Yellow Fever Initiative
ONE INJECTION, FULL PROTECTION

yellow fever

# Detection and investigation of serious adverse events following yellow fever vaccination

## Guidance from an informal consultation of experts

18–19 November 2008
Geneva, Switzerland

World Health Organization

WHO/HSE/GAR/ERI/2010.2

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# Abbreviations

| | |
|---|---|
| ADEM | acute disseminated encephalomyelitis |
| AEFI | adverse events following immunization |
| ALT | alanine aminotransferase (also known as serum glutamic-pyruvic transaminase, SGPT) |
| AND | associated with neurological disease |
| AST | aspartate aminotransferase (also known as serum glutamic-oxaloacetic transaminase, SGOT) |
| AVD | associated with viscerotropic disease |
| BCG | Bacille-Calmette-Guérin |
| CSF | cerebrospinal fluid |
| CMV | cytomegalovirus |
| CPK | creatine phosphokinase |
| EBV | Epstein-Barr virus |
| ECG | electrocardiogram |
| EEG | electroencephalogram |
| ELISA | enzyme-linked immunosorbent assay |
| EMG | electromyography |
| EPI | Expanded Programme on Immunization |
| ERI | Epidemic Readiness and Intervention Team |
| GAVI | The GAVI Alliance (Global Alliance for Vaccines and Immunization) |
| HIV | human immunodeficiency virus |
| ICD | International Classification of Diseases |
| IgE | immunoglobulin E |
| IgG | immunoglobulin G |
| IgM | immunoglobulin M |
| RT-PCR | reverse transcriptase-polymerase chain reaction |
| PRNT | plaque-reduction neutralization testing |
| WHO | World Health Organization |
| YF-AEFI | adverse events following immunization against yellow fever |

# Preface

Yellow fever vaccine has been in use for more than 70 years and, as is the case for most vaccines, has been associated with occasional mild side-effects, such as low-grade fever or local discomfort at the site of injection. Recently, the description of clinical syndromes related to yellow fever vaccination of previously non-immunized travellers has led to the recognition of rare but serious adverse events following immunization (AEFI). To improve understanding of these new clinical entities, it is necessary to develop and standardize procedures for surveillance, detection and investigation of serious adverse events following yellow fever vaccination.

The World Health Organization (WHO) convened a meeting of experts to initiate discussion, with the aim of developing guidance to countries embarking on active surveillance of yellow fever AEFI. The Meeting was held on 18–19 November 2008, in Geneva, Switzerland, at WHO headquarters.

The present report reflects the guidance provided through collaborative work between WHO and recognized experts during and after the informal consultation. Further work will be required to formalize operational guidance for surveillance of serious adverse events after field experience has served to validate some of the proposals presented herein.

# Background

The yellow fever virus, which causes a haemorrhagic fever, was at one time the source of high-mortality epidemics in Africa, the Americas, and Europe. With the introduction of a live attenuated vaccine in the 1930s and large-scale immunization and mosquito vector-control programmes, the transmission of yellow fever declined. Since the late 1980s, however, there has been a resurgence of yellow fever, with West Africa being most affected. In response to this challenge, population immunity against yellow fever has been strengthened through the Expanded Programme on Immunization (EPI), outbreak response, and preventive mass vaccination in countries at risk in Africa and South America (WHO, 2005).

The yellow fever vaccine contains live attenuated virus of the 17D strain,[1] which confers immunity for at least 10 years in more than 92% of those vaccinated. The vaccine should not be administered to children aged less than 6 months, pregnant women, persons with a severe allergy to eggs or severely immunocompromised persons. Children aged 6–8 months should only be vaccinated when the risk of yellow fever virus transmission is very high.

Yellow fever vaccines from four manufacturers[2] have been pre-qualified by the World Health Organization (WHO) and fulfil the following requirements:

- The WHO technical specifications (e.g. potency, stability) of the vaccine have been examined;
- Vaccine production conforms to standards for good manufacturing practice; and
- The vaccine has been approved by national regulatory authorities of the country of manufacture.

Used worldwide, these vaccines have long been considered amongst the safest and most effective vaccines available, and reports of adverse events following immunization (AEFI) are rare (Struchiner et al., 2004). First recognized in 2001 serious adverse events related to vaccination include yellow fever vaccine-associated viscerotropic disease, neurological diseases, or severe hypersensitive reactions. Most of the adverse events following yellow fever immunization (YF-AEFI) reported in the scientific literature are viscerotropic cases, which mimic yellow fever disease, often with fatal multi-organ failure.

Acute viscerotropic disease following yellow fever vaccination was first described in 2001 (Chan et al., 2001; Vasconcelos et al., 2001; Hayes, 2007). Since then, surveillance for YF-AEFI has been established by the Centers for Disease Control and Prevention (CDC) in the United States of America (USA), the Robert Koch Institute (Germany) and the Ministry of Health of Brazil to further assess the adverse events reported and understand the relationship between these adverse events and the 17D

[1] 17D vaccines are produced from two viral sub-strains, 17D-204 and 17DD, which are equally effective.

[2] Sanofi Pasteur, France; The Pasteur Institute, Senegal; and BioManguinhos, Brazil, Chumakov Institute, Russian Federation.

vaccines. The available data suggest that the incidence of reported adverse events ranges from 0 to 0.21 cases per 100 000 vaccine doses in regions where yellow fever is endemic, and from 0.09 to 0.4 cases per 100 000 doses in populations not exposed to the virus. These estimates are consistent with data from Africa, where the reported rates of serious adverse events following yellow fever vaccination campaigns in 2007 and 2008 were 0.02 cases per 100 000 vaccine doses in Mali, 0.06 cases per 100 000 vaccine doses in Senegal. The highest incidence of viscerotropic disease – 0.4 cases per 100 000 vaccine doses administered – has been reported in vaccinated travellers in the USA.

Neurological (or neurotropic) disease in vaccinated travellers in the USA is estimated to occur with a frequency of 0.8 cases per 100 000 vaccine doses administered.

With the emergence of these newly recognized syndromes, it is critical to establish high-quality surveillance for detection and monitoring of adverse events following immunization, particularly in the case of population vaccination in the absence of an outbreak of yellow fever. Since 2007, all preventive mass-vaccination campaigns must be designed with enhanced surveillance for AEFI in order to monitor the safety of the vaccine in different contexts.

## Purpose

The present document is designed to provide guidance for the surveillance and investigation of serious adverse events in the context of campaigns for preventive vaccination against yellow fever in countries of the WHO African Region.

The emphasis of this report is on detecting and differentiating distinct syndromes of serious AEFI with supporting laboratory data, and excluding other possible etiologies for such syndromes by the use of geographically-appropriate differential diagnoses and laboratory tests.

This operational guide will help national immunization programmes and laboratories to:

- Implement active surveillance for serious adverse events following immunization (AEFI) at peripheral level in resource-poor settings for 30 days after preventive mass vaccination with yellow fever vaccine.
- Determine the clinical and laboratory criteria necessary to confirm or exclude a serious reaction to yellow fever vaccine, and discount other possible etiologies through geographically-appropriate differential diagnoses.
- Gather and verify evidence for a possible association between vaccine and clinical disease to support evidence-based decision-making regarding yellow fever vaccination.

## Who will use this operational guide?

This operational guide will be useful for the coordinators of immunization campaigns, members of national expert committees, laboratory personnel and other health workers involved in yellow fever immunization and management of adverse events, particularly after large-scale preventive immunization campaigns.

# Defining the problem

## The need for surveillance of serious AEFIs

For every AEFI identified, the relationship between the event and vaccination must be explored. An adverse event may occur coincidentally and have no connection with vaccination. It is therefore necessary to develop a differential diagnosis for the case in order to rule out other possible causes of the observed manifestations. In other situations, despite an investigation, it is not possible to determine the cause of the adverse event.

