CDC
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Pan American Health Organization
Regional Office of the World Health Organization

Preparedness and Response for

Chikungunya Virus

Introduction in the Americas



PREPAREDNESS AND RESPONSE FOR **CHIKUNGUNYA VIRUS** INTRODUCTION IN THE AMERICAS

PAHO/CDC

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PREFACE

Chikungunya fever (CHIK) is an emerging, mosquito-borne disease caused by an *alphavirus*, Chikungunya virus (CHIKV). The disease is transmitted predominantly by *Aedes aegypti* and *Ae. albopictus* mosquitoes, the same species involved in the transmission of dengue.

Traditionally, CHIKV epidemics have shown cyclical trends, with inter-epidemic periods ranging from 4 to 30 years. Since 2004, CHIKV has expanded its geographical range, causing sustained epidemics of unprecedented magnitude in Asia and Africa. Although areas in Asia and Africa are considered to be endemic for the disease, the virus produced outbreaks in many new territories in the Indian Ocean islands and in Italy. This recent reemergence of CHIKV has heightened the world's public health awareness and concern about this virus.

Controlling the spread of arthropod-borne viruses (arboviruses) in the Americas has not been very successful. Dengue continues to ravage many areas in the Americas, reaching as far north as the United States and as far south as Argentina. During the first trimester of 2010, several dengue virus outbreaks in the Region occurred at unprecedented rates for this time of the year, especially in Central America and the Caribbean.

West Nile virus, another arbovirus recently introduced to the Americas, is now endemic in the Region. Over the last decade, West Nile virus has evolved epidemiologically and has expanded its geographic range in the Region from Canada to Argentina; in 2007, human and equine cases were detected in Argentina. Moreover, in 2010, three laboratory-confirmed cases of a related

arbovirus, the Saint Louis encephalitis virus, were reported in children from 6 to 8 years old in Argentina (the city of Buenos Aires and the province of Buenos Aires).

Although indigenous transmission of CHIKV does not occur in the Americas now, the risk for its introduction into local vector mosquito populations is likely higher than had previously been thought, especially in tropical and subtropical areas where *Ae. aegypti*, one of the main vectors of CHIKV, has a broad distribution. The broad distribution of competent vectors, coupled with the lack of exposure to CHIKV of the human population in the Americas, places this Region at risk for the introduction and spread of the virus. The resulting large outbreaks would likely tax existing health-care systems and the public health infrastructure, and could potentially cripple some of society's functioning.

Between 2006 and 2010, 106 laboratory-confirmed or probable cases of CHIKV were detected among travelers returning to the United States, compared to only 3 cases reported between 1995 and 2005. There also have been nine imported CHIK cases reported in the French territories in the Americas since 2006-three in Martinique, three in Guadeloupe, and three in Guyana. To date, none of the travel-related cases have led to local transmission, but these cases document an ongoing risk for the introduction and possible sustained transmission of CHIKV in the Americas.

There is no specific treatment for CHIKV infection, nor any commercially available vaccine to prevent it. Pending the development of a new vaccine, the only effective means of prevention are to protect individuals against mosquito bites. It should be noted, however, that the only available method for preventing an ongoing transmission of a possible CHIKV epidemic, namely the control of its vectors, has rarely been achieved and never has been sustained.

Given these factors, the Pan American Health Organization (PAHO), with the support of the Division of Vector-borne Diseases of the United States Centers for Disease Control and Prevention (DVBD, CDC), created a working group and convened a meeting in Lima, Peru, on July 21–23, 2010, to discuss the threat this virus represents and to examine measures that might be taken to mitigate this

threat (see Appendix I for additional details on the meeting). These preparedness guidelines are the result of this collaboration.

These guidelines are intended to be adapted by each Member Country. They are designed to increase awareness about the threat and to provide the necessary tools to put in place the best possible strategies to prevent the importation of CHIKV into the Region, or to control it if introduced. These guidelines provide guidance on how to detect an outbreak of the disease, conduct pertinent epidemiological investigations, and prevent or mitigate the spread of the disease throughout the Region.

We encourage everyone working to apply these guidelines to take into account all the knowledge available and their own country's capability to cope with the introduction of CHIKV. Steps should be taken now to put in place the necessary measures that will decrease the impact that this new arbovirus could have in our Region.

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The publication of these guidelines was possible thanks to funding from the U.S. Centers for Disease Control and Prevention.

ABBREVIATIONS AND ACRONYMS

Ae.	*Aedes*
BSL-3	Biosafety level 3
CAREC	Caribbean Epidemiology Centre
CBC	Complete blood count
CDC	United States Centers for Disease Control and Prevention
CHIK	Chikungunya fever
CHIKV	Chikungunya virus
CIRE	Les Cellules de l'Institut de veille sanitaire en région
CPE	Cytopathic effects
CSF	Cerebrospinal fluid
Ct	Cutoff
DEET	N,N-Diethyl-meta-toluamide
DVBD	Division of Vector-borne Diseases
EEE	Eastern equine encephalitis
ELISA	Enzyme-linked immunosorbent assay
FAQ	Frequently asked question
GIS	Geographic Information System
HHS	U. S. Department of Health and Human Services
HI	Hemaglutination Inhibition
HSD/IR/D	Area of Health Surveillance and Disease Prevention and Control / Alert and Response and Epidemic Diseases / Program for Dengue
HSD/IR/V	Area of Health Surveillance and Disease Prevention and Control / Alert and Response and Epidemic Diseases / Program for Viral Diseases
IFA	Immunofluorescence assay
IgG	Immunoglobulin G

IgM	Immunoglobulin M
IHR	International Health Regulations
IRS	Indoor residual sprays
IT	Insecticide-treated
IVM	Integrated vector management
JE	Japanese encephalitis
JIC	Joint Information Center
MAC-ELISA	IgM antibody capture enzyme-linked immunosorbent assay
NAT	Nucleic acid amplification testing
NGO	Non-Governmental Organization
NMRC	United States Naval Medical Research Center
NMRCD	United States Naval Medical Research Center Detachment
NSAID	Non-steroidal anti-inflammatory agent
PAHO	Pan American Health Organization
PCR	Polymerase chain reaction
Pfu	Plaque-forming unit
PHEIC	Public Health Emergency of International Concern
PIO	Public Information Officer
PRNT	Plaque reduction neutralization test
PWR/PAN	Pan American Health Organization, World Health Organization Representative/Panama
RELDA	Red de laboratorios de dengue de las Américas
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase-polymerase chain reaction
SFV	Semliki Forest virus
SLE	St. Louis encephalitis
SMS	Short Message Service
TMB	Tetramethylbanzidine base
THAN	Travel health alert notice
VEE	Venezuelan equine encephalitis
WEE	Western equine encephalitis
WHO	World Health Organization
WHOCC	World Health Organization Collaborating Centre
WHOPES	World Health Organization Pesticide Evaluation Scheme

1. BACKGROUND AND RATIONALE

Since 2004, Chikungunya virus (CHIKV) has been causing large epidemics of chikungunya fever (CHIK), with considerable morbidity and suffering. The epidemics have crossed international borders and seas, and the virus has been introduced into at least 19 countries by travelers returning from affected areas. Because the virus has been introduced into geographic locations where the appropriate vectors are endemic, the disease could establish itself in new areas of Europe and the Americas. The possibility that CHIKV could become established in the Americas has heightened awareness of the need to develop guidelines for the prevention and control of CHIK in PAHO's Member Countries. This document is meant to serve as a guideline that individual countries can use as the basis for their CHIKV surveillance, prevention, and control programs.

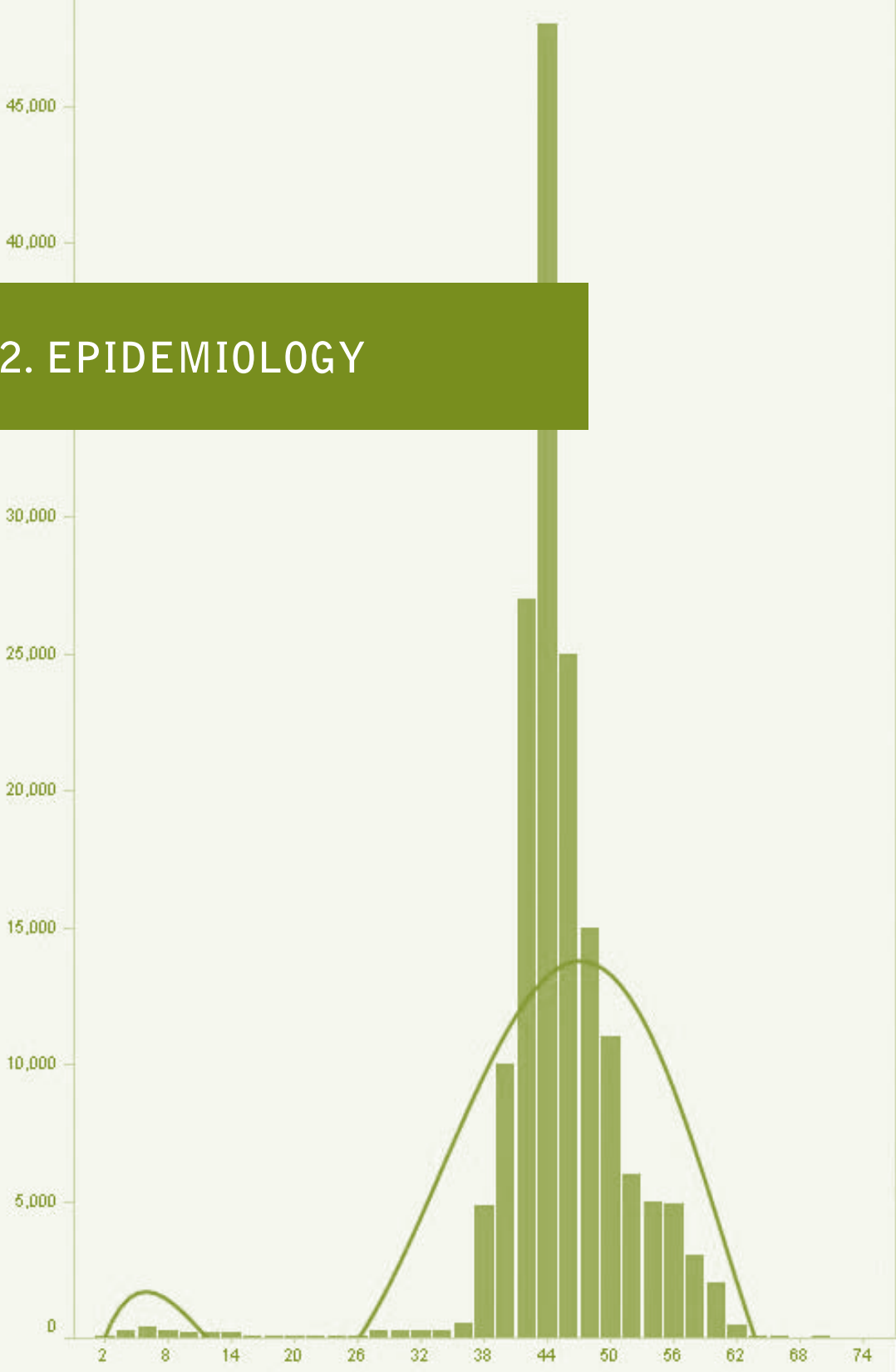
TARGET OF THESE GUIDELINES:

These guidelines are intended to be used by health workers, public health officials, and vector control specialists at the national, district, and sub-district levels.

OBJECTIVES:

General objectives are the prevention, detection, and timely response to outbreaks of CHIK through surveillance, case detection, investigation, and the launching of public health actions.

2. EPIDEMIOLOGY



CHIKV is an RNA virus that belongs to the *Alphavirus* genus in the family *Togaviridae*. The name *chikungunya* derives from a word in Makonde, the language spoken by the Makonde ethnic group living in southeast Tanzania and northern Mozambique. It roughly means "that which bends," describing the stooped appearance of persons suffering with the characteristic painful arthralgia.

Epidemics of fever, rash, and arthritis resembling CHIK were reported as early as the 1770s. However, the virus was not isolated from human serum and mosquitoes until an epidemic in Tanzania in 1952–1953.[1] Subsequent outbreaks occurred in Africa and Asia, many of them affecting small or rural communities. In Asia, however, CHIKV strains were isolated during large urban outbreaks in Bangkok, Thailand, in the 1960s and in Calcutta and Vellore, India, during the 1960s and 1970s.[2, 3]

Recent Outbreaks

After the initial identification of CHIKV, sporadic outbreaks continued to occur, but little activity was reported after the mid-1980s. In 2004, however, an outbreak originating on the coast of Kenya subsequently spread to Comoros, La Réunion, and several other Indian Ocean islands in the following two years. From the spring of 2004 to the summer of 2006, an estimated 500,000 cases had occurred.

The epidemic spread from the Indian Ocean islands to India, where large outbreaks occurred in 2006. Once introduced in India, CHIKV spread to 17 of the country's 28 states, infecting more than 1.39 million people before the end of the year. The outbreak in India continued into 2010, with new cases appearing in areas that had not been affected in the epidemic's early phase. Viremic travelers also spread outbreaks from India to the Andaman and Nicobar Islands, Sri Lanka, the Maldives, Singapore, Malaysia, Indonesia. Concern over the spread of CHIKV peaked in 2007, when the virus was found to be spreading autochthonously (human-to-mosquito-to-human) in northern Italy after being introduced by a viremic traveler returning from India.[4] The attack rates in communities that have been affected in the recent epidemics ranged from 38%–63%, and in many of these countries cases continue to be reported, albeit at reduced levels. In 2010, the virus continued to cause illness in India, Indonesia, Myanmar, Thailand, and the Maldives; it also has resurged in La Réunion. In 2010, imported cases also were identified in Taiwan, France, and the United States. These cases were infected viremic travelers returning from Indonesia, La Réunion, and India, respectively.

During the recent outbreaks, individuals viremic with CHIKV were found in the Caribbean (Martinique), the United States, and French Guiana.[5] All of them had returned from areas with endemic or epidemic CHIKV transmission; thus, these cases were not due to autochthonous transmission. All of these areas have competent mosquito vectors and naïve hosts, however, and thus could support endemic transmission of CHIKV in the Americas. Given these factors, CHIKV has the capacity to emerge, re-emerge, and quickly spread in novel areas, which makes heightened surveillance and preparedness a priority.

Transmission Dynamics

Vectors

There are two main vectors of CHIKV, *Aedes aegypti* and *Ae. albopictus*. Both mosquito species are widely distributed throughout the tropics with *Ae. albopictus* also present at more temperate latitudes. Given the vectors' distribution throughout the Americas, the entire Region is susceptible to the virus' invasion and spread.

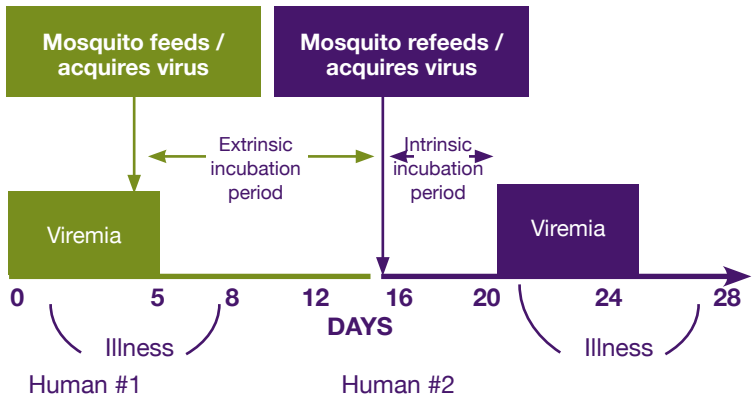
Reservoirs

Humans serve as the primary CHIKV reservoir during epidemic periods. During inter-epidemic periods, several vertebrates have been implicated as potential reservoirs, including non-human primates, rodents, birds, and some small mammals.

Incubation Periods

Mosquitoes acquire the virus from a viremic host. Following an average extrinsic incubation of 10 days, the mosquito is then able to transmit the virus to a naïve host, such as a human. In humans bitten by an infected mosquito, disease symptoms typically occur after an average intrinsic incubation period of three-to-seven days (range: 1–12 days) (Figure 1).

Figure 1. **Extrinsic and intrinsic incubation periods for Chikungunya virus.**



Susceptibility and Immunity

All individuals not previously infected with CHIKV (naïve individuals) are at risk of acquiring infection and developing disease. It is believed that once exposed to CHIKV, individuals will develop long lasting immunity that will protect them against reinfection.

Summary of Epidemiology Section

- CHIKV is an RNA virus that belongs to the *Alphavirus* genus in the family *Togaviridae*.
- The attack rates in communities that have been affected in the recent epidemics ranged from 38%–63%.
- The two major vectors of CHIKV are *Ae. aegypti* and *Ae. albopictus*; both mosquitoes are widely distributed throughout the tropics and *Ae. albopictus* is present at more temperate latitudes.
- CHIK is not known to be circulating in the Americas; however, the risk of introduction is high due to travel importation, competent vectors (same vectors as dengue), and population susceptibility.



3. CLINICAL

3A. Clinical Presentation of Acute Disease

Following the bite of a mosquito infected with CHIKV, most individuals will present with symptomatic disease after an incubation period of three to seven days (range: 1–12 days). Not all individuals infected with the virus develop symptoms, however. Serosurveys indicate that between 3% and 28% of persons with antibodies to CHIKV have asymptomatic infections.[6–7] Individuals acutely infected with CHIKV, whether clinically apparent or asymptomatic, can contribute to the spread of the disease if the vectors that transmit the virus are present and active in the same location.

CHIKV can cause acute, subacute, and chronic disease. Acute disease is most often characterized by sudden onset of high fever (typically greater than 102°F [39°C]) and severe joint pain.[8–10] Other signs and symptoms may include headache, diffuse back pain, myalgias, nausea, vomiting, polyarthritis, rash, and conjunctivitis (Table 1).[11] The acute phase of CHIK lasts for 3–10 days.

Table 1. **Frequency of acute symptoms of CHIKV Infection.**[a]

Symptom or sign	Frequency range (% of symptomatic patients)
Fever	76–100
Polyarthralgias	71–100
Headache	17–74
Myalgias	46–72
Back pain	34–50
Nausea	50–69
Vomiting	4–59
Rash	28–77
Polyarthritis	12–32
Conjunctivitis	3–56

[a]Table compiled from a number of different studies.[8, 9, 12-17]

- Fever typically lasts from several days up to a week. The fever can be continuous or intermittent; a drop in temperature is not associated with worsening of symptoms, however. Occasionally, the fever may be associated with relative bradycardia.

- Joint symptoms are usually symmetric and occur most commonly in hands and feet, but they can affect more proximal joints. Swelling can also be seen and is often associated with tenosynovitis. Patients are often severely incapacitated due to pain, tenderness, swelling, and stiffness. Many patients cannot perform normal tasks or go to work, and many will be confined to bed due to these symptoms.

- Rash usually occurs two to five days after onset of fever in approximately half of all patients. It is typically maculopapular, involving the trunk and extremities, but can also include palms, soles, and face. The rash can also present as a diffuse erythema that blanches with pressure. In infants, vesiculobullous lesions are often the most common skin manifestation.

There are no significant pathognomonic hematologic findings seen with CHIKV infections. Abnormal laboratory findings can include mild thrombocytopenia (>100,000/mm^3), leukopenia, and elevated liver function tests. Erythrocyte sedimentation rate and C-reactive protein are usually elevated.

