TECHNICAL REPORT
TENTH ROUND
2023-2024

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

July, 2025

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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; Plasmodium malariae,* and mixed slides (P.f./P.v.).
- Slides with different parasite densities: low, medium and high density.
- Stages: asexual and sexual stages of *P. vivax, P. falciparum,* and *P. malariae*.
- 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*, *P. malariae,* or mixed infections.
3. Stage: Refers to detection of asexual and sexual stages (*P. vivax*, *P. falciparum,* and *P. malariae* gametocytes).
4. Parasite density: Refers to quantitative detection of parasites, 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 ±25% 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: 50 - 100 %. Unacceptable < 50%

RESULTS

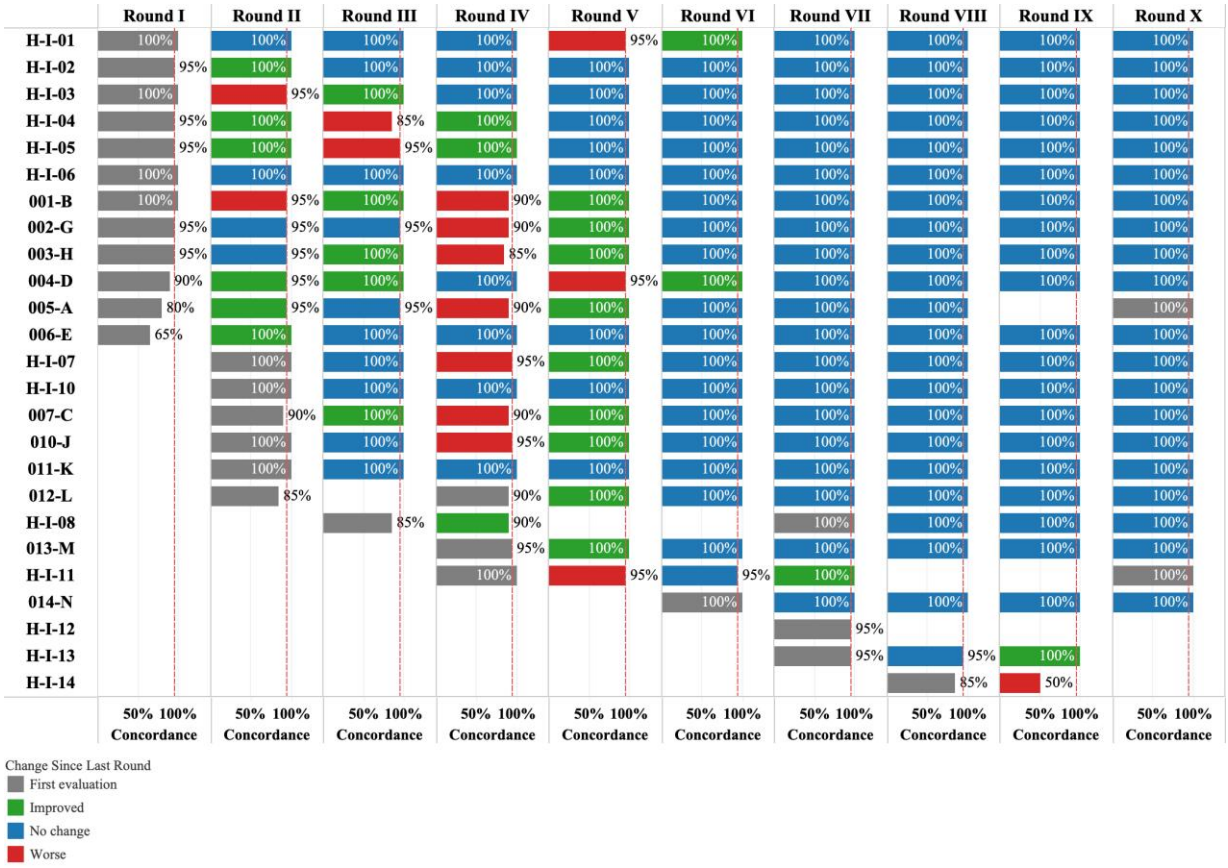
Twenty-two reference laboratories from the Region of the Americas agreed to participate in this tenth round: ten from Mesoamerica and the Caribbean and 12 from South America. The analysis and results of the current report represents the 22 National Reference Laboratories that participated in this exercise in the 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 quick results for each of the four 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 the tenth round. In this report, laboratories are identified by their codes to ensure anonymity of results.