After investigation, AEFIs are classified (WHO, 1999) as:

- Programme error;
- Injection reaction;
- Vaccine reaction;
- Coincidental; or
- Unknown.

An adverse event is also classified as "serious" or "non-serious". A serious adverse event includes "any untoward medical occurrence that results in death, hospitalization or prolongation of hospitalization, persistent or significant disability/incapacity, or is life-threatening" (ICH, 1994).

Serious AEFIs are rare and in most cases (aside from an immediate hypersensitivity reaction or an injection-site reaction), there is inadequate historical or clinical information and laboratory investigation of suspect cases to confirm a link to administration of the vaccine.

This operational guide focuses on serious reactions to the yellow fever vaccine, including:

- Viscerotropic disease;
  - — Multi-organ system failure mimicking wild-type yellow fever with similar mortality rates;
- Neurological disease, which can manifest as:
  - — Neurotropic disease: vaccine-virus invasion of the central nervous system; or
  - — Autoimmune disease: post-immunization autoimmune-related illnesses involving the central and/or peripheral nervous system.
- Severe hypersensitivity reactions, including anaphylaxis;
- Any post-vaccination death occurring within 30 days after the end of a yellow fever vaccination campaign and for which the circumstances and clinical signs lead investigators to suspect a vaccine reaction.

## Objectives

The specific objectives of the guidance provided are to assist programme managers to:

- Develop standard procedures to identify and investigate serious adverse events during and following preventive mass-vaccination against yellow fever;
- Identify, classify and report serious AEFIs;
- Detect viscerotropic and neurological disease following yellow fever immunization;
- Detect severe hypersensitivity reactions;
- Document the occurrence of viscerotropic disease, neurological disease and severe hypersensitivity reactions to yellow fever vaccine in resource-poor settings.
- Evaluate the relationship between the serious adverse event and the vaccine;
- Rule out non-vaccine-related causes of the serious adverse event;
- Determine whether the serious adverse event is isolated or part of a cluster;
- Inform the relevant authorities about the occurrence of a serious adverse event;
- Obtain additional information about the pathogenesis and pathophysiology of vaccine-related syndromes and propose appropriate case management.

## Methods

The target population for surveillance of adverse events in the context of campaigns for mass vaccination against yellow fever includes all individuals vaccinated. The surveillance period begins on day zero (the day of vaccination) and continues for 30 days after the end of the vaccination campaign. Surveillance includes routine reporting of AEFIs and active case-finding through review of hospital charts and registries and consultations with hospital staff. Each administrative level in the country has responsibilities and activities related to active surveillance for AEFI, and diagnosis and classification are performed at each level (see Figure 1). The present guide suggests building upon existing surveillance systems to enhance the detection of cases of YF-AEFI.

# Section 1

## Field surveillance and data collection for serious adverse events following yellow fever immunization: peripheral level

### 1.1 Case detection based on existing notification systems

Surveillance of adverse events following immunization (AEFI) is described in the immunization-safety surveillance guidelines published by the World Health Organization (WHO, 1999); this document recommends that all immunization programmes report certain AEFIs, including anaphylaxis, seizures and infection.

Figure 1 presents the typical tasks involved in AEFI surveillance that are carried out at each administrative level. Personnel involved in monitoring reportable events include health workers providing immunization services or clinical treatment of AEFI in health centres, hospitals or special treatment facilities; parents who report AEFI affecting their children; and researchers conducting clinical studies or field trials. Their roles are outlined in Annex I.

Where possible, early detection of AEFIs is organized at the peripheral level, that is, all administrative subunits within the district, including villages, communities and neighbourhoods. The main objective of detection and early investigation of serious AEFIs is to detect severe illness quickly, to hospitalize the patient and obtain the necessary specimens (Figure 2).

A reporting form for AEFIs (Annex II) must be completed by the health worker and sent to the next level (usually the district manager) by the quickest means (e.g. fax, e-mail, telephone).

All hospitalizations and deaths occurring within 30 days after vaccination and for which circumstances and clinical signs lead the health worker to suspect a vaccine reaction must be reported immediately (within 24 hours) to the next administrative level.

### 1.2 Classification: serious versus non-serious

Once an AEFI is detected, commonly at the peripheral level, it must be classified by the health worker as either serious or non-serious (Box 1) (ICH, 1994).

**BOX 1**

**Classification of an adverse event following immunization (AEFI) as "serious" or "non-serious"**

**Serious:** any untoward medical occurrence that is life-threatening or results in death, hospitalization or prolongation of hospitalization, persistent or significant disability or incapacity, a congenital anomaly or birth defect.

**Non-serious:** any adverse event that is not serious.

*National authorities will determine the administrative level responsible for case classification.*

## 1.3 Reporting of adverse events following immunization

District managers are responsible for ensuring that routine data collection, case investigation, and reporting are planned and implemented in all health units and immunization sites (WHO, 1999).

A health worker reports data concerning an AEFI on the AEFI reporting form (Annex II) sends it to the next administrative level (usually district level). All vaccines given concurrently must be documented at this time.

When the AEFI is determined to be serious, a second detailed report form should be completed and sent within 24 hours (Annex III).

The standard AEFI surveillance system in place as part of the Expanded Programme on Immunization (EPI) must be enhanced with active case-finding during yellow fever mass-vaccination campaigns. This active surveillance is meant to increase the sensitivity of case detection in resource-poor settings. The active system is based at the intermediate level and will be discussed in Section 2.

Tools for case detection, to be used at the peripheral health centre level, are listed in Box 2.

**BOX 2**

**Tools for case detection at the peripheral health centre**

1. Guidelines for surveillance of adverse events following immunization (AEFI)
2. Categories of adverse events (*Annexes IV*, *V*)
3. Roles of health workers (*Figure 1*, *Annex I*)
4. List of reportable events
5. Simple case definitions for AEFI against yellow fever (YF-AEFI)
6. Definition of serious versus non-serious adverse event (*Box 1*)
7. Report forms for AEFI (*Annexes II*, *III*)
8. Guidelines for collection and storage of specimens (*Annexes VI*, *VII*)
9. Decision tree for early detection of YF-AEFI (*Figure 2*)

***Figure 1. Surveillance and classification of adverse events following immunization (AEFI): tasks to be accomplished, by administrative level***

