REGIONAL FOREIGN ASSESSMENT PROGRAM OF THE PERFORMANCE OF THE MICROSCOPIC DIAGNOSIS OF LEISHMANIASIS

Regional Leishmaniasis Program Neglected, Tropical and Vector-Borne Transmittable Diseases Transmittable Diseases and Health Analysis

Pan-American Health Organization PAHO/WHO

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OVERVIEW

The Pan-American Health Organization/World Health Organization presents the following technical and operational guidelines for the development of the Regional Foreign Assessment Program in the Performance of Microscopic Diagnosis of Leishmaniasis in the Americas.

The program is lead and coordinated by the Parasitology Group of the Colombian National Institute of Health, Ministry of Health and Social Welfare of Colombia, and the Regional Program of Leishmaniasis PAHO/WHO.

The purpose of the program is to establish the technical procedure for the organization, design and assessment of National Reference Laboratories in the countries of the region, for the microscopic diagnosis of leishmaniasis, with the goal of maintaining an efficient quality management system, and contributing to the strengthen the diagnosis monitoring of this parasitosis in Latin America and the Caribbean, endemic areas in the region.

ACKNOWLEDGMENTS

We are thankful for the work and leadership of the Colombian National Institute of Health, and the support and collaboration of the Ministry of Health and Social Welfare of Colombia; the Oswaldo Cruz Institute, Oswaldo Cruz Foundation – Brazil; the International Center of Medical Training and Research – CIDEIM, the Pan-American Health Organization in Honduras and Colombia, in the development and revision of this document and the implementation of the Program for Direct External Evaluation of Performance (PEED), as well as the contribution of the Regional Malaria Program – CDE-VT-PAHO/WHO for sharing their experience on the subject.

1. INTRODUCTION

Parasitosis like leishmaniasis are the subject of increasing interest, as much for their impact on public health as the severe problem that they pose to human rights. It is estimated that they are the greatest cause of the burden of disease among individual infectious diseases. They are directly related with poverty, but they are also influenced by environmental and climatic factors.

Leishmaniasis is present on all five continents and is endemic in 98 countries and three territories. Approximately 350 million people are at risk of contracting one of its various clinical forms. It is estimated that globally there are 1.3 million new cases and 20,000 – 30,000 deaths each year. Approximately 300,000 cases of the visceral form of the disease (90% in Bangladesh, Brazil, Ethiopia, India, Nepal, South Sudan and Sudan) and close to 95% of cases of cutaneous leishmaniasis occur in the Americas, the Mediterranean Basin, the Middle East and Central Asia.

In the Americas, a total of 940,396 new cases of cutaneous (CL) and mucosal leishmaniasis (ML) were reported by 17/18 endemic countries from 2001-2017, with an annual average of 55,317 cases. This historic series of 17 years shows that 2015 had the lowest number of new cases (46,074) in the region, mostly given by a case reduction of 45%, 42% and 35% in Costa Rica, Panama and Colombia, respectively. In 2016, there was an increase of cases in the region, despite a 35% reduction in Brazil (Figure 1).

