HSD/CD/M/001-11[1]

EXTERNAL QUALITY ASSURANCE PROGRAM FOR MALARIA MICROSCOPY DIAGNOSIS

REGIONAL MALARIA PROGRAM
PREVENTION AND CONTROL OF COMMUNICABLE DISEASES
HEALTH SURVEILLANCE AND DISEASE PREVENTION AND CONTROL
PAN AMERICAN HEALTH ORGANIZATION

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Contents

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Background

The first component of the Global Malaria Control Strategy is access to timely, adequate diagnosis and treatment. This strategic component has been emphasized in the efforts undertaken within the framework of malaria surveillance, prevention, and control activities in the Americas.

Implementing policies that guarantee access to adequate treatment requires the existence of a health system that provides timely access to reliable diagnosis. Quality in the preparation and reading of thick blood films for malaria requires procedures and tools for promoting and monitoring the quality of diagnosis, based on the structure of the laboratory network.

The countries of the Region have been engaged in decentralization processes in which malaria diagnosis has been moved from a centralized, vertical structure to various actors in the public and private health services network. The multiplicity of actors, high turnover of human resources, and greater complexity in the structure of the services network impose ever-greater challenges on national malaria programs and the directors of national laboratory networks in the countries to guarantee diagnostic quality.

Quality control of malaria diagnosis in the countries of the Region is marked by the predominance of indirect evaluation activities (the periodic shipment of material from local laboratories to intermediate laboratories and, in some cases, from that level to a national reference laboratory). This monitoring system is only partially functional in some countries, and the prevailing methodology permits the introduction of biases into the evaluations. In the majority of cases, the system demands excessive time and human resources without necessarily meeting the quality objectives. The need for an External Quality Assurance Program (EQAP) for national reference laboratories is the reason for this protocol. This program will not only improve malaria diagnosis at the reference centers, but permit the transfer of skills and the upgrading of resources in the countries through South-South cooperation.

Objective

The objective is to define the technical procedure for the organization, design, and evaluation of the performance of the national reference laboratories in the countries of the Region in microscopic malaria diagnosis, with a view to maintaining an efficient system of quality control and strengthening the monitoring of malaria diagnosis in the Region of the Americas.

Scope

This applies to the national reference laboratories of the countries of the Region that, voluntarily and in writing, have agreed to participate in the external evaluation of the quality of microscopic diagnosis of malaria, which will be carried out through the shipment of slide sets. The laboratories of the participating countries should extend this evaluation methodology using slide sets to the intermediate levels (major regional laboratories) of their countries to strengthen the national network for surveillance of malaria diagnosis.

Design

This will be executed by organizational levels, as follows:

Stage 1

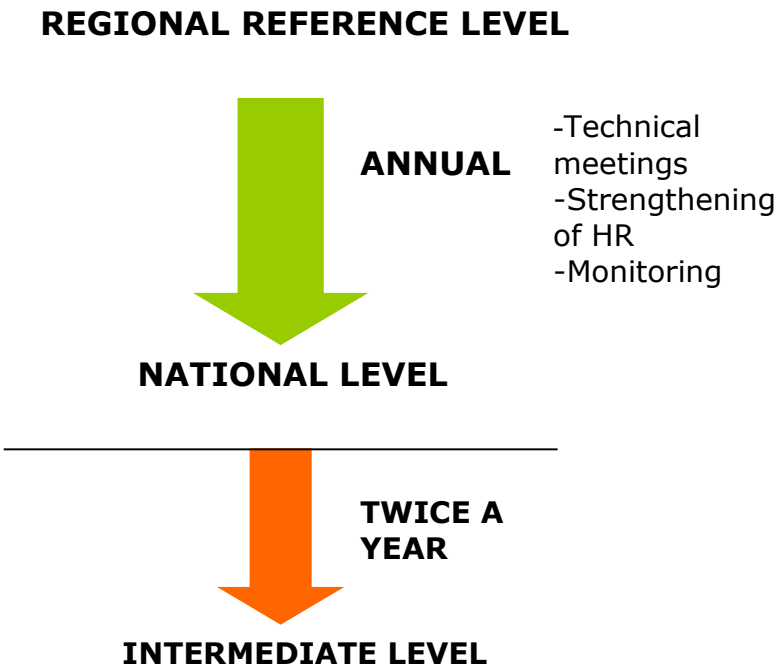
Regional Reference Level: National Institute of Health of Lima and National Health Laboratory of Honduras

National Level: National reference laboratories of the countries of the Region

Stage 2

Intermediate Level: Major Regional Laboratories

The process will be executed by organizational levels, based on the diagram below:



Organization:

The preparation and shipment of the sets will be divided geographically between the two regional reference laboratories to facilitate a more effective and timely response.

