



HSD/CD/M/001-11

**PROGRAM FOR EXTERNAL
PERFORMANCE EVALUATION IN
MICROSCOPIC DIAGNOSIS OF MALARIA**

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

November 2010

Contents

Acknowledgements.....	2
Contents	3
Background.....	3
Objective.....	4
Scope.....	5
Design.....	5
Organization:	6
Regional Reference Center for South America	7
Regional Reference Center for Central America and the Caribbean.....	8
Methodology	8
Preparation of the slide sets.....	8
Cross-checking the slide sets.....	9
Frequency of evaluation.....	9
Shipment methods for the slide sets.....	9
Commitments by participating institutions.....	9
Regional Reference Level	9
National Level	9
Statistical model for analysis of results	10
Quality standards.....	10
- Concordance of results:	11
- Concordance of species:.....	11
- Concordance of parasite densities:.....	11
- Concordance of stages:	11
Period for responses from the evaluation laboratory	11
Proof of participation	12
Methods for implementation of the recommendations	12
Implementation of the recommendations	12
Storage of the reports	13
References	13

Definitions	14
Annexes	16
Form for evaluation of results.....	16

Acknowledgements

We are grateful for the support and collaboration of the regional reference centers, namely the Malaria Laboratory of the National Institute of Health, Ministry of Health of Peru and the Malaria Laboratory of the National Public Health Laboratory, Ministry of Health of Honduras, in the preparation of this document and the implementation of the External Performance Evaluation Program (EPEP), and for the input from Public Health Laboratory Services of the Pan American Health Organization (PAHO/HSD/IR/LAB).



This program is being executed thanks to the support and collaboration of the U.S. Agency for International Development (USAID), under USAID/PAHO agreement N° 527 A-00-08-00026-00.



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 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 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 Performance Evaluation Program (EPEP) 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:

REGIONAL REFERENCE LEVEL



ANNUAL

- Technical meetings
- Strengthening of HR
- Monitoring

NATIONAL LEVEL



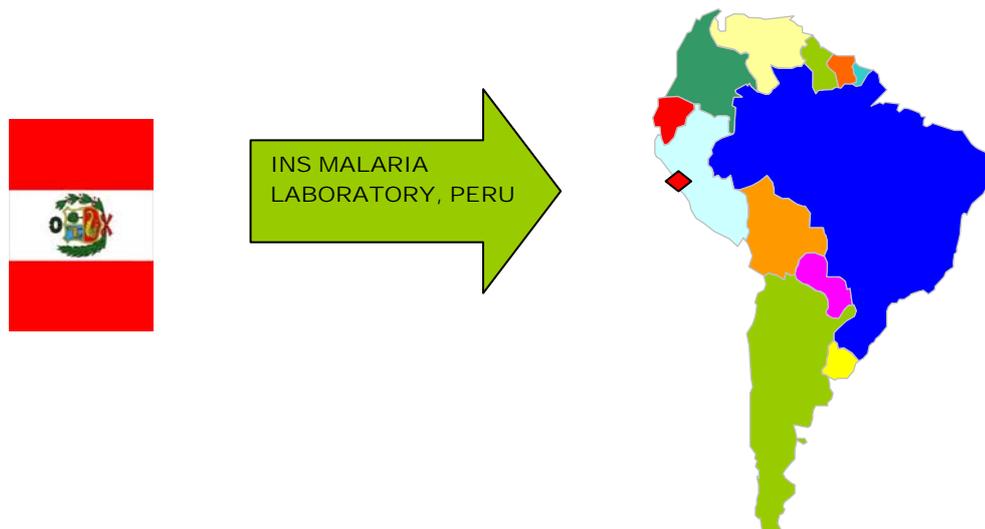
**TWICE A
YEAR**

INTERMEDIATE LEVEL

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



Regional Reference Center for Central America and the Caribbean



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 EPEP (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 EPEP 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.gov.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 or discordance is based on the percentage of slides evaluated correctly out of the total number of slides:

$$\% \text{ Concordance} = \frac{\text{Number of correct evaluations} \times 100}{\text{Total number}}$$

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: Not less than 100%
- Concordance of the species: Not less than 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 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⁾

- 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.

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:

In the first year, before the slide sets are shipped, a workshop will be held with all the representatives of the national laboratories participating in this quality control program, with a view to standardizing the criteria for evaluating the slides in terms of procedures and quality standards.

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 Performance Evaluation Program.