Rarely, severe forms of the disease can occur with atypical manifestations (see Section 3B). Fatalities related to CHIKV infection are thought to be uncommon. However, an increase in crude death rates was reported during the 2004–2008 epidemics in India and Mauritius.[18, 19]



A. Edematous rash of the face



B. Edematous poylarthritis of the hands



C. Erythema that blanches with pressure



D. Periarticular swelling and joint effusion in knees



E. Maculopapular rash in trunk and extremities



F. Maculopapular rash in extremities, including palms



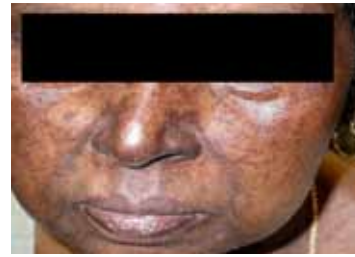
G. Bullous lesions in infant leg



H. Infant with maculo-papular rash, petechial spots and erythema of upper and lower limbs associated with edema of the extremities



I. End of the acute stage. Swollen hands and fine desquamation



J. Hyperpigmentation



K. Tenosynovitis in hands



L. Tenosynovitis in ankle



M. Elbow hygroma



N. Swollen and stiff hands in a 55-year-old man who was infected 5 years earlier

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(B) Pr. Fabrice Simon. Department of Infectious Diseases and Tropical Medicine. Laveran Military Teaching Hospital. Marseille, France. **Previously published in:** Simon F. et al. Chikungunya infection: an emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. Medicine (Baltimore). 2007 May ;86(3):123-37.

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3B. Atypical Manifestations

Although most CHIKV infections result in fever and arthralgias, atypical manifestations can occur (Table 2). These manifestations can be due to the direct effects of the virus, immunologic response to the virus, or drug toxicity.

Table 2. **Atypical manifestations of CHIKV infection.**

System	Clinical manifestations
Neurological	Meningoencephalitis, encephalopathy, seizures, Guillain-Barré syndrome, cerebellar syndrome, paresis, palsies, neuropathy
Ocular	Optic neuritis, iridocyclitis, episcleritis, retinitis, uveitis
Cardiovascular	Myocarditis, pericarditis, heart failure, arrhythmias, hemodynamic instability
Dermatological	Photosensitive hyperpigmentation, intertriginous aphthous-like ulcers, vesiculobullous dermatosis
Renal	Nephritis, acute renal failure
Other	Bleeding dyscrasias, pneumonia, respiratory failure, hepatitis, pancreatitis, syndrome of inappropriate secretion of antidiuretic hormone (SIADH), hypoadrenalism

Adapted from Rajapakse et al. [20]

Some of the atypical manifestations are more in common in certain groups. For instance, meningoencephalitis and vesiculobullous dermatosis are observed more frequently in children and infants, respectively.[21, 22]

3C. High-risk Groups

CHIKV can affect women and men of all ages. Clinical presentation is thought to vary by age, however, with the very young (neonates) and the elderly being at greater risk for more severe disease.[23-26] In addition to age, comorbidities (underlying diseases) have also been identified as a risk factor for poor disease outcome.[8, 23, 24, 27]

Most CHIKV infections that occur during pregnancy will not result in the virus being transmitted to the fetus.[25, 28] There have been rare reports of spontaneous abortions following CHIKV infection in the mother, however.[26] The highest transmission risk appears to be when women are infected during the intrapartum period[29] The vertical transmission rate is as high as 49% during this period. Infants are typically asymptomatic at birth and then develop fever, pain, rash, and peripheral edema. Those infected during the intrapartum period may also develop neurologic disease (e.g., meningoencephalitis, white matter lesions, brain swelling, and intracranial hemorrhage), hemorrhagic symptoms, and myocardial disease.[30] Laboratory abnormalities included raised liver function tests, reduced platelet and lymphocyte counts, and decreased prothrombin levels. Neonates who suffer from neurologic disease often develop long-term disabilities.[31] There is no evidence that the virus is transmitted through breast milk.[25]

Older adults are more likely to suffer from severe atypical disease and death. Individuals >65 years had a 50-fold higher mortality rate when compared to younger adults (<45 years old).[23] Although it is unclear why older adults are at increased risk for more severe disease, it may be due to the frequency of concomitant underlying diseases or decreased immunlogic response.[23]

3D. Differential Diagnosis

Fever with or without arthralgia is a very common manifestation of several other diseases. CHIK may not have the typical manifestations or it may coexist with other infectious diseases such as dengue fever or malaria. Diseases that can be considered in the differential diagnoses may vary based on pertinent epidemiologic features such as place of residence, travel history, and exposure (Table 3).

Table 3. **Diseases or agents in the differential diagnosis of CHIK.**

Disease or agent	Presentation
Malaria	Periodicity of fever and alteration of consciousness
Dengue fever	Fever and two or more of the following, retro-orbital or ocular pain, headache, rash, myalgia, arthralgia, leucopenia, or hemorrhagic manifestations. See section and table below for more information on dengue
Leptospirosis	Severe myalgia localized to calf muscles with conjunctival congestion/or subconjunctival hemorrhage with or without jaundice or oliguria. Consider history of contact with contaminated water
Alphaviral infections (Mayaro, Ross River, Barmah Forest, O'nyong nyong, and Sindbis viruses)	Similar clinical presentation as CHIK; utilize travel history and known areas of Mayaro in the Americas

(Continued)

Table 3. **Diseases or agents in the differential diagnosis of CHIK.** (Cont.)

Disease or agent	Presentation
Post-infectious arthritis (including rheumatic fever)	Arthritis of one or more, typically larger joints due to an infectious disease such as Chlamydia, shigella, and gonorrhea. Rheumatic fever is seen more commonly in children as migratory polyarthritis predominantly affecting large joints. Consider antistreptolysin O (ASO) titer and history of sore throat with Jones criteria for rheumatic fever
Juvenile rheumatoid arthritis	Abrupt onset of fever and subsequent joint involvement in children

Overlap and Confusion with Dengue Fever:

CHIK has to be distinguished from dengue fever, which has the potential for much worse outcomes, including death. The two diseases can occur together in the same patient. Observations from previous outbreaks in Thailand and India have characterized the principal features distinguishing CHIK from dengue fever. In CHIK, shock or severe hemorrhage is very rarely observed; the onset is more acute and the duration of fever is much shorter. In CHIK, maculopapular rash also is more frequent than in dengue fever (Table 4). Although people may complain of diffuse body pain, the pain is much more pronounced and localized to the joints and tendons in CHIK, in comparison of dengue fever.

Table 4. **Comparison of the clinical and laboratory features of chikungunya and dengue virus infections.[a]**

Clinical and laboratory features	Chikungunya virus infection	Dengue virus infection
Fever (>102°F or 39°C)	+++	++
Myalgias	+	++
Arthalgias	+++	+/-
Headache	++	++[b]
Rash	++	+
Bleeding dyscrasias	+/-	++
Shock	-	+
Leukopenia	++	+++
Neutropenia	+	+++
Lymphopenia	+++	++
Elevated hematocrit	-	++
Thrombocytopenia	+	+++

[a] Mean frequency of symptoms from studies where the two diseases were directly compared among patient seeking care; +++ = 70-100% of patients; ++ = 40-69%; + = 10-39%; +/- = <10%; - = 0% [32, 33]

[b] Often retroorbital

Table modified from Staples et al.[34]

3E. Subacute and Chronic Disease

After the first 10 days, most patients will feel an improvement in their general health and joint pain. Following this period, however, a relapse of symptoms can occur, with some patients complaining of various rheumatic symptoms, including distal polyarthritis, exacerbation of pain in previously injured joints and bones, and subacute hypertrophic tenosynovitis in wrists and ankles. This is most common two to three months after their illness onset. Some patients can also develop transient peripheral vascular disorders, such as Raynaud's syndrome. In addition to physical symptoms, the majority of patients will complain of depressive symptoms, general fatigue, and weakness.[13]

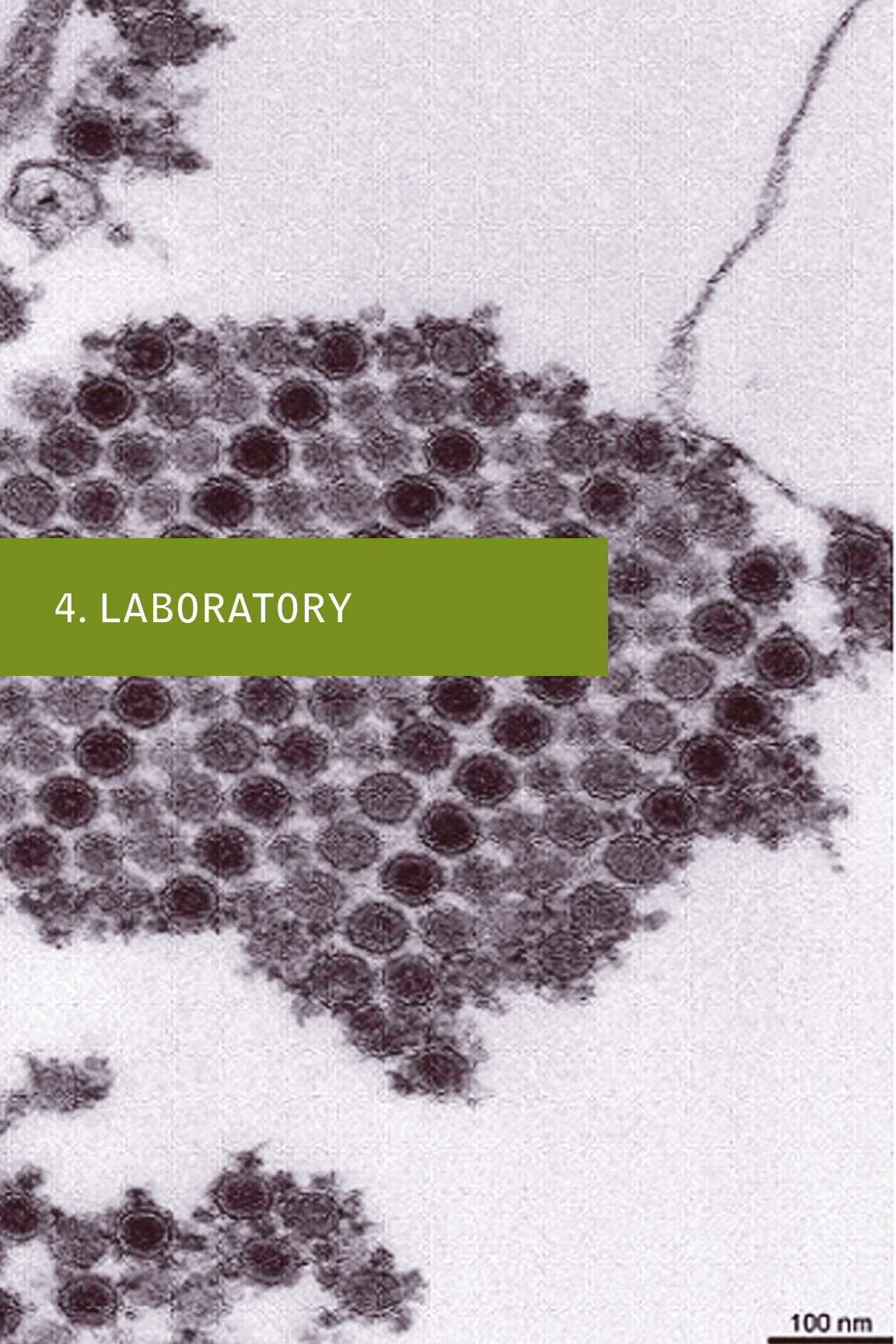
Chronic disease is defined by symptoms that persist for more than three months. The frequency of persons reporting persistent symptoms varies substantially by study and the time that had elapsed between symptom onset and follow-up. Studies from South Africa note that 12%–18% of patients will have persistent symptoms at 18 months and up to 2 to 3 years later.[35, 36] From more recent studies in India, the proportion of patients with persistent symptoms at 10 months was 49%.[37] Data from La Réunion have found that as many as 80%–93% of patients will complain of persistent symptoms 3 months after disease onset; this decreases to 57% at 15 months and to 47% at 2 years[38, 39] (F. Simone, Dept of Infectious Diseases and Tropical Medicine, Laveran Military Hospital, Marseilles, France, *personal communication*).

The most common persistent symptom is inflammatory arthralgias in the same joints that were affected during the acute stages. Usually, there is no significant change in laboratory tests and x-rays of the affected areas. However, some individuals will go onto develop destructive arthropathy/arthritis resembling rheumatoid or psoriatic arthritis.[40] Other symptoms or complaints of the chronic phase of the disease can include fatigue and depression.[6] Risk factors for non-recovery are older age (> 45 years), pre-existing joint disorders, and more severe acute disease.[38, 41]

Summary of Clinical Section

- Acute stage is symptomatic in most people and causes acute fever, distal polyarthralgias, and occasional rash.
- Severe and lethal forms are more frequent among patients older than 65 years and/or with underlying chronic diseases.
- Maternal-fetal transmission is possible among pregnant women, with the highest risk for severe infection in the neonates during the antepartum period.
- Most patients initially will have severe and incapacitating joint symptoms; many will go on to develop long-lasting rheumatism, fatigue, and depression resulting in an impaired quality of life for months to years.

4. LABORATORY



4A. Types of Laboratory Tests Available and Specimens Required

Three main types of laboratory tests are used for diagnosing CHIK: virus isolation, reverse transcriptase-polymerase chain reaction (RT-PCR), and serology. Samples collected during the first week after onset of symptoms should be tested by both serological (immunoglobulin M [IgM] and G [IgG] ELISA) and virological (RT-PCR and isolation) methods. Specimens are usually blood or serum, but in neurological cases with meningoencephalitic features, cerebrospinal fluid (CSF) may also be obtained. Limited information is available for the detection of virus by isolation or RT-PCR from tissues or organs. In suspected fatal cases, virus detection can be attempted on available specimens.

Selection of the appropriate laboratory test is based upon the source of the specimen (human or field-collected mosquitoes) and the time of sample collection relative to symptom onset for humans.

Virus Isolation

Virus isolation can be performed on field collected mosquitoes or acute serum specimens (≤8 days). Serum obtained from whole blood collected during the first week of illness and transported cold (between 2°–8°C or dry ice) as soon as possible (within 48 hours) to the laboratory can be inoculated into a susceptible cell line or suckling mouse. CHIKV will produce typical cytopathic effects (CPE) within three days after inoculation in a variety of cell lines, including Vero, BHK-21, and HeLa cells. Virus isolation can be performed in T-25 flasks or shell vials (see Appendix A). Recent data suggest that isolation in shell vials is both more sensitive and produces CPE earlier than conventional isolation in flasks[42] CHIKV isolation must be confirmed either by immunofluorescence assay (IFA), using CHIKV-specific antiserum, or by RT-PCR of the culture supernatant or mouse brain suspension. Virus isolation must only be carried out in biosafety level 3 (BSL-3) laboratories to reduce the risk of viral transmission.

RT-PCR

Several RT-PCR assays for the detection of CHIKV RNA have been published. Real time, closed system assays should be utilized, due to their increased sensitivity and lower risk of contamination. The Arboviral Diagnostic Laboratory within DVBD, CDC routinely utilizes the published assay in Appendix B,[43] which demonstrates a sensitivity of less than 1 pfu or 50 genome copies. Serum from whole blood is used for PCR testing as well as virus isolation.

Serological Tests

For serological diagnosis, serum obtained from whole blood is utilized in enzyme-linked immunosorbent assay (ELISA) and plaque reduction neutralization testing (PRNT). The serum (or blood) specimen should be transported at 2°–8°C and should not be frozen. Serologic diagnosis can be made by demonstration of IgM antibodies specific for CHIKV or by a four-fold rise in PRNT titer in acute and convalescent specimens. IgM antibodies specific for CHIKV are demonstrated by using the IgM antibody capture ELISA (MAC-ELISA),[44] followed by the PRNT (detailed protocols for IgM and IgG ELISAs shown in Appendix C). As of 2010, there were no World Health Organization (WHO)

validated commercial IgM ELISAs available. PRNT is required to confirm the MAC-ELISA results, since cross-reactivity in the MAC-ELISA between some members of the Semliki Forest virus (SFV) serogroup has been observed. PRNT testing, whether used to confirm the MAC-ELISA or to demonstrate a four-fold rise in acute/convalescent specimens, should always include other viruses within the SFV serogroup (e.g., Mayaro virus) to validate specificity of reactivity. In situations where the PRNT assay is not available, other serological tests (e.g. hemaglutination inhibition [HI]) can be used to identify a recent alphavirus infection; however, PRNT is required to confirm a recent CHIKV infection.

An acute phase serum should be collected immediately after the onset of illness and the convalescent phase serum 10–14 days later. CHIKV-specific IgM and neutralizing antibodies normally develop towards the end of the first week of illness. Therefore, to definitively rule out the diagnosis, convalescent samples should be obtained on patients whose acute samples test negative.

Collection, Storage, and Transportation of Samples

Proper collection, processing, storage, and transportation of the specimens are essential aspects of the laboratory diagnosis.

Collection of samples for serology, isolation and molecular diagnosis:

Sample: Serum
Time of collection: Acute, within the first eight days of illness; convalescent, 10–14 days after acute specimen collection.

To collect serum:

- Aseptically collect 4–5 ml of venous blood in a tube or a vial.
- Allow blood to clot at room temperature, centrifuge at 2,000 rpm to separate serum. Collect the serum in a clean dry vial.
- All clinical samples should be accompanied by their clinical and epidemiological information.

Other types of specimens for laboratory investigation:

Specimens:

- CSF in meningo-encephalitis cases.
- Synovial fluid in arthritis with effusion.
- Autopsy material – serum or available tissues.

[Note: Mosquitoes collected in the field will also be handled using the same techniques described here]

Transportation of Samples:

- Transport specimens to the laboratory at 2°–8°C (icebox) as soon as possible.
- Do not freeze whole blood, as hemolysis may interfere with serology test results.
- If a delay greater than 24 hours is expected before specimens can be submitted to the laboratory, the serum should be separated and stored at refrigerated temperature.
- Serum samples for virus isolation and molecular diagnosis should be stored frozen (at –20°C for short-term storage or at –70°C for long-term storage).

4B. Laboratory Surveillance

Prior to identification of CHIKV in a country, laboratory surveillance should be conducted on three sets of samples, as follows: 1) dengue-negative specimens where the patient exhibits severe joint pain; 2) samples with clinically compatible illness from new geographic areas without active dengue circulation; 3) clusters of febrile illness with severe joint pain. The following table (Table 5) outlines the ideal tests to be performed in various epidemiological settings.

Table 5. **Laboratory surveillance for Chikungunya virus by epidemiologic scenario.**

Epidemiological scenario	Testing to be performed	Samples to test
No signs of transmission	IgM ELISA, IgG ELISA	All samples from patients exhibiting clinically compatible illness
Suspect CHIKV illness	IgM ELISA, IgG ELISA, real-time RT-PCR, virus isolation, PRNT	All samples from patients exhibiting clinically compatible illness
Continued transmission	IgM ELISA, IgG ELISA, real-time RT-PCR; limited virus isolation	Subset samples from classical CHIK cases, as determined by lab constraints and epidemiological status; Samples from all atypical or severe cases should be tested
Periodic outbreaks (once CHIKV has been detected in an area) or active surveillance in areas near CHIKV transmission	IgM ELISA, IgG ELISA, real-time RT-PCR; limited virus isolation	Subset of samples from classical CHIK cases, as determined by lab constraints and epidemiological status; samples from all atypical or severe cases should be tested

During the initial introduction of CHIKV into a new region, comprehensive testing should be completed to confirm that CHIKV is the etiological agent. After CHIKV has been identified, limited testing (not testing all specimens or performing fewer assay types) can be considered depending upon the capacity of the lab and the epidemiological situation.