Regional Reference Center for South America

INS MALARIA LABORATORY, PERU

Regional Reference Center for Central America and the Caribbean

LNSP MALARIA LABORATORY, HONDURAS

Methodology

Preparation of the slide sets

These slide sets will be prepared by the regional reference laboratories using the following guidelines, which are based on the PAHO/WHO guidelines for malaria diagnosis and the development of the EQAP (see references):

- Slides with preparations of thick blood film and of thin blood film will be included.
- Each slide set will contain 20 slides.
- Slide sets should be prepared that are uniform in terms of the characteristics of the slides (species, parasitemia) to ensure that the evaluation is comparable when sets of the same type are used to evaluate different laboratories.
- The slides should include the species present in the Region and differential diagnoses by including mixed infections, different parasite densities, and negative samples.
- Each slide will be assigned a code as follows:
  For example: P-I-01-XX. The first letter (P) is the initial of the country responsible for the preparation of the slide; (I) the Roman numeral is the number of the set to be sent; and (01) is the number of the slide, from 1 to 20. The code (XX) is the code that the regional reference laboratory assigns to the participating laboratory.
- Each slide set sent out will be accompanied by a form for recording the results of the examination of the set.
- The reference laboratories will prepare a brief summary for each slide.

Cross-checking the slide sets

For proper standardization of the sets to be sent to the countries, there will be an initial cross-check between the regional reference laboratories using the same guidelines. Once these results are in, the sets will be sent to the participating laboratories in each country.

Frequency of evaluation

Annual.

Shipment methods for the slide sets

For each set of slides, both the initial shipment between the regional reference laboratories and the shipments to the national reference laboratories of the different countries of the Region, will be by international air mail. Since the slides meet the requirements of the relevant international guidelines [(4)] for materials **exempt** from the regulations governing the transport of infectious substances, they can be sent in normal packages marked “fragile.”

Commitments of participating institutions

Regional Reference Level

- Guarantee the sustainability of the EQAP for malaria diagnosis through the preparation of slide sets and system monitoring and management.
- Comply with the technical standards for malaria diagnosis contained in the PAHO/WHO guidelines.
- In the case of unanticipated events, hold technical meetings or obtain direct assistance from the regional reference centers and PAHO to respond to these events.

National Level

- The Director or head of the National Institutes of Health or Reference Laboratories of each country in the Region agree in writing to participate voluntarily in the program and provide all the facilities necessary for the execution of the program.
- The analyst(s) of the national malaria reference laboratory in charge of the activity review the slide sets received and respond within the stipulated time.
- Promote the participation of intermediate levels in the evaluation programs to strengthen the national laboratory network.

Deadline for participants’ response

The laboratory being evaluated should submit its results to the respective regional reference laboratory no more than 10 working days after receiving the slide set.

The results should be sent electronically on the form designed for this purpose; participants will have an access code for a specific Web page for submitting the results. For official submission, a preprinted copy of the results containing the signature of the head of the laboratory must be sent to the following addresses:

For laboratories in South America: National Institute of Health, Lima, Peru: malaria@ins.gob.pe
For laboratories in Central America and the Caribbean: National Laboratory of Honduras, Tegucigalpa, Honduras: laboratoriomalariahon@gmail.com

Statistical model for analysis of results

The evaluation of concordance is based on the percentage of slides evaluated correctly out of the total number of slides:

% Concordance = Number of correct evaluations x 100 / Total number

Quality standards

To judge the acceptable degree of concordance for the laboratory evaluated, the following quality standards should be used:

- Concordance of the presence or absence of the parasite: 95% - 100%
- Concordance of the species: 95% - 100%
- Concordance of parasitemia: 80% - 100%
- Concordance of stages of the parasite: 80% - 100%

Evaluation criteria

- Concordance of results:

This refers to assessment of the results on recognition of the slides with positive or negative findings for the presence of *Plasmodium*.

