Pan American Health Organization

World Health Organization
REGIONAL OFFICE FOR THE Americas

Meeting Report

Advisory group meeting to review and update PAHO guidelines for laboratory diagnosis of yellow fever virus infection

PAHO HQ, Washington, DC

June 07-08, 2018

Health Emergencies Department

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Executive Summary

Due to the recent yellow fever outbreak in Brazil and the continuous threat of its dissemination in the American region, an advisory group meeting was held to review and update the Pan American Health Organization/World Health Organization guidelines for laboratory diagnosis of yellow fever virus infection. The main topics discussed were the historical aspects and background of yellow fever infections and outbreaks in the region, laboratory diagnosis of yellow fever virus infections, surveillance and regional algorithms of yellow fever infection in non-human primates and vectors. The challenge of yellow fever diagnostic in the Region due to circulation of other endemics flavivirus was also discussed. The recommended laboratory algorithm for the detection of yellow fever virus infections was updated. The need for active and passive surveillance systems for yellow fever cases in human, non-human primates, and mosquitos was emphasized. To enhance and strengthen laboratory capabilities to detect and diagnose yellow fever infection in a timely manner, it was recommended to continuously provide technical support through laboratory trainings and workshops, as well as regularly perform external quality assurance programs with the laboratories of the Americas region.

Introduction

Despite the availability of an effective vaccine, yellow fever virus (YFV) remains one of the most important threats to public health in the Americas. Between 2000 and mid-2016, sporadic cases and outbreaks were officially reported in the American Region, mostly concentrated in Peru, Brazil, Colombia, and the Plurinational State of Bolivia. One of the major outbreaks of the last 40 years started in December 2016. It began in Minas Gerais, Brazil, and eventually spread to three regions of the country. By the time the Ministry of Health declared the end of the outbreak on September 6, 2017, 777 confirmed cases and 261 deaths were officially notified. However, in January 2018, an increase of cases was reported by the Brazilian Government, signaling a new outbreak which is currently ongoing. As of March 28, 2018, 1131 cases have been confirmed, distributed in the states of Minas Gerais, Sao Paulo, Rio de Janeiro, Espirito Santo and the Federal District. Additionally, with over 4,800 epizootics in non-human primates (665 confirmed), the impact on the sylvatic ecosystems is still to be evaluated.

Given the epidemiological situation, laboratory surveillance and early detection of yellow fever transmission/circulation in the Americas region needs to be strengthened. Accurate guidelines should be developed, considering the challenging context of co-circulation with endemic flaviviruses and in the light of new knowledge.

To this end, the PAHO Health Emergencies Department (PHE) coordinated this meeting with subject matter experts to review and identify the most relevant topics to be updated in the new laboratory surveillance guidelines, including detection algorithms, available techniques, and diagnosis in both humans and non-human primates.

Objectives

- Review the current epidemiological situation of yellow fever in the Americas
- Review the existing guidelines for surveillance and laboratory diagnosis of yellow fever virus infection
- Propose and update recommendations for the laboratory diagnosis of yellow fever virus infection

Summary

Session 1: Introduction and background

Dr. Pedro Vasconcelos, Dr. Sylvain Aldighieri, Mrs. Alba María Ropero, Dr. Andrea Vicari, Dr. Jairo Mendez-Rico

Surveillance and current situation

- YFV infection outcomes in humans range from asymptomatic infections (~53%) to mild (~34%) and severe disease (~13%). Although approximately 50% of severe infections are fatal, differences might be observed depending on the clinical capacity to recognize and manage the case.
- YF outbreaks have occurred in Africa recently, notably in Angola in 2015/2016 and in the Democratic Republic of Congo in 2016. Fractional dose of yellow fever vaccine was first used in this context.
- Factors that may have contributed to the re-emergence and spread of YFV in Southeast Brazil since December 2016 include: large populations of mosquito vectors and non-human primates (NHP), climate, changes in land use and deforestation, low vaccination coverage, and simultaneous circulation of viremic humans and NHPs.
- Monitoring of epizootics is essential for YF alert and response.
- From 2015-2018, 1,948 YF cases were reported in the Americas, mostly in the Brazilian states of Rio de Janeiro, São Paulo, Minas Gerais, and Espírito Santo. Cases have also been reported from 6 additional countries and territories in the Americas region.
- Canopy loss with the maintenance of sylvatic corridors may contribute to YFV spread in Southeast Brazil.
- Data from Brazil's current epidemic points towards a peri-urban YFV transmission by sylvatic vectors.
- Triggering transmission by *Aedes aegypti* likely requires combination of several human cases introduced in an urban environment, vector densities higher than for dengue, chikungunya or Zika viruses, and low vaccination coverage.
- The WHO Eliminating Yellow Fever Epidemics (EYE) strategy is a global, comprehensive framework for optimized YF control for the 2017-2026 period that builds on lessons learned and partner consultation.
- The EYE strategic objective is to protect at-risk populations, to prevent international spread, and to contain outbreaks rapidly.