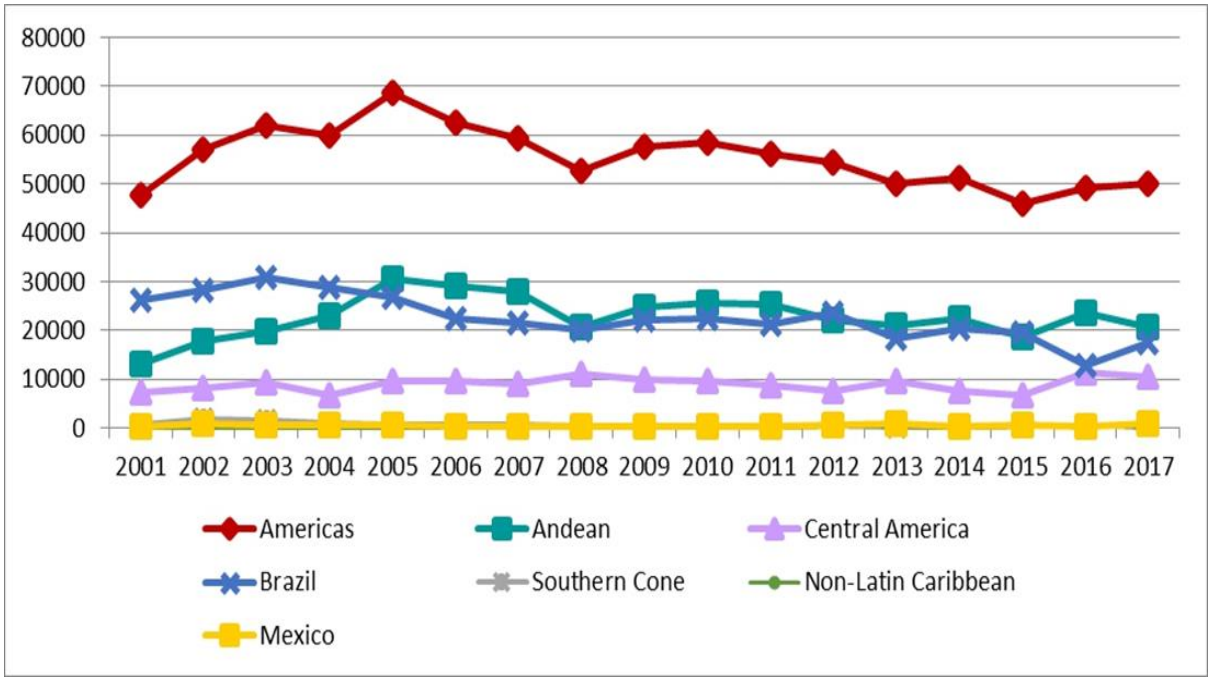


Figure 1. Number of cutaneous and mucosal leishmaniasis cases in the region, subregions and selected countries of the Americas, 2001-2017Source: SisLeish-PAHO/WHO: Data reported by the National Leishmaniasis Programs/Surveillance Services. Accessed on: November 2018.

In 2017, 49,959 cases were reported to the Pan-American Health Organization (SisLeish – PAHO/WHO) by 17 endemic countries, seeing that French Guyana continues reporting directly to France. In general, there was a decrease in the number of cases in 9 endemic countries, nevertheless, the total number of cases in the region has maintained stable compared to 2016 due to the increase in Brazil (38%), Costa Rica (94%), Mexico (88%) and Ecuador (36%).

From the total of the 2017 cases, 72.6% were reported by Brazil (17,526), Colombia (7,764), Peru (6,631) and Nicaragua (4,343). The incidence rate of the region was 22.51 cases per 100,000 population, resulting in an increase of 17,3% compared to 2016 (21.71 cases/100,000 pop.) the highest rates were reported by Nicaragua (140/100,000 pop.), Suriname (121/100,000 pop.) and Costa Rica (51,7/100,000 pop.). Five countries presented an expressive increase in the incidence rate compared to the previous year: El Salvador (9.63/100,000 pop.), Argentina (10,27/100,000), Mexico (11.5/100,000 pop.), Ecuador (22.6/100,000 pop.) and Costa Rica (51.7/100,000 pop.).

The CL cases were registered in 210 (61%) units of the first subnational administrative political level (states, departments, regions or provinces, according to each national political division) and in 2,895 (24%) units of the second administrative level (municipalities, cantones, provinces, districts, etc.). 20.2% (10,081) of cases were reported in 332 (11.3%) international borders units, highlighting Argentina, Costa Rica and Guatemala with over 36% of CL cases occurring in border zones.

Figure 2 presents the regional analyses of the average CL number of cases and incidence from 2015- 2017, normalized and stratified in the triennium-composite indicator, disaggregated at the second subnational administrative level.