- Concordance of species:

This refers to assessment of the results for the identification of each species present on the positive slides in the set. In the case of slides with mixed parasites, all species should be identified; if the laboratory being evaluated identifies only one of the species, only half of the value of the correctly evaluated slide will be counted.

- Concordance of stages:

This refers to assessment of the results on identification of the sexual and asexual stages of *Plasmodium* present on the positive slides.

- Concordance of parasitemia:

This refers to assessment of the results on recognition of the exact quantity of parasites on the positive slide, expressed in parasites per microliter, considering that the differences between readings should not exceed 50%, as established in the practical guide for in vivo studies of the effectiveness of antimalarial drugs in the Americas [3]

Deadline for responses from the evaluating laboratory

The report will be generated automatically once the results are entered into the system. At the same time, the evaluating laboratories will issue an official report with a compilation of the results, the related analysis, and the principal recommendations. The laboratories evaluated will be listed with their assigned codes. This general report will be available to all. However, detailed results for the slides evaluated by each laboratory will be available only to that laboratory. This report will be sent no more than one month after the results are received from the national laboratories being evaluated. A copy will be sent to the officer responsible for the PAHO Regional Malaria Program in Washington, D.C.

Certificate of participation

The participants in this evaluation will receive a certificate of participation once they have submitted their results. This will be generated automatically when the results are reported. This certificate will be provided whether the percentage of concordance is high or low and will be attached to the report of the results.

A certificate of approval will be issued to participating laboratories that have obtained scores that meet the standards of quality for concordance described above. It will also be sent along with the official report.

Methods for implementing the recommendations

The evaluating laboratory should provide assistance to laboratories with results outside the margin of acceptability. The following activities will be considered:

The evaluated laboratories that have low concordance results will receive technical support from the regional reference laboratories to improve their capacity for microscopic malaria diagnosis. For decision-making on improvements or changes in this external quality control program, there will be annual technical meetings, with the location rotating among the participating countries.

Implementation of the recommendations

Laboratory personnel responsible for microscopic reading of the slide sets of thick blood film who have low concordance results should participate in a program run by the regional reference laboratory to upgrade their technical competencies. A visit by regional reference laboratory personnel to the laboratory evaluated can also be included when warranted.

Storage of the reports

The reports will be stored as follows: Each country will keep a copy of the results of the evaluated laboratories; the evaluating laboratories should keep electronic and printed copies of the results and respective reports; and at the regional level, the PAHO Regional Malaria Program in Washington, D.C. will keep a copy of the official reports from the two evaluating laboratories.

The electronic files will be stored as long as the software is still in use. There will be annual backups of all files, with backups stored for a period of no less than five years.

The printed reports will be kept for a period of no more than three years. There will be an annual evaluation of the External Quality Assurance Program.

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Definitions

1. **Analyst**: A person who conducts qualitative and quantitative studies using specialized methods for diagnostic purposes.
2. **Concordance**: Percentage of conformity of the results of a given test obtained by different laboratories or analysts.
3. **Disconcordance**: Percentage of disagreement of the results of a given test obtained by different laboratories or analysts.
4. **Evaluated Laboratory**: This is the laboratory that is evaluated by a supervisory laboratory appropriate to the level of complexity.
5. **Evaluating Laboratory**: This is the laboratory that evaluates the quality of diagnostic procedures performed by laboratories at an immediately lower level
6. **External evaluation:** A periodic, objective, and retrospective system for comparing different laboratory results through assessments organized by an independent external entity.
7. **Malaria**: Disease caused by the infection of human erythrocytes with the parasite of the genus *Plasmodium* corresponding to any of the four species circulating in the world: *P. vivax*, *P. falciparum*, *P. ovale* and *P. malariae*.
8. **Microscopist**: A person who interprets prepared stained slides of thick blood smears and reports the results for malaria diagnosis, using a microscope.
9. **Negative Samples:** Samples from clinically healthy people with negative microscopic diagnosis of thick blood film.
10. **Positive Samples:** Whole blood samples with parasites of any of the four species of the genus *Plasmodium*.
11. **Quality control:** Actions taken during a given process to ensure that the results, products, or services can be delivered.
12. **Quality management system:** Total program of standards, processes, and procedures that continuously ensures that services, products, or results are reliable and timely.
13. **Repeatability**: Degree of agreement between the results of successive measurements or tests of a single characteristic, obtained with the same method, by the same observer, with the same instruments for measurement or testing, in the same laboratory and at sufficiently short intervals of time.
14. **Replicability:** Degree of agreement between independent results obtained with

the same method and the same sample, but different analysts, different equipment, or different laboratories.