The results of round X for the first parameter evaluated, concordance of results, as illustrated in Figure 1, was: of the 22 participating laboratories, all attained 100% concordance. For this parameter, a concordance $\geq$ 95% is deemed as acceptable.

Figure 1. Percentage concordance and round comparison for Results parameter.



As no labs had problems identifying slides as positive or negative, the specificity and sensitivity for all 22 laboratories evaluated was 100% (Table 1) and all laboratories had a Kappa index of 1.00. A Kappa (K) index value greater than 0.8 shows good concordance among evaluators of the slides; it demonstrates that all laboratories have good concordance with the regional reference laboratories for the results parameter, as shown in Table 1.

Table 1. Specificity, Sensitivity & Kappa for Results parameter.

Result			
Laboratories	**Specificity**	**Sensitivity**	**Kappa**
006-E	100%	100%	1.00
005-A	100%	100%	1.00
001-B	100%	100%	1.00
004-D	100%	100%	1.00
002-G	100%	100%	1.00
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	100%	100%	1.00
H-I-06	100%	100%	1.00
H-I-05	100%	100%	1.00
H-I-07	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-08	100%	100%	1.00
H-I-11	100%	100%	1.00
013-M	100%	100%	1.00
014-N	100%	100%	1.00

As can be observed in Figure 2, the results for the second parameter evaluated, species concordance, in round X were: 15 of the 22 participating laboratories obtained an acceptable result (percentage greater than or equal to 95%), while the remaining seven had concordance rates below the required standard (one with 93%, four with 89%, one with 86%, and one with 79%).

Comparing these results with the previous round, it can be observed that, while most of the participating laboratories either maintained or improved their concordance rates for this parameter, there was an increase in the number of labs reporting lower concordance than last round. Eight demonstrated a decline, while eleven maintained the same concordance and one improved. Two participating laboratories did not participate in the previous round.

As seen in Table 2, one laboratory had a Kappa index below 0.8 for both *P. falciparum* and *P. vivax*, indicating problems with the identification of these species. Analyzing the data using

sensitivity and specificity, it can be observed that 19 out of 22 participating laboratories had no problems with identifying positive slides for *P. falciparum* (sensitivity) while the other three laboratories had sensitivities of 88%, 88%, and 86%. Only one laboratory had problems reading negative slides for this species with a specificity of 77% (see Table 2). Although some of these laboratories belong to non-endemic countries for *P. falciparum*, the results of this evaluation demonstrate high levels of sensitivity and specificity for the diagnosis of this species. For *P. vivax,* 20 out of 22 laboratories presented good results for both positive and negative slides, as indicated by a Kappa index of 0.8 or higher. The other laboratories mainly had problems identifying positive slides of this species, with a sensitivity of 78%. Unlike previous rounds, this round also included one slide of *P. malariae* in the panels. Sixteen out of 22 laboratories made no errors in identifying this species. Four laboratories failed to correctly identify the positive slide, and another two laboratories identified this species on negative slides.

Figure 2. Percentage concordance and round comparison for species type.