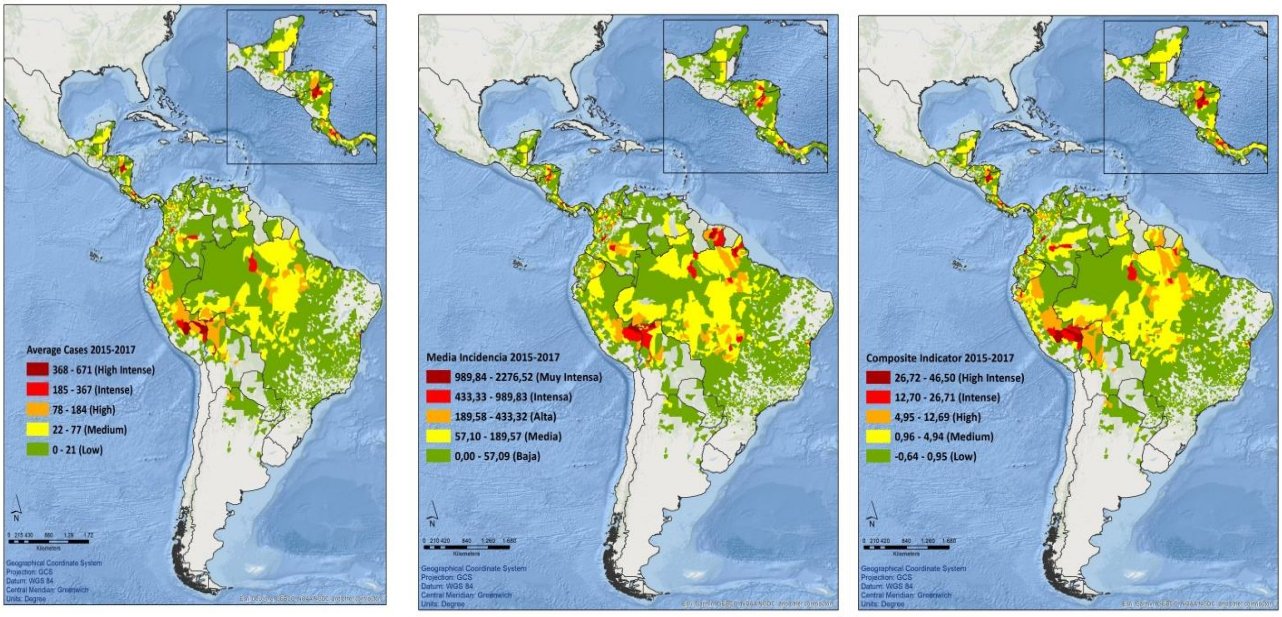


Figure 3. Average of CL number of cases and incidence, and composite indicator* stratified by risk of transmission at the second subnational administrative level, Americas, 2015-2017.**

Source: SisLeish-PAHO/WHO: Data reported by the National Leishmaniasis Programs/Surveillance Services. Accessed on: November 2018.

*CILc: Cutaneous leishmaniasis composite indicator, representing the average of cases and incidence (cases/100,000 pop.) of the 2015-2017 triennium.

** Guyana is not represented in the figure because their data is only available at the first administrative level (Regions).

With leishmaniasis as with any parasitosis, it is paramount that the etiological agent is detected and identified. Which diagnostic tools are used depends on the clinical form. However, in the majority of cases, identification is difficult because of the low number of circulating parasites.

The clinical presentations vary according to the type of parasite, the immune response of the host, the number and area of the lesions, the environmental conditions, the presence of secondary infections, the nutritional state of the patient and how advanced the disease is.

The clinical spectrum, immunological and histological, is very broad and includes from sparse lesions that can resolve spontaneously, to diffuse, mucosal lesions with organs such as the liver and the spleen being compromised and also the lymph nodes and bone marrow, as occurs with visceral leishmaniasis, which tends to be confused with other diseases which are found in the same geographical areas of the country. These aspects reinforce the importance of carrying out an early and appropriate laboratory diagnosis in order to provide the required treatment.

Great effort is taking place in the region, in order to ensure the decentralization of the direct examination diagnostic methodology of leishmaniasis, and be nearer to the areas of transmission. Furthermore, efforts are being made to facilitate and guarantee access to early treatment for which it is necessary to offer an effective and trusted diagnosis.

The taking, preparation and reading of samples requires specific procedures and materials which allow for the certification of the quality of leishmaniasis diagnosis in the laboratory network. In general, the countries have defined procedures, although there are important differences between them when analyzed in a regional context. This caused the countries to request the development of standardized procedures for diagnosis, quality control and performance evaluation, with the goal of improving and guaranteeing the quality of exam results in the countries of the region.

Currently, the internal quality control of leishmaniasis diagnosis is being done in some countries of the region by way of indirect evaluation, where 10% of negative samples and 100% of positive samples are sent to the regional laboratories, although in some situations the national laboratories are the ones doing the quality control of diagnostics of the regional or local laboratories. In areas where there are a great number of confirmed cases of leishmaniasis, the control of the positive samples, are not determined to be positive, due to an overload of activities. This introduces biases into assessments, furthermore there is no regularity in the percentage of slides to evaluate the reading, nor in the process of recording the samples.

Quality control allows the results of the diagnosis carried out in routine service to be known, with the goal of taking the necessary measures to correct the causes which do not conform to the diagnostic and thereby improve the quality of the diagnosis.