15. **Reproducibility:** The extent to which the method can be performed under the same conditions by several laboratories.
16. **Sample set**: Slide samples of thick blood smears that are prepared in the laboratory. The set includes different species, stages, and parasite densities.

ANNEXES

I. Reading criteria

The Formula for Quantification of Malaria Parasites (Parasitemia) is:

$$\frac{\text{Number of parasites}}{\text{Number of white blood cells (WBC)}} \times 6000$$

Use the following criteria to quantity of parasites found (parasitemia):

1. If after counting 200 WBC, 10 or more parasites are observed, apply the formula using 200 as the number of white blood cells (WBC) in the denominator.

2. If after counting 200 WBC, 9 or fewer parasites are observed, continue counting up to 500 WBC and apply formula using 500 as the number of white blood cells (WBC) in the denominator.

3. If you count more than 500 parasites and less than 200 WBC, stop counting when you reach the last field. Apply the formula using the actual number of white blood cells (WBC) counted, in the denominator

4. To report a negative slide, 500 fields need to be examined

II. Results format report (automatic)

PROGRAMA DE EVALUACION EXTERNA DEL DESEMPEÑO (PEED)
EN EL DIAGNOSTICO POR GOTA GRUESA Y FROTIS DE MALARIA
POR COLORACION GIEMSA

Fecha 07/01/2013
Hora: 12:14:53p.m.

Laboratorio: XXXXXXXX
Codigo del Panel: ING2012
Fecha Recibo: 03/02/2012
Fecha Proceso: 02/03/2012

RESULTADO LABORATORIO EVALUADO

Codigo	Resultado	Vivax Asexuado	Vivax Sexuado	Falciparum Asexuado	Falciparum Sexuado
P-I-01	VF	80		544	128
P-I-02	N				
P-I-03	V	128	32		
P-I-04	VF	144	48	1760	288
P-I-05	N				
P-I-06	V	288	48		
P-I-07	N				
P-I-08	F			544	
P-I-09	V	272	32		
P-I-10	N				
P-I-11	V	128	16		
P-I-12	F			544	
P-I-13	F			8360	40
P-I-14	N				
P-I-15	F				48
P-I-16	F			368	
P-I-17	V	720	48		
P-I-18	VF	672	208	496	48
P-I-19	F			192	
P-I-20	N				

RESULTADO DEL PANEL

Codigo	Resultado	Vivax Asexuado	Vivax Sexuado	Falciparum Asexuado	Falciparum Sexuado
P-I-01	VF	95	89	535	24
P-I-02	N				
P-I-03	V	95	12		
P-I-04	VF	377	81	1543	24
P-I-05	N				
P-I-06	V	343	23		
P-I-07	N				
P-I-08	F			428	12
P-I-09	V	300	12		
P-I-10	N				
P-I-11	V	42			
P-I-12	F			561	
P-I-13	VF	24		7042	
P-I-14	N				
P-I-15	F				23
P-I-16	F			217	
P-I-17	V	907	12		
P-I-18	VF	477	188	653	35
P-I-19	F			47	
P-I-20	N				

Parámetros evaluados	Porcentaje
Concordancia de Resultado	100.00 %
Concordancia de Especie	96.43 %
Concordancia de Estadio	86.31 %
Concordancia de Densidad	47.02 %

Las bases para la evaluación de los resultados son las siguientes:

Concordancia en el Resultado	Aceptable	95 - 100 %	No aceptable	< 95%
Concordancia en la Especie	Aceptable	95 - 100 %	No aceptable	< 95%
Concordancia en el Estadio	Aceptable	80 - 100 %	No aceptable	< 80%
Concordancia en la Densidad Parasitaria	Aceptable	80 - 100 %	No aceptable	< 80%

Para el análisis de la Concordancia en la Densidad Parasitaria, se considerará concordante si el número de parasitos reportados es ± 50% de los resultados de la Densidad Parasitaria en el panel asignado por el laboratorio evaluador

Firma y Sello del responsable : .....

Nombre del responsable : .....