- In-country and international investigation capacity, maintaining an emergency vaccination stockpile and coordinating control interventions (vaccination, case management, vector control, community mobilization) are essential for immediate outbreak response.
- As the vaccine demands exceeds the current production capacity, countries need to optimize vaccination delivery.
- Several global and regional YF guidelines exist, in particular the 1998 and 2010 WHO documents and the 2005 PAHO Control of YF Field Guide. More recent recommendations on YF vaccination are also available (WHO 2013 and 2017).
- The institutional framework for guideline production requires that guidelines are as evidence-based as possible, using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.
- Key questions that need to be addressed in the updated YF guidelines will be defined soon with inputs from experts.
- Subsequently, systematic literature reviews and ad-hoc expert meetings will be conducted, and the proposed guidelines will be sent to external peer-review.
- The International Health Regulations (IHR) 2005 urge State Parties to establish mechanisms to provide a reliable and timely identification and characterization of infectious agents.
- PAHO technical cooperation aims at strengthening the capacity to detect and characterize (re)emerging viral pathogens through the generation of guidelines and recommendations, the identification of technical gaps, and the support to the implementation of diagnostic assays in public health laboratories.
- For YFV infection detection, recommended protocols for both RT-PCR and IgM ELISA are available in the Americas.
- Essential reagents for these protocols have been distributed to 20 countries in the American region, while in-country missions for their implementation have been conducted in 15 countries.
- Two regional courses on molecular diagnosis, with a focus on YFV, have trained 24 delegates from 15 countries.
- PAHO's response to the outbreak in Brazil included: the deployment of a virologist to the affected states, the training of nine state laboratories, several meetings to update national laboratory algorithms for the detection of YFV in humans and NHPs, and two trainings on YF histopathology and immunohistochemistry.

Session 2: Laboratory diagnosis of yellow fever virus infection

Dr. Jorge Boshell, Dr. Erin Staples, Dr. Cristina Domingo, Dr. Ana Bispo, Dr. Edgar Parra

Clinical diagnosis and histopathology of yellow fever

- Three distinct clinical phases characterize YF: infection, remission, and intoxication.
- Poor prognosis symptoms include early jaundice, arterial hypotension, Faget sign, gastrointestinal hemorrhage ("black vomit"), and oliguria/anuria.
- YF differential diagnose include toxic or viral hepatitis, malaria (*P. falciparum*), typhoid fever, rickettsial infection, viral hemorrhagic fevers, and leptospirosis.
- Histopathology is an important tool to understand and diagnose YF.
- Underreporting of YF is common in many areas of the world.

Major challenges and limitations

- There is a lack of data regarding IgM antibodies persistence in wild-type YF; however, published data shows that vaccine-induced IgM antibodies can persist for up to 5-6 years (44% of IgM-positive vaccinees).
- Differentiating wild-type disease from adverse events following immunization is challenging and currently requires sequencing.
- Three real time RT-PCR assays that differentiate wild-type from vaccine YFV strains have been recently developed but need additional validation.
- Flavivirus infection/vaccination generate cross-reactive antibodies that limit the utility of both IgM EIAs and PRNTs in areas where multiple flavivirus have recently caused outbreaks or are endemic and in areas where YF vaccine is used.
- Despite good sensitivity and specificity, RT-PCR results should be repeated if there is clustering or low likelihood of occurrence.
- In general, all test results should be interpreted according to the epidemiologic and clinical context.