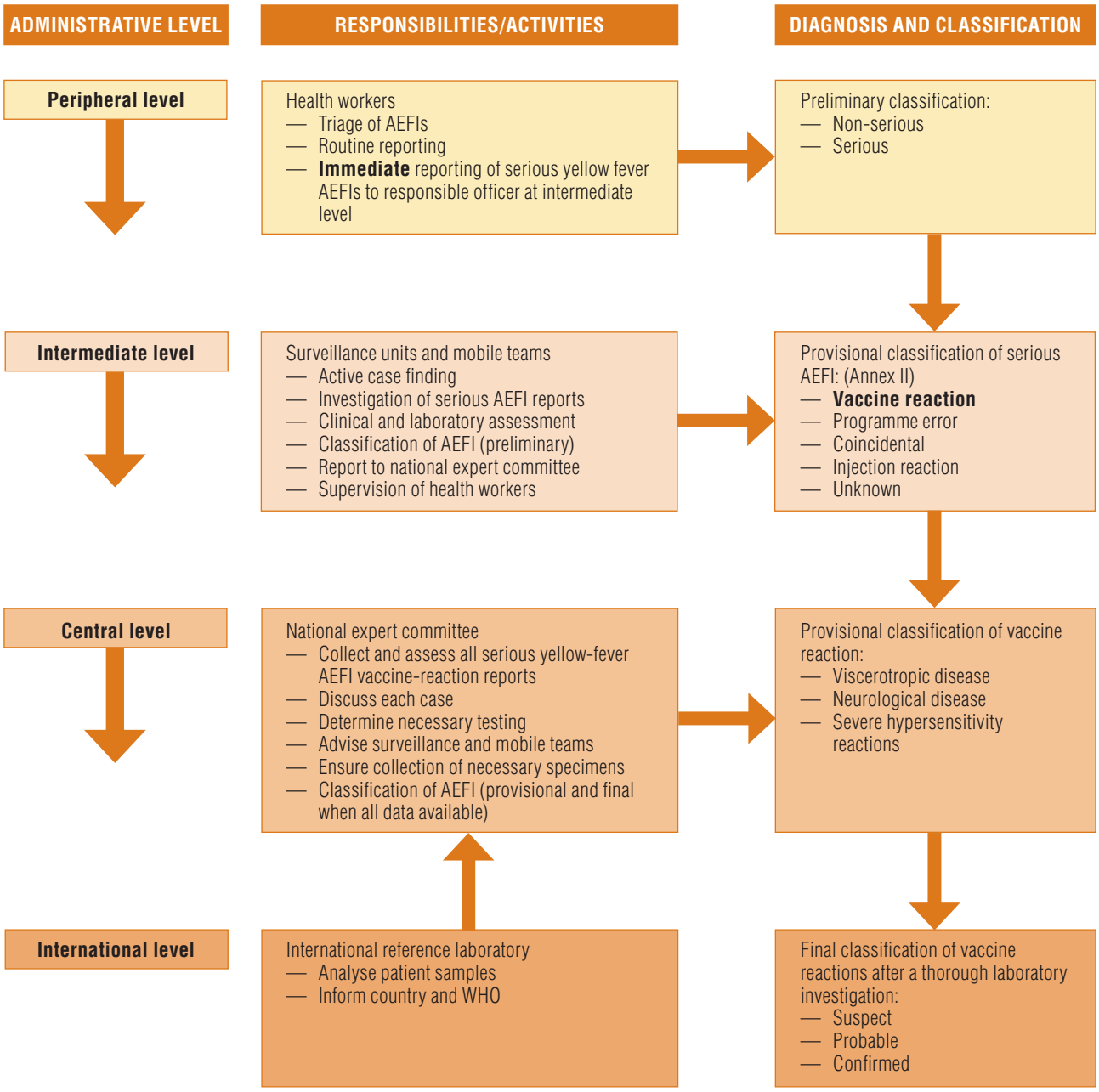
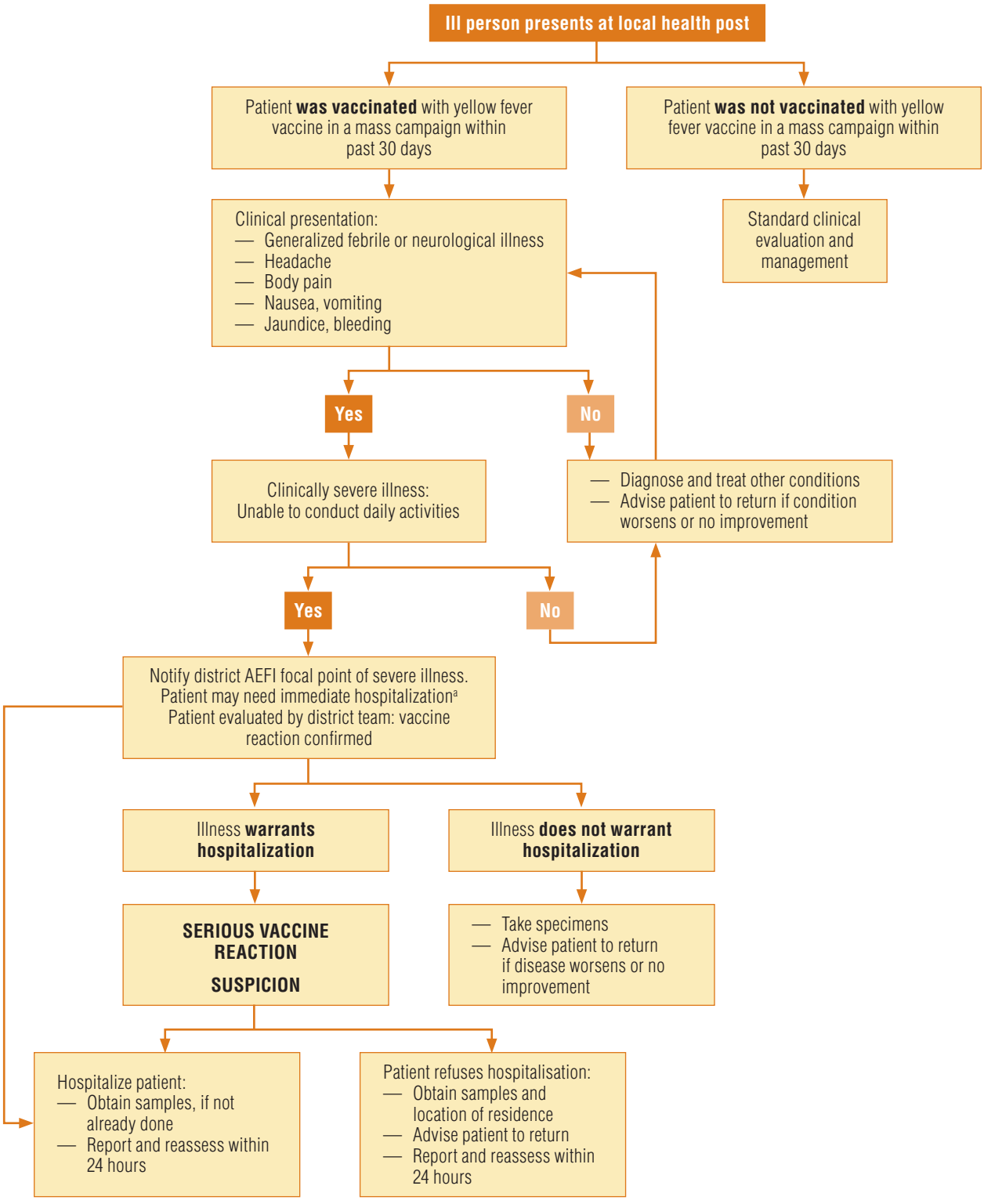


***Figure 2. Algorithm for early detection of adverse effects following immunization (AEFI) during a campaign of mass vaccination against yellow fever: peripheral level***




[a] If yellow fever vaccine reaction suspected and district team will take > 24 hours to arrive:
— Take biological specimens at health post
— Take vaccine details and sample

# Section 2

## Surveillance and primary investigation of serious adverse events: intermediate and referral facilities

All cases identified as serious adverse events must be referred to the intermediate level, where further investigation and analysis will take place. The intermediate level is the administrative unit between the peripheral level (district or the local government areas) and the central level. In some countries, the intermediate level may be the region, state, department, province etc, according to the country involved.

At the intermediate level, surveillance activities include ongoing routine surveillance of adverse events using existing health structures in the zones covered by the vaccination campaign and supplementary active case-finding of serious AEFIs by trained personnel.

Annex V provides the clinical and laboratory characteristics of the main serious AEFIs under surveillance (viscerotropic, neurological and severe allergic reactions).

### 2.1 Active case-finding

Active case-finding should be established at the intermediate administrative level in the geographical area targeted for immunization during the vaccination campaign and for 30 days after the last day of the campaign. Active case-finding requires the mobilization of trained personnel to communicate with health centres, district hospitals and referral hospitals to determine whether serious adverse events (including deaths) have occurred, and to follow-up persons hospitalized for serious AEFI.

All reportable events require completion of the case report forms (Annexes II, III).

Any post-vaccination death or hospitalization occurring within 30 days after the end of the vaccination campaign and for which circumstances and clinical signs give reason to suspect a vaccine reaction must be reported and evaluated immediately (within 24 hours) (Figure 3). Arrangements must be made for immediate transport of corpses to the closest facility where organ samples may be obtained or autopsy performed within 72 hours.