4C. Interpretation and Reporting of Results

Figure 2 shows typical viremia and antibody response in humans and Table 6 describes the typical results of testing samples at various time points.

Figure 2. **Viremia and immune response following Chikungunya virus infection.**

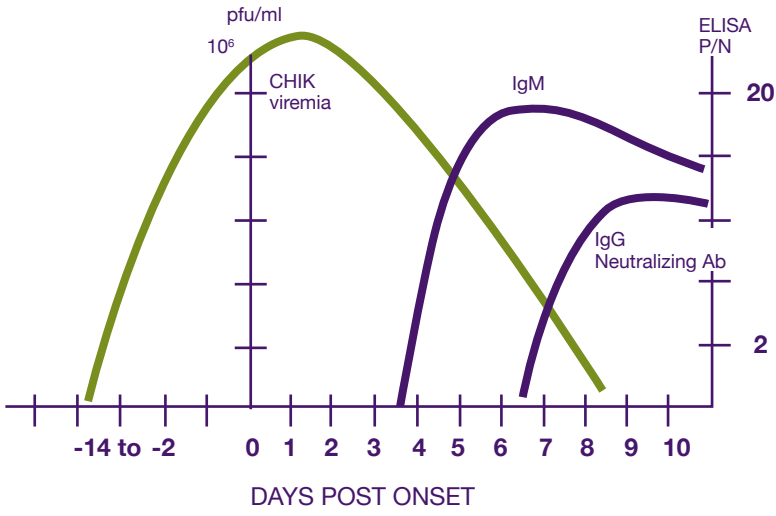


Table 6. **Typical results of samples tested at various time points post-infection.**

Days post illness onset	Virus testing	Antibody testing
Day 1-3	RT-PCR = Positive Isolation = Positive	IgM = Negative PRNT = Negative
Day 4-8	RT-PCR = Positive Isolation = Negative	IgM = Positive PRNT = Negative
>Day 8	RT-PCR = Negative Isolation = Negative	IgM = Positive PRNT = Positive

The following laboratory test results would confirm a recent CHIKV infection:

- Isolation of CHIKV, including confirmatory identification (either IFA, RT-PCR, or sequencing).
- Detection of CHIKV RNA by real time RT-PCR.
- Identification of a positive IgM result in a patient with acute symptoms of CHIK, followed by the demonstration of CHIKV-specific antibody determined by PRNT with viruses in the SFV serogroup.
- Demonstration of seroconversion or a four-fold rise in PRNT, HI, or ELISA titers (again using other SFV serogroup viruses) between acute and convalescent specimens.

Autochthonous cases should be reported to WHO, in collaboration with an epidemiologist, according to International Health Regulations (IHR) (see section 6F).

4D. Laboratory Network for Diagnosing CHIKV

Currently DVBD, CDC can provide diagnostic testing for CHIKV infection. Reagents and consultations can also be provided by CDC and the Public Health Agency of Canada. Depending on the availability of resources and the epidemiologic situation, PAHO and CDC will be working together in the near future to improve CHIKV detection in the Region by providing training and reagents to existing dengue (RELDA) and other arbovirus laboratories in the Americas. Furthermore, proficiency testing is planned to ensure testing quality in the Region. A contingency plan will be developed to ensure that all laboratories capable of performing testing in the Americas have an adequate supply of reagents and protocols.

Summary of Laboratory Section

- Both molecular and serologic techniques are available for the laboratory diagnostic evaluation of CHIKV infection.
- During an outbreak, laboratories will need to develop, with other public health partners, sample triage plans to avoid laboratory overload.
- Laboratories have a key role in the surveillance for CHIKV introduction and spread; ongoing training of laboratories for CHIK detection is needed throughout the Region.
- Collaboration is important, in order for network partner (public health) labs to be able to share materials.
- Reference laboratories in the Region will play a significant role in reagent production and in providing laboratory confirmation of suspected CHIK cases.



5. CASE MANAGEMENT

5A. Treatment

There is no specific antiviral drug treatment for CHIK. Symptomatic treatment is recommended after excluding more serious conditions like malaria, dengue, and bacterial infections.

Acute Disease

Treatment is symptomatic or supportive, comprised of rest and the use of acetaminophen or paracetamol to relieve fever, and ibuprofen, naproxen, or another non-steroidal anti-inflammatory agent (NSAID) to relieve the arthritic component of the disease. Using aspirin is not advised because of the risk of bleeding in small number of patients and the risk of developing Reye's syndrome in children younger than 12 years of age. In patients with severe joint pains that are not relieved by NSAID, narcotics (e.g., morphine) or short-term corticosteroids can be used after evaluating the risk-benefit of these treatments. Patients should be advised to drink plenty of fluids to replenish fluid lost from sweating, vomiting, and other insensible losses.

Subacute and Chronic Disease

While recovery from CHIK is the expected outcome, convalescence can be prolonged (sometimes up to a year or even more) and persistent joint pain may require pain management, including long-term anti-inflammatory therapy. Although an older study suggested that chloroquine phosphate offered some benefit,[45] a recent double-blind, placebo-controlled randomized trial found it to be of no real value treating joint symptoms.[46] Disabling peripheral arthritis that has a tendency to persist for months, if refractory to other agents, may occasionally respond to short-term corticosteroids.[38] To limit the use of oral corticosteroids, local injections (intra-articular) of corticosteroids or topical NSAID therapy can be used. In patients with refractory joint symptoms, alternative therapies such as methotrexate can be evaluated. In addition to pharmacotherapy, cases that have prolonged arthralgia and joint stiffness may benefit from a program of graduated physiotherapy. Movement and mild exercise tend to improve morning stiffness and pain, but heavy exercise may exacerbate symptoms.

5B. Patient Isolation Recommendations

To prevent the infection of others in the household, the community, or the hospital, a patient with acute CHIK should avoid being bitten by *Ae. aegypti* or *Ae. albopictus* mosquitoes during the viremic phase, which is usually the first week of illness. As these mosquitoes bite during daytime, from dawn to dusk or even after dark in the presence of artificial light, staying under an insecticide-treated (IT) bednet or remaining in place with intact screens is highly recommended. Furthermore, physicians or health care workers who visit CHIK-infected patients at home should take care to avoid being bitten by mosquitoes by using insect repellent and wearing long sleeves and pants.

One hospital-associated infection of CHIK has been identified in a health-care provider who had an accidental needle stick from a patient with CHIK.[47] Several laboratory workers also have contracted CHIKV infection after handling infected blood.[48] These exposures indicate that direct contact transmission can occur. However, other modes of transmission, such as through respiratory droplets or particles, have not been documented.

5C. Health Care and Hospital Surge Capacity

At the peak of one recent outbreak, 47,000 suspected cases were identified in a single week among a population of 766,000.[27] There also can be an accumulation of patients with symptoms who seek more long-term care. With that potential volume of cases per week, huge demands are likely to be placed on the health care system during outbreaks of the disease. A number of steps similar to those for pandemic influenza preparedness should be considered by health care facilities preparing for and during a CHIK outbreak. Triage systems should be considered at various levels of health care to facilitate the flow of patients during an outbreak.

Prior to the introduction of CHIKV, the following should be considered (adapted from PAHO and U. S. Department of Health and Human Services (HHS) Influenza Pandemic Plan[49, 50]):

- Develop and implement methods for identifying and investigating potential introduction of CHIKV within existing surveillance systems (e.g., surveillance system for dengue).
- Inform health care providers and public health officials about the potential threat of CHIKV, and educate them about the clinical presentation, diagnosis, and management of cases at health care facilities.

- Develop planning and decision-making structures for responding to a potential outbreak at health care facilities.

- Develop institutional plans to address disease surveillance, hospital communications, education and training, triage and clinical evaluation, facility access, occupational health, surge capacity (beds and access to care), supply chain, and access to critical inventory needs.

Following the introduction of CHIKV into an area, health care facilities should:

- Activate institutional plans with assistance from the Ministry of Health.

- Ensure rapid and frequent communication within health care facilities and between health care facilities and health departments.

- Implement surge-capacity plans that address staffing, bed capacity, consumable and durable supplies, and continuation of essential medical services (see section on Health care Planning in the PAHO and HHS Pandemic Influenza Plan for further considerations[49, 50]).

Effective triage systems at various levels of health care may help to decrease the potential burden of a CHIK outbreak on the health care system. Regardless of the level of medical care available at the triage location, a key measure that needs to be considered at all levels of health care is the institution of appropriate mosquito control measures in the immediate area. If this is not done, patients acutely ill with CHIK can serve as a source of subsequent infections for other patients and for health care workers via mosquito transmission. Furthermore, consideration should be given to establishing areas where patients with suspected CHIK infection are seen and, if necessary, hospitalized (e.g., establish CHIK wards with screens and/or bednets). Finally, consideration should be given to the safety of health care workers. During a previous outbreak, up to one-third of health care workers became infected, further taxing already overburdened and stretched resources.[9]

'Guiding principles for managing acute stage of the disease' has been previously described in detail in WHO's "Guidelines on Clinical Management of Chikungunya Fever". [51] Key information, including triage considerations, from that document is summarized here.

Who should seek medical care?

- Anyone with neurologic signs or symptoms including irritability, drowsiness, severe headaches, or photophobia.
- Anyone with chest pain, shortness of breath, or persistent vomiting.
- Anyone with a fever persisting for more than five days (indicative of another illness like dengue).
- Anyone who develops any of the following, especially once the fever subsides:
  - intractable severe pain,
  - dizziness, extreme weakness, or irritability,
  - cold extremities, cyanosis,
  - decreased urine output, and
  - any bleeding under the skin or through any orifice.
- Women in the last trimester of pregnancy, newborns, and persons with chronic underlying disease, as they or their offsprings are at risk for more severe disease.

Triage at point of first contact (Primary or ambulatory/urgent care)

- Rule out other illnesses by history, clinical examination, and basic laboratory investigations, including but not limited to complete blood count (CBC), liver function tests, and electrolytes. Be careful to evaluate if patient has warning signs for severe dengue or malaria. If these signs are present, refer patient immediately to a hospital.
- Assess for dehydration and provide proper rehydration therapy as needed.
- Evaluate hemodynamic status and stabilize and immediately refer patients with delayed capillary refill, narrow pulse pressure, hypotension, oliguria, altered sensorium, or bleeding manifestations.
- Treat symptomatically (paracetamol/acetaminophen).
- For those with prolonged joint pain (after three days of symptomatic treatment) consider more aggressive pain management, such as morphine and short-term corticosteroids.

- Consider referral for patients with increased risk of a poor outcome (persons older than 60 years, those with chronic disease, pregnant women, and newborns).

Triage at the secondary level (district or local hospital)

- Treat symptomatically (according to previous treatments).
- Investigate person for renal failure, neurologic signs and symptoms, hepatic insufficiency, cardiac illness, thrombocytopenia, and malaria.
- Evaluate hemodynamic status and assess for dehydration; provide proper supportive care and rehydration therapy as needed.
- Consider cerebral spinal tap if meningitis is suspected.
- Collect blood for serologic testing of CHIK and other diseases in the differential diagnosis (e.g., dengue).
- Review history of present illness and evaluate if patient has warning signs for severe dengue. If present, administer supportive care in a unit that can monitor vital signs on an hourly basis during the critical phase.
- Refer patients with any of the following conditions to a higher level health center: pregnancy, oliguria/anuria, refractory hypotension, significant clinical bleeding, altered sensorium, meningoencephalitis, persistent fever of more than one week's duration, and signs of decompensation of underlying diseases.

Triage at the tertiary care level (advanced care centers or centers with infectious disease specialists)

- Ensure that all the above-mentioned procedures have been completed and that a comprehensive medical team is available to assist in managing patients with severe or atypical disease.
- Collect blood sample for serology and/or RT-PCR (see Laboratory section for more specific on CHIK testing).

- Consider the possibility of other rheumatic (e.g., rheumatoid arthritis, gout, rheumatic fever) or infectious diseases (e.g., viral or bacterial meningoencephalitis).
- Treat serious complications (e.g., bleeding disorder with blood components, acute renal failure with dialysis).
- Assess disability and recommend rehabilitative procedures.
- Given the severity of the pain and the potential long-term pain with CHIK, pain management and psychological assistance should be made available and consideration given to develop chronic pain management protocols, teams, and centers. Autopsies should be considered on all deceased patients, with involvement of pathologists.

5D. Blood, Organ, and Tissue Safety

Blood-borne transmission is possible. There are documented cases that include infection of laboratory personnel handling infected blood and of a health care worker drawing blood from an infected patient.[47, 48] These cases support the belief that CHIKV is able to be transmitted through blood products.

To determine the impact of CHIKV on blood supply safety consider: 1) incidence of viremia among blood donors (which may vary depending on the time of the outbreak); 2) clinical impact on transfusion recipients who become infected; 3) availability of measures to reduce transfusion transmission (e.g., nucleic acid amplification testing (NAT) or photochemical pathogen inactivation treatment); 4) availability of an alternative blood supply (from non-affected areas); and 5) the cost incurred by adopting these measures.[52]

In addition to asking the local health-care community to promote optimal use of blood components, possible considerations for blood safety in areas with CHIKV introduction could include:[53]

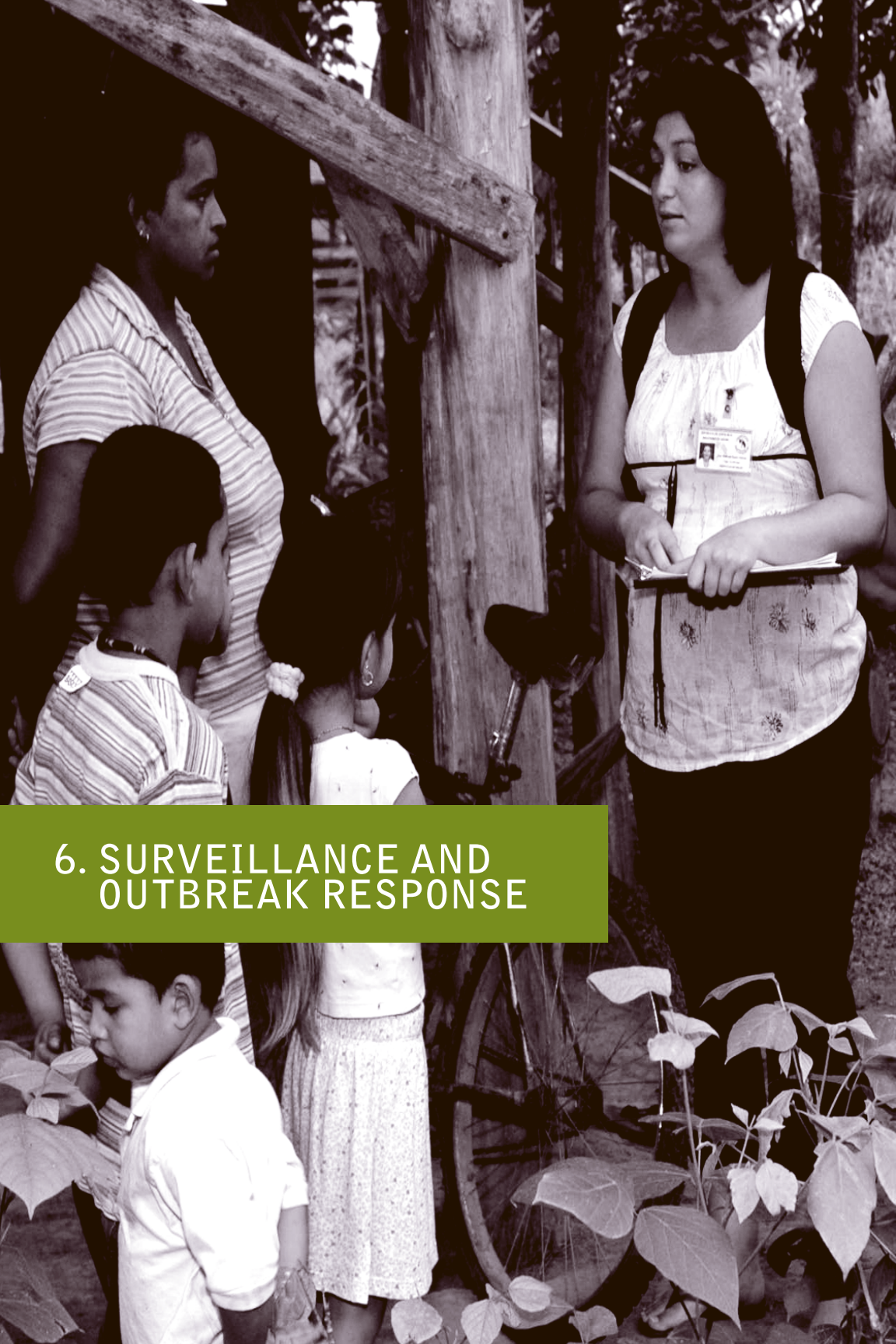
- Continue to obtain blood donations from local persons until an unacceptable incidence or prevalence[a] of infection is reached in the community.
- Screen blood donors for symptoms prior to donation.
- Asking donors to report any illness they experience after donating blood, while holding on to the blood donations for several days (e.g, 2-5 days) prior to releasing it.
- If feasible, cease all blood donations in an area of known CHIKV infections and import blood products from uninfected areas.
- Institute screening (e.g., NAT) of the blood supply for CHIKV. This will require a preexisting platform and regulatory clearance, and is unlikely to be available in most areas.

Similar measures should be considered for organ and tissue (grafts) transplantations.

[a] To be determined by blood banks and public health officials in the area.

Summary of Case Management Section

- Treatment for CHIK is supportive, using anti-pyretics, optimal analgesics, and fluids.
- Acutely infected patients need to be protected against mosquito bites to prevent further disease spread at home, in the community, and in the health care facility.
- Because CHIK will place a large burden on the community, including on all levels of the health care system, well-established protocols and plans need to be developed in advance to assist in the triage, care, and rehabilitation of patients.



6. SURVEILLANCE AND OUTBREAK RESPONSE

The main objective of surveillance is to detect, in a timely manner, cases of CHIK in the Americas. Early detection will allow for proper response and characterization of the outbreak and identification of the viral strains circulating.

6A. Modes of Surveillance

Multiple modes of surveillance can be considered to determine if CHIK may have been introduced to an area, to track the disease once introduced, or to follow the disease once it has been established.

1. Preparedness phase

Reinforce existing febrile syndromic surveillance sentinel sites so they can detect CHIK cases. A percentage of patients presenting with fever and arthralgia or fever and arthritis with no known etiology (e.g., negative test for malaria or dengue), should be tested for CHIK at the national reference laboratory (See Section 4 for more details on proposed laboratory surveillance testing). To ensure adequate laboratory testing and surveillance capacity, laboratories should be aware of the laboratory network set up for testing and eventual distribution of supplies.

2. Response phase

Introduction

Once an autochthonous case of CHIK is detected, an in-depth epidemiologic investigation must be conducted to:

- Track viral spread.
- Monitor for possible introduction into surrounding areas.
- Describe key epidemiologic and clinical features.
- Assess clinical severity and impact on society (e.g., days missed from work, school closures, etc.).
- Identify risk factors for infection or severe disease.
- Identify circulating CHIKV lineages.