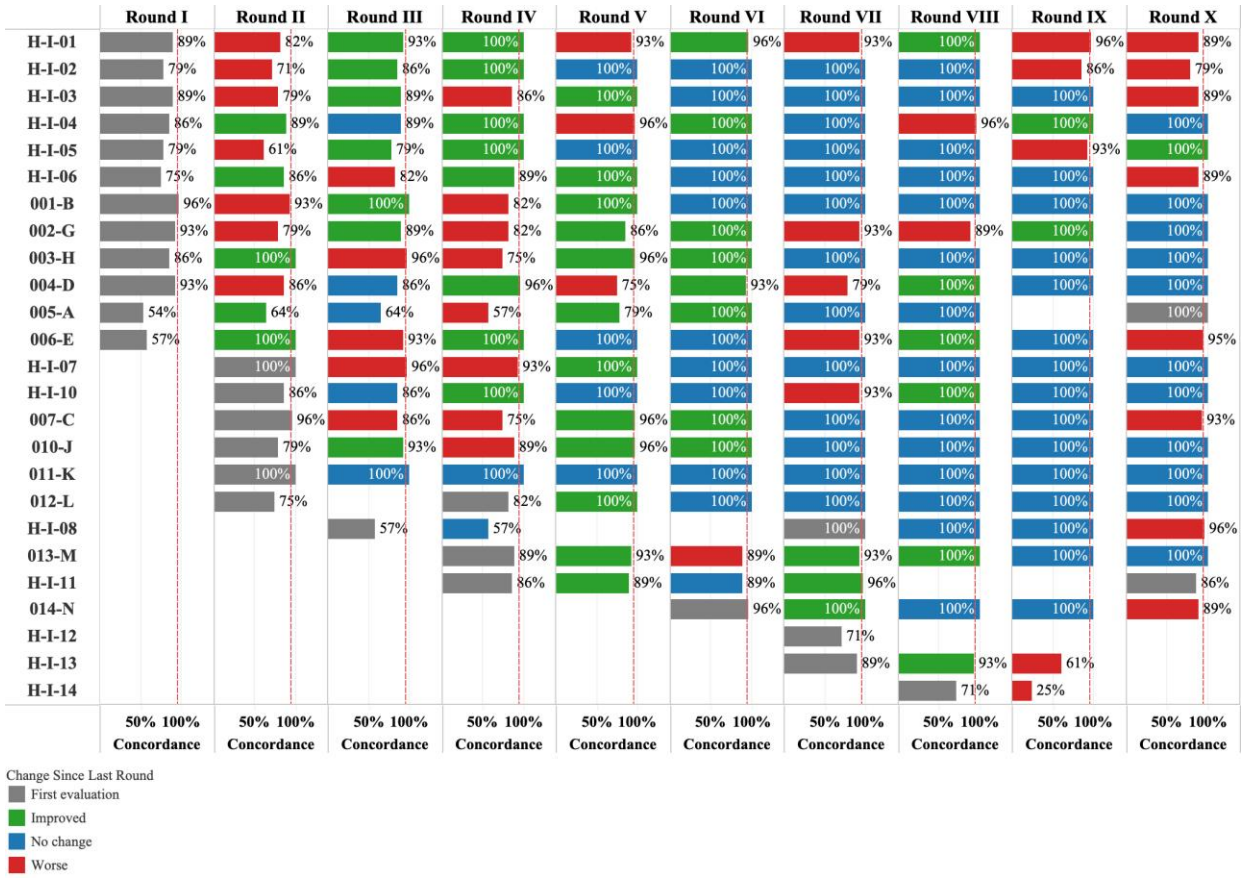


Table 2. Specificity, Sensitivity & Kappa for species type.