#### *2.1.1 Enhanced surveillance in intermediate and referral facilities*

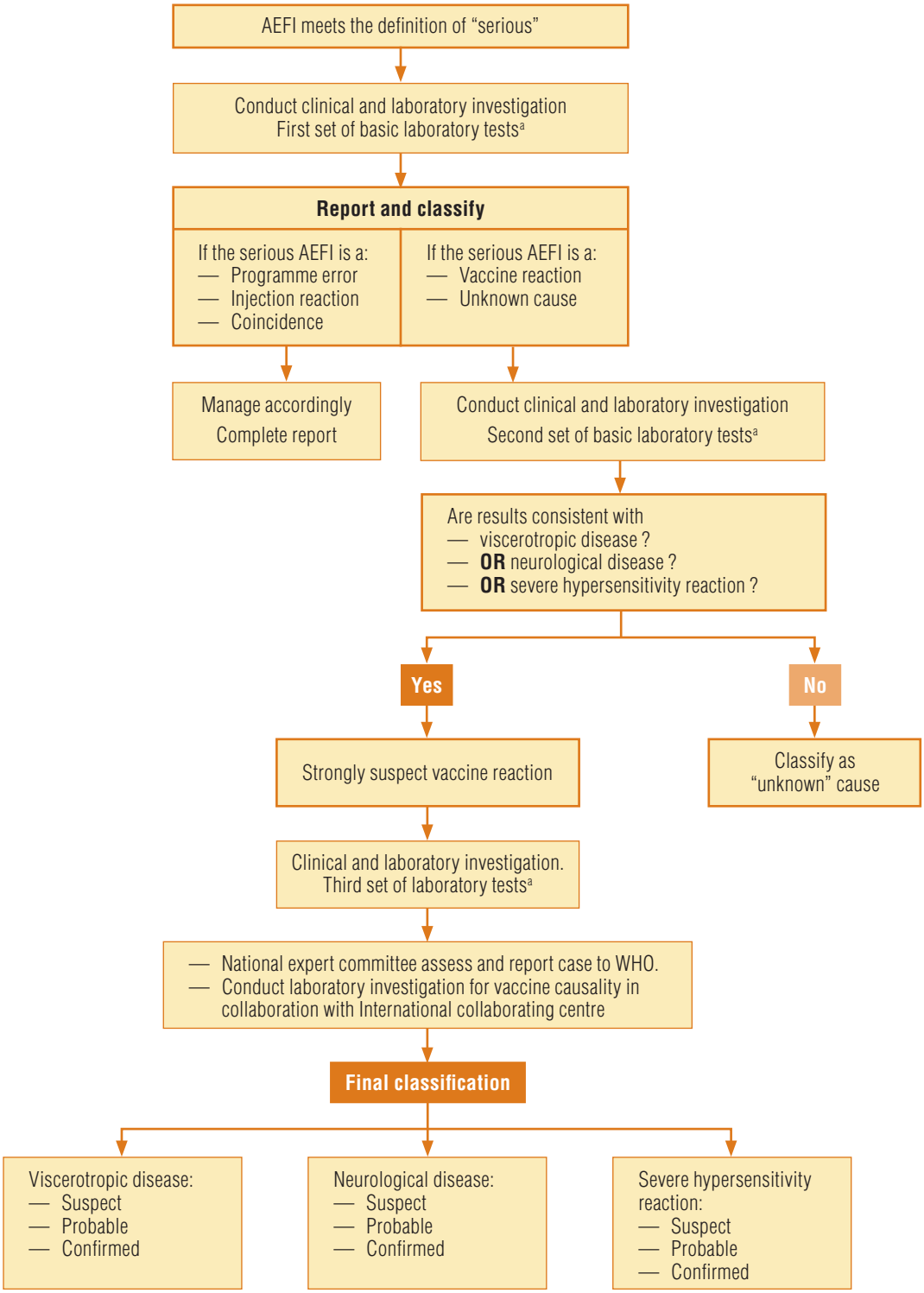
Trained personnel should be organized into surveillance units acting for the referral and intermediate health facilities (e.g. district hospitals) selected by the national authorities to carry out active case-finding. Members of these surveillance units must review hospital registries and patient charts and consult with hospital staff to identify cases of serious adverse events occurring within the 30-day period of the vaccination campaign.

#### *2.1.2 Surveillance system units and mobile teams: definition and role*

Where appropriate and feasible, mobile teams are created from among the staff of the surveillance system unit to ensure active support to lower-level health units and to complete case investigations (Box 3).

Annex VIII details the main symptoms giving reason to suspect an AEFI, which are to be used during active case research.

***Figure 3. Decision tree for serious adverse events following immunization (AEFI) against yellow fever: intermediate level***




[a] See Table 1.

**BOX 3**

**Role of surveillance system units and mobile teams**

*Surveillance system unit and mobile teams:*

A group of trained and supervised clinicians based in selected referral hospitals whose task is to identify and investigate adverse events following immunization (AEFI) against yellow fever. Members of the surveillance system unit can form teams that mobilize to districts when necessary.

The role of the surveillance unit and mobile surveillance team is to:

- Perform active case-finding;
- Review hospital registries and patient charts;
- Consult with hospital staff;
- Coordinate clinical and laboratory assessments;
- Classify AEFIs as serious or non-serious;
- Investigate serious AEFI reports;
- Assist with specimen collection and transport of corpses;
- Report AEFI cases to the national expert committee.

The surveillance of serious AEFIs requires daily communication and collaboration between mobile teams acting for peripheral and intermediate levels. The time frame (≤ 30 days from the end of the vaccination campaign) and definition of serious AEFI must be clear.

## 2.2 Classification of AEFI

### *2.2.1 Classification by severity*

Each AEFI must be classified initially by the peripheral level as serious or non-serious (Figure 1, Box 1).

### *2.2.2 Classification by cause*

- Programme error: an adverse event caused by an error in vaccine preparation, handling or administration (WHO, 1999).
- Injection reaction: an adverse event caused by anxiety about, or pain from the injection rather than the vaccine itself.
- Vaccine reaction: an adverse event caused or precipitated by the vaccine when given correctly, caused by the inherent properties of the vaccine.
- Coincidental: an adverse event that happens after immunization but is not caused by the vaccine – a chance association.
- Unknown: the cause of the adverse event cannot be determined.

### *2.2.3 Classification of an AEFI as a serious vaccine reaction*

Reliable classification of an AEFI as a serious vaccine reaction will require:

- Case definitions for viscerotropic disease, neurological disease and hypersensitivity reactions (Annex IV).
- Review of charts, reports and hospital registries looking for symptom complexes consistent with viscerotropic disease, neurological disease and hypersensitivity reactions (Annexes V, VIII).
- Laboratory data supporting diagnosis of viscerotropic or neurological disease or hypersensitivity reactions (Table 1).

- Ruling out other causes of viscerotropic or neurological disease and hypersensitivity reactions (differential diagnosis) (Box 5, Annex IX).
- Completion of reporting forms with supporting patient information, history, physical examination, laboratory data or pathology (in case of death) and vaccine information.

## 2.3 Investigation

For serious yellow fever vaccine reactions only, further investigation will be required by the yellow fever vaccine surveillance teams; Table 1 provides details of biological specimens to be collected and laboratory analyses to be carried out.

Annex IX provides a differential diagnosis for serious AEFI syndromes.

## 2.4 Case management

As yet, there are no standardized treatment protocols for patients with viscerotropic or neurological disease. Cases are treated symptomatically.

Tools to be used for surveillance of AEFI at the intermediate level are listed in Box 4 and a list of the main diseases to be considered for differential diagnosis is given in Box 5.