Available protocols for molecular diagnosis: Advantages and disadvantages

- Including molecular detection in the YF diagnosis is essential as a positive result by RT-PCR unequivocally confirms the case.
- Currently, there are no validated commercial assays for the molecular detection of YFV.
- *In silico* approaches can be used to evaluate published molecular detection assays.
- YFV primers and probes must be checked periodically for the presence of mismatches. This highlights the need of sequencing circulating strains early during an outbreak.

- The Domingo et al., 2012 assay (PubMed [PMID]: 23052311, (one of the assays recommended in the American region) performs well in terms of sensitivity and specificity and is robust and versatile. Commercially-available ready-to-use kits based on this protocol are currently under evaluation.
- Although YF vaccine is considered safe, rare vaccine-associated adverse events can occur showing viscerotropic symptoms, which can overlap with those of wild-type disease.
- Two real time RT-PCR assays were developed to differentiate wild-type vs. vaccine YFV: a single-target assay and a dual-target assay (PMID: 28949285).
- Assay specificity was evaluated using 39 high-titer flavivirus cell culture isolates. All isolates were negative.
- The sensitivity of the dual-target assay was identical to Domingo et al. 2012. Nevertheless, it is recommended to use the diagnosis primers first as screening.
- The assays are limited to vaccine and wild-type American YFV strains (genotypes I and II). West African wild-type strains can't be differentiated from vaccine strains by these assays.

Implementation of immunohistochemistry for yellow fever

- The collection and fixation of high quality tissue samples from fatal cases suspected of YF is important for histopathology and immunochemistry (IHC) studies. Fixed samples can also be used for molecular detection of YFV after deparaffinization.
- Classical histopathological findings of YFV infection in the liver include necrosis (midzonal or panacinar), the presence of Councilman bodies, and portal or lobular inflammatory infiltration.
- YFV antigens detected by IHC are usually midzonal. Other patterns might appear being more difficult to interpret (particularly in cases with severe necrosis or prolonged life support where the amount of antigen might be lower).
- Optimal IHC results are obtained in a few hours using the CDC protocol (CDC YFV 17DD primary antibody combined with the MACH 4 detection system).
- Critical steps for IHC include: fixation in 10% neutral buffered formalin, deparaffinization, and antigen retrieval. Adhering to the incubation times and avoiding sample dehydration throughout the procedure are also key.
- Histopathological findings in diseases included in the YF differential diagnosis such as dengue and acute hepatitis are usually different than those observed in YF cases.

Session 3: Laboratory surveillance and regional algorithms:

Dr. Andre Abreu, Dr. Nestor Sosa, Dr. Amy Lambert, Dr. Jairo Mendez-Rico, Dr. Lionel Gresh

Role of the public health laboratories in the yellow fever surveillance: challenges and lessons learned in Brazil and Panama

BRAZIL

- In the last decades, yellow fever has been registered beyond the limits of the area considered endemic (Amazon region).
- Human cases and epizootics in nonhuman primates (NHP) have recently been recorded in a wide area of the national territory.
- Between December 2016 up to June 2017, 779 human cases, 262 deaths, and 1,659 epizootics were confirmed.
- During the seasonal period starting July 2017 up to May 2018, 6,589 suspected human cases were reported, of which 1266 were confirmed, and 415 were fatal. Additionally, 7,412 epizootics were reported, of which 752 were laboratory confirmed.
- The 2016-2018 outbreak in Brazil affected principally the states of Minas Gerais, Sao Paulo, Rio de Janeiro, Espírito Santo.
- The diagnosis of yellow fever virus can be achieved through serology, hemagglutination inhibition assay, PRNT, RT-qPCR, sequencing, viral isolation, histopathology, and immunohistochemistry.
- The standardization and implementation of the immunohistochemistry protocol in routine diagnostic procedures is essential.
- There is a need to implement wild-type vs. vaccine strains diagnostic methods and quality control programs.
- It is essential to increase operational capacity and to expand the laboratory diagnostic network. Technology transfer between laboratories is important to maintain and strengthen international technical cooperation. Public Health Laboratories should be classified as “Critical Infrastructure” and should be fully integrated in the surveillance system.