Laboratories	*P. falciparum*			*P. vivax*			*P. malariae*		
	Specificity	**Sensitivity**	**Kappa**	**Specificity**	**Sensitivity**	**Kappa**	**Specificity**	**Sensitivity**	**Kappa**
006-E	100%	100%	1.00	100%	88%	0.89	95%	100%	0.64
005-A	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
001-B	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
004-D	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
002-G	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
003-H	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
H-I-02	77%	86%	0.59	100%	78%	0.79	100%	100%	1.00
H-I-01	100%	100%	1.00	91%	89%	0.80	100%	0%	0.00
H-I-03	100%	100%	1.00	91%	89%	0.80	100%	0%	0.00
H-I-04	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
H-I-06	100%	100%	1.00	91%	89%	0.80	100%	0%	0.00
H-I-05	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
H-I-10	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
H-I-07	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
011-K	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
010-J	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
012-L	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
007-C	100%	88%	0.89	92%	100%	0.90	100%	100%	1.00
H-I-08	100%	100%	1.00	100%	89%	0.90	100%	100%	1.00
H-I-11	100%	100%	1.00	91%	78%	0.69	100%	0%	0.00
013-M	100%	100%	1.00	100%	100%	1.00	100%	100%	1.00
014-N	100%	88%	0.89	100%	88%	0.89	95%	100%	0.64

Results for the third parameter evaluated, stage concordance, as observed in Figure 3, show that 21 of the 22 participating laboratories obtained acceptable results (≥80% concordance). However, 11 labs reported a decrease in concordance from the previous round while seven reported improvement and two reported no change.

A more detailed analysis of the results by species and stage concordance is shown in Table 3. Regarding *P. vivax,* challenges were greater in the detection of sexual stages where in nine of the 22 participating laboratories obtained Kappa indices of less than 0.8. For, the asexual stage, 19 of the 22 laboratories obtained a Kappa index ≥0.8 and only three obtained indices below this rate.

For *P. falciparum,* there were 20 of 22 participant laboratories with Kappa indices equal to or greater than 0.8 for sexual stages or gametocytes, and 17 laboratories with Kappa indices equal to or greater than 0.8 for asexual stages. For *P. malariae*, 16 of the 22 labs made no errors with either the asexual or sexual stage, while the other six made errors in both stages.

Figure 3. Percentage concordance and round comparison for stage type.

Table 3a. Specificity and Sensitivity for stage type.

Laboratories	*P. vivax* asexual		*P. vivax* sexual		*P. falciparum* asexual		*P. falciparum* sexual		*P. malariae* asexual		*P. malariae* sexual	
	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity
006-E	100%	88%	100%	75%	86%	100%	100%	100%	95%	100%	95%	100%
005-A	100%	100%	100%	88%	86%	100%	100%	100%	100%	100%	100%	100%
001-B	100%	100%	100%	100%	100%	83%	100%	100%	100%	100%	100%	100%
004-D	100%	100%	100%	100%	86%	100%	100%	100%	100%	100%	100%	100%
002-G	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
003-H	92%	100%	100%	88%	100%	100%	100%	100%	100%	100%	100%	100%
H-I-02	100%	78%	100%	100%	77%	86%	82%	67%	100%	100%	100%	100%
H-I-01	91%	89%	92%	88%	100%	100%	100%	100%	100%	0%	100%	0%
H-I-03	91%	89%	92%	63%	100%	100%	100%	100%	100%	0%	100%	0%
H-I-04	100%	100%	85%	100%	100%	100%	100%	100%	100%	100%	100%	100%
H-I-06	91%	89%	85%	86%	100%	100%	100%	100%	100%	0%	100%	0%
H-I-05	100%	100%	92%	100%	100%	100%	100%	100%	100%	100%	100%	100%
H-I-10	100%	100%	92%	88%	100%	100%	100%	100%	100%	100%	100%	100%
H-I-07	100%	100%	92%	100%	100%	100%	88%	100%	100%	100%	100%	100%
011-K	100%	100%	92%	100%	93%	100%	100%	100%	100%	100%	100%	100%
010-J	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

Laboratories	*P. vivax* asexual		*P. vivax* sexual		*P. falciparum* asexual		*P. falciparum* sexual		*P. malariae* asexual		*P. malariae* sexual	
	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity
012-L	100%	100%	100%	50%	100%	83%	100%	100%	100%	100%	100%	100%
007-C	92%	100%	100%	100%	93%	83%	100%	100%	100%	100%	100%	100%
H-I-08	92%	88%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
H-I-11	91%	78%	100%	57%	100%	100%	100%	100%	100%	0%	100%	0%
013-M	100%	100%	100%	88%	100%	100%	100%	100%	100%	100%	100%	100%
014-N	100%	88%	100%	75%	100%	100%	100%	83%	95%	100%	95%	100%