This document presents the guidelines to establish the Program for Direct External Evaluation of Performance (PEED) for the microscopic diagnosis of leishmaniasis at a regional level, which will allow for, not only the strengthening of the laboratory diagnostics in the region, but also will permit the exchange of information and technical capabilities.

The Program for Direct External Evaluation of Performance (PEED) for the microscopic diagnosis of leishmaniasis lead by the Regional Reference Laboratory – The Colombian National Institute of Health, have as a proficiency testing scheme, a simultaneous inter-laboratory comparison which is qualitative and continuous, for the parasitological diagnosis of cutaneous leishmaniasis.

2. OBJECTIVE

- To establish technical procedures to carry out the evaluation of the National Reference Laboratories of the countries in the region for the parasitological diagnosis or direct examination, with the aim of improving quality and strengthening the diagnosis of leishmaniasis in the Americas.

2.1 SPECIFIC OBJECTIVES:

- Promote among laboratory personnel, the continual improvement, in terms of pre-analytic, analytic and post-analytic quality through the use of tools that allow the identification of technical errors and thereby find the best strategies to impact the quality of the diagnosis of this parasitosis.
- Incentivize laboratory personnel to create strategies that react to the need for continual improvement of processes in their diagnostic methods through the results obtained by PEED.
- Contribute to the quality strengthening of the analytic processes for laboratory personnel, with the aim of offering service provision with high standards of quality to our users.

3. SCOPE

The program applies to the National Reference Laboratories of the countries in the region, who have, voluntarily and in writing, accepted to participate in the external evaluation of the parasitological diagnosis or direct examination quality of leishmaniasis. The laboratories of the participant countries must replicate this methodology of evaluation by panels at the intermediate level (Regional Laboratories) in their countries, in order to strengthen the leishmaniasis diagnosis network at a national level.

4. PLAN

The performance evaluation will be executed by levels of organization, in accordance with the following stages:

STAGE 1:

- **Regional Reference Level:** The Colombian National Institute of Health
- **National Level:** National Reference Laboratories of the Countries in the region

STAGE 2:

- **Intermediate Level:** Regional Laboratories

The process will be executed by levels of organization in accordance with the following scheme:

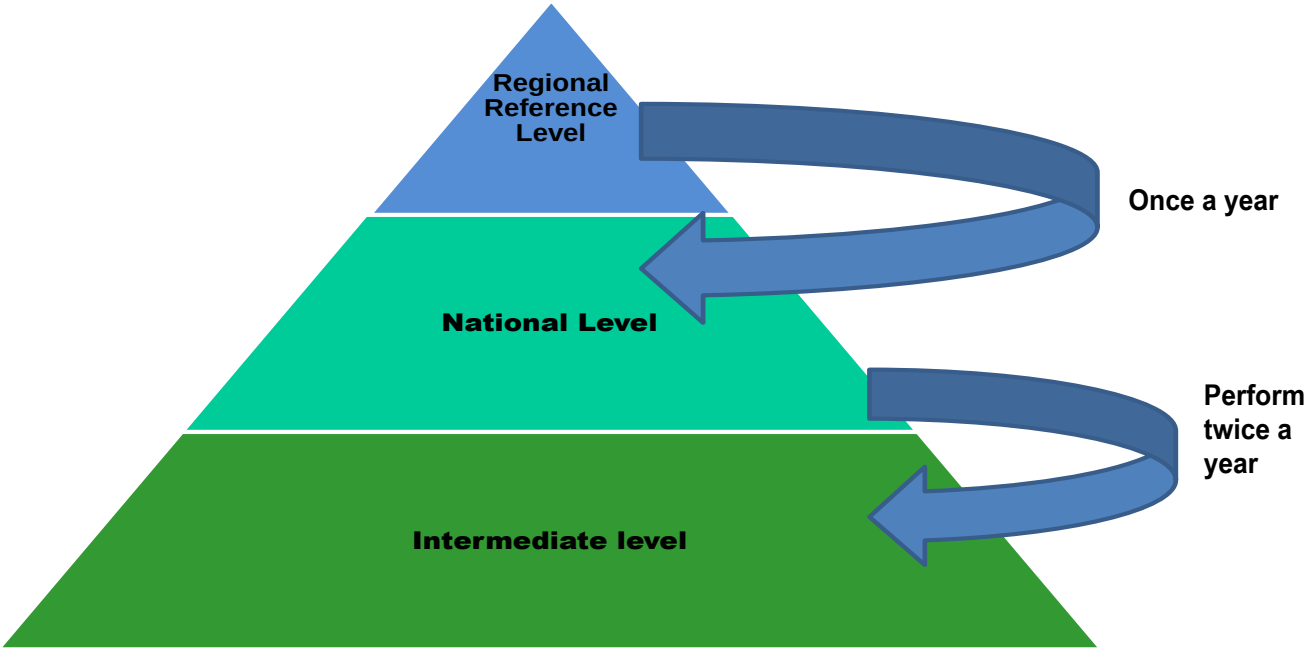


Figure 3. Source: prepared by the Parasitological Group in the Colombian National Institute of Health, 2018.

5. STATEMENT OF CONFIDENTIALITY

The Program for Direct External Evaluation of Performance (PEED) in the microscopic diagnosis of leishmaniasis - Colombian National Institute of Health, declares that the results and information obtained through the evaluation of each of the participants are confidential and will only be given to each participant, although non-personalized results can be made known in reports, technical publications and to organisms or entities that specialize in health at an international level for relevant purposes as appropriate, but participants will be informed with anticipation. Likewise, participants may voluntarily renounce the statement of confidentiality for recognition or regulatory purposes. Once a participant has completed his or her enrollment, the program will automatically assign them a random number which is the means of identification for tracking in reports of results and general reports issued by the Regional Reference Library.

6. GENERAL PROGRAM INFORMATION

The National Reference Laboratories of the countries in the region have voluntarily and in writing accepted to participate in the external evaluation of the parasitological diagnosis or direct examination quality of leishmaniasis. The program is generated by the Parasitology Group of the Colombian National Health Institute, whose proficiency testing scheme is by qualitative, continuous, and simultaneous inter-laboratory comparison for the parasitological diagnosis of cutaneous leishmaniasis.

7. DOCUMENTATION

In the current cycle the following documentation will be available to the participants:

1. The PEED program protocol which describes the operation of the program, statistical methodology, test items, test item management, delivery characteristics, results and evaluation of the program.

2. Enrollment instructions which describe the procedures that must be completed to enroll in the PEED program online.

3. 2019 Calendar of the PEED parasitological cycle.

4. Results matrix available on the web page.

5. Checklist – The participant will verify the contents of the packet and the condition of the contents and notify on the form “Supply and Inspection of the packet” by email to peedleishdirectoreg@ins.gov.co their receipt and approval.

6. Evaluation Report – Performance of the laboratory for the respective round.

8. PERIODICITY

There will be an annual cycle with a single dispatch of reference material, made up of one round that consists of ten (10) test items.

9. TEST ITEMS

The test items available for the inter-laboratory exercise consist of: material obtained through skin scrapings of the lesions by the direct swab method; the PEED Direct Leishmaniasis Examination provides ten (10) test items (slides) for direct swabs with three appositions for each one, colored with Romanowsky derived pigments (Field, Wright or Giemsa). Ten (10) homogeneous and stable slides, individually marked with non- repeating codes will be sent in the following way:

The first three letters (LHI) signify **L**eis**H**maniasis **I**nternational

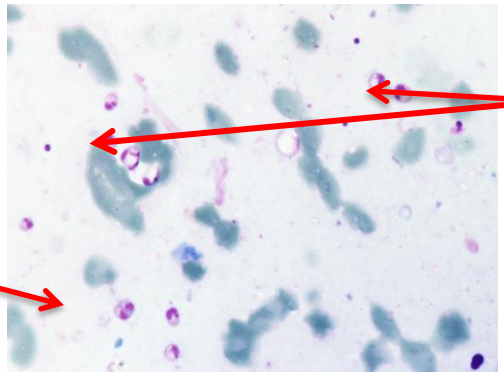
The next two digits **(01)** correspond with the number of the slide.

Followed by a dash to separate the last two digits **(19)** for the current year.