**BOX 4**

**Tools for surveillance of adverse events following immunization (AEFI): intermediate level**

- Guidelines for AEFI surveillance
- Decision tree (*Figure 3*)
- Categories and classification of adverse events (*Box 1*)
- Roles of health workers (*Figure 1*; *Annex I*)
- Case investigation reporting forms for AEFI (*Annexes II, III*)
- Case definitions for clinical syndromes associated with viscerotropic disease, neurological disease and hypersensitivity reactions (*Annex IV*)
- Differential diagnosis for clinical syndromes associated with viscerotropic disease, neurological disease and hypersensitivity reactions (*Annex IX*)
- Guidelines for collection and storage of specimens (Annexes VI, *VII*)
- Kits for blood sampling (*Annex VII*)

**BOX 5**

**Differential diagnosis: main diseases to be ruled out[a]**

Laboratory tests should be performed to rule out the following diseases:

- Wild-type yellow fever
- Leptospirosis
- Louse-borne relapsing fever
- Malaria
- Viral hepatitis, especially the fulminating form of hepatitis B and C
- Dengue haemorrhagic fever
- Other viral haemorrhagic fevers, particularly those with severe hepatic manifestations (e.g. Rift Valley fever, Congo-Crimean haemorrhagic fever)

[a] See also Annex IX

## *Table 1. Recommended laboratory tests for suspected serious adverse events following immunization against yellow fever*

### A. ALL SUSPECTED SERIOUS ADVERSE EVENTS

**First set of essential basic laboratory tests**

| SPECIMEN | LABORATORY TESTS | CLINICAL RATIONALE |
|---|---|---|
| Blood | Complete blood count & platelets | Basic workup, rule out bacterial infection, clinical baseline |
| Blood | Thin/thick smear | Rule out malaria, borrelia |
| Urine | Urine analysis | Proteinuria, haemorrhage |
| Cerebrospinal fluid (CSF)[a] | Turbidity | Basic workup, rule out bacterial infection, clinical baseline |
| | Cell count | |
| | Protein | Rule out bacterial meningitis |

### B1. SUSPECTED ACUTE VISCEROTROPIC DISEASE

**Second set of laboratory tests for clinical assessment and differential diagnosis[b]**

| SPECIMEN | LABORATORY TESTS | CLINICAL RATIONALE |
|---|---|---|
| **Essential[c]** | | |
| Blood | Complete blood count & platelets | Rule out other etiologies |
| | Blood culture | Rule out bacteraemia |
| Serum | Serum transaminases | Assess liver enzymes and function |
| | Direct & indirect bilirubin | Assess liver enzymes and function |
| | Alkaline phosphatase | Assess liver enzymes and function |
| | Gamma glutamyl transferase | Assess liver enzymes and function |
| | Viral hepatitis B/C tests | Rule out other viral hepatitis |
| | Blood urea nitrogen | Assess renal function |
| | Creatinine | Assess renal function |
| | Amylase | Assess pancreatic inflammation |
| | Creatine phosphokinase | Assess rhabdomyolysis |
| | Prothrombin time, partial thromboplastin time | Coagulation panel |
| Urine | Urine analysis | Assess rhabdomyolysis |
| | Urine antigen | Rule out leptospirosis |
| Saliva | PCR | Detect yellow fever virus |
| Stool | PCR | Detect yellow fever virus |
| Other fluids | PCR | Detect yellow fever virus |
| **Desirable** | | |
| Serum | Yellow fever IgM, IgG antibodies (acute and convalescent) | Confirm yellow fever vaccination or infection |
| | PCR/viral culture | Rule out wild type virus yellow fever infection |

### B2. SUSPECTED NEUROLOGICAL/NEUROTROPIC DISEASE

**Second set of laboratory tests[b]**

| SPECIMEN | LABORATORY TESTS | CLINICAL RATIONALE |
|---|---|---|
| **Essential[c]** | | |
| CSF | Turbidity | Rule out bacterial infection |
| | Gram stain antigen detection or culture if available | Rule out bacterial infection |
| | Cell count: erythrocytes, leukocytes & differential | Rule out bacterial infection |
| | Glucose | Rule out bacterial infection |
| | Protein | Rule out bacterial infection |
| Blood | Thin/thick smear | Rule out malaria |
| **Viral testing desirable** | | |
| CSF (paired with serum) | PCR/culture within first 7 days | Confirm presence of yellow fever virus |
| | Yellow-fever IgM & confirmatory PRNT (titre) | Confirm presence of yellow fever virus vaccine reaction |
| Serum | PCR/culture | Confirm presence of yellow fever virus |
| | Antibody testing[d] | Rule out infection with other viruses |
| Stool | Viral culture[d] | Polio, enterovirus |

### C. SUSPECTED VISCEROTROPIC DISEASE, NEUROLOGICAL DISEASE OR HYPERSENSITIVITY REACTION

**Third set of laboratory tests focus on yellow-fever virus, to determine vaccine causality[b]**

| SPECIMEN | LABORATORY TESTS | CLINICAL RATIONALE |
|---|---|---|
| Various | See Annexes X ,VI, VII | Yellow fever vaccine virus identification and differential diagnosis |

CSF, cerebrospinal fluid; PRNT, plaque-reduction neutralization testing; PCR, polymerase chain reaction.

[a] CSF where possible and indicated by clinical picture.

[b] Standard operating procedures for collection, storage and transport of second and third set of laboratory tests to reference laboratories: see Annexes X ,VI, VII.

[c] Where this test is not available, arrange transport of specimen to national reference laboratory.

[d] Minimum differential diagnosis: Box 5. See more extensive differential diagnosis in Annex IX.

# Section 3

## Further investigation, processing and analysis of data on serious AEFIs: intermediate and central level

### 3.1 Investigation of serious "vaccine reaction" following yellow fever vaccination

All serious AEFIs reported by passive or active surveillance must be re-assessed by a team of trained health providers. The mobile teams/surveillance units will complete the report form with all the clinical data available.

Each report form should include clinical symptoms, treatment administered, the progress of the investigation, the clinical and laboratory findings, the clinical course of the event, and the conclusions of the investigation in terms of diagnosis and putative causal link with the vaccination (Annex II). At the referral hospital where the patient is admitted and treated, laboratory analyses must be performed to verify the clinical syndrome and to rule out other diagnoses. When laboratory facilities are inadequate at the intermediate level, serum samples must be sent to the national reference laboratory or to an international reference laboratory.

### 3.2 Clinical syndromes and physical findings

There are three serious adverse events classified as "vaccine reaction" that have been noted after vaccination against yellow fever (Annex V).

- Yellow fever vaccine-associated viscerotropic disease (AVD)
- Yellow fever vaccine-associated neurological disease (AND)
- Severe hypersensitivity reactions following yellow fever vaccination.

#### *3.2.1 Yellow fever vaccine-associated viscerotropic disease*

The clinical presentation of viscerotropic disease has been described as:

- A non-specific febrile syndrome that mimics yellow fever wild-type disease, typically progresses to hypotension or shock and multi-organ failure associated with jaundice and/or bleeding.
- Early signs/symptoms include fever, myalgias, arthralgias, weakness/fatigue, diarrhoea, vomiting and headache.
- Late signs/symptoms include severe abdominal pain, jaundice, hepatic insufficiency, bleeding, renal failure, hypotension/shock and dyspnea/hypoxia.
- There is generally multi-organ involvement and there may be clinical and laboratory evidence of liver dysfunction, renal impairment, respiratory distress, third-space sequestration, rhabdomyolysis, thrombocytopenia, myocarditis, haemorrhage and disseminated intravascular coagulation.
- In contrast to wild-type yellow fever, the activity of hepatic aminotransferases may be only mildly elevated, and haemorrhage may be mild or absent (Hayes, 2007).
- While not diagnostically conclusive, the number of leukocytes tends to be normal or there may be leukopenia, progressing to leukocytosis.

- In all cases there is neutrophilia with a shift to the left.[1]
- Lymphopenia and pancytopenia have been reported in some cases.

