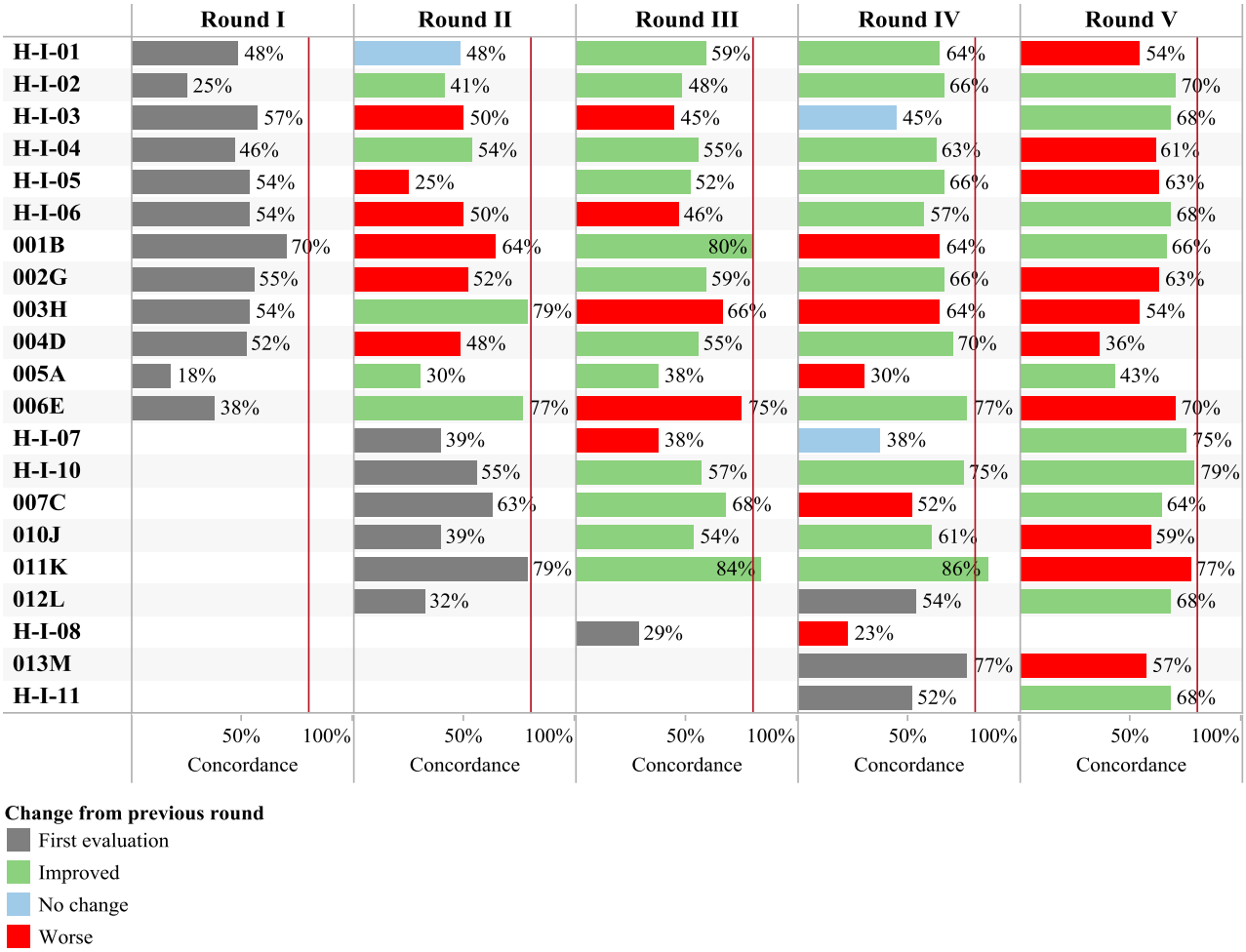
Laboratories	*P. vivax* asexual		*P. vivax* sexual		*P. falciparum* asexual		*P. falciparum* sexual		Kappa			
	NPV	PPV	NPV	PPV	NPV	PPV	NPV	PPV	*P. vivax* asexual	*P. vivax* sexual	*P. falciparum* asexual	*P. falciparum* sexual
010-J	100%	89%	100%	100%	92%	100%	100%	100%	0.90	1.00	0.90	1.00
012-L	100%	100%	79%	100%	100%	100%	100%	100%	1.00	0.69	1.00	1.00
007-C	100%	89%	100%	78%	92%	100%	87%	100%	0.90	0.79	0.90	0.76
H-I-11	100%	89%	92%	100%	100%	89%	92%	88%	0.90	0.89	0.90	0.79
013M	100%	89%	100%	78%	91%	89%	87%	100%	0.90	0.79	0.80	0.76

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

For the fourth parameter evaluated, parasite density, results show substantial improvement for all the participating laboratories (figure 4). Although this parameter still needs strengthening, in this round the concordance rates obtained by almost all laboratories have been higher than those in the previous rounds. In this parameter the difference of ±50% to the assigned value of the parasite density in each slide is taken into account. See Annex 1 for the details of the formulas used in the NETLab system for the calculation of concordance rates.

The major problem observed in this parameter was the in correct use of the formula for calculation of parasite density by parasites per microliter of blood (p/µl). This is due to the fact that some laboratories are still using the 'plus' system which had been previously established for estimating parasite density. Currently, several of the national laboratories evaluated are implementing the counting of parasites per microliter (p/µl) and a noticeable improvement since the first round has been observed for most of these.

Figure 4. Percentage of parasite density concordance.



CONCLUSIONS

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

This program will also allow the 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 again the slides received 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 (9) 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 parasitaemia

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.