These efforts will be the basis for developing effective control measures.

Active, passive, and laboratory surveillance should be used to calculate and monitor indicators such as: incidence, rate of spread, rate of hospitalization (per infections), proportion of severe disease, mortality ratios, and disability rates.

Sustained transmission

Once the virus has been identified throughout a country, scaling back of the level of testing and active surveillance can be considered (e.g., testing only a fraction of suspect cases or testing severe or atypical cases, newborns, cases identified in new regions) to avoid unnecessary costs in resource-limited settings. However, ongoing surveillance should be continued to monitor changes in the epidemiology and ecology of CHIKV transmission. Any changes in surveillance at the national level should be readily communicated to other surveillance and prevention partners, such as vector control specialists, to ensure the quality and uniformity of the data collected.

6B. Case Detection

Clinicians should consider CHIK in the differential diagnosis for individuals who are presenting with fever and arthralgias that are not explained by another etiology or have an atypical presentation, e.g., an atypical dengue presentation with more severe joint pain or conjunctivitis. The index of suspicion should be heightened for a traveler or someone having contact with a traveler who has recently returned from an area with ongoing CHIKV infections (to obtain updated information on location of CHIK outbreaks visit http://www.who.int/csr/don/en/index.html or http://wwwnc.cdc.gov/travel/default.aspx).

Laboratory personnel should consider CHIK if there is a low proportion of samples that are seropositive for an etiology that has a similar clinical presentation, like dengue, or if there are a number of synovial fluid samples that are sterile on bacterial culture.

Public health authorities should be alerted to small clusters of disease (fever and arthralgia or arthritis) associated with a traveler returning from a CHIK endemic area or an increase in the number of hospital visits for fever and arthralgia or arthritis occurring in a localized area in a short time.

6C. Case Definition

Suspect case: a patient with acute onset of fever >38.5ºC (101.3ºF) and severe arthralgia or arthritis not explained by other medical conditions, and who resides or has visited epidemic or endemic areas within two weeks prior to the onset of symptoms.

Confirmed case: a suspect case with any of the following CHIK specific tests:

- Viral isolation.
- Detection of viral RNA by RT-PCR.
- Detection of IgM in a single serum sample (collected during acute or convalescent phase).
- Four-fold increase in CHIKV-specific antibody titers (samples collected at least two to three weeks apart).

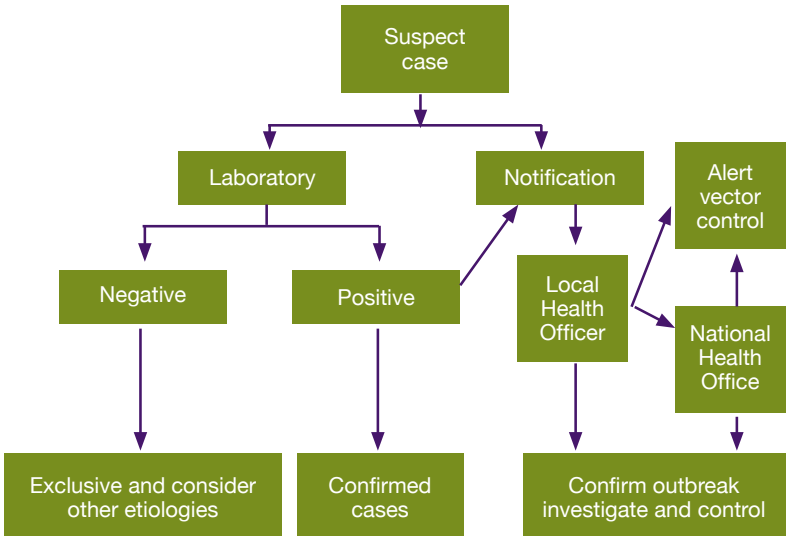
During an epidemic, all patients need not be subjected to confirmatory tests as above. An epidemiologic link can be sufficient.

An evaluation of the sensitivity and specificity for clinical criteria for CHIKV infection was done during a large outbreak of the disease.[54] The combination of fever and polyarthralgias had the best sensitivity and specificity at 84% and 89%, respectively, and allowed for the correct classification 87% of individuals with serologically confirmed CHIKV infection.

6D. Case Reporting

CHIK is not a notifiable disease in most countries. However, depending on the epidemiologic situation, each country must determine independently when CHIK should be a disease of mandatory reporting. Occurrence of suspect cases could indicate a possible outbreak and, therefore, should be immediately reported to the nearest health authority in accordance with the IHR guidelines. Prior to the introduction of CHIK into an area, clinicians should report any suspect or confirmed travel-related cases to local public health officials who, in turn, should report them to a regional level and then on to a national level, where the information should be summarized and shared with stakeholders (Figure 3). In addition, other key partners, such as vector control management teams, should be notified.

Figure 3. **Scheme for notification of a suspected outbreak of CHIK.**



6E. Epidemiologic Reports

Ideally, epidemiologic reporting should be established at the national level, with the support of local and regional public health officials. The types and number of epidemiologic reports will likely evolve during the course of the outbreak to reflect the types of surveillance that are performed in an area.

Following the introduction of CHIK into an area, a line list of suspect and laboratory confirmed cases should be kept and updated daily. Reporting should be coordinated at the national level by establishing a web-based line list, if possible, that contains a few required variables and additional variables as needed. A standardized case report form, including demographic, epidemiologic and laboratory information, should be developed quickly and shared with key partners to help facilitate the collection of information (See Appendix D for an example). At the national level, there should be clearly defined cutoffs in

terms of presenting and closing the data on a daily basis. In addition to case count by location and timing, reporting on disease severity (hospitalization, mortality), number of hospital beds occupied per day, and trends in cases based on syndromic surveillance can be considered as ways to present the data. The national level data should be communicated back to the collecting districts, as well as to the press and other public health and partner agencies that participate in the control efforts (see Section 8, "Risk and Outbreak Communication" for more detail). Once a country has identified autochthonous transmission within its borders, it should activate its emergency operations center ("sala de situación") to serve as a source for rapid communication and decision making.

6F. International Health Regulations and Border Measures

International Health Regulations

A single imported case (i.e., a traveler) of CHIKV into the Americas would not necessarily constitute a public health emergency of international concern (PHEIC) under IHR,[55] although this case should be thoroughly investigated to minimize the risk of CHIK establishment in the country. However, suspicion of autochthonous CHIKV transmission in the Americas will meet PHEIC criteria and should be reported per IHR (see Appendix E for an example). Such an event would have a serious public health impact because of its potential to cause an epidemic with high attack rates among an immunologically naïve population, and because vectors are sufficiently abundant to potentially support permanent establishment of the virus and year-round transmission. The event would also be unusual for the Americas, since it would signal the appearance of a previously absent pathogen and a significant risk of international spread given the amount of travel between countries in the Region. Although CHIKV does not have a high mortality rate, it has high morbidity rates associated with persistent arthralgias that can lead to disability and a reduction in productivity. The establishment of CHIKV in a Member Country could also affect key national income sources, such as tourism. For example, La Réunion Island observed a 60% decline in tourism after its CHIKV outbreak.[56]

Any Member Country should thoroughly investigate any suspect CHIK case detected without a travel epidemiologic link to another country and rule out indigenous CHIKV transmission. PAHO recommends that Member Countries consider making the reporting of CHIK mandatory to enable and promote a timely response.

Border Measures

Closing borders due to suspected CHIKV cases would be counterproductive and it is not recommended by WHO. It also is inconsistent with the IHR, which emphasize detection and containment at the new source of transmission, rather than control at borders of entry. The costs associated with screening for CHIK at ports of entry outweigh the benefits. It is insufficiently sensitive and specific and too expensive to be a tool for preventing CHIKV introduction and spread. The anticipated prevalence among travelers coming from areas of the world with CHIKV activity is low, symptoms are non-specific, and screening would yield a low positive predictive value. The reported experience of entry screening for CHIKV in Taiwan validates this point. During 2006, more than 11.7 million passengers arrived in Taiwan. Of these passengers, 6,084 were identified as having fever using thermal infrared imaging cameras; laboratory testing of passengers detected 44 cases of dengue fever, 13 cases of shigellosis, 1 case of malaria, 1 case of paratyphoid fever, and 1 case of CHIK (JW Hsieh, Centers for Disease Control, Ministry of Health, Taiwan,*personal communication*, 2007).

Even disregarding the issue of cost and complexity of implementation, port of entry screening activities are unlikely to prevent or delay the importation of CHIKV. There is no evidence to support that requiring pilots or ship captains to complete health declarations, asking passengers to complete screening questionnaires, taking temperature measurements and engaging in other entry screening modalities effectively prevent CHIKV introduction and spread into the Americas. Member Countries should use their scarce public health resources on activities more likely to achieve intended results, including implementing sustainable vector control efforts, enhancing clinical surveillance for CHIKV disease, educating the public, and considering assisting affected Member Countries. For similar reasons, exit

screening is not recommended if Member Countries in the Americas confront CHIKV outbreaks within their borders.

Some jurisdictions outside the Americas have instituted mosquito abatement activities at international airports and spraying adulticides in the passenger cabins of arriving international flights as part of efforts aimed at preventing dengue importation. However, virus-infected mosquitoes arriving in passenger aircraft are not considered as significant sources of most arboviral importations. For arboviruses with a human-mosquito-human transmission cycle, the most important source of viral importation is the viremic traveler. In a region like the Americas, where competent vectors are already present in the majority of countries, mosquito abatement and vector surveillance efforts predominantly focused on international airports and seaports can be implemented by national authorities to prevent CHIKV importation, but PAHO does not recommend them. The exception would be if cases were being detected close to an international airport or seaport, or if suspected cases worked in or around these ports of entry. Routine vector control efforts consistent with IHR Article 22, which calls for eliminating vectors at facilities used by travelers at points of entry, should be implemented, but are not intended as a principal means of preventing CHIKV importation.

Similarly, in the presence of CHIK cases and local virus transmission, there is no need to place any restrictions on baggage, cargo, containers, goods, or postal parcels beyond usual practices; this will avoid unnecessary interference with international traffic in the absence of any identified public health benefit. It is advisable, however, to establish communications between public health authorities and conveyance operators (sea and air, cargo and passenger) and other port-based organizations, in case there is a need to implement a CHIKV communication campaign.

Countries may elect to distribute Travel Health Alert Notices (THANs) to international travelers if there is concern that CHIKV transmission is likely or if ongoing transmission has been detected. This information would offer guidance to travelers on how to reduce their risk of contracting CHIKV, steps to take for

reducing the likelihood that they will be bitten by mosquitoes, or seeking early diagnosis if they develop signs and symptoms compatible with CHIK fever. These messages could be relayed through online reservation systems, travelers' health clinics, travelers' health Web sites, and postings at international ports when outbreaks are occurring.

It will be important to monitor air travel patterns between countries where CHIKV is circulating and every other country or area in the Americas, in order to identify locations most at risk to virus introduction. In a preliminary analysis that was limited exclusively to direct flight data, scheduled commercial flight data shows that countries importing CHIKV had 23 times more total scheduled passenger seats originating from countries with CHIKV activity than did non-importing countries (CDC, unpublished). Subsequent analyses using passenger-specific data, which includes travel connections and actual passenger volume, could provide more accurate information on which to base a risk assessment of CHIKV importation.

Summary of Surveillance and Outbreak Response Section

- Epidemiological surveillance is key to the timely detection of cases and appropriate and rapid response with active participation from all stakeholders.
- CHIK surveillance should be built upon existing dengue surveillance (highlighting differences in clinical presentation).
- If autochthonous transmission of CHIK is identified, it must be reported immediately as a PHEIC under IHR.



7. VECTOR SURVEILLANCE AND CONTROL

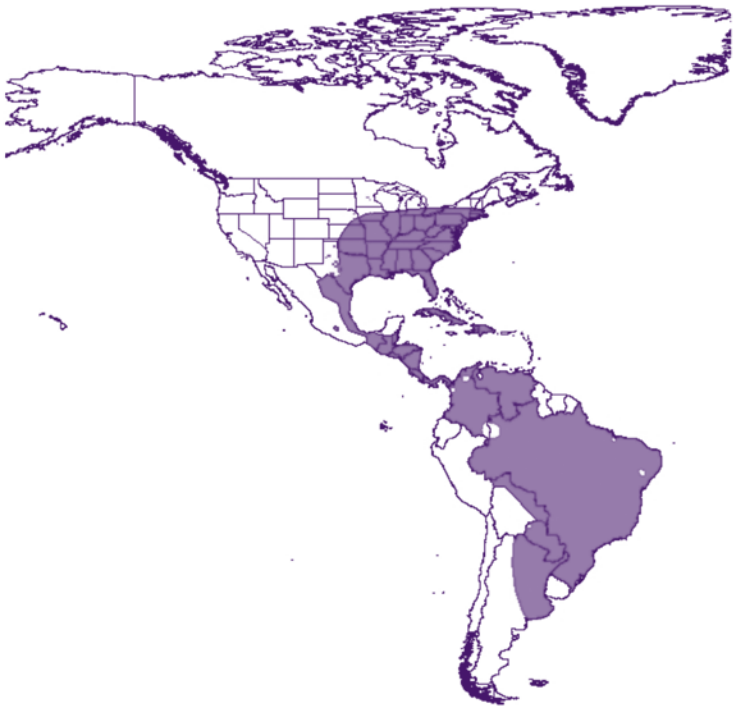
In the absence of an effective CHIKV vaccine, the only tool available to prevent infection is reduction of human-vector contact. The primary vectors of CHIKV are *Ae. aegypti* and *Ae. albopictus*. *Ae. aegypti* is the principal vector in areas of Africa where the virus is considered to be endemic. However, *Ae. albopictus* was incriminated during recent epidemics, following introduction of the virus into temperate Europe[17] and some tropical areas of the Indian Ocean.[27, 57] These outbreaks were associated with an adaptation of CHIKV strains to *Ae. albopictus*.[58, 59] Both *Ae. aegypti* and *Ae. albopictus* are present in the Americas (Figures 4 and 5). *Ae. aegypti* will likely be the most important vector in urban areas, and *Ae. albopictus* will likely play a more significant role in temperate areas and areas where it is already well established. Both mosquitoes could support the introduction of CHIKV strains into a variety of geographic areas in the Region. Therefore, vector control planning efforts should focus on suppression of both *Ae. aegypti* and *Ae. albopictus* populations to prevent the likelihood of CHIKV establishment and to lay the foundation for emergency interventions in the event of an outbreak.

Figure 4. **Distribution of *Ae. aegypti* in the Americas.[a]**



[a] Adapted from Arias, 2002.[60]

Figure 5. **Approximate distribution of *Ae. albopictus* in the Americas.[a]**



[a] Adapted from Benedict et al. 2007.[61]

There are some significant differences between *Ae. aegypti* and *Ae. albopictus* that must be considered in developing surveillance and control procedures. *Ae. aegypti* is more closely associated with humans and their homes, and feeds preferentially on humans. Adult *Ae. aegypti* rest indoors, and its larval habitats are most frequently containers on the household premises. *Ae. albopictus* feeds readily on humans, but also utilizes a broader range of bloodmeal hosts;[62] its larvae occur in peridomestic habitats as well as surrounding natural habitats. *Ae. albopictus* can overwinter in the egg stage and, therefore, can occupy more temperate climates[63] than *Ae. aegypti.* These species have distinct morphological features, and the identification of specimens collected during surveillance and control programs in the Americas can be readily accomplished.[64, 65]

An effective, operational dengue control program provides the basis for CHIKV preparedness, because the biology and control procedures for *Ae. aegypti* are similar to those for *Ae. albopictus*. Surveillance and control recommendations developed for dengue management[66] as a component of the Integrated Management Strategy for Dengue Prevention and Control (IMS-Dengue) may be utilized and intensified in order to respond to a CHIKV introduction. Successful control programs require well trained professional and technical staff and sufficient funding. In addition, an independent quality assurance program should be incorporated into the integrated vector management (IVM) scheme.

To be successful, the CHIKV IVM program must include intersectoral participation (collaboration) at all levels of government and among health, education, environment, social development, and tourism agencies. IVM programs also benefit from participation of non-governmental organizations (NGOs) and private organizations. CHIKV control program must communicate with and mobilize the entire community.[67] In fact, the community's participation is an essential component of IVM.[68] To be effective, an IVM strategy must be developed and in place before CHIKV is introduced.

7A. Reducing the Risk of CHIKV

Components of an IVM program to reduce CHIKV risk include:

1. Vector Surveillance and Identification of High Risk Areas

In areas where dengue is endemic, a retrospective analysis of Dengue virus transmission during previous years should be conducted during the CHIKV planning phase to indicate the areas where CHIKV is expected to circulate (given the similarity in transmission cycles of these viruses). Areas can be stratified in terms of risk of transmission.[69] Stratification is then used to assign resources and priorities. For example, controlling or preventing CHIKV transmission in neighborhoods that traditionally have produced many cases of dengue should inhibit virus amplification and virus spread to nearby neighborhoods.

The program must have the ability to systematically collect surveillance data on relative densities of *Ae. aegypti* and *Ae. albopictus*. Surveillance methods for *Ae. aegypti* and *Ae. albopictus* are varied and include methods to monitor egg production, larval sites, pupal abundance, and adult abundance. These methods are reviewed in Chapter 5 of the WHO Dengue Guidelines.[66] New traps and sampling methods are being developed that may provide more accurate surveillance data.[70, 71] Programs must be able to detect and identify hidden and difficult to control larval sites (e.g., cryptic locations such as septic tanks, storm drains, sump pumps, and vacant lots) and other productive sites, as well as the readily identified and commonly found larval habitats.

2. Personal Protection

Individuals may reduce the likelihood of infection by the use of personal repellents on skin or clothing. DEET (N,N-diethyl-m-toluamide) and picaridin (also known as KBR3023 or Bayrepel™) are effective repellents widely available in the Americas. Infants and others sleeping or resting during the day should use bednets to avoid infection from *Ae. aegypti* and *Ae. albopictus*, both of which are day biting mosquitoes. It is of particular importance that individuals who are

potentially infected with CHIKV during an outbreak rest beneath an IT bednet to avoid mosquito bites and further spread of infection. Use of IT bednets has the additional benefit of killing mosquitoes that come into contact with the net, which may reduce vector-human contact for other household members.[72] A number of pesticide products may be used to safely treat bednets (Table 6), or long-lasting pretreated nets can be obtained commercially.

Table 6. **WHO recommended insecticide products for treatment of mosquito nets.**[a]

1. Conventional treatment:		
Insecticide	**Formulation**[b]	**Dosage**[c]
Alpha-cypermethrin	SC 10%	20–40
Cyfluthrin	EW 5%	50
Deltamethrin	SC 1%; WT 25%; and WT 25% + Binder[d]	15–25
Etofenprox	EW 10%	200
Lambda-cyhalothrin	CS 2.5%	10–15
Permethrin	EC 10%	200–500

2. Long-lasting treatment:		
Product name	**Product type**	**Status of WHO recommendation**
ICON® MAXX	Lambda-cyhalothrin 10% CS + Binder Target dose of 50 mg/m^2	Interim

[a]Adapted from http://www.who.int/whopes/Insecticides_ITN_Malaria_ok3.pdf

[b]EC = emulsifiable concentrate; EW = emulsion, oil in water; CS = capsule suspension; SC= suspension concentrate; WT = water dispersible tablet

[c]Milligrams of active ingredient per square meter of netting.