### 3.2.2 Yellow fever vaccine-associated neurological disease

Three neurological syndromes associated with yellow fever vaccine have been defined and fall into two categories:

- Neurotropic disease: meningoencephalitis;
- Autoimmune disease:
  - — Acute disseminated encephalomyelitis; and
  - — Guillain-Barré syndrome.

Neurotropic disease is secondary to infection of the central nervous system with the vaccine virus. The clinical presentation of neurotropic disease may include fever, headache, focal neurological findings, mental status changes and seizures.

In contrast, autoimmune neurological disease is due to immunization-induced antibodies and/or autoreactive T-cells, which cross-react with self-proteins within the central or peripheral nervous system. The clinical presentation of autoimmune disease may include limb weakness, absent tendon reflexes, cranial nerve abnormalities, altered mental status and ataxia (McMahon et al., 2007).

Establishing a clinical diagnosis requires that cerebrospinal fluid (CSF) be obtained: findings in neurotropic disease reflecting inflammation of the CNS include CSF pleocytosis and elevated protein and yellow fever specific IgM antibodies, as demonstrated by specific neutralization testing. In autoimmune disease, an elevated level of CSF protein is the more prominent finding, and while yellow fever IgM antibodies can sometimes be detected, they are frequently absent.

### 3.2.3 Severe hypersensitivity reactions following vaccination against yellow fever

Severe hypersensitivity reactions may include anaphylactic shock, anaphylactoid reactions, urticaria and exanthems without evident pruritus.

*Note:* Among YF-AEFI reported in Brazil, three types of hypersensitivity reactions were characterized:
- — anaphylactic shock;
- — hypersensitivity reactions occurring ≤ 2 hours after vaccination;
- — delayed, serum-sickness type reactions and erythema multiforme following vaccination against yellow fever.

### 3.2.4 Other serious adverse events

Severe gastroparesis has been described following vaccination against yellow fever.

## 3.3 Information and samples required for all serious vaccine reactions

The following information and samples are necessary to assess whether the serious adverse event following immunization, such as viscerotropic and neurological disease, may be due to the yellow fever vaccine:

- Patient information and vaccine information (Annexes II and III). The temporal relationship between the vaccination and the onset of symptoms is important in order to establish the possible causal relationship between the vaccine and the adverse event (Annex V).

[1] A "shift to the left" refers to the presence of increased proportions of younger, less well-differentiated neutrophils and neutrophil-precursor cells in the blood.

- Additional clinical information and laboratory findings to rule out other diagnoses (Figure 3, Annex IX)
- In the referral hospital, a minimum set of laboratory tests must be performed in order to:
  - — Assess a potential causal relationship of the event to the vaccine; and
  - — Rule out other diagnoses, including but not limited to the diseases listed in Box 5.

    See Annex IX for specific differential diagnoses for viscerotropic and neurological disease.
- Post-mortem investigation: in order to investigate a possible link between the vaccine and the AEFI, an autopsy should be performed within 72 hours of death. If an autopsy is refused or not possible, efforts should be made to obtain a liver biopsy using a viscerotome.

# Section 4

## Final classification of serious adverse events following immunization against yellow fever into "suspect", "probable" and "confirmed" cases: central and international level

After local assessment of the serious vaccine-related adverse event, a detailed description of the case is presented to a national expert committee responsible for classification into "suspect", "probable" and "confirmed" cases for viscerotropic and neurological (or neurotropic) disease. The case definitions are presented in Annex IV.

The central level of the Ministry of Health is generally the national level, but in some cases (e.g. large country), could be represented by another administrative area (state, region). In this exercise, it is represented by the national expert committee. The international level is represented by the international WHO reference laboratory for AEFI and the technical support of WHO.

### 4.1 National expert committee: central and international level

The expert committee on YF-AEFI is designated by the authorities of the ministry of health and should include clinical, pharmacological and public-health experts from the ministry of health, WHO and the academic network of experts on yellow fever.

#### *4.1.1 Roles and responsibilities of the national yellow fever vaccine adverse events monitoring expert committee*

The national expert committee is responsible for the coordination and monitoring, surveillance and investigation of AEFIs related to yellow fever vaccine during and 30 days after a preventive campaign of vaccination against yellow fever. The final aim is to ensure classification of all suspected adverse events, once all clinical and laboratory data are available, with a particular focus on suspected serious adverse events.

The committee is responsible for the following tasks:

- Developing guidelines for surveillance and management of suspected YF-AEFI.
- Ensuring the finalization of all documents and tools for surveillance and investigation of YF-AEFI.
- Proposing methods and procedures for:
  - Active surveillance and case-finding for YF-AEFI;
  - Investigation for all serious suspected cases of YF-AEFI;
  - Specimen collection, handling and transport for all serious suspected YF-AEFI;
  - Clinical case management for suspected YF-AEFI.
- Supervise all investigations of suspected cases of serious YF-AEFI;
- Ensure and monitor data collection for all suspected cases;
- Ensure final classification of all suspected serious cases identified and investigated;
- Draft and share the final report for AEFI surveillance;

- Communicate the findings to the relevant partners and authorities (ministry of health, national regulatory authority, WHO, relevant vaccine manufacturer).

### 4.1.2 National yellow fever vaccine adverse events monitoring expert committee

It is recommended that membership of the committee does not exceed 10–14 persons. In some cases, one person can serve more than one function. The committee should comprise the following members:

1. Senior representative of the ministry of health, the Department of Disease Control, and/or the EPI programme;
2. A representative/expert of the national regulatory authority, and/or unit responsible for pharmacovigilance/post-marketing surveillance;
3. A communications specialist or journalist;
4. An epidemiologist and/or representative of national disease surveillance system;
5. A virologist/senior laboratory expert;
6. A pathologist;
7. A neurologist;
8. A gastroenterologist;
9. An infectious-disease clinician and/or an internist and/or emergency-medicine specialist;
10. A public-health physician/specialist;
11. WHO EPI focal person and/or surveillance officer;
12. Others specialists if necessary.

It can also be helpful for a representative of the United Nations Children's Fund (UNICEF) to attend.

### 4.1.3 Meetings of the expert committee

The expert committee should begin work 2 months before the implementation of the vaccination campaign and continue for 3 months afterwards, until all suspected cases have been investigated and classified and the final report presented. All health workers involved in the campaign will need information on this activity during their training.

The objectives of the first meeting are as follows:

- To brief committee members on surveillance of yellow fever vaccine adverse events;
- To share experience from other countries in the region;
- To review a draft guide on surveillance and data-collection tools and plan for their completion before the campaign;
- To draft a work plan for subsequent activities.

## 4.2 Investigation by the reference laboratory: international level

### *4.2.1 Differential diagnosis*

The role of the reference laboratory at the international level involves the identification of yellow fever vaccine virus and differentiation from other health conditions that might have similar clinical presentations to yellow fever vaccine-associated viscerotropic and neurological disease.

- The differential diagnosis varies with geographical area of occurrence of the adverse event (Annex IX).
- The differential diagnosis for non-infectious diseases resembling neurological and viscerotropic disease is not addressed in this operational guide.

### *4.2.2 Standard operating procedures for collection, storage and transport of samples to be sent to a WHO reference laboratory*

Standard operating procedures for collection, storage and transport of samples may vary according to the WHO reference laboratory.

Current recommendations for collection and handling of samples to be sent to a WHO reference laboratory are summarized below. Annex XI gives a patient admission flowchart that summarizes the recommendations for samples to be collected from the patient and storage thereof. Annex VII describes specimens required for diagnosis. Collection of specimens after death and after autopsy is summarized in Table 2 and in Annex VI.

### *4.2.3 Standard operating procedures for collection, storage and transport of autopsy samples to be sent to a WHO reference laboratory*

It is recommended that an autopsy be performed within 72 hours of death, following the procedures described below.