PANAMA

- Routine entomological surveillance and vector control are currently performed in Panama. Entomological surveys are done in April, August, and December utilizing the Breteau Index (BI) and the House Index (HI). Fumigation occurs every 2 weeks in High and Moderate index areas using Deltametrine 0.27% and Cyfluthrin 1.5%. Mass media educational campaigns are performed routinely and are done more active during the rainy season.

- Regarding epidemiological surveillance, there are 18 sentinel sites connected with surveillance system and cases are reported according to case definitions (as per national guidelines).
- With regards to laboratory diagnosis in Panama, while dengue laboratory diagnosis has been decentralized, the laboratory diagnosis of Chikungunya, Zika and other Arboviruses is centralized at the Gorgas Institute.
- As part of quality control procedures, 10% of negative samples for Dengue, Chikungunya and Zika are evaluated with RT-PCR and a viral culture is performed to detect another possible flaviviruses, alphaviruses, and phleboviruses. Additionally, selective seroprevalence studies for arboviruses are performed by Gorgas investigators in response to outbreaks as well as selective mosquito and phlebotomus captures to detect viruses, in response to outbreaks or as part of research projects.
- Regarding preparedness, laboratory trainings in the clinical-epidemiology topic are done routinely. Additionally, laboratory proficiency panels have been routinely performed since 2009 as well as capacity building courses.
- Current innovations were presented, which include the Electronic Data Capture and Geo-referencing (IBM Health Corps-Gorgas Project), the evaluation of the RIDL mosquitos (Oxitec/Intrexon Gorgas Project), and in the future, *Wolbachia sp* infected mosquitos (Collaboration with Eliminate Dengue and Monash University).

YFV laboratory diagnosis recommendation from the WHO Collaborating Center

- CDC is addressing the issues that challenge YF diagnosis such as the need to make ELISA testing more transferable, stable, and less time consuming.
- To that end, CDC is developing a transferable, easy-to-use MAC ELISA kit as well as the working on the development and implementation of a vaccine-specific real-time assay.
- There is a need to work towards making ELISA more transferable, stable, and less time consuming.
- Molecular testing is developing into the preferred frontline approach given capacities and limitations on serological approaches (IgM persistence, cross-reactivity, need for PRNT confirmation of limited utility in flavivirus-endemic areas).
- There is a need for molecular assays to be validated and to have lyophilized reagents to allow increased sample throughput.
- CDC has published a molecular diagnostic assay for yellow fever detection and differentiating between infections with wild-type YFV and the vaccine strain in the same assay.

PAHO Algorithms: Discussion and agreement on the regional recommendations

- The PAHO yellow fever laboratory algorithms were presented and discussed with all the participants.
- Regarding the laboratory algorithm for the detection of yellow fever virus, the need to obtain a unique regional algorithm that is easily adaptable by each country was emphasized.

- It was also emphasized that each country and laboratory should be able to align this algorithm with their already established laboratory protocols.
- A concern was raised regarding the number of days post-symptom presentation. The algorithm presented stated that viral detection was possible for 15 days post-symptom onset. However, most of the laboratories already have established protocols for collection and analysis of samples to be done within 10 days of symptom onset. In this regard, it was recommended to change the number of days from symptom onset to 10 days and add a footnote stating that detection of YF RNA beyond 10-15 days has been reported in certain studies.
- It was emphasized that is essential to integrate laboratory results with clinical history. Even a positive result, especially from ELISA IgM should be carefully evaluated in the light of the clinical history and epidemiological context from which the patient sample was taken.
- It was emphasized that cross-reactivity between flaviviruses in serological assays remains a major concern.