ITEM	PROGRAM	ID	YEAR	FINAL
1	LHI	1	19	LHI01-19
2	LHI	2	19	LHI02-19
3	LHI	3	19	LHI03-19
4	LHI	4	19	LHI04-19
5	LHI	5	19	LHI05-19
6	LHI	6	19	LHI06-19
7	LHI	7	19	LHI07-19
8	LHI	8	19	LHI08-19
9	LHI	9	19	LHI09-19
10	LHI	10	19	LHI010-19

The key parameter to be determined in the test items is the morphological identification of the amastigote forms of *Leishmania sp* intra or extracellularly, with all of its characteristics:

- **Nucleus:** dark violet-blue color
- **Kinetoplast:** intense violet color
- **Cytoplasm:** light blue color
- Well defined **cellular membrane**



Photograph 1. Taken by the Parasitological Group – DRSP of the Colombian National Institute of Health.

A result is **POSITIVE** when at least one amastigote is clearly observed.

A result is **NEGATIVE** when no amastigotes are observed after reviewing **ALL OF THE FIELDS OF ALL OF THE SLIDES.**

As such, the test items have or don't have the **presence of the parasite.**

10. TEST ITEM MANAGEMENT

Each assessment packet will be sent by certified international airmail. The material will comply with the definitions and requirements of materials exempt from regulations for the transport of infectious substances according to the corresponding international standard. They will be dispatched in triple packaging always guarding the fragility of the contents.

Upon receipt of the panel, each participant must verify that the panel is in the necessary condition to be read. In such case that there is any alteration or defect, the participant should immediately report via the link *"Suministro e Inspeccion del Paquete"* **Trámites y Servicios, Programas externa del desempeño / programas Regionales / documentación del programa,** which can be found on the web page, additionally evidentiary photographs of the state of the packet may be attached to an email and sent to peedleishdirectoreg@ins.gov.co so that the necessary corrective measures may be taken and ensure that a new packet is sent.

NOTE: remember that the completion of the form *"Suministro e Inspeccion del paquete"* is part of the administrative traceability, consequently it is obligatory to complete and send the form to the Regional Reference Library. If it is not reported by the established deadline, it will be assumed that: the reference material was received as requested and thus late claims will not be accepted.
Once the test items have been received, they must be kept in a cool place, free of humidity and out of any exposure to direct sunlight.

Before initiating the processing of the samples the following should be taken into account:

The microscope to be used for reading the slides, should be in optimum hygienic and metrological condition in order to guarantee the correct observation of the parasites.

The personnel involved in the analysis of the samples must demonstrate competencies in line with the test method and must comply with biosecurity regulations.

NOTE: the test item should be analyzed under the same conditions established in the evaluated laboratory, as a routine sample and under no circumstances should the test item have special conditions.

11. TEST METHODOLOGY

As a general principle, the PEED Regional Direct Examination of Leishmania recommends to the participants that the methods employed in the analysis of the proficiency test items should be those used in the routine analytical work; if possible these methods should be standardized internally by each laboratory.

12. UNIT OF MEASUREMENT

The evaluated variable in this program is a qualitative variable such that the results must be reported in terms of presence or absence, they should also be selected from a pre-established list in the results matrix.

PARAMETER	VALUE
Identification of amastigotes of ***Leishmania sp***	Present or absent

13. EVALUATION CRITERIA

The test items evaluation criteria is present/absent of amastigote forms of Leishmania sp., in the examined sample.

14. SUBMISSION OF RESULTS

Without exception the results of the participants should be completed and sent **ONLY** in the matrix found in the Excel file, available in the *Plataforma Control de Calidad* **(PCC)** of the PEED direct examination link: aplicacionesproduccion.ins.gov.co/pcc/:

Once the results matrix has been completed by the participant it will be a confidential record, so the requested information must be provided, without leaving anything blank and with the code assigned by the PCC.

They must be sent only on the date established in accordance with the calendar. Likewise, not sending the results will indicate that the participant will be excluded from the current annual cycle.

15. EVALUATION METHODOLOGY

Statistical methodology for the evaluation of consistency between Laboratories

Measures of consistency for qualitative variables:

Cohen's Kappa Test – (Kappa's index of agreement)

Cohen's Kappa Test attempts to measure the level of agreement between two methods or evaluators that classifies the patient, or the sample (or the result of an observation) according to a series of possibilities (categories) that are mutually exclusive. The simplest case is when the qualitative variable is dichotomous (two possibilities) and the classification of the judges or experts (for example two dichotomous clinicians), where one of them is the judge of reference (gold) who is the point of reference for all possible comparisons.

This situation can be represented in a table of frequencies:

		Method B Rival Judge		
Method A		Positive	Negative	
Judge of reference (gold)	Positive	a	c	f_1
	Negative	b	d	f_2
		c_1	c_2	n

The simplest measurement of agreement is the proportion of agreement with the total subjects: Po = (a+d)/n.