[d]K-O TAB 1-2-3

3. Household Prevention

The use of intact screens on windows and doors will reduce entry of vectors into the home, and mosquito proofing water storage vessels will reduce oviposition sites and local production. Within a household, use of IT bednets[72] and IT curtains[73] also reduce vector-human contact.

The number of adult mosquitoes in a home may be reduced by using commercially available pyrethroid-based aerosol sprays and other products designed for the home, such as mosquito coils and electronic mat vaporizers. Aerosol sprays may be applied throughout the home, but areas where adult mosquitoes rest (dark, cooler areas) must be targeted, including bedrooms, closets, clothing hampers, etc. Care should be taken to emphasize proper use of these products when advocating their application to the public, in order to reduce unnecessary exposure to pesticides.

4. Neighborhood and Community Prevention

Neighborhood and community prevention for a CHIKV introduction in the Americas should be based on methods developed for dengue control, utilizing effective strategies to reduce the densities of vector mosquitoes.[66] A fully operational dengue control program will reduce the probability that a viremic human arriving in the Americas will be fed upon by *Ae. aegypti* or *Ae. albopictus* mosquitoes, thereby leading to secondary transmission and potential establishment of the virus.

Dengue programs for controlling *Ae. aegypti* have traditionally focused on control of immature mosquitoes, often through the community's involvement in environmental management and source reduction. It is essential that community involvement be incorporated into an IVM program.[74, 75]

Vector Control Procedures

The WHO Dengue Guidelines[66] provide information on the main methods of vector control, and they should be consulted when establishing or improving existing programs. The program should be managed by experienced professional vector control biologists to assure that the program uses current pesticide recommendations, incorporates new methods of vector control, and includes resistance testing. Prevention programs should utilize the methods of vector control found in Appendix F, as appropriate.[66, 74]

7B. Response to CHIKV Introduction

Immediately upon confirmation of the first autochthonous CHIKV case, the health department should inform the IVM program regarding the onset date and location of the case. Vector control procedures must be intensified to effectively reduce the abundance of infected vectors in order to halt transmission in the areas of the case(s).

Simultaneously, emergency response committees at local and national levels should be informed of the situation and activated. Initial efforts should focus on containing virus transmission and preventing expansion (Appendix G). If virus containment fails, or if cases are not detected until the outbreak has spread over a large geographic area, intensive vector control efforts will need to be expanded to a larger scale program.

Summary of Vector Surveillance and Control Section

- Epidemiological surveillance is key to the timely detection of cases Early detection of disease will increase the likelihood of containing transmission of CHIKV in the area.
- Successful IVM for CHIKV requires trained experts in medical entomology and vector control, sufficient resources, and a sustained commitment.
- Current dengue control programs in the Region should be utilized and improved to prevent CHIKV transmission.
- Vector surveillance and control activities and methodologies must be validated and continually evaluated to measure efficacy.



8. RISK AND OUTBREAK COMMUNICATION

8A. Risk Communication for CHIKV Introduction or Outbreaks

Effective communication to the community and various stakeholders is crucial to avoid confusion and misinformation and to engage people in steps to reduce the risk of disease. Under IHR, risk communication for public health emergencies includes the range of communication capabilities through the preparedness, response, and recovery phases of an outbreak.[55] Messages should encourage informed decision making, positive behavior change, and the maintenance of trust in public authorities. As CHIKV is new to the Americas, the media, the public, and many officials will need to be educated about the disease, its mode of transmission, the lack of specific therapeutic treatment, means of symptomatic and supportive treatment, and the adoption of control measures. Risk communication messaging can emphasize that the risk of CHIKV infection can be reduced, and that it is typically a self-limiting disease.

8B. Risk Communication Strategies by Phase and Target Audience

Appendix H gives an example of a model risk communication plan with strategies organized by preparedness, response, and recovery phases of an emergency. The plan defines various target audiences that should be considered in developing a country-specific risk communication plan.

Risk communication should be organized across multiple agencies and should target the media, the public health community, community-based organizations, the private sector, and civil society institutions.

Structure and Coordination

Ideally, an emergency response to a CHIKV outbreak will use an Incident Command System that provides structure for collaboration. In Latin America, the equivalent is the Emergency Operational Committee (or COE in Spanish). A key component in emergency operations is the establishment of a Joint Information Center (JIC) that allows for coordination of messages from local, state, national, and international partners. Information about setting up and running a JIC can be found online at: http://www.fema.gov/emergency/nims/PublicInformation.shtm

As part of the emergency operations structure, communication staff should work closely with other operational components (epidemiology, vector control, etc.). All groups should meet regularly ensure that they are in agreement on key data points, including number of cases, geographic factors, and messages. Lack of coordination on these points will help create confusion and undermine confidence in the management of the response.

Strategies by Phase: Preparedness Phase

The primary activities during the preparedness phase are to develop a communications plan and to create strategic partnerships. During this phase, potential activities may include:

- Informing key stakeholders about preparedness materials, such as these guidelines.
- Developing basic response materials, such as fact sheets and frequently asked questions, will facilitate a rapid response to a CHIKV introduction and reduce misinformation. Information channels may include printed materials, websites and other electronic and social media, the mass media, short message service (SMS) text messages, inter-personal communication through group meetings, schools, and utilization of traditional or folk media.
- Working with partners to develop strategies to guide care seeking, travel (national and international), and prevention/risk reduction.
- Communication with journalists and news agencies to provide baseline information on CHIKV and on the national preparedness and response plan.
- Networking with key personnel at potential information points, such as arrivals and departure locations (airports, ports, borders) and public facilities (health care facilities, educational centers, workplaces, nursing homes, shopping malls, churches, public transport sites, stadiums, among others).
- Anticipating sensitive issues can allow for preemptive preparation of responses and strategies. Sensitive topics related to CHIKV may include concern over safety of community and household pesticide use, any restrictions involved in a containment response, large numbers of persons seeking care at health care facilities, and the cost of control measures.

Strategies by Phase: The Response Phase

During the response phase, the communication plan is put into action; in particular, communications with the mass media, health care providers, and other key audiences are intensified.

The Mass Media

Effective communication through the mass media can help maintain clear information regarding the outbreak and the public health response. Information should be communicated via an appropriate, trained national-level spokesperson. Use of a consistent spokesperson can build trust and avoid the release of potentially conflicting messages from various sources. Information in the mass media can also reinforce the key behavioral outcomes that can help reduce risk during an outbreak. Content in the electronic and print media should be regularly monitored (on a daily basis during an intense outbreak), in order to make any necessary adjustments to the strategies and messages conveyed to the population.

Response to media inquiries should be timely and accurate, and should include promotion and prevention issues. Messaging for media responses should be coordinated through the JIC. Sensitive issues should be addressed promptly and transparently, following best crisis and risk communication principles: http://www.bt.cdc.gov/cerc/

It is useful to employ multiple channels to disseminate accurate information on the disease and its prevention. These may include advertising and other social marketing tools (e.g., TV, radio, printed media, the Web, outdoor billboards, and social networks, such as Twitter, Facebook, or YouTube). Relying on multiple channels may be especially important when the outbreak engenders confusion and controversy.

Health Care Provider Communication

Because CHIKV is a new disease in the Region, many health care providers probably will have little specific information available on diagnosis and care for CHIK patients. Mechanisms for rapid communication with care providers should be established, such as dedicated health care provider websites, health alert network notices, and communication via professional associations. Ideally, basic materials can be prepared in advance of an outbreak. Specific communication strategies should reflect the actual availability of electronic media to health care providers throughout the Region. See Appendix H for further details.

Strategies by Phase: The Recovery Phase

During the recovery phase, the main activities include guiding the general population on the sustainment of appropriate public health measures, and informing the public when the risk of disease transmission has been reduced. At this point there also is an opportunity to evaluate and assess the effectiveness of risk communication efforts. A summary evaluation at the end of the outbreak will provide valuable insight for future responses. For further details refer to Appendix H.

8C. Specific Behavioral Strategies for CHIKV Risk Reduction

Specific strategies for effective personal, household, and community primary prevention are discussed in section 7 (Vector Surveillance and Control). Messages regarding control measures should be developed in collaboration with vector control staff, and should emphasize specific steps that households must consider to optimize potential control measures (e.g., leaving windows open during fogging, which materials to remove from the home in the event of indoor residual application, what the larvicide looks like and how long to leave in place, etc.).

Advance research into knowledge, attitudes, and practices regarding repellent and household control measures may yield benefits in understanding barriers to use and potential for misapplication. Even if it is not feasible to conduct this research in advance, rapid qualitative assessment in affected areas can yield insights to increase the effectiveness of prevention messages.

Communication on prevention should target specific behaviors that offer the best likelihood of reducing risk. Feasible strategies will vary by location, depending on a given community's resources, attitudes, control program capacity, and ecology. Key messages for personal and household prevention can include:

Community Strategies

- Encouraging support of and compliance with governmental control efforts such as environmental sanitation, larviciding and adulticiding.
- Advocacy for household and neighborhood source reduction (e.g., trash clean-up, control of water storage, etc.).

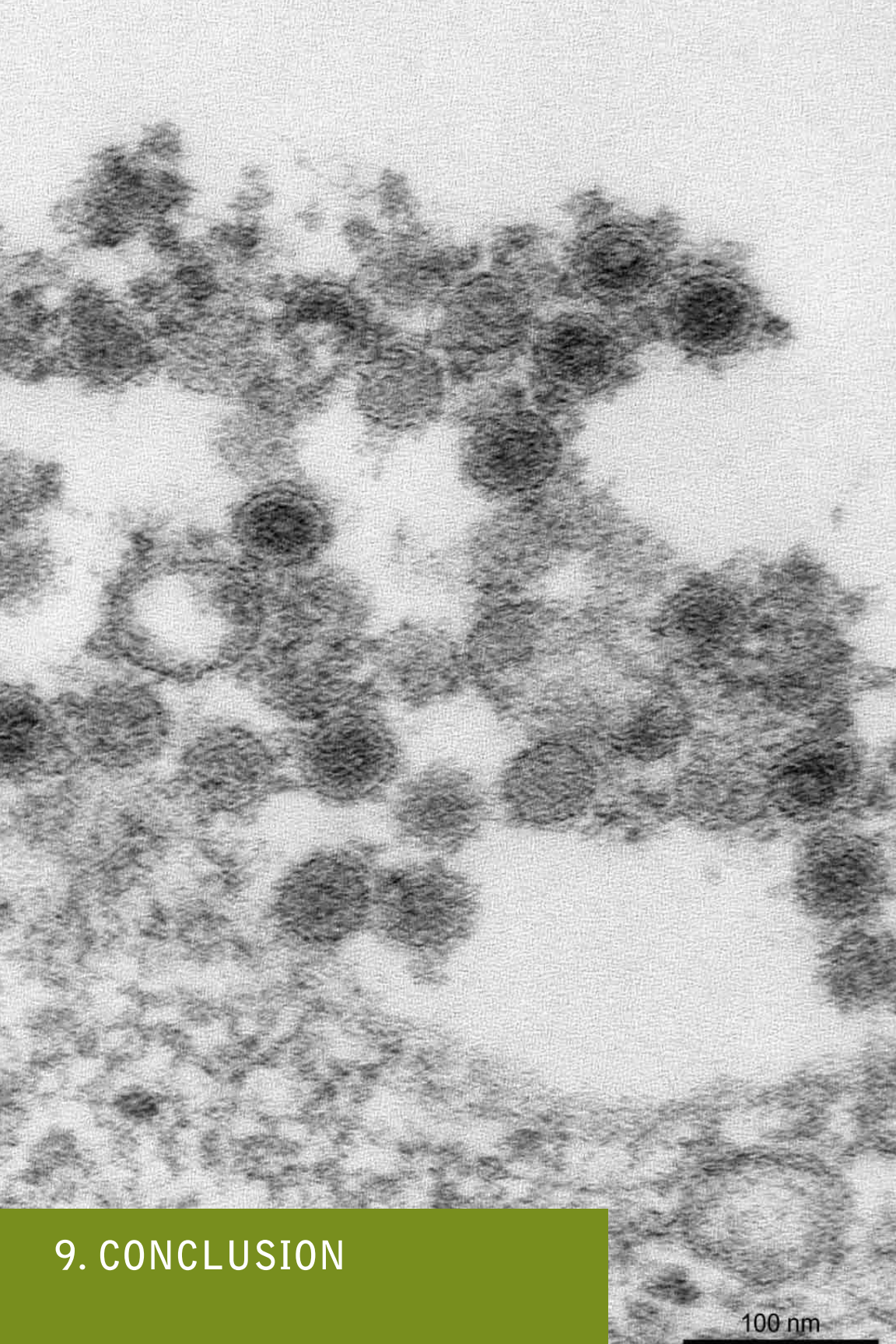
Household and Personal Strategies

- Use of personal prevention such as clothing, repellents, and insecticide-treated materials:
  - Encouraging the use of long sleeves and pants may be reasonable in areas where temperatures are moderate, particularly during evening when *Aedes* mosquitoes are often still seeking a bloodmeal. This recommendation may be less practical in tropical zones.
  - Repellents for use on skin and clothing are now sold widely throughout the Americas. A significant outbreak may increase interest in these tools, and authorities should be prepared to provide guidance and to rely on creative strategies to increase use.

- Methods to reduce human-vector contact include use of household insecticides and installation of screening. Where feasible, screening material can be installed over windows even without the use of expensive frames (stapling in place or using wooden frames).
- It may be useful to specify active ingredients or even brand names for recommended repellent and/or household insecticides, as a way to reduce use of ineffective and possibly dangerous materials.

Summary of Risk and Outbreak Communication Section

- Communications are an integrated, coordinated effort involving all disciplines and components for preparation and response.
- Timely communication with stakeholders is crucial for enlisting the community's participation and to avoid confusion and misinformation.
- As CHIKV is novel in the Americas, the media, the public and many officials will need to be educated about the disease, the mode of transmission, the lack of specific therapeutic treatment, means of symptomatic and supportive treatment, and effective control measures.



9. CONCLUSION

Currently, CHIKV is not known to be circulating in the Americas. The risk of introduction is high, however, due to travel importation, competent vectors (same vectors as dengue), and population susceptibility. Given the likelihood that CHIKV will be introduced in the Region, advance preparation is essential. The timely detection of cases and an appropriate and rapid response with active participation of all stakeholders will be necessary to minimize the risk of importation and sustained transmission in the Region.

These guidelines for the preparedness and response CHIKV introduction in the Americas were developed to increase awareness of the disease and to provide the necessary information to institute the most appropriate strategies to prevent the importation and spread of CHIKV in the Region. Each Member Country is encouraged to use and adapt these guidelines to detect an outbreak of the disease early, to conduct pertinent epidemiologic investigations, and to prevent or mitigate the expansion of the disease in the Americas.

10. APPENDICES

Appendix A. Viral Isolation Protocol (for Cell Culture)

Introduction	The optimal method for determining specific etiology of an arbovirus infection requires isolation of the virus from a specimen obtained from the patient during the acute stage of the disease and the demonstration of a rise in titer of an antibody to the isolate during convalescence. For a number of reasons, the successful isolation of most arboviruses from patient specimens is the exception, whether because the specimen to be examined is not collected early enough, is not properly handled, or is not expeditiously transmitted to the virus laboratory for inoculation. The viremia for many arbovirus infections in humans, if detectable at any stage, ceases by the time of or soon after onset of symptoms—a stage when antibody is often demonstrable. Because some circulating virus may be recoverable and the antibody may be absent, or present in low titer, the acute-phase blood specimen should be collected immediately upon suspicion of a viral etiology. Delay of an hour or so can compromise the chance of virus isolation; the allowable time depends upon the type of viruses involved. Certain arboviruses, like CHIKV, produce a viremia of sufficient magnitude and duration that the viruses can be isolated from blood during the acute phase of illness, e.g., 0 to 5 days after onset. Viral isolates can be recovered by biopsy or at autopsy from the viscera of patients with acute disease.

Introduction	For isolation from brain, samples should be taken from several areas, including the cortex, brain nuclei, cerebellum, and brain stem. Neurotropic arboviruses sometimes can be isolated from CSF obtained by lumbar puncture during the acute stages of encephalitis or aseptic meningitis. Alphaviruses, like CHIKV, have been isolated from joint fluid of patients with acute polyarthritis. Under certain circumstances arboviruses have been recovered from urine, milk, semen, and vitreous fluid.
Principle	Susceptible cell culture systems are available for the attempted isolation of the presumed etiologic agent of an illness or disease. Following successful isolation, the isolate may be positively identified and an antigen prepared from this isolate or the virus itself may be used to test the patient's serum for the presence of antibodies to the viral isolate. If antibodies are detected, this exercise confirms that the isolate was the causative agent of the illness or disease. In certain instances, serum from a patient may not be available. Under those circumstances, one relies on reisolation of the causative virus from the same original specimen. Reisolation should always be attempted, however, whether serum is available from the patient or not.
Materials and Reagents	Vero cell culture monolayers or other suitably susceptible cell cultures C6/36 cell cultures-cloned *Ae. albopictus* mosquito cell.
Procedure	Available tissues or fluids should be divided for viral isolation, electron microscopy, and for immunohistochemical examination. Tissues should be collected aseptically and rapidly transported to the laboratory in viral transport. The aliquot for viral isolation should be immediately frozen at –70° C in a mechanical freezer or stored on dry ice. Samples for viral isolation should be kept frozen continuously, avoiding freeze-thaw cycles that inactivate virus.

(Continued)

Appendix A. Viral Isolation Protocol (for Cell Culture) (Cont.)	
Procedure	The aliquot for electron microscopy should be minced and place directly in glutaraldehyde. Autolytic changes occur rapidly and tissues should be fixed as quickly as possible. A portion of the sample should be fixed in buffered formalin or, preferably, embedded in freeze-media and frozen, to prepare sections for immunohistochemical examination. Processed specimens should be inoculated into cell cultures with a minimum of delay. Sera from patients with acute febrile illnesses can be used undiluted for virus isolation or at dilutions of 1:10 and 1:100 in a protein containing diluent. It is important to inoculate unknown specimens at two or preferably more dilutions (undiluted to 10^{-2}). Shell vial cultures or 25cm^2 flask cultures of Vero are inoculated and observed for the production of CPE during 10–14 days. For shell vials, a total volume of 400 µl is inoculated, followed by centrifugation at 100 x g for one hour at 37°C. A portion of the cell supernatant can be collected and tested for the presence of virus by either targeted RT-PCR or consensus RT-PCR assays. Alternatively cells are harvested and spot slides are prepared for IFA examination using monoclonal dengue type-specific antibodies.
Controls	Uninoculated Vero and C6/36 cells
Interpretation	Positive virus isolation, reisolation, and definitive identification define the etiologic agent of the patient's illness. If paired sera or a convalescent serum from that patient are available, the identified viral isolate is tested serologically with the patient's sera to verify antibody response to that virus.

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Appendix B. Real-Time Reverse Transcriptase-Polymerase Chain Reaction Protocol

Real time RT-PCR can be performed using a number of commercially available kits. Either the BioRad iScript 1 Step RT-qPCR (#170-8895) or the QIAGEN QuantiTect Probe RT-PCR kit (#204443) is currently used by the Arboviral Diagnostic Laboratory at DVBD, CDC. The two kits are nearly identical in the reaction setup, with one exception: the volume of enzyme used in the QIAGEN kit is 0.5 µl per reaction, instead of 1.0 µl; the volume of water in the master mix is adjusted by 0.5 µl to account for this. The setup shown below is for the QIAGEN kit. Note also that the volume of RNA added per reaction below is 10 µl but can be increased or decreased with the appropriate adjustment of total volume with water.