References

1. Basic malaria microscopy. Part I Learner's guide. Second Edition Geneva WORLD HEALTH ORGANIZATION. 2010
2. Basic malaria microscopy. Part II Tutor's guide. Second edition. Geneva WORLD HEALTH ORGANIZATION. 2010
3. Guía práctica revisada para estudios de eficacia de los medicamentos antimaláricos en las Américas. HSD/CD/M/005-10. Organización Panamericana de la Salud. 2010.
4. Guía sobre la reglamentación relativa al transporte de sustancias infecciosas 2009–2010. WHO/HSE/EPR/2008.10. Organización Mundial de la Salud. 2009
5. Guía para la implementación de un sistema de gestión de calidad en el diagnóstico microscópico de malaria: Estandarización de procedimientos y herramientas sobre el control de calidad y la evaluación externa del desempeño en las redes de laboratorio. OPS/DPC/CD/M/393/06
6. Manual de Procedimientos de Laboratorio para el Diagnóstico de Malaria. Instituto Nacional de Salud. 2003. Lima: INS. Serie de Normas Técnicas No. 39. ISSB: 9972-857-37-9.
7. Alger J, Matute ML, Mejía RE. Manual de Procedimientos Operativos Estándar para el Diagnóstico Microscópico de la Malaria. Secretaría de Salud, Tegucigalpa, 2006.
8. Manual de Procedimientos Bioseguridad en Laboratorios de Ensayo, Biomédicos y Clínicos. Serie de Normas Técnicas No. 18. Lima, 2005.
9. Manual para el diagnóstico microscópico de malaria. 4ta. Edición. Ginebra: OMS, 1975. Publicación científica N° 276. p.1-105
10. New Perspectives Malaria Diagnosis Report of a joint WHO/USAID informal consultation. Copyright; 2000.
11. Antonio Martí Veciana. NTP 656: Materiales de referencia. Utilización en el laboratorio de higiene industrial.
12. INDECOPI 2001. Norma Técnica Peruana requisitos generales para la competencia de laboratorios de ensayo y calibración NTP- ISO/IEC-17025-2001.
13. WHO (2000). Bench aids for the diagnosis of malaria infections, 2nd ed. Geneva, World Health Organization.

14. WHO (2006). Informal consultation on quality control of malaria microscopy WHO HEADQUARTERS, GENEVA, 3 MARCH 2006.
15. WHO (2005a). Malaria light microscopy: creating a culture of quality. Manila, Philippines, World Health Organization (Report of the WHO WPRO-SEARO biregional workshop on quality assurance for malaria microscopy. Regional Office for the Western Pacific and Regional Office for South-East Asia- Kuala Lumpur, Malaysia 18–21 April 2005-).

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

Form for evaluation of results

Concordance of Results						
Code	Participant Laboratory		Reference Centre		C	D
	Negative	Positive	Negative	Positive		
H-I-01-23	X		X		✓	
H-I-02-23	X		X		✓	
H-I-03-23	X		X		✓	
H-I-04-23		X	X			✓
H-I-01-23		X	X			✓
H-I-01-23		X		X	✗	
H-I-01-23		X		X	✓	
H-I-01-23		X	X			✓
H-I-01-23	X		X		✓	
H-I-01-23		X		X	✓	
H-I-01-23		X		X	✓	
H-I-01-23		X		X	✓	
H-I-01-23	X			X		✓
H-I-01-23	X		X		✓	
H-I-01-23	X		X			✓
H-I-01-23	X			X	✓	
H-I-01-23		X		X	✓	
H-I-01-23		X		X	✓	
Concordance of results %					75	25
Reference Value: Not less than 100%						

Conclusion: Based on the results, the diagnosis by the participating laboratory is below the reference value.

Conclusion: Based on the results, the diagnosis by the participating laboratory reaches the reference value.

Print



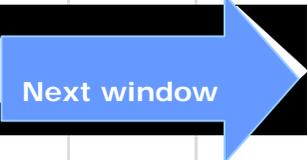
Concordance of species

Code	Participating Laboratory						Reference Center						C	D
	Pv	Pf	Pm	Mixed Infections			Pv	Pf	Pm	Mixed Infections				
				Pv/Pf	Pv/Pm	Pf/Pm				Pv/Pf	Pv/Pm	Pf/Pm		
H-I-01-23													✓	
H-I-02-23													✓	
H-I-03-23													✓	
H-I-04-23	X													✓
H-I-01-23	X	X												✓
H-I-01-23		X						X					✓	
H-I-01-23		X						X					✓	
H-I-01-23	X													✓
H-I-01-23													✓	
H-I-01-23			X						X				✓	
H-I-01-23	X						X						✓	
H-I-01-23	X						X						✓	
H-I-01-23		X						X					✓	
H-I-01-23								X					✓	✓
H-I-01-23													✓	
H-I-01-23													✓	
H-I-01-23													✓	✓
H-I-01-23							X						✓	
H-I-01-23				X						X			✓	
H-I-01-23					X						X		✓	
Concordance of species %														
Reference value: Not less than 100%														

Conclusion: Based on the results, the diagnosis from the participating laboratory is below the reference value.

Conclusion: Based on the results, the diagnosis from the participating laboratory reaches the reference value.

Print



FINAL REPORT

Code	Negative	Positive	Species			Parasite Density																	
			P. vivax	P. falciparum	P. malariae	Mixed Infections			<i>Plasmodium vivax</i>					<i>Plasmodium falciparum</i>					<i>Plasmodium malariae</i>				
						Pv/Pf	Pv/Pm	Pf/Pm	ASex	Sex	WBC	ASex/ul	Sex/ul	ASex	Sex	WBC	ASex/ul	Sex/ul	ASex	Sex	WBC	ASex/ul	Sex/ul
H-I-01-23																							
H-I-02-23																							
H-I-03-23																							
H-I-04-23																							
H-I-05-23																							
H-I-06-23																							
H-I-07-23																							
H-I-08-23																							
H-I-09-23																							
H-I-10-23																							
H-I-11-23																							
H-I-12-23																							
H-I-13-23																							
H-I-14-23																							
H-I-15-23																							
H-I-16-23																							
H-I-17-23																							
H-I-18-23																							
H-I-19-23																							
H-I-20-23																							

Laboratory Official Responsible			
Given Names	1 st Surname	2 nd Surname	Signature
		Date of Report	DAY MONTH YEAR