- Perform the autopsy as quickly as possible (within 72 hours) to prevent tissue lysis. The autopsy protocol is completed to help the medical examiner by furnishing the patient's history.
- Collect blood samples via cardiac puncture. Five 1 mL specimens of serum are needed for testing. The blood should be transported on wet or dry ice and stored at –70 °C or in liquid nitrogen.
- Collect two 1 g samples from each organ for pathology; for example, specimens from the brain with meninges, specimens from each lobe of the lung, specimens from the two adrenal glands, spleen, kidney, lymph node and thymus. In each case, the samples should be representative of the area and investigated for the suspected pathology. There should be enough formalin to cover the specimens.
  - — DO NOT FREEZE FORMALIN SPECIMENS. The specimens of RNAlater® stabilization should be flash-frozen at –70 °C.
  - — Send specimens separately in the appropriate solution, in an individual zip-sealed bag for each kind of specimen.
- Collect a specimen from two lymph nodes as near as possible to the injection site. One will be directly frozen at –70 °C and the other preserved in formalin. (DO NOT FREEZE sample in formalin).
- Label all specimens with the name of the patient and the autopsy protocol number.

***Table 2. Recommendations for collection of specimens to be sent to a WHO reference laboratory***

| SAMPLE | COLLECTION AND USE | NOTES |
|---|---|---|
| 1. Serum (necessary) | | |
| Sample 1 (at onset) | Five 1 mL samples of serum at onset of symptoms<br>Transported in dry or wet ice and stored at –70 °C or liquid nitrogen<br>Use for acute titres for detection of IgM and IgG antibodies to yellow fever 17D virus, isolation of yellow fever virus (>7 days after vaccination) for genome detection and to rule out leptospirosis, hepatitis A, B and C, EBV (Ministry of Health in Togo, 2007), CMV and rickettsial disease as appropriate. | It may be necessary to obtain clinically appropriate smaller volumes from small children. In general, 0.5 mL of serum is the minimum amount for laboratory testing for yellow fever.<br>Additional samples may be needed to rule out other etiologies (see Annex IX). |
| Sample 2 (at 21–35 days) | Five 1 mL samples at 21–35 days after the onset of symptoms. If it is not possible to obtain the blood sample at 21–35 days, it may be taken up to 6 months after onset. Use for detection of the viral genome by quantitative RT-PCR and specific yellow fever serology (IgM, IgG neutralizing antibodies). | |
| 2. Cerebrospinal fluid (if clinically indicated) | Obtain a minimum of five 1 mL samples for viral testing at onset of symptoms. Transport in dry or wet ice and store at ≤ 70 °C or liquid nitrogen. Specimens are to be used for viral isolation, detection of the viral genome by quantitative RT-PCR and yellow fever specific serology (IgM). | CSF should only be collected if clinically indicated for confirmation of suspected neurological disease. |
| 3. Urine (if clinically indicated) | Obtain a minimum of 5 mL at the onset of symptoms. Collect in a single tube, transport in dry or wet ice and store at ≤ 70 °C or liquid nitrogen. Useful for detection of virus by RT-PCR. | |
| 4. Peritoneal or pleural fluid (if clinically indicated) | Obtain a minimum of one 0.2 mL specimen at onset of symptoms. Transport in dry or wet ice and store at ≤ 70 °C or liquid nitrogen. Useful for detection of virus by RT-PCR. | |
| 5. Stool (where possible) | Obtain a 1 g specimen at onset of symptoms. Transport in dry or wet ice and store at ≤ 70 °C or liquid nitrogen. Useful for detection of virus by RT-PCR. | |
| 6. Saliva (where possible) (Sejvar, 2007) | Obtain 0.2 mL in one tube at onset of symptoms. Transport in dry or wet ice and store at ≤ 70 °C or liquid nitrogen. Useful for detection of yellow fever virus by RT-PCR and for differentiation from enteroviral infection in the case of neurotropic disease. | DO NOT FREEZE SPECIMENS at –20 °C |
| 7. Tissue (post mortem) | The following organs should be preserved if possible: liver* (mandatory), kidney (mandatory), brain (for YF-AND), lung, heart, intestine, lymph node (mesenteric), adrenal gland, spleen and thymus.<br>In the case of neurological disease, a sample of brain tissue should be obtained if possible.<br>Collect formalin-fixed paraffinated and RNAlater®-stabilized tissue.[a]<br>RNAlater® buffer: obtain 1 gr of fresh tissue and place in 2 mL cryovials. Transport on wet or dry ice and store at –70 °C or in liquid nitrogen.<br>The RNAlater®-stabilized tissues will be used for:<br>— YF 17D virus isolation (titration) from tissue;<br>— YF 17D quantitative and 17D RT-PCR virus amplification in tissue.<br>Paraffinated samples: 1 g of paraffinated tissues should be stored and transported at ambient temperature or at 2–8 °C. The tissues will be used for:<br>— Immunocytochemistry for yellow fever antigen.<br>Formalin-fixed: 1 g of formalin-fixed tissues should be stored and transported at ambient temperature or at 2–8 °C.<br>The formalin-fixed tissues will be used for:<br>— Histopathology (e.g. liver, thymus);<br>— Immunohistochemistry for yellow fever antigen in visceral tissue. | Tissue samples are necessary for confirmation of a case of viscerotropic disease.<br>*Liver biopsy can aid in diagnosis; however, it should not be performed on living patients because of the significant risk of haemorrhage.<br>DO NOT FREEZE SPECIMENS AT –20 °C. |

CMV, cytomegalovirus; EBV, Epstein-Barr virus; RT-PCR, reverse transcriptase polymerase chain reaction.

[a] RNAlater®: a reagent that stabilizes RNA.

- Send the pathology specimens to the WHO reference laboratory and include the following documents: the summary from the clinical history, the conclusions of the investigation, the laboratory-test request forms and the autopsy report with the cause of death (classified according to ICD-10[1]).

### 4.2.4 Identification of the international reference laboratory

The contact information for the WHO reference laboratory where specimens (serum, CSF, organ specimens for pathology) are sent should be readily available. Collection, handling and storage must be consistent with the requirements of the designated international reference laboratory. The international reference laboratory for forensic and auxiliary tests will send the results to the immunization programme of the ministry of health.

[1] International Classification of Disease, Tenth Revision (http://www.who.int/classifications/icd/en/).

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18–19 November 2008

World Health Organization, Geneva, Switzerland

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# Annexes

# Annex I

## Expertise necessary for effective immunization safety

| PERSON/ENTITY | ROLE |
|---|---|
| Peripheral health workers | Detect and report event (to district office) |
| District level supervisor | Completes an AEFI report, if adverse event meets criteria; forward to province level |
| Province level AEFI investigator | Assesses AEFI report and investigates AEFI if it meets criteria; produces regular line listing of reports received, and the conclusion of the investigation if conducted; forward to regional/national assessor |
| Regional/national assessor | A person (with a deputy for periods of absence) with designated responsibility for immunization safety at regional/national level; reviews information on provincial AEFI returns; conducts regular analysis of AEFI and feeds results back down the system; provides support to provincial investigator; spokesperson for immunization safety. |
| Regional/national immunization safety committee | Composed of national regulatory authority representative, EPI manager, paediatrician, infectious-disease physician, neurologist, immunologist, epidemiologist, and possibly a pharmacologist/toxicologist – reviews overall pattern of reports and investigations; provides the causality assessment on investigations which have not reached conclusions; provides quality control on system (can be part of national immunization advisory group). In addition, the system needs defined procedures; case definitions; clear guidelines and standard forms for reporting and investigating; forms for line listings; and AEFI database for comprehensive analysis (from lowest practicable level in system up to national level). |

AEFI, adverse event following immunization; EPI, Expanded Programme on Immunization; NRA, national regulatory authority.