YFV External Quality Assurance Program: Results and next steps

- The results of the pilot External Quality Assurance (EQA) program, which was performed in the laboratories of the American region was presented.
- The panels were sent to 27 laboratories in 15 countries, and results from 20 laboratories in 15 countries has been received so far.
- Out of the 20 laboratories that sent their results: 18 laboratories stated use of the QIAamp Viral RNA Mini Kit, while 2 laboratories stated that they used Invitrogen PureLink Viral RNA/DNA kits. The detection protocols that were used are: Domingo et al., J Clin Microbiol 50, 4054-60 (2012), (18 laboratories); Johnson et al., CDC (Unpublished), (7 laboratories); Rojas et al., Am J Trop Med Hyg (2018) (1 laboratory); and Domingo et al., J Clin Microbiol 50, 4054-60 (2012) (lyophilized reagents) (1 laboratory).
- Additionally, 17 of the 20 laboratories obtained 100% concordance, 12 laboratories obtained 100% concordance for the positive samples and 3 laboratories obtained a Ct value higher than the expected.
- No particular association between detection protocol and concordance was detected.
- Even though most laboratories in the region performed well, additional training and support will be provided to laboratories with lower performance.
- The pilot program will be extended to all countries in the Americas (national and sub-national public health laboratories performing YFV detection). The program will be implemented on a regular basis.

Session 4: Surveillance of YFV infection in non-human primates and vectors

Dr. Enrique Perez, Dr. Renato Souza, Dr. Daniel Garkauskas, Lic. Irma Lopez, Dr. Livia Caricio

Surveillance in non-human primates: Current guidelines and recommendations

- The main objective in non-human primates (NHP) and vector surveillance is the early detection of the circulation of the virus, even in the enzootic cycle (primates and vectors) to undertake, in a timely manner, measures to prevent and control yellow fever and to avoid human cases and outbreaks of yellow fever.
- There are two types of NHP surveillance: passive surveillance which investigates suspicious areas; and active surveillance, which operates within “silent zones”.
- After the investigation of suspicious cases, possible results include the laboratory confirmation of epizootics for Yellow Fever which occur when the laboratory results are conclusive for Yellow Fever in at least one animal.
- Another possible result could be finding an epidemiological link within the confirmed epizootics.
- Surveillance in both NHP and vectors is essential to monitor possible viral activity of yellow fever in endemic areas, allowing the early detection of the virus and facilitating the risk assessment.
- The main objectives and challenges in surveillance of yellow fever in NHP is to understand the viral circulation of sylvatic yellow fever as a biological process in an evolutionary and ecological context.
- Contextualizing laboratory surveillance in NHP as a tool to understand disease ecology is important. It is essential to understand the role of the laboratory in the process of surveillance in NHP.
- Through NHP surveillance investigations in Brazil, an important discovery regarding structural corridors was made.
- These structural corridors are an important ecological factor regarding disease transmission.
- Additionally, using these structural corridors, it was possible to map out high risk areas of disease transmission.

Virus detection in mosquitos: recommendations for the surveillance and laboratory detection

- One main objective of the entomological surveillance is the anticipation and early identification of the occurrence of arboviruses through the simultaneous monitoring in humans, animals and vectors.
- Timely determination and alert of possible risks and response to emerging and reemerging arboviruses is needed. Entomological surveillance is essential for timely detection of circulating virus and detection of possible disease outbreaks.

- In Brazil, there are two types of entomological surveillance: passive and active. Passive entomological surveillance allows detection of viruses in vectors, defining the transmission cycle and subsidizing the planning of control actions. It is essential for suspected events to be reported and investigated to determine epidemiological linkage. On the other hand, active surveillance allows early detection of viral circulation, subsidizing the planning of prevention and control actions before the occurrence of human cases / epizootics. This form of surveillance is performed in a systematic and continuous manner, independent of the incidence of cases in humans and epizootics.
- The entomological surveillance should be routinely performed as part of the vector control strategies.
- Entomo-virologic surveillance is essential to assess the risk. Even if there is no current circulation of yellow fever in the country, the risk of having imported cases and introduction of the virus is a Public Health should be a major concern.

Role of the public health laboratories in the surveillance of YFV in non-human hosts

- The components for yellow fever surveillance are: surveillance of human cases; surveillance of vaccine coverage; surveillance of epizootics of non-human primates; and entomological surveillance.
- The roles of the national reference laboratories for arboviruses on yellow fever related activities are to participate in the national team for the surveillance of yellow fever; to collaborate and promote training and capacity building of the epidemiological and laboratory surveillance network; to support in laboratory diagnosis; to participate in the laboratory network for yellow fever (humans, primates, and vectors); to evaluate and develop new diagnostic investigation protocols; and to promote and implement new generation sequencing (metagenomic and genomic analysis).
- Instituto Evandro Chagas (IEC) is performing experimental infection in mosquitoes and developing molecular diagnostic protocols for entomo-virologic surveillance.
- Immunopathogenic studies on YFV infection are being performed at IEC, as well as evaluation of the protective role of the yellow fever vaccine in sequential infection with ZIKV

Session 5: Final Remarks

The meeting concluded with a discussion on the availability and distribution of reagents as well as next steps, including future trainings and activities. The need for continuous laboratory trainings and workshops to strengthen timely laboratory diagnosis and detection of yellow fever was emphasized. The full list of final conclusions and recommendations can be found below.