Component	Vol. per reaction	10 Reactions
RNase free water	13.2 µl	132 µl
2X Ready mix	25 µl	250 µl
primer 1 (100 µM stock)	0.5 µl	5 µl
primer 2 (100 µM stock)	0.5 µl	0.5 µl
FAM/ probe (25 µM stock)	0.30 µl	3.0 µl
enzyme	0.5 µl	5 µl

Prepare a reagent “master mix” according to the number of reactions desired. The master mix should be prepared in a “clean room” that is physically separated from all other laboratory activities and that has dedicated reagents and equipment (e.g., pipettes). For 10 samples make a 10X master mix (see above) by multiplying the volumes of all individual reagents by 10. Combine the reagents in the above order in an RNase free centrifuge tube **on ice**. Divide the master mix into 10 portions of 40 µl each into either 0.2 ml optical (specifically for TaqMan assays; emission fluorescence is read through the cap) PCR tubes or a 96 well optical PCR plate. Finally add 10 µl of the individual RNA sample to each tube or well. All samples are tested in duplicate wells. **Include several “NO RNA”** negative controls (NTC) by adding water instead of any RNA. Include a positive control or a dilution series of known quantities of positive control RNA if setting up a quantitative assay.

Cycling conditions (QIAGEN conditions for Real Time RT-PCR):		
1 cycle:		**45 cycles:**
50°C for 30 min	(RT reaction)	95°C 15 sec
95°C for 15 min	(enzyme activation)	60°C 1 min

(Continued)

Appendix B. Real-Time Reverse Transcriptase-Polymerase Chain Reaction Protocol (Cont.)

Interpretation

The following algorithm is used by the Arboviral Diagnostic Laboratory at DVBD, CDC to evaluate the TaqMan results.

Positive:	• Cutoff (Ct) value 38 in duplicate wells
Equivocal:	• Ct value 38 in one of two wells
Negative:	• Ct values >38 in duplicate wells

All positive and equivocal samples are repeated with a second set of primer/probes for confirmation. A positive result in any of the negative controls invalidates the entire run. Failure of the positive control to generate a positive result also invalidates the entire run.

I. RNA EXTRACTION

Avoiding Contamination and Working with RNA

- Maintain physically separated work areas; one should be dedicated to **pre-amplification RNA work** (RNA extraction and RNA addition) and the other to **Master mix** production.
- Utilize dedicated or separate equipment within pre- and post-amplification areas, especially pipets and centrifuges.
- Always wear gloves, even when handling unopened tubes.
- Quickly open and close tubes and avoid touching any inside portion.
- Use RNase free plastic disposable tubes and pipet tips.
- Use aerosol block pipet tips.
- Use RNase free water.
- Prepare all reagents on ice.

1. Solid phase samples (mosquitoes or tissues) are first homogenized in an isotonic buffer to produce a liquid homogenate. RNA is extracted from liquid specimens (CSF or serum) without any pre-treatment as described below. Tissue specimens (~10mm^3) are homogenized in 1 ml of BA-1 diluent using TenBroeck tissue grinders. Mosquito specimens are homogenized in TenBroeck tissue grinders or by using the copper clad steel bead (BB) grinding technique. With both techniques the homogenates are clarified by centrifugation in a microcentrifuge (e.g., Eppendorf) at maximum speed for 5 minutes to pellet any particulate material.

(Continued)

Appendix B. Real-Time Reverse Transcriptase-Polymerase Chain Reaction Protocol (Cont.)

2. Extract RNA from 140 µl of the liquid specimen (CSF, serum, or clarified homogenate) using the QiAmp viral RNA kit (QIAGEN part # 52904). Follow the manufacturer's protocol exactly. **NOTE: For mosquito specimens add one additional wash with AW1**. Extract at least two negative controls and two positive controls along with the test specimens. The positive controls should differ in the amount of target RNA present (i.e., a pre-determined high positive and a low positive). The volume of sample extracted can be greater or less than the standard volume stated in the QIAGEN protocol (140 µl) with the appropriate adjustments to all other volumes in the protocol.

Appendix C. IgM and IgG Serologic Assay Protocols

IgM ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Introduction	Assays that detect viral specific immunoglobulin M (IgM) are advantageous because they detect antibodies produced during the first few days after onset of clinical symptoms in a primary infection. This obviates the need for convalescent-phase specimens in many cases. IgM capture is the optimal approach to IgM detection: it is simple, sensitive, and is applicable to serum and cerebrospinal fluid (CSF) samples from a variety of animal species (e.g., human, equine, avian). False-positive reactions due to rheumatoid factor are minimized.
Principle	IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) provides a useful alternative to immunofluorescence for documentation of a serologic response. ELISA is less subjective than immunofluorescence and large numbers of samples can be processed. The principle of ELISA is similar to that of immunofluorescence. Anti-IgM (the capture antibody) is coated on 96-well plates in the Arboviral Diagnostic Laboratory at DVBD, CDC. This is followed sequentially by the patient's serum, then known non-infectious viral antigen. The presence of antigen is detected by using enzyme-conjugated anti-viral antibody, and a colorimetric result is generated by the interaction of the enzyme and a chromogenic substrate. This constitutes the MAC-ELISA.
Safety	The procedure should be performed under laboratory safety conditions that take into consideration the potential infectious nature of the serum specimens involved. Lab coat, gloves, and a laminar flow hood is recommended.

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgM ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Materials and Reagents	**Coating buffer:** Carbonate/bicarbonate buffer pH 9.6, 1.59g Na_2CO_3 + 2.93g $NaHCO_3$ diluted in 1L water. **Wash buffer:** Phosphate buffered saline (PBS), 0.05% Tween 20, pH 7.2. PBS is available in powdered form from multiple commercial sources. **Blocking buffer:** PBS/5% milk/ 0.5% Tween 20. **Stop solution:** 1 N H_2SO_4. **Coating antibody:** Goat anti-human IgM, Kirkegaard and Perry Laboratories cat# 01-10-03. **Viral antigen:** Sucrose-acetone extracted suckling mouse brain viral antigens, non-infectious, previously titrated. **Normal antigen:** Sucrose-acetone extracted suckling mouse brain antigen from mock-infected animals. **Detecting antibody conjugate:** Horseradish peroxidase conjugated monoclonal antibody, previously titered. **Substrate:** 3,3'5, 5' tetramethylbenzidine base (TMB-ELISA), Gibco cat# 15980-0414. **Plates:** Immulon II HB flat-bottomed 96 well plates, Dynatech Technologies cat# 3455. Microplate washer Microplate reader

Materials and Reagents	Incubator Single and multi-channel pipettors Reagent reservoirs Ziploc bags, paper towels
Clinical specimens	Acute and convalescent human serum and/or cerebrospinal fluid (CSF) specimens Previously tested antibody-positive and antibody-negative human sera for controls Note: Store all diagnostic specimens at 4°C prior to testing, and -20°C after all anticipated testing has been completed. Avoid repeated freeze-thaw cycles.
Procedure	Note: The following procedure includes information on quality control and interpretation. Each serum specimen is tested in triplicate on both viral and normal antigens. Eight test specimens can be analyzed per plate. CSF specimens are usually tested only singly. 1. Using a fine-tipped permanent marker, number and label the plates. Identify the location of each clinical specimen (S1–S8) by using the appropriate laboratory code number. *To keep timing of reagent addition consistent, process plates in the order that they are numbered during all steps of the procedure.* Plates should be kept in an enclosed, humidified environment during all incubation times with the exception of the coating step. A large ziploc bag containing a moist paper towel works well for this purpose.

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgM ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Procedure	2. Coat the inner 60 wells of 96 well plates with 75 µl well of goat anti-human IgM diluted 1:2000 in coating buffer pH 9.6. I**ncubate at 4°C overnight.** 3. Dump out the coating antibody and blot plates on paper towels. Block plates with 200µl blocking buffer per well. **Incubate at room temperature for 30 minutes.** **4.** Wash wells 5X with wash buffer by using an automatic plate washer. Wells should be filled to the top each cycle. 5. Add 50µl per well of the patient's serum (S) diluted 1:400 in wash buffer to a block of six wells, or add patient's CSF undiluted to two wells only, so that the CSF will be tested singly against the viral and normal antigens. Note: CSF can be diluted to a maximum of 1:5 in wash buffer if necessary. Add positive control human serum (Ref) diluted in wash buffer according to a previous titration, and a negative human serum control (N) diluted 1:400 in wash buffer to a block of six wells each. Incubate plates for **1 hour at 37°C** in a humidified chamber. 6. Wash 5X. 7. Dilute viral antigen in wash buffer according to a previous titration. Add 50µl per well to the left three wells of each serum block. To the right three wells of each block, add 50µl per well of normal antigen diluted in wash buffer to the same concentration as the viral antigen. **Incubate overnight at 4°C** in a humidified chamber. 8. Wash 5X.

Procedure	9. Add 50µl per well of horseradish peroxidase-conjugated monoclonal antibody, broadly cross-reactive for the appropriate viral antigenic group, diluted in blocking buffer, according to a previous titration. **Incubate for one hour at 37°C in a humid chamber**. 10. Turn on plate reader to warm up, and remove TMB-ELISA from refrigerator. 11. Wash plates 5X **twice**. Turn the plates 180° in the washer after the first series of five cycles. This promotes consistent results. 12. While the plate is at room temperature, add 75µl per well of TMB substrate to all wells. Immediately cover plates to block out light. Incubate at room temperature for **10 minutes**. A blue color will develop in antibody-positive wells. 13. Add 50µl per well of stop solution to all wells, including the outer rows of wells on the plate (the plate reader itself should be set to zero on some of these wells). The wells that were blue will now change to a yellow color. Allow plates to sit at room temperature for one minute. Read plates in microtiter plate reader by using a 450 nm filter.

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgM ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Practical considerations	1. Plates can be coated and kept at 4°C for up to a week. 2. Undiluted control sera can be stored at 4°C for up to two weeks. 3. Reconstituted, undiluted viral and normal antigens can be stored at –20°C for an undefined period of time. 4. Test and control sera can be diluted to the working dilutions and refrigerated one day prior to use. Antigens and conjugate *must be diluted to the working dilutions immediately prior to use.* The MAC-ELISA should be restandardized periodically. This should occur when new lot numbers of reagents are introduced, and at the very least, once a year. It is recommended that the mean optical density of the positive control serum reacted on the viral antigen be set to approximately at 1.0. The normal control serum reacted on the viral antigen should be around 0.2 (this varies). The standardization of reagents is normally achieved via titration, always comparing the optical densities of the reagents when reacted on viral and normal antigen.
Results	Before the results can be calculated for each clinical specimen, the test must be determined to be **valid**. For a **valid** test the following must be true:

Results	$\frac{\text{Mean OD of the positive control serum reacted on viral antigen (P)}}{\text{Mean OD of the negative control serum reacted on viral antigen (N)}}$ must be greater than or equal to 2.0. **This is the P/N of the positive control.** Test validity must be determined for each plate. Results for the clinical specimens may only be determined if the test is valid. If the test is not valid, then that plate must be repeated. If the test still fails after a repeat, then one or more of the reagent or test parameters was likely in error, and troubleshooting should be performed. To determine whether the clinical specimens (S1–S8) contain IgM to the viral antigen (which would indicate recent infections with that virus) the following must be calculated: $\frac{\text{Mean OD of the test specimen reacted on viral antigen (P)}}{\text{Mean OD of the negative control serum reacted on viral antigen (N)}}$ **This is the P/N of the test specimen.** For a specimen to be considered IgM-positive to the test virus, the P/N must be greater than or equal to 2.0. **In addition** the value of P for the test specimen must be greater than or equal to twice the mean OD of the test specimen reacted on normal antigen. If this requirement is not met, non-specific background is being generated, and the result **must** be reported as uninterpretable.

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgM ANTIBODY CAPTURE ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Interpretation	All patient P/N values greater than or equal to 2.0 should be reported as presumptive IgM-positive (see paragraph below), as long as they meet the requirements listed above. In the event that an early acute CSF or serum is negative by this test, a convalescent serum specimen must be requested and tested before that patient is reported as negative for serological evidence of recent viral infection. Without testing of a convalescent specimen, a negative result may reflect testing of an acute-phase specimen obtained before antibody has risen to detectable levels. In most patients, IgM is detectable eight days post-onset of symptoms from an alpha-, flavi-, or California group virus infection. IgM persists for at least 45 days, and often for as long as 90 days. The positive P/N cut-off value of 2.0 is empirical, based on experience and convention. P/N values that lie between 2.0 and 3.0 should be considered suspect false-positives. Further tests should be performed to determine the status of these specimens. It should be stressed that the P/N value for a specimen at the screening dilution of 1:400 is not an indication of absolute antibody concentration, i.e., the P/N value is not quantitative.

Interpretation	It is further recommended that for sera, all positive results should be confirmed by titration using 6, 2-fold dilutions of the serum specimens compared to a similar titration of the negative control serum. Linear curves indicate true seropositivity. Flat or undulating titration curves indicate false-positive results.
References	• Tsai, TH: Arboviruses, In Rose NR, Marcario EC, Fahey JL, Friedman H, and Penn GM, (Eds): Manual of Clinical Laboratory Immunology, 4th Edition, American Society for Microbiology: 606-618, 1976. • Diagnosis of Infections caused by Viruses, Chlamydia, Rickettsia, In Koneman EW, Allen SD, Janda WM, Schreckenberger PC, and Winn Jr. WC , (Eds): Diagnostic Microbiology, 4th Edition, JB Lippicott Co: 956-1074, 1992. • Monath, TP, Nystrom, RR, Bailey, RE, Calisher, CH, and Muth, DJ:Immunoglobulin M antibody capture enzyme-linked immunosorbent assay for diagnosis of St. Louis encephalitis. J Clin Microbiol. 1984;20:784-790. • Martin, DA., Muth, DA., Brown, T., Karabatsos, N., and Roehrig, JT. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays (MAC-ELISA) for routine diagnosis of arboviral infections. J Clin Microbiol. 2000 May; 38(5): 1823-6.

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgG ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Introduction	Immunoglobulin G (IgG) is less virus-specific than IgM, appears in serum slightly later in the course of infection than IgM, and remains detectable until long after IgM ceases to be present. Using the IgG-ELISA in parallel with the IgM Antibody Capture Enzyme-linked immunosorbent assay (MAC-ELISA), one can observe the relative rises and falls in antibody levels in paired serum samples. The test is simple and sensitive. It is applicable to serum specimens but not generally to CSF samples. False-positive reactions due to rheumatoid factor are minimized.
Principle	The IgG-ELISA provides a useful alternative to immunofluorescence for identification of a viral isolate or documentation of a serologic response. IgG-ELISA is less subjective than immunofluorescence and large numbers of samples can be processed. Viral group-reactive monoclonal antibody is coated on a 96-well plate, followed sequentially by known viral antigen, patient serum, enzyme-conjugated human IgG, and lastly substrate for the conjugate used. This constitutes the IgG-ELISA used at the Arboviral Diagnostic Laboratory, DVBD, CDC.

Safety	The procedure should be performed under laboratory safety conditions that take into consideration the potential infectious nature of the serum specimens involved. Lab coat, gloves, and a laminar flow hood is recommended.
Materials and Reagents	**Coating buffer:** Carbonate/bicarbonate buffer pH 9.6, 1.59g Na2CO3 + 2.93g NaHCO3 diluted in 1L water. **Wash buffer:** Phosphate buffered saline (PBS), 0.05% Tween 20, pH 7.2. PBS is available in powdered form from multiple commercial sources. **Blocking buffer:** 3% goat serum, 1% Tween-20, in PBS. **Coating antibody:** Group-specific monoclonal antibody, previously titrated. **Viral antigen:** Sucrose-acetone extracted suckling mouse brain viral antigens, non-infectious, previously titrated. **Normal antigen:** Sucrose-acetone extracted suckling mouse brain antigen from mock-infected animals. **Detecting antibody conjugate:** Alkaline phosphatase-conjugated goat anti-human IgG Fcγ portion, previously. titrated (Jackson Immunoresearch cat# 109-055-098) **Substrate:** 3 mg/ml p-nitrophenyl phosphate, disodium (Sigma 104, Sigma diagnostics cat# 104-105) in 1M Tris (base) pH 8.0 (note: the Tris requires considerable conc. HCl for the pH adjustment). **Stop solution:** 3M NaOH.

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgG ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Materials and Reagents	**Plates:** Immulon II HB flat-bottomed 96 well plates. Dynatech Technologies cat# 3455. Microplate washer Microplate reader Incubator Single and multi-channel pipettors Reagent reservoirs Ziploc bags, paper towels
Clinical specimens	Acute and convalescent human serum Note: Store all diagnostic specimens at 4°C prior to testing, and at -20°C after all anticipated testing has been completed. Avoid repeated freeze-thaw cycles.

Procedure	Note: The following procedure includes information on quality control and interpretation. Each serum specimen is tested in triplicate on both viral and normal antigens. Eight test specimens can be analyzed per plate. 1. Using a fine-tipped permanent marker, number and label the plates. Identify the location of each clinical specimen (S1–S8) by using the appropriate laboratory code number. T*o keep timing of reagent addition consistent, process plates in the order that they are numbered during all steps of the procedure.* Plates should be kept in an enclosed, humidified environment during all incubation times with the exception of the coating step. A large ziploc bag containing a moist paper towel works well for this purpose. 2. Coat the inner 60 wells of 96 well plates with 75µl/well of the appropriate group-reactive monoclonal antibody diluted in coating buffer according to prior titration. I**ncubate at 4°C overnight.** 3. Dump out the coating antibody and blot plates on paper towels. Block plates with 200µl blocking buffer per well. **Incubate at room temperature for 30 minutes.** 4. Wash wells 5X with wash buffer by using an automatic plate washer. Wells should be filled to the top each cycle. 5. Dilute viral antigen in wash buffer according to a previous titration. Add 50µl per well to the left three wells of each serum block. To the right three wells of each block, add 50µl per well of normal antigen diluted in wash buffer to the same concentration as the viral antigen. **Incubate overnight at 4°C** in a humidified chamber. 6. Wash 5X. 7. Add 50µl per well of the patient's serum (S) diluted 1:400 in wash buffer to a block of six wells. Add positive control human serum (Ref) diluted in wash buffer according to a previous titration, and a negative human serum control (N) diluted 1:400 in wash buffer to a block of 6 wells each. I**ncubate plates for one hour at 37°C** in a humidified chamber.

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgG ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Procedure	8. Wash 5X. 9. Add 50µl per well of alkaline phosphatase-conjugated goat anti-human IgG diluted in blocking buffer, according to prior titration. **Incubate for one hour at 37°C** in a humid chamber. 10. Turn on plate reader to warm up and dissolve substrate tablets in tris buffer about 15 minutes prior to adding it to the plates. 11. Wash plates 5X **twice**. Turn the plates 180° in the washer after the first series of five cycles. This promotes consistent results. 12. While the plate is at room temperature, add 75µl per well of Sigma 104 substrate to all wells. Immediately cover plates to block out light. Incubate at room temperature for **30 minutes.** A yellow color will develop in antibody-positive wells. 13. Add 35µl per well of stop solution to all wells, including the outer rows of wells on the plate (the plate reader itself should be set to zero on some of these wells). Reactive wells will remain a yellow color. Allow plates to sit at room temperature for one minute. Read plates in microtiter plate reader by using a 405 nm filter.