However, even if no relation exists between the two methods of classification (or evaluation), it is probable that some level of agreement between the two methods will be found by sheer chance. So, if method A classifies the patient or the sample with a positive result, and the same is true for method B, it is probable that an average of 50% agreement will be found.

Assuming that Method A is a scientific method of diagnostic classification and method B is the classification of the judge or expert in training, it is also probable that a certain level of agreement will be found in part due to chance.

With the goal of determining what level of observed concordance is greater than what can be expected due to simple chance, is defined by the Kappa index of agreement in the following way:

$$\kappa = \frac{P_o - P_e}{1 - P_e}$$

In the previous formula, Po is the proportion of observed agreement (in x times 1) and Pe is the proportion of expected agreement due to simple chance. In the case of perfect agreement the proportion of concordance will be 1, because 1-Pe represents the margin of agreement possible not attributable to chance.

From this margin we observe probably only one part Po-Pe, unless there is perfect agreement Po=1.

So in the case of perfect agreement the Kappa value is 1; if the observed agreement is equal to the expected agreement, the Kappa value is 0; and in cases where the observed is less than the expected the Kappa index is less than zero.

To calculate Pe, the expected agreement, the derivation is the following: in accordance with the previous table the probability that method A classifies a subject or sample as positive can be estimated as f1/n; while the corresponding probability of method B is estimated as c1/n. Considering that the two methods of classification are independent of each other, the probability that they classify the same subject as positive is a result of the two probabilities (independent events).

Applying the same reasoning, we can calculate the probability that agreement be produced between the methods when classifying a subject as negative and so the probability of any agreement between the two classifications is the sum of both values, which is:

$$P_e = \frac{f_1 \cdot c_1 + f_2 \cdot c_2}{n^2}$$

The Kappa coefficient was originally proposed by Cohen (1960) in cases of two evaluators or two methods, and is often known as Cohen's Kappa, and was generalized for cases of more than two evaluators by Fleiss, and thus it is sometimes known as the Fleiss' Kappa Index. Landis and Koch proposed limits for assessing the level of agreement in accordance with the Kappa index:

KAPPA	LEVEL OF CONCORDANCE
< 0	No agreement
0 - 0,2	Poor
0,2 - 0,4	Weak
0,4 - 0,6	Moderate
0,6 - 0,8	Good
0,8 - 1	Very Good

The extensive use of the kappa index of agreement in medical literature is probably due to the ease of calculating results as much as to its clear interpretation.

16. RESULTS REPORT

A partial report will be issued at the time that the participant enters the results on the web page.

Subsequently, a technical report corresponding to the cycle of assessment will be issued by the Regional Reference Laboratory, which will contain the following aspects:

- Results of the participants
- Results of the homogeneity studies
- Results of the stability studies
- Assigned values
- Test method used
- Statistical method

Additionally, the report will include a database, which will consolidate all results obtained from participating laboratories.

The report will be published in the PPC of the Regional Program for Direct External Evaluation of Performance (PEED) for the microscopic diagnosis of Leishmaniasis. Participants will be informed of its availability via email.

The established dates for the issuance of the report can be found on the calendar.

17. PRE-ENROLLMENT AND ENROLLMENT

With the aim of providing the best service to the participants of PEED, the Regional Reference Laboratory offers enrollment through the Quality Control Platform (PCC), available on the webpage of the INS: www.ins.gov.co, using the following steps in “Tramites y Servicios”, followed by “Programas de Evaluación Externa de la Calidad”. Information on the enrollment process can be found in the “PEED Enrollment Instructions” published on the webpage.