Final Conclusions and Recommendations

- It is necessary to have Regional algorithms, based on the diagnostic platforms already available in the laboratory response network.
- The algorithms should be efficient to detect and confirm the suspected cases in a timely manner. Each Country and Member State will align their established surveillance protocols with the recommended algorithms.
- It was agreed that there will be one algorithm for Yellow Fever specifically, and another algorithm integrating the other arboviruses in the Region.
- YFV differential diagnosis should include other arboviral diseases, leptospirosis, malaria, viral hepatitis, and other hemorrhagic fevers (depending on the epidemiological profile in the country, territory or area).
- Articulation between all the components of the surveillance system should be prioritized. Epidemiological context and clinical background should be particularly considered to declare outbreaks or to confirm cases in new areas or territories.
- The need for active and passive surveillance of yellow fever cases in human, non-human primates, and mosquitos was emphasized.
- Because of the sensitivity and specificity of the YFV molecular detection, serum samples taken up to 10 days since the onset of symptoms will be initially tested by PCR. A positive result in a suspected case (matching case definition) will be consider as a confirmed case of YF.
- PCR negative samples will be tested by ELISA IgM. Nevertheless, cross-reactivity in serology assays remains a major issue especially in areas where co-circulation of diverse flaviviruses has been demonstrated. Long lasting YFV IgM (either post vaccine or natural infection) should be considered.
- Additional workshops and training to address new laboratory techniques including immunohistochemistry and use of tissue sample as well as the fundamentals of cell culture and viral isolation should be a priority.
- In relation to quality control and external quality assurance programs, it was agreed to continue to perform EQA for the laboratories in the region regularly.
- It was agreed that EQA panels should be done for Yellow Fever and other arboviruses.
- It was agreed to promote new testing kits after they have obtained validation through workshops and trainings.
- It was recommended that standardized material, primers, probes, and testing kits should be available and distributed to the laboratories in the Region.

Annex 1 : List of Participants

Participant	Institution	Country
María Alejandra Morales	Instituto Nacional de Enfermedades Virales Humanas (INEVH)	Argentina
Pedro Vasconcelos	Instituto Evandro Chagas (IEC)	Brazil
Ana María Bispo,	Fundación Oswaldo Cruz (FIOCRUZ)	Brazil
Lívia Caricio	Instituto Evandro Chagas (IEC)	Brazil
André de Abreu	Coordenação Geral de Laboratórios (CGLAB) / Minsitério da Saude	Brazil
Renato de Souza	Instituto Adolfo Lutz (IAL)	Brazil
Jorge Boshell	Banco de Huesos y Tejidos, Cosme y Damián	Colombia
Edgar Alberto Parra	Instituto Nacional de Salud	Colombia
Cristina Domingo	Robert-Koch-Institut	Germany
María Paquita García	Instituto Nacional de Salud	Peru
Irma López	Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE)	Mexico
Juan Miguel Pascale	Instituto Conmemorativo Gorgas de Estudios de la Salud (ICGES)	Panama
Néstor Sosa	Instituto Conmemorativo Gorgas de Estudios de la Salud (ICGES)	Panama
Cynthia Vázquez	Laboratorio Central de Salud	Paraguay
Amy Lambert	Centers for Disease Control and Prevention (CDC) – Fort Collins	United States of America
Erin Staples	Centers for Disease Control and Prevention (CDC) – Fort Collins	United States of America
Sylvain Aldighieri	Pan American Health Organization (PAHO)	United Nations
Andrea Vicari	Pan American Health Organization (PAHO)	United Nations
Enrique Pérez	Pan American Health Organization (PAHO)	United Nations
Jairo Méndez-Rico	Pan American Health Organization (PAHO)	United Nations
Alba María Ropero	Pan American Health Organization (PAHO)	United Nations
Juliana Leite	Pan American Health Organization (PAHO)	United Nations
Lionel Gresh	Pan American Health Organization (PAHO)	United Nations
Mariana Leone	Pan American Health Organization (PAHO)	United Nations