Practical considerations	1. Plates can be coated and kept at 4°C for up to a week. 2. Undiluted control sera can be stored at 4°C for up to two weeks. 3. Reconstituted, undiluted viral and normal antigens can be stored at –20°C for an undefined period of time. 4. Test and control sera can be diluted to the working dilutions and refrigerated one day prior to use. Antigens and conjugate **must be diluted to the working dilutions immediately prior to use.** The IgG-ELISA should be restandardized periodically. This should occur when new lot numbers of reagents are introduced, and at the very least, once a year. It is recommended that the mean optical density of the positive control serum reacted on the viral antigen be set to approximately. 1.0. The normal control serum reacted on the viral antigen should be around 0.2 (this varies). The standardization of reagents is normally achieved via titration, always comparing the optical densities of the reagents when reacted on viral and normal antigen.
Results	Before the results can be calculated for each clinical specimen, the test must be determined to be valid. For a valid test the following must be true: $\frac{\text{Mean OD of the positive control serum reacted on viral antigen (P)}}{\text{Mean OD of the negative control serum reacted on viral antigen (N)}}$ must be greater than or equal to 2.0. **This is the P/N of the positive control.**

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgG ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Results	Test validity must be determined for each plate. Results for the clinical specimens may only be determined if the test is valid. If the test is not valid, then that plate must be repeated. If the test still fails after a repeat, then one or more of the reagent or test parameters was likely in error, and troubleshooting should be performed. To determine whether the clinical specimens (S1-S8) contain IgG to the viral antigen (which would indicate either recent or past infections with that virus) the following must be calculated: $\frac{\text{Mean OD of the test specimen reacted on viral antigen (P)}}{\text{Mean OD of the negative control serum reacted on viral antigen (N)}}$ **This is the P/N of the test specimen.** For a specimen to be considered IgG-positive to the test virus, the P/N must be greater than or equal to 2.0. In addition the value of P for the test specimen **must be** greater than or equal to twice the mean OD of the test specimen reacted on normal antigen. If this requirement is not met, non-specific background is being generated, and the result must be reported as uninterpretable.

Interpretation	All patient P/N values greater than or equal to 2.0 should be reported as presumptive IgG-positive (see the explanatory paragraph on the following page), as long as they meet the requirements listed above. **Interpretations of IgG-ELISAs should always be made in the context of the corresponding MAC-ELISA, and the date of collection with respect to onset of symptoms.** A positive IgG-ELISA result on its own cannot distinguish a recent from a past infection, due to the persistence of IgG from past infections. IgG is also more cross-reactive than IgM, which means that a positive result by the IgG-ELISA may in fact indicate the presence of antibody to a related virus. In most cases, IgG is detectable 12 days post-onset of symptoms from an alpha-, flavi-, or California group virus infection and persists for long periods of time, possibly for years. Some examples of common scenarios are listed below: 1. A positive IgG-ELISA result with a positive MAC-ELISA result would indicate the presence of a recent infection. 2. A negative IgG-ELISA result with a positive MAC-ELISA result in an acute specimen would indicate a recent infection in which the IgG antibody had not yet risen to detectable levels. 3. A positive IgG-ELISA result and a negative MAC-ELISA result from a specimen timed between approximately 8 and 45 days post-onset of symptoms would suggest the occurrence of a past infection (remember that IgG to a virus is often cross-reactive with other viruses from the same genus).

(Continued)

Appendix C. IgM and IgG Serologic Assay Protocols (Cont.)

IgG ENZYME-LINKED IMMUNOSORBENT ASSAY PROTOCOL

Interpretation	4. For a single late specimen (obtained later than 45 days post-onset of symptoms) yielding a positive IgG-ELISA result and a negative MAC-ELISA result, the distinction between the current infection and past infections cannot be made. 5. A negative IgG-ELISA result plus a negative MAC-ELISA result indicates the lack of any recent or past infections with the test virus if the sample was collected >7 days post illness onset. These results on a more acute sample cannot rule out the infection as the antibody response may not have had time to form. The positive P/N cut-off value of 2.0 is empirical, based on experience and convention. P/N values that lie between 2.0 and 3.0 should be considered suspect false-positives. Further tests should be performed to determine the status of these specimens. It should be stressed that the P/N value for a specimen at the screening dilution of 1:400 is not an indication of absolute antibody concentration, i.e., the P/N value is not quantitative.

References	• Tsai, TH: Arboviruses, In Rose NR, Marcario EC, Fahey JL, Friedman H, and Penn GM, (Eds): Manual of Clinical Laboratory Immunology, 4th Edition, American Society for Microbiology: 606-618, 1976. • Diagnosis of Infections caused by Viruses, Chlamydia, Rickettsia, In Koneman EW, Allen SD, Janda WM, Schreckenberger PC, and Winn Jr. WC , (Eds): Diagnostic Microbiology, 4th Edition, JB Lippicott Co: 956-1074, 1992. • Johnson, AJ., Martin, DA., Karabatsos, N., and Roehrig, JT. Detection of anti-arboviral immunoglobulin G by using a monoclonal antibody-based capture enzyme-linked immunosorbent assay. J Clin Microbiol. 2000 May; 38(5): 1827-31.

Appendix D. Example of a Case Report Form

Basic Data

Last name _____ First name: _____

Sex: ( ) male ( ) female
Date of birth: ____/____/____/ age: |__|__| years |__|__|months |__|__|days
Occupation: _____
Address: _____
Zipcode: |__|__|__|__|__|__|__| telephone number: |__|__|__|__|__|__|__|

Clinical Information

Clinical history number: _____
Date of symptom onset: ____/____/____/ Epidemiological week: |__|__|
Number of days with symptoms: ____/____/ Date of first medical consult: ____/____/____/
Date of hospitalization: ____/____/____/
Death: Yes ( ) No ( ) Date: ____/____/____/

Symptoms

	Yes	No		Yes	No
Fever	☐	☐	Myalgia	☐	☐
Arthritis	☐	☐	Back pain	☐	☐
If yes, where:			Headache	☐	☐
Hands	☐	☐	Nausea	☐	☐
Feet	☐	☐	Mucosal bleeding	☐	☐
Ankles	☐	☐	Vomiting	☐	☐
Other	☐	☐	Asthenia	☐	☐
Arthralgia	☐	☐	Meningoencephalitis	☐	☐
Periarticular edema	☐	☐			
Skin manifestations	☐	☐			

If yes, describe: _____
Other _____

Clinical diagnosis _____

Laboratory information

Blood sample testing for CHIKV infection:

Date of collection: ____/____/____/

Serology - IgM	Yes ☐	No ☐		
Result:	Positive ☐	Negative ☐	Date of result	____/____/____/
Serology - IgG	Yes ☐	No ☐		
Result:	Positive ☐	Negative ☐	Date of result	____/____/____/
RT-PCR	Yes ☐	No ☐		
Result:	Positive ☐	Negative ☐	Date of result	____/____/____/
Viral isolation	Yes ☐	No ☐		
Result	Positive ☐	Negative ☐	Date of result	____/____/____/

(Continued)

Appendix D. Example of a Case Report Form (Cont.)

Epidemiological information

History of travel within the previous 30 days prior to symptom onset: Yes No
If yes, where: Country _____ City _____
Place of residence:
Community _____ Locality _____

Blood or blood products received within the previous 30 days prior to symptoms onset
Yes No

Final classification:

Discarded: I__I
Confirmed: I__I
Suspected: I__I
Date of notification: _____/_____/_____/

Name of reporting personnel: _____

Appendix E. Report for an Event or Outbreak of Public Health Importance

NOTIFICATION_____Region_____

A case or outbreak of *[EVENT OF HEALTH]* has occurred in the town *[LOCATION],* commune, region [NAME Municipalities and Regions] on *[MONTH and YEAR or time period].*

As of *[Report Date], [CASE NUMBER]* of *[EVENT OF HEALTH]* presenting with *[MAIN SIGNS AND SYMPTOMS]* were seen at *[INSTITUTION OR SECTOR OR OTHER COMMUNITY].* These cases are occurring in an areas with an approximate population of *[N ° OF INHABITANTS or exposed population].*

The cases have occurred between the *[START DATE, WEEK EPIDEMIOLOGICAL]* and *[DATE TO END* or *TODAY].* The affected area is mainly [urban or rural] and has previously presented occasional outbreaks of *[PREVIOUS OUTBREAKS].*

The most striking feature of the cases is *[FEATURE PERSON: SEX, AGE, OR OTHER TO DEFINE CHARACTERISTICS of people affected].*

Of all the cases, *[# DECEASED]* died and *[# HOSPITALIZED]* requiring hospitalization. Cases either dying or requiring hospitalization have *[COURSE TYPE: DEATH, HIGH WITHOUT COMPLICATIONS, AFTERMATH, ETC].*

[# samples] sample(s) of *[SPECIMEN TYPE]* have been taken and sent to [LAB] for processing where testing is ongoing or has confirmed [Etiologic agent].

Epidemiological research indicates that the outbreak was produced by *[POSSIBLE MECHANISM, SOURCE, exposure factor].*

The control actions have been taken are as follows: *[ACTION]*

Note: The immediate notification must include whatever is possible to complete the learning of the outbreak. Once investigated, it is sent back the full format to regional World Health Organization office.

Appendix F. Vector Control Procedures

There are a number of vector control procedures that should be considered to mitigate the risk of CHIKV expansion in an area (Table F1).

Table F1. Vector control procedures

Environmental management • Reduce larval habitats • Manage (wash/cover) containers • Discard/recycle containers • Reduce human-vector contact • Install window screens	**Adult mosquito control** • Use of IT bednets • Use of IT curtains • Lethal ovitraps • Space sprays • Indoor residual treatments
Larval control • Source reduction • Chemical control • Biological control	**Resistance testing** **Operational research and efficacy evaluation**

Chemical Control of Larval Habitats

If potable water vessels cannot be screened or covered, they should be cleaned regularly or treated to stop larval production according to WHO Pesticide Evaluation Scheme (WHOPES) recommended practices for potable water.[66] Potential larval habitats that do not contain water intended for human consumption may be treated with larvicides listed in Table F2.

Table F2. WHO-recommended compounds and formulations for control of mosquito larvae in container habitats.[a]

Insecticide	Formulation[b]	Dosage[c]	WHO hazard classification of active ingredient[d]
Organophosphates			
Pirimiphos-methyl	EC	1	III
Temephos	EC,GR	1	U
Insect growth regulators			
Diflubenzuron	DT,GR, WP	0.02-0.25	U
rs-methoprene[e]	EC	1	U
Novaluron	EC	0.01-0.05	NA
Pyriproxyfen[e]	GR	0.01	U
Biopesticides			
Bacillus thuringiensis[e] israelenses	WG	1-5 mg/L	U
Spinosad	DT,GR,SC	0.1-0.5	U

[a] WHO recommendations on the use of pesticides in public health are valid only if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available at http://www.who.int/whopes/quality/en/. Instructions must always be followed when using insecticides.

[b] DT=tablet for direct application; GR=granule; EC=emulsifiable concentrate; WG=water-dispersible granule; WP=wettable power; SC=suspension concentrate.

[c] mg/L of active ingredient for control of container-breeding mosquitoes.

[d] Class II=moderately hazardous; Class III=slightly hazardous; Class U=Unlikely to pose an acute hazard in normal use; NA=not available.

[e] Can be used at recommended dosages in potable water.

Space Sprays for Adult Mosquito Control

Space sprays for *Ae. aegypti* and *Ae. albopictus* control are most effective when the inside of houses and associated yards are individually treated with handheld sprayers. Repeated applications are required to kill newly emerging adults. In an epidemic response, space sprays should be carried out with handheld sprayers whenever possible, or with truck-mounted sprayers to increase speed of coverage, every two to three days.[66] Attention to resistance testing, calibration of equipment, droplet size, and timing of application are all critical to effective use of these tools.[66] Large-scale truck and airplane based application of pesticides is generally not effective in controlling *Ae. aegypti* when used alone.[76] Large-scale space spraying must be used as a component of an IVM program to be effective. Table F3 provides information on insecticides suitable to *Ae. aegypti* and *Ae. albopictus* control.

Table F3. Examples of insecticides for cold aerosol or thermal fog application against mosquitoes.[a]

Insecticide	Chemical	Dosage of active ingredient (g/ha)		WHO hazard classification of active ingredient[c]
		Cold aerosols	Thermal fogs[b]	
Fenitrothion	Organophosphate	250–300	250–300	II
Malathion	Organophosphate	112–600	500–600	III
Pirimiphos -methyl	Organophosphate	230–330	180–200	III
Bioresmethrin	Pyrethroid	5	10	U
Cyfluthrin	Pyrethroid	1–2	1–2	II
Cypermethrin	Pyrethroid	1–3	–	II
Cyphenothrin	Pyrethroid	2–5	5–10	II
d,d-trans-Cyphenothrin	Pyrethroid	1–2	2.5–5	NA
Deltamethrin	Pyrethroid	0.5–1.0	0.5–1.0	II
D-Phenothrin	Pyrethroid	5–20	-	U
Etofenprox	Pyrethroid	10–20	10–20	U
λ Cyhalothrin	Pyrethroid	1.0	1	II
Permethrin	Pyrethroid	5	10	II
Resmethrin	Pyrethroid	2–4	4	III

[a] Adapted from: Pesticides and their application for the control of vectors and pests of public health importance.[77] Label instructions must always be followed when using insecticides.

[b] The strength of the finished formulation when applied depends on the performance of the spraying equipment used.

[c] Class II=moderately hazardous; class III=slightly hazardous; class U=unlikely to pose an acute hazard in normal use; NA=not available.

Indoor Residual Sprays for Adult Mosquito Control

Traditionally, Indoor Residual Sprays (IRS) have been used most successfully against malaria vectors (Table F4). IRS treatment should be effective against *Ae. aegypti*, which rests indoors, though it may be difficult to apply operationally. Generally, all interior walls and ceilings of a house are treated. For control of *Ae. aegypti*, it is important to treat bedrooms, closets, the undersides of beds, and other dark areas where *Ae. aegypti* adults rest before and after taking a bloodmeal. Residents should be informed that IRS are safe when applied according to the label, but that individuals with health concerns, such as those with asthma or allergies, should take measures to reduce or eliminate exposure during the application process.

Resistance Testing

Frequent application of the same insecticide or class of insecticide may select for individual mosquitoes that are able to survive pesticide applications.[78] Resistance is a heritable change in the sensitivity of a mosquito population to an insecticide that may lead to failure of the pesticide to yield the expected degree of control.

The insecticides available for use as adulticides are limited, and fall into three chemical classes: organophosphates, carbamates, and pyrethroids. Some products for larviciding have different modes of actions, such as insect growth regulators and microbials tools.[78] However, the most commonly used product for controlling larvae of *Ae. aegypti* in containers is the organophosphate temephos. Resistance to temephos has been detected in multiple *Ae. aegypti* populations in the Americas[79, 80] and poses a serious threat to *Ae. aegypti* control. Little information is available about resistance in *Ae. albopictus* populations in the Region.

Control programs must include a resistance monitoring program[81–83] (additional references are available at http://www.who.int/whopes/resistance/en/) to assess efficacy and to establish a pesticide rotation plan to mitigate the development of resistance.

Table F4. WHO recommended insecticides for use as indoor residual sprays.[a]

Insecticide compounds and formulations[b]	Class group[c]	Dosage g a.i./m²	Mode of action	Duration of effective action (months)
DDT WP	OC	1–2	contact	>6
Malathion WP	OP	2	contact	2-3
Fenitrothion WP	OP	2	contact & airborne	3-6
Pirimiphos-methyl WP & EC	OP	1–2	contact & airborne	2-3
Bendiocarb WP	C	0.1–0.4	contact & airborne	2-6
Propoxur WP	C	1–2	contact & airborne	3-6
Alpha-cupermethrin WP & SC	PY	0.02–0.03	contact	4-6
Bifenthrin WP	PY	0.025–0.05	contact	3-6
Cyfluthrin WP	PY	0.02–0.05	contact	3-6
Deltamethrin WP, WG	PY	0.02–0.025	contact	3-6
Etofenprox WP	PY	0.1–0.3	contact	3-6
Lambda-cyhalothrin WP, CS	PY	0.02–0.03	contact	3-6

[a] Available at (http://www.who.int/whopes/Insecticides_IRS_Malaria_09.pdf).

[b] CS = capsule suspension; EC = emulsifiable concentrate; SC = suspension concentrate; WG = water dispersible granule; WP = wettable

[c] OC = Organochlorines; OP = Organophosphates; C = Carbamates; PY = Pyrethroids

Note: WHO recommendations on the use of pesticides in public health are valid only if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available on WHO's homepage at http://www.who.int/whopes/quality/en/.

Supervision, Safety, and Quality Assurance

Continuous monitoring and supervision are required to ensure that staff are adequately trained and are following appropriately technical guidelines for pesticide application and personal safety.[77] IVM programs must include a quality assurance program designed to monitor the effectiveness of the control activities. A quality assurance program should monitor applicator performance and control outcomes. Control failures may be due to misapplication, incomplete coverage, or insecticide resistance, and must be corrected immediately. Quality assurance efforts should be continuous, systematic, and independent.

Appendix G. Vector Control for CHIKV Containment

Virus containment efforts should be initiated upon discovery of a CHIKV case or cluster (introduced or autochthonous transmission), simultaneous with activating the local emergency response capacity. The purpose of containment is to eliminate the newly introduced CHIKV and to prevent its spread by implementation of intensive vector control measures. This concept has been applied to contain the invasion and spread of dengue viruses in non-endemic areas.[84] Even if CHIKV spreads into a country's urban area, containment should be considered a primary strategy to avoid its spread elsewhere in that country and into neighboring countries. Application of vector control measures should start at homes of detected CHIKV cases (or at a suspected site of infection) and should be applied to the entire neighborhood. Because of delays in case detection and notification, it is likely that CHIKV may have already spread to other parts of the neighborhood.[85] Request the involvement of local authorities to gain access to closed or abandoned properties. The entire emergency containment operation needs to be conducted rapidly, so human and other resources devoted to this effort should be matched to the size of the containment area. Malaria control personnel and others with suitable training may be utilized to accomplish goals of the containment effort. The following actions are recommended to contain an introduction of CHIKV:

1. In addition to participating in a national communication effort, immediately inform the community (residents, schools, churches, businesses, etc.) of the CHIKV introduction. Topics should include mode of spread, symptoms, advice to consult a physician if symptoms appear, and community involvement to eliminate standing water from containers and to allow health inspectors into homes for application of anti-mosquito measures. Prepare the community so that CHIKV containment operations can be conducted more efficiently and rapidly in residential and commercial properties, as well as in public spaces and parks.

2. Conduct indoor and outdoor insecticide applications to eliminate adult mosquitoes. Details on insecticides, dosages, and precautions can be found in Table F3 and in WHO publications.[66, 77, 86]

3. Simultaneously conduct container elimination/protection and larviciding to eliminate the production of new mosquitoes. Special attention should be given to cryptic or subterranean bodies of water that can produce *Aedes* mosquitoes, such as roof gutters, drains, wells, elevated water tanks, water meters, and even septic tanks.[87] Water storage containers and animal drinking pans should be cleaned (by scrubbing and rinsing) and protected with tight covers. Some containers, such as useful implements (paint trays, buckets) and bottles should be stored in a way to prevent them from collecting water (e.g., upside-down, under a roof). Large objects that accumulate rain water (boats, cars) should be properly covered. Containers that cannot be prevented from holding water for any reason should be treated with a larvicide. For example, containers holding water for animal or human consumption require the application of larvicides that have been licensed in the country for that particular purpose. WHO's approved larvicides used to treat potable water-storage containers are provided in Appendix F, in the section "Chemical control of larval habitats". Pesticides should always be used following their label specifications. For other larvicides that can be applied to containers holding non-potable water, see Table F2. [66, 77, 86, 88]

4. Alternatively, or concurrently with source reduction, residual insecticides can be applied to containers holding non-potable water (to inner/outer walls) to kill the larvae and pupae and to nearby outdoor surfaces to kill landing or resting adult mosquitoes. This type of insecticide application is done with hand-held compression sprayers and much care has to be taken to avoid spraying near unprotected water-storage containers or pets.[66, 77]

5. Monitor houses and buildings in the neighborhoods that are being treated and implement special control rounds after working hours, weekends, and holidays to assure that nearly 100% of homes and businesses are treated.