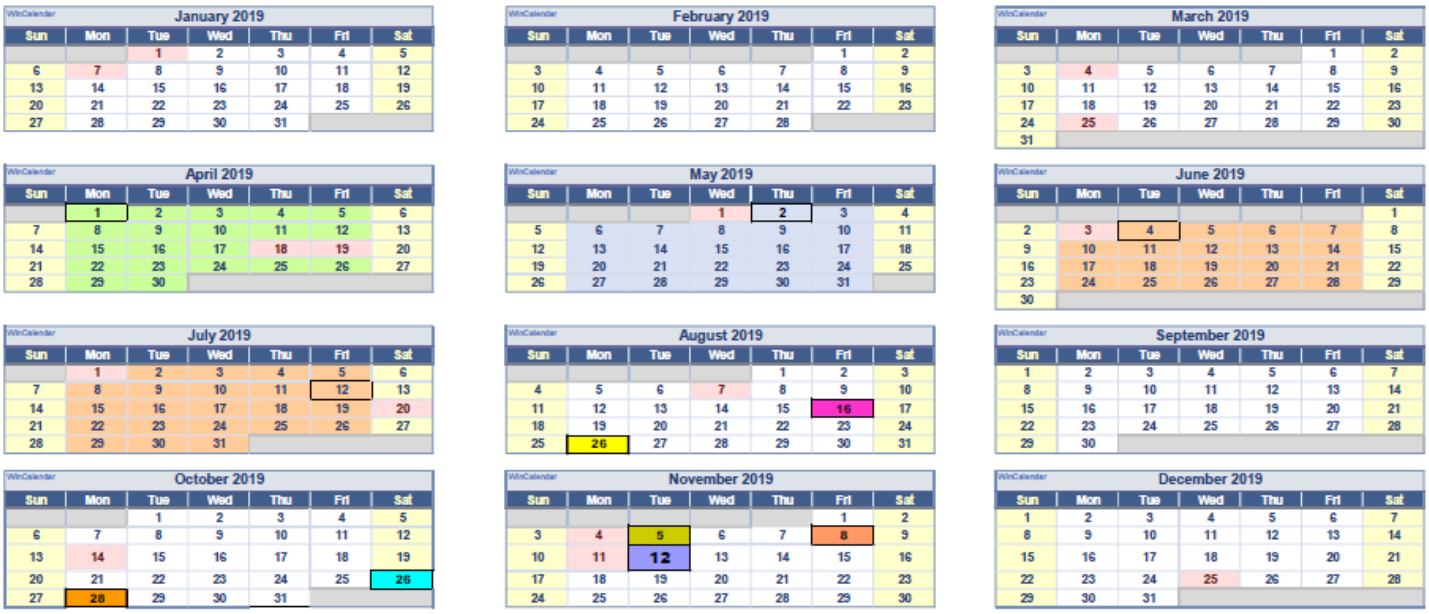
Once pre-enrollment has been completed it is the responsibility of the Regional Reference Laboratory to validate the pre-enrollment information and thereby confirm the final program enrollment of each participant.

In order to insure the timely reception of the panels, it is of utmost importance that each participant laboratory completes the enrollment process in its entirety.

Note: Consult the calendar in order to be aware of the dates established by the Regional Reference Laboratory.

18. CALENDAR 2019

Calendar PEED 2019



DATE	ACTIVITY
1 abr.	Obtaining Material and preparing panels
2 may.	Invitation to the countries to participate in the 2019 cycle
4 jun.	Registration of Participants Cycle 2019
16 ago.	Expert Consensus
26 ago.	Shipping of Test Panels Cycle 2019
26 oct.	"Last day to send the completed form of Supply and Inspection of the Package "
28 oct.	Deadline for receipt of results cycle 2019
5 nov.	Publication of Results
8 nov.	Deadline for claims by participants
12 nov.	Final Report and Closing Cycle 2019

19. INSTITUTIONAL COMMUNICATION CHANNELS

1. To improve the communication, information will only be received through the available official channels at National Health Institute of Colombia:

Schedule of attention: Monday to Friday 8:30 am to 4:30 pm

National Free Line: 018000113400
Virtual Channel: peedleishdirectoreq@ins.gov.co
Telephone:
(+571) 2207700 Ext: 1322, 1545, and 1409 Technical consulting, report of results, Group

2. For the attention of request, complaints, appeals and consultations. The communication channels are:

Virtual channel

- ✓ contactenos@ins.gov.co
- ✓ www.ins.gov.co

Communication link to citizen/System of request, complaints and appeals

- ✓ Chat attention to citizen

- ✓ Link information to citizen on citizen attention

- Phone channel
  - ✓ Call center (+571) 2207700
  - ✓ National free line 018000113400
  - ✓ Cell phone and land line, please dial 100 option 7
- Goverment display TIC Ministry: land line (+571) 5953525 option 7
- National free line:018000952525

20. PROFICIENCY TEST PROVIDER

Parasitology Group – Regional Reference Laboratory
Sub-directorate of the National Reference Laboratory of the Networks Directorate in Public Health,
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National Institute of Health Avenida
Calle 26 No. 51-20
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