Annex 2 : Agenda

Session 1: Introduction and background

Time	Title	Presenter
9:00 AM	General overview of the workshop objectives and program	PAHO
9:15 AM	The global situation of yellow fever: what have we learned from the last outbreaks?	Pedro Vasconcelos IEC/BRA
10:00 AM	Yellow fever in the Americas: an epidemiological update	Sylvain Aldighieri PAHO
10:20 AM	Eliminating Yellow Fever Epidemics: The EYE strategy	Alba María Ropero PAHO
10:35 AM	*Coffee break (Lobby)*	
10:40 AM	Updating of the Yellow Fever Regional guidelines: working process	Andrea Vicari PAHO
11:10 AM	Yellow fever laboratory network in AMRO: Cornerstone of Regional surveillance and response, 2017-2018	Jairo Méndez PAHO
11:40 PM	Discussion	
12:20 PM	*Lunch (Chess Room on the 3rd floor, inside the cafeteria)*	

Session 2: Laboratory diagnosis of yellow fever virus (YFV) infection

Time	Title	Presenter
2:00 PM	Clinical diagnosis and histopathology of yellow fever	Jorge Boshell COL
2:30 PM	CDC field experience in laboratory diagnosis of YFV infection: Challenges and limitations	Erin Staples CDC
3:00 PM	Review of available protocols for molecular diagnosis: Advantages and disadvantages	Cristina Domingo IRK
3:30 PM	*Coffee break (Lobby)*	
3:45 PM	Review of available protocols and methods for serology diagnosis	Jane Basile CDC
4:15 PM	Natural infection vs. Vaccine derived: Differential diagnosis and laboratory options	Ana Bispo Fiocruz
4:45 PM	Implementation of immunohistochemistry for yellow fever: challenges for an accurate interpretation	Edgar Parra INS/COL
5:15 PM	Discussion: defining the recommended assays for YFV infection diagnosis	
6:00 PM	*Welcome Reception (PAHO's Patio located by the lobby)*	

Session 3: Laboratory surveillance and regional algorithms

Time	Title	Presenter
9:00 AM	Summary of day 1 discussions	PAHO
9:15 AM	Role of the public health laboratories in the yellow fever surveillance: challenges and lessons learned in Brazil	Andre Abreú CGLAB
9:45 AM	Experience in Arbovirus integrated surveillance in Panama	Nestor Sosa ICGES/PAN
10:15 AM	*Coffee break (Lobby)*	
10:30 AM	YFV laboratory diagnosis algorithm: Recommendation from the WHO Collaborating Center (CDC)	Amy Lambert CDC
11:00 AM	PAHO algorithms: Discussion and agreement on the regional recommendations	PAHO
11:45 AM	YFV External Quality Assurance Program: Results and next steps	Lionel Gresh PAHO
12:15	*Lunch (Chess Room on the 3rd floor, inside the cafeteria)*	

Session 4: Surveillance of YFV infection in non-human primates and vectors

Time	Title	Presenter
1:30 PM	Surveillance in non-human primates: Current guidelines and recommendations	Enrique Perez PAHO
2:00 M	Challenges for the surveillance in non-human hosts: experiences from the field	Renato Souza IAL
2:30 PM	Virus detection in mosquitoes: Recommendations for the surveillance and laboratory detection	Daniel Garkauskas MoH/Brazil Irma López InDRE/MEX
3:00 PM	Role of the public health laboratories in the surveillance of YFV in non-human hosts	Livia Caricio IEC/BRA
3:30 PM	*Coffee break (Lobby)*	

Session 5: Final remarks

Time	Title	Presenter
3:45 PM	Availability and distribution of reagents	PAHO / WHO CC
4:15 PM	Concluding remarks and following steps	
5:00 PM	*Workshop adjourns*	