Outbreak Intervention

Controlling an epidemic of CHIKV or a series of outbreaks over a larger geographic scale requires the following:

1. Activating a command center (Emergency Operations Center), either physical or virtual, where epidemiologists, entomologists and vector control specialists, educators, media communicators, etc., can jointly plan, work, and evaluate progress throughout the epidemic. Epidemiological services need to be organized so that daily, detailed reports are sent to all authorized personnel in the affected areas (states, municipalities). To be successful, it will be necessary to establish an efficient system of communications, allowing for feed-back reports and the receipt of acknowledgements (by e-mail, fax, telephone, etc.).

2. Orienting the population at large through the media on the possibility of resulting infection with CHIKV and on how families and communities can contribute to the abatement of the epidemic. Educational materials on specific actions to prevent or control CHIKV transmission should be elaborated and distributed by various media (TV, radio, newspapers, local organizations, schools, clinics, etc.). It is important to report daily (to the press) which communities or neighborhoods are being affected by CHIKV, so that residents and local authorities are aware of imminent risk of infection and can take appropriate actions (e.g., proper use of repellents, elimination of all standing water, organizing clean-up campaigns, etc.). Dissemination of this information needs to be done in a way that no personal information or identifiers are released to the public at any time.

3. Ensuring that infected and febrile persons are protected from mosquito bites by using bednets at home and in hospitals.

4. Orienting vector control operations through real-time epidemiological and entomological assessments of CHIKV transmission, indicating the specific areas that need to be treated. In areas where dengue is endemic,

knowledge from a retrospective analysis of dengue virus transmission or previous experience with dengue viruses should be used to guide vector control operations.

5. Applying effective vector control measures. An epidemic is generally a series of smaller outbreaks occurring simultaneously in several different places within a country (neighborhoods, cities, municipalities, states), where the number of disease cases is unusually large. This means that epidemic control measures may need to be applied concurrently in several locations. Large-area control of mosquito populations over short periods by spraying insecticides from truck- or aircraft-mounted equipment has not proven effective in reducing dengue transmission. Large-scale outdoor application of pesticides may be beneficial when used in conjunction with other control measures as part of an integrated mosquito control program.[76] Therefore, effective vector control measures to be applied during an epidemic are similar to those recommended for area-wide CHIKV containment (above) and dengue virus outbreaks.[66] The main difference is that they should be simultaneously applied in many areas to abate individual outbreaks.

   a. Geo-reference each CHIKV case to the level of operational control areas. In the case of endemic areas, conduct the retrospective epidemiological study at this level, so that stratification serves operational purposes. Use Geographical Information System (GIS) to map operational units, make and distribute maps of disease incidence, and spatially monitor the epidemic.
   b. Divide the target area (e.g., state, municipality) into relatively uniform areas (operational control areas) that will be treated using an area-wide approach (neighborhoods with 2,000–5,000 persons; census areas, zip-codes, etc.). All premises, businesses and other areas (parks, cemeteries, abandoned lots, areas along creeks, illegal dumps, etc.) will be simultaneously treated within a few days. This operational division of the space should be conducted well in advance of an eventual introduction of CHIKV.

c. Area-wide vector control measures imply having sufficiently trained personnel, equipment, and supplies to treat the environment where *Aedes* mosquitoes are being produced. By significantly reducing mosquito adults (using adulticides) and the production of new adult mosquitoes (source reduction and elimination, larvicides) in a particular area, the transmission cycle could be interrupted, and CHIKV could be driven to extinction. This scenario is possible only if the number of biting mosquitoes is dramatically reduced for the length of time it takes for humans and vectors to become clear of CHIKV. For this reason, vector control measures need to achieve a very high efficiency, as measured by the elimination of an extremely large proportion of vector mosquitoes.

Limitations of Vector Control

Vector population reduction and the associated reduction of vector-human contact should be correlated with reduced virus transmission and reduced human disease. In order to interrupt an outbreak, however, vector population reduction must be immediate, substantial, and sustained. Adult mosquitoes will continue to emerge and replace adult mosquitoes killed by adulticides. Therefore, it is essential to maintain IVM programs with complete coverage and repeated treatments. In addition to the presence of mosquito control professionals and an active IVM program, it is important to maintain the support and cooperation of all members of society.[67]

Appendix H. Model of Risk and Outbreak Communication Plan

Target audience	Preparation phase	Response phase	Recovery phase
Government authorities	• Prepare briefing to authorities on the risk of introduction of CHIKV, in coordination with subject matter experts. • Train spokespersons on this subject. • Develop a plan for risk and crisis communication. • Coordinate with the media and other social stakeholders.	• Activation of communication plan.	• Evaluation and adjustment of communication plan.

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)			
Target audience	**Preparation phase**	**Response phase**	**Recovery phase**
Public health and emergency response authorities	• Establish protocol for use of incident management/ emergency operations approach if not in place. • Conduct exercises to allow communication responders to know emergency response structure and their roles.	• Establish JIC within Emergency Operations Center. • Establish regular meetings of Public Information Officers (PIOs) and strategic communication staff for all agencies involved and regular meeting schedule with other key elements of operational response.	• Conduct "lessons learned" assessment of communications response and use of emergency response structure.

Medical personnel	• Develop and provide information via websites, booklets, pamphlets, and pocket guides. • Participate in conferences addressing risk factors, case presentations, diagnostics, and risk factors. • Develop frequently addressed questions (FAQs) addressing differences between CHIKV and dengue, if applicable. • Establish hotline infrastructure for clinical support.	• Implement response plan(s). • Provide updated, easy-to-access information concerning epidemiology of outbreak, risk factors, case definition, diagnostics, etc. • Update information flow as necessary. • Activate and staff an information hotline for clinical support.	• Continue to provide updates. • Continue to support the clinical hotline. • Provide information concerning sequelae. • Evaluate communication with the clinical community; gather "lessons learned". • Provide final response report.

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)			
Target audience	**Preparation phase**	**Response phase**	**Recovery phase**
Hospitals	• Develop and provide information for preparedness planning, patient management. • Develop a handbook or pocket guide addressing the type of information that should be shared with CHIKV patients, patients' families, hospital personnel, and hospital-associated personnel (emergency medical personnel).	• Implement contingency plans with hospitals. • Gather information from the hospitals to support information and counseling for CHIKV patients, patients' families, hospital personnel, and associated personnel (emergency medical personnel, Red Cross, paramedics, fire services, public safety, etc.).	• Evaluate the communications plan. • Gather information for "lessons learned". • Provide final report to the hospital community.

Hospitals		• Use gathered information to facilitate communications with other sectors and the general population concerning the status of hospital operations and medical care support locations.	

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)			
Target audience	**Preparation phase**	**Response phase**	**Recovery phase**
Associations of health professionals and medical sciences	• Collaborate with associations to educate members via lectures, newsletters, social networking, and websites to address risk factors, case definition and diagnostics, treatment, and sequelae. • Provide the associations with FAQ sheets. • Work with associations to provide prevention messages to the general population.	• Intensify communication with the medical sciences and health-professional associations with respect to health care services and look for disease patterns and trends.	• Evaluate the timeliness of information provided to the associations, as well as the timeliness of transfer of the information to the association's membership.

Laboratory – government and private laboratories	• Develop and provide information addressing sample management, tests, procedures, and materials in both electronic and hardcopy formats via video conferences, workshops, etc.	• Activate information channels for the timely gathering of information to support the decision cycles at the operational level, including the health care services.	• Evaluate communications with the laboratory system. • Continue to gather information from laboratories. • Gather “lessons learned”.

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)			
Target audience	**Preparation phase**	**Response phase**	**Recovery phase**
Vector control personnel	• Vector control personnel and communicators work together to develop and provide information concerning possible CHIKV vectors and integrated vector management, in both electronic and hardcopy formats via video conferences, workshops, etc.	• Activate communication plan with health professionals and other entities. • Gather information concerning the effectiveness of ongoing integrated vector management activities, if appropriate. • Provide updated information to health professionals concerning protection and prevention.	• Evaluate communications actions for vector control and gather “lessons learned”. • Gather information concerning best practices for vector management.

Local and regional health department personnel; epidemiologists	• Health department staff, epidemiologists, and communicators work together to develop and provide information to be used by public health partners and the media to address the surveillance methods, analysis of data, and development of messages for the general population.	• Activate information channels for the timely gathering of information to support decision cycles at the operational level, including the health care services.	• Evaluate communications with health departments and epidemiologists. • Gather “lessons learned”.

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)			
Target audience	**Preparation phase**	**Response phase**	**Recovery phase**
Blood banks	• Provide information to blood bank managers concerning risks associated with CHIKV. • Develop and provide information concerning blood-product management and risks, as well as preparation for donor shortages. • Develop donor screening guidelines and procedures. • Develop fact sheets for donors and prospective donors for distribution in the blood banks.	• Establish active communication with blood banks to address shortages of supplies and of donors within restricted areas, in order to inform the general population as well as the media. • Coordinate with implementation of donor screening guidelines and procedures in areas affected by CHIKV emergence.	• Evaluate the effectiveness of recommendations that blood banks provide to blood donors. • Develop a communications plan to support lifting of restrictions for donations within a previously restricted area.

Travelers associations, businesses, and organizations	• Outreach to those traveling to regions at risk for CHIKV, describing symptoms and prevention of the disease, using official and business websites and factsheets, and other means (such as closed circuit TV, message boards, and public service announcements).	• Request travel and tourism industry operators to intensify the communication activities included in the travelers information plan. • Provide updates concerning disease status and preventive and protective actions.	• Evaluate the timeliness of response by the travel industry. • Gather “lessons learned”.
Maritime, land, and air transportation industry and authorities (ports)	• Develop THANs before the event for use by port authorities, customs and transportation security agencies. • Provide the industry and authorities with information concerning IHR requirements.	• Request maritime, land, and air industry and port representatives to intensify their communications activities as appropriate for the response. • Provide updates concerning disease status and preventive and protective actions.	• Evaluate the timeliness of response by the travel industry. • Gather ”lessons learned”.

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)			
Target audience	**Preparation phase**	**Response phase**	**Recovery phase**
Civil authorities, government officials	• Engage in advocacy to gain the support needed for effective preparation and response. • Keep channels open with local, regional, and national levels of government. • Designate and train spokespersons, providing function-specific information appropriate for the level of responsibility.	• Implement the communications plan with the other government authorities, updating spokespersons information. • Include appropriate representatives in the JIC.	• Evaluate the effectiveness of preparation and response communications activities conducted with authorities and officials. • Gather “lessons learned”.

General population	• Use multiple channels to inform the general public of the potential for CHIKV risk and means of prevention and protection. • Plan for use of hotlines; support local hotlines as appropriate. • Develop health education materials, such as website pages, posters, pamphlets, handbills, billboards, SMS text messaging and social media, and on-line social networking. • Consider the use of interpersonal communication through group meetings, in schools; make optimal use of traditional/folk media.	• Special campaigns may be carried out via the mass media, including in local newspapers/ magazines, radio, and TV, as well as through outdoor publicity, such as billboards. • Monitor communication channels. Assess delivery of the messages. • Increase efforts to garner support of insecticide use and other control measures, as needed. • Develop location-specific messaging and update as appropriate. • Open hotlines, and support local hotlines as appropriate.	

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)			
Target audience	**Preparation phase**	**Response phase**	**Recovery phase**
The media	• Develop and maintain relationships with the media that will support communications activities. • Provide training, participate in interviews, and develop public service announcements to advice media partners and prepare them for potential CHIKV activity. • Prepare spokespersons. Spokespersons must be technically and politically credible and willing to interact with the press on short notice.	• Establish a permanent channel of information with the media for regular communications, including briefings and interviews. • Disseminate regular reports from the JIC concerning the status of outbreak to provide a consistent message. • Monitor press reports and coverage. Conduct analysis of reports for appropriateness and relevance and adjust messaging/ strategies accordingly.	• Continue to provide updates to the media, including appropriate messaging as the risk of transmission is reduced. • Evaluate implementation of the communications plan to introduce necessary adjustments to it. • Gather “lessons learned”.

Faith-based communities	• Develop and provide information for use within religious media networks, during services, and among outreach groups.	• Collaborate with leadership to advance protection and prevention efforts and vector management.	• Evaluate involvement in the communications plan for the preparation and response to CHIKV.
Nongovernmental organizations (NGOs), humanitarian groups, community-based health organizations, and other civil society organizations	• Collaborate with these organizations on outreach to organize, sensitize, and educate their communities.	• Collaborate with leadership to advance protection and prevention efforts and vector management.	• Evaluate involvement in the communications plan for the preparation and response to CHIKV.

(Continued)

Appendix H. Model of Risk and Outbreak Communication Plan (Cont.)			
Target audience	**Preparation phase**	**Response phase**	**Recovery phase**
Educational system	• Collaborate with the educational system to develop lessons, teaching materials, and content that will raise awareness of CHIKV, as well as sanitation and other preventive measures. • Seek to have lessons on CHIKV risks and response in the school curriculum, as a way to promote and expand awareness; the students will become communications multipliers.	• Collaborate with leadership to advance protection and prevention efforts and vector management.	• Evaluate involvement in the communications plan for the preparation and response to CHIKV.

Private sector, business	• Collaborate with the private sector in preparing its plan to organize, sensitize, and educate their organizations, employees, and customers. • Seek to involve the private sector in the government's efforts in communications activities for preparation and prevention.	• Collaborate with the private sector to intensify its communications activities and to further the government`s communication initiatives addressing protection and prevention efforts and vector management. • Provide updates to the private sector concerning the response.	• Evaluate involvement of private sector in the communication plan for preparation and response to CHIKV. • Gather "lessons learned".

Appendix I. Meeting of the Technical Advisory Group of Preparedness and Response for Chikungunya Virus Introduction in the Americas

Objectives

The aim of this meeting was to assemble a technical advisory group to review and adapt a preliminary draft of "Preparedness and Response for Chikungunya Virus Introduction in the Americas". The technical advisory group included experts in various fields from the Americas, including epidemiologists, clinicians, entomologists, laboratory personnel, and communication specialists. After discussing the document's various chapters, these experts submitted changes, additions, and rewrites that they considered appropriate to make the guidelines factual and relevant to all countries in the Region. The guidelines are meant to be a useful tool that can be adapted and applied by each Member Country in establishing the most appropriate strategies for the prevention and control of Chikungunya virus in the Americas.

Agenda

Wednesday, 7/21/10

8:30 – 9:00	Reception for participants
9:00 – 9:30	Welcome and presentation of the meeting's objectives and working dynamics CDC (Roger Nasci) and PAHO/WHO (Otavio Oliva, Luz Maria Vilca)
9:30 – 10:15	Chikungunya Virus: Clinical and Epidemiological aspects. CDC (Ann Powers)
	Break
10:45 – 11:30	Laboratory Diagnosis of Chikungunya virus: An overview CDC (Robert Lanciotti)

11:30–12:30 Impact of a Chikungunya outbreak on public health: Experience of La Réunion
FRANCE, Laveran Military Hospital (Fabrice Simon)

Lunch

13:30–14:30 Chikungunya cases identified in the Americas: USA, Canada, and French Territories
CDC (Erin Staples)
CANADA, National Microbiology Laboratory, WHOCC (Michael Drebot)
FRENCH TERRITORIES (Philippe Quenel)

14:30 – 15:15 Round table: Control of *Aedes aegypti* in the Americas: what has worked and what has not
CDC (Roberto Barrera)
BRAZIL Ministry of Health (Irma Braga)
CDC (Harry Savage)
PAHO/WHO (José Luis San Martín)
PAHO/WHO (Chris Frederickson)

Break

15:45 – 16:15 Assignment of work groups and review of goals for groups (five working groups were formed for reviewing the draft guideline "Preparedness and Response for Chikungunya Virus Introduction in the Americas"):

- Epidemiological Aspects (Epidemiology, Surveillance, and Outbreak Response chapters)
- Clinical aspects (Clinical, Case Management chapters)
- Laboratory (Laboratory Chapter)
- Entomology (Vector Surveillance and Control chapters)
- Communications (Communications Chapter)

16:15 – 17:45 Groups meet to decide on approach (Coordinator, Presenter)

Thursday, 7/22/10

8:30 – 10:00 Working groups (Cont.)

Break

10:30 – 12:30 Working groups (Cont.)

Lunch

13:30 – 15:00 Working groups (Cont.)

Break

15:30 – 17:00 Make changes to draft guidelines (edit manuscript) and meet to draft presentations of proposed changes

Friday, 7/23/10

8:30 – 09:00 Groups meet to finalize draft presentation of proposed changes

Break

9:30 – 11:30 Group presentations

11:30 – 12:30 Group presentations

Lunch

13.30 – 14.30 Additional changes to the draft guideline (final edits to manuscript)

Break

15.00 – 16.00 Wrap-up and next steps

CDC (Roger Nasci) and PAHO/WHO (Otavio Oliva)

List of Participants

Pan American Health Organization:

Dr. Otavio Oliva (HSD/IR/V)
Dr. José Luis San Martín (HSD/IR/D)
Dr. Luz Ma. Vilca (HSD/IR/V)
Ms. Olivia Brathwaite (PWR/PAN)
Ms. Vivien Lewis (HSD/IR/V)

Participants by Working Group:

A. Epidemiological surveillance:

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Dr. Yeni Herrera, Peru (Ministry of Health)
Dr. Philippe Quenel, Martinique. (CIRE, Institut de Veille Sanitaire)
Dr. Joel Montgomery, Peru (NMRCD)
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B. Laboratory:

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Dr. César Cabezas, Peru (Instituto Nacional de Salud)
Dr. Erick Halsey, Peru (NMRC, Virology Department)
Dr. Otavio Oliva, USA (PAHO, WDC)

C. Entomology:

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Dr. Harry Savage, USA (Arboviral Diseases Branch, DVBD, CDC)
Dr. Roberto Barrera, Puerto Rico (Dengue Branch, CDC, WHOCC)
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Dr. José Luis San Martín, Panama (PAHO, Panamá)

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Dr. Ernesto Pleites, El Salvador (Ministry of Health, Hospital Nacional de Niños Benjamín Bloom)

Dr. Rivaldo Venancio Da Cunha, Brazil (Secretariat of Health)

Dr. Fabrice Simon, France (Department of Infectious Diseases and Tropical Medicine. Laveran Military Hospital)

Dr. Iris Villalobos Chacon, Venezuela (Secretaria de Salud)

Dr. Roser Gonzalez, USA (PAHO, WDC)

E. Social Communication:

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Dr. Carmen Pérez: Puerto Rico (Dengue Branch, CDC, WHOCC)

Mr. Lee Smith, USA (Division of Global Migration and Quarantine, CDC)

Dr. Marco Fidel Suárez, Bolivia (PAHO/WHO Bolivia)

Dr. Emily Zielinski-Gutierrez, USA (DVBD, CDC) (Final revision)

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