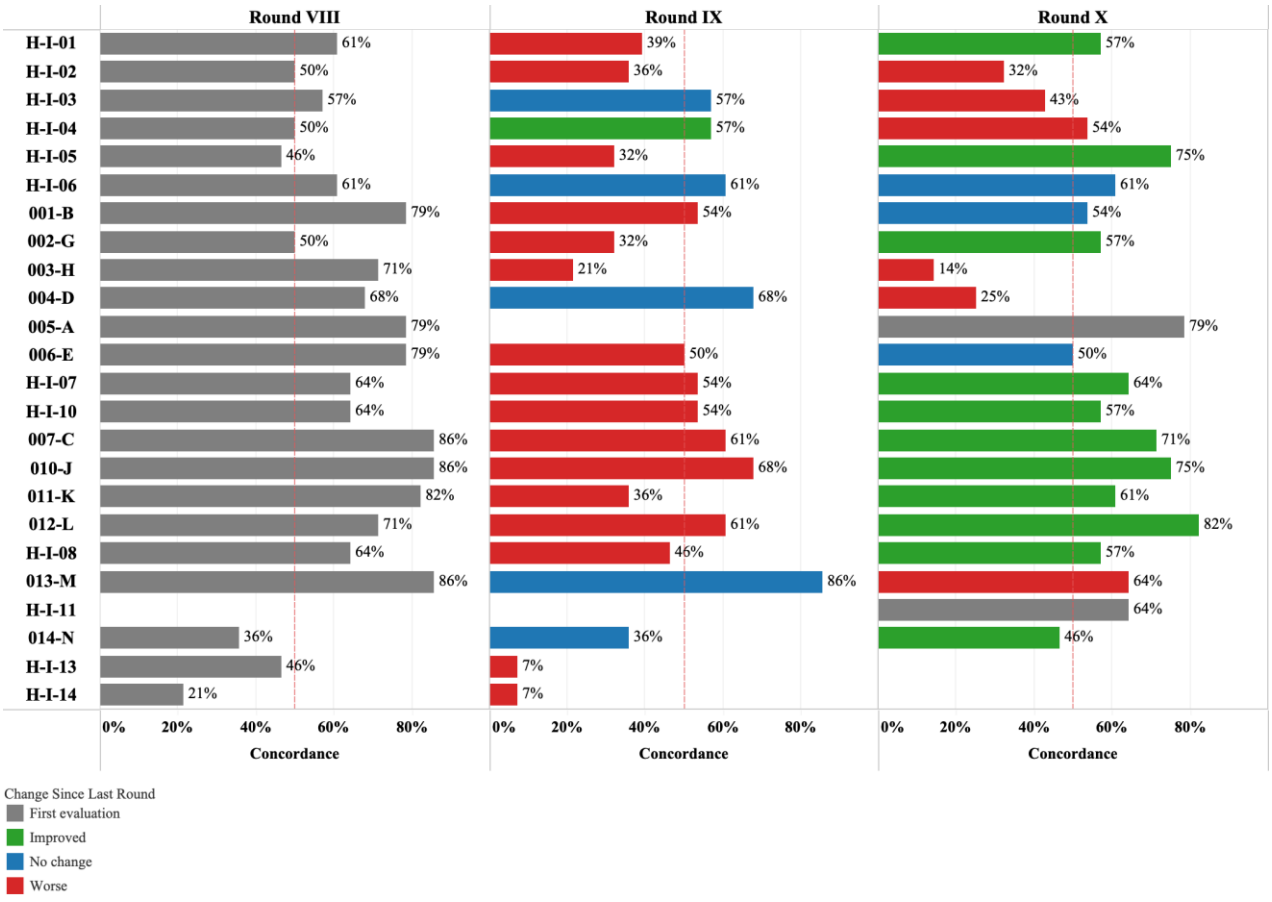
Table 3b. Kappa for stage type

Laboratories	*P. vivax* asexual	*P. vivax* sexual	*P. falciparum* asexual	*P. falciparum* sexual	*P. malariae* asexual	*P. malariae* sexual
	Kappa	Kappa	Kappa	Kappa	Kappa	Kappa
006-E	0.89	0.78	0.78	1.00	0.64	0.64
005-A	1.00	0.89	0.78	1.00	1.00	1.00
001-B	1.00	1.00	0.88	1.00	1.00	1.00
004-D	1.00	1.00	0.78	1.00	1.00	1.00
002-G	1.00	1.00	1.00	1.00	1.00	1.00

Laboratories	*P. vivax* asexual	*P. vivax* sexual	*P. falciparum* asexual	*P. falciparum* sexual	*P. malariae* asexual	*P. malariae* sexual
	Kappa	Kappa	Kappa	Kappa	Kappa	Kappa
003-H	0.89	0.89	1.00	1.00	1.00	1.00
H-I-02	0.79	1.00	0.59	0.38	1.00	1.00
H-I-01	0.80	0.79	1.00	1.00	0.00	0.00
H-I-03	0.80	0.57	1.00	1.00	0.00	0.00
H-I-04	1.00	0.79	1.00	1.00	1.00	1.00
H-I-06	0.80	0.68	1.00	1.00	0.00	0.00
H-I-05	1.00	0.90	1.00	1.00	1.00	1.00
H-I-10	1.00	0.79	1.00	1.00	1.00	1.00
H-I-07	1.00	0.90	1.00	0.69	1.00	1.00
011-K	1.00	0.89	0.89	1.00	1.00	1.00
010-J	1.00	1.00	1.00	1.00	1.00	1.00
012-L	1.00	0.55	0.88	1.00	1.00	1.00
007-C	0.90	1.00	0.76	1.00	1.00	1.00
H-I-08	0.79	1.00	1.00	1.00	1.00	1.00
H-I-11	0.69	0.63	1.00	1.00	0.00	0.00

Laboratories	*P. vivax* asexual	*P. vivax* sexual	*P. falciparum* asexual	*P. falciparum* sexual	*P. malariae* asexual	*P. malariae* sexual
	Kappa	Kappa	Kappa	Kappa	Kappa	Kappa
013-M	1.00	0.89	1.00	1.00	1.00	1.00
014-N	0.89	0.78	1.00	0.88	0.64	0.64

Figure 4. Percentage of concordance and round comparison for parasite density



CONCLUSIONS

This program has made it possible to identify strengths and weaknesses in participant laboratories, which will be addressed individually with each one.

This program also has allowed the standardization of the processes for malaria microscopic diagnosis 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 reestablishment 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 (https://netlabv2.ins.gob.pe/login).

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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*, *P. falciparum, or P. malariae*; 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

For each slide, the software divides the number of correctly identified positive stages by the total number of positive stages plus the number of stages incorrectly identified as positive. Correctly identified negative stages are not counted.

This round included six stages (asexual and sexual stages of *P. falciparum*, *P. vivax,* and P. *malariae*), and 0.17, 0.25, 0.33, 0.5, 0.67, 0.75, 0.83, or 1 point 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

For *P. vivax* and *P. malariae*, parasitaemia is reported as the total parasite density for both the asexual and sexual stages. For *P. falciparum*, parasitaemia is reported as the parasite density of the asexual stage. For slides with a single species, the software awards one point if the density reported by the evaluated lab is within 25% (above or below) of the value reported by the reference laboratory. For slides with two species, the software awards 0.5 points for each species with matching densities (within 25%). Species that are correctly identified as negative (and no density is reported) are not counted.

The software awards points when the reference panel has fewer than 50 parasites (in any species) and the evaluated laboratory enters any amount between 01 and 75.

Up to 1, 0.5, or 0 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.