# Annex II

## Notification report for an adverse event following immunization against yellow fever (YF-AEFI)

| HEALTH FACILITY INFORMATION | PATIENT INFORMATION |
|---|---|
| Region: ..... | Surname: ..... |
| District: ..... | First name: ..... |
| Health facility: ..... ..... | Address and contact information: ..... ..... ..... ..... |
| If general infirmary, specify unit: ..... | |
| Health worker: ..... | Age: ..... years Sex: ☐ M ☐ F |
| Date of notification: ...../...../..... | |

### VACCINATION INFORMATION

**Vaccination card**: ☐ Yes ☐ No

If No, other source of information: .....

**Place of vaccination/village/vaccination point**:

.....

**Date of vaccination**: ...../...../.....

**Vaccine**: .....

**Mode of administration**: ☐ subcutaneous ☐ intramuscular

**Site of administration**: ☐ Right arm ☐ Other: .....

| | MANUFACTURER | BATCH NUMBER | EXPIRY DATE |
|---|---|---|---|
| Vaccine | | | ...../...../..... |
| Solvent | | | ...../...../..... |

### DESCRIPTION OF POST-VACCINATION REACTIONS OBSERVED

**Date of onset of initial symptoms**: ...../...../.....

**Fever**: ☐ Yes ☐ No *If Yes, specify*: ..... °C Date of fever peak: ...../...../.....

**Headaches**: ☐ Yes ☐ No

**Local reaction at injection site**: ☐ Yes ☐ No *If Yes, specify*: .....

☐ Pain ☐ Redness ☐ Swelling/oedema ☐ Skin lesion, *if Yes, purulent*? ☐ Yes ☐ No

☐ Other local reaction: .....

**Skin or mucous tissue reaction:** ☐ Yes ☐ No *if Yes, specify* Date of onset: ...../...../.....

- Rash/itching: ☐ Yes ☐ No, *if Yes,* Site .....
- Eczema: ☐ Yes ☐ No, *if Yes,* Site .....
- Conjunctivitis: ☐ Yes ☐ No
- Other skin/mucous tissue reaction: .....

**Swelling/oedema:** ☐ Yes ☐ No

*If Yes*, Date: ...../...../..... Site: .....

**Respiratory problem**: ☐ Yes ☐ No, *if Yes*, date: ...../...../.....

Specify: .....

**Gastrointestinal problem**: ☐ Yes ☐ No, *if Yes*, date of onset: ...../...../.....

☐ Nausea ☐ Vomiting ☐ Diarrhoea ☐ Stomach pain

☐ Other: .....

**Anaphylactic shock (collapsus)**: ☐ Yes ☐ No **Muscle pain**: ☐ Yes ☐ No

**Jaundice**: ☐ Yes ☐ No, *if Yes*, date of onset: ...../...../.....

**Neurological involvement**: ☐ Yes ☐ No, *if Yes*, specify type: .....

Date of onset: ...../...../.....

**Mental status change**: ☐ Yes ☐ No, *if Yes*, specify: .....

**Seizures**: ☐ Yes ☐ No

**Viscerotropic disease**: ☐ Yes ☐ No, *if Yes*, describe:

.....

**Haemorrhage**: ☐ Yes ☐ No, *if Yes*, describe:

.....

Other signs observed or other laboratory test results: .....

.....

**Presumed diagnosis**: .....

## TREATMENT AND CLINICAL COURSE

**Treatment(s) administered**: .....

.....

**Patient hospitalized**: ☐ Yes ☐ No, *if Yes*, duration: ..... days

**Status of patient on discharge**: ☐ Cured ☐ In remission ☐ Other: .....

**Patient cured**: ☐ Yes ☐ No, *if Yes*, date: ...../...../.....

**Sequelae**: ☐ Yes ☐ No, *if Yes*: .....

**Patient deceased**: ☐ Yes ☐ No, *if Yes*, date: ...../...../.....

**Cause of death**: .....

# Annex III

## Evaluation form for a serious adverse event following immunization against yellow fever (YF-AEFI)

Notification number: .....

Presumed diagnosis: .....

Date of investigation: ...../...../..... (DD/MM/YYYY)

Place of investigation: .....

### DEMOGRAPHIC DATA

| Family name: ..... | First name(s): ..... | Age: ..... Year ..... Months | Gender: ☐ F ☐ M |
|---|---|---|---|
| District of residence: ..... | | Village: ..... | |
| Address: ..... | | Contact (Tel.): ..... | |
| Date of vaccination: ...../...../..... | Vaccine manufacturer and lot no.: ..... | Diluent manufacturer and lot no.: ..... | Date of onset of AEFI: ...../...../..... |

### TYPE OF SEVERE AEFI SUSPECTED (check box)

☐ Encephalitis
☐ Encephalopathy
☐ Cranial nerve abnormalities
☐ Guillain-Barré syndrome
☐ Thrombocytopenia
☐ Rhabdomyolysis
☐ Kidney failure
☐ Liver failure
☐ Death
☐ Anaphylactic shock/reaction
☐ Septicaemia :
☐ Other: .....

### STATUS OF PATIENT

☐ Alive ☐ Comatose ☐ Recovered ☐ Lost to follow-up

☐ Deceased date: ...../...../..... (DD/M/YY)

**1. Clinical examination**: ☐ Yes ☐ No

If yes, please provide clinical description:

.....

.....

.....

.....

**2. Results of additional investigations**:

| General tests | Specific tests |
|---|---|
| ..... | ..... |
| ..... | ..... |
| ..... | ..... |
| ..... | ..... |

**3. Results of laboratory tests carried out before the investigation**:

.....

.....

.....

.....

**4. Treatment(s) before investigation**:

.....

.....

.....

.....

**5. Specimens for laboratory analysis**:

| Specimen | Date | Time |
|---|---|---|
| Blood : | Date: ...../...../..... | Time: ..... h ..... min |
| Cerebrospinal fluid, aspect: | Date: ...../...../..... | Time: ..... h ..... min |
| Urine | Date: ...../...../..... | Time: ..... h ..... min |
| Stool | Date: ...../...../..... | Time: ..... h ..... min |
| Saliva | Date: ...../...../..... | Time: ..... h ..... min |
| Tissues | Date: ...../...../..... | Time: ..... h ..... min |
| Vaccine | Date: ...../...../..... | Time: ..... h ..... min |
| Solvent | Date: ...../...../..... | Time: ..... h ..... min |

**6. Laboratory tests performed**:

| Specimen | Laboratory test(s) considered | Laboratory test(s) requested | Date specimen was received at laboratory(s) |
|---|---|---|---|
| Blood | | | ...../...../..... |
| Cerebrospinal fluid | | | ...../...../..... |
| Urine | | | ...../...../..... |
| Stool | | | ...../...../..... |
| Saliva | | | ...../...../..... |
| Tissues | | | ...../...../..... |
| Vaccine | | | ...../...../..... |
| Solvent | | | ...../...../..... |

**7. Conclusions of investigation** (based on information available at the time of the investigation):

Provisional diagnosis .....

Alternative explanations possible .....

Classify the adverse event:

1. Programme error ☐ Yes ☐ No

   ***If yes***, specify reason (*tick as appropriate*):

   ☐ Defective cold chain ☐ Vaccine reconstitution error

   ☐ Incorrect injection technique ☐ Non-sterile handling

2. Injection reaction ☐ Yes ☐ No
3. Vaccine reaction (suspected) ☐ Yes ☐ No

   ***If yes***, assess likelihood of vaccine-relatedness: ☐ Very likely ☐ Likely ☐ Possible

4. Coincidental ☐ Yes ☐ No
5. Unknown ☐ Yes ☐ No

Explain conclusion:

.....

.....

.....

.....

Remedial action taken:

.....

.....

.....

.....

Further investigations/actions recommended:

.....

.....

.....

.....

**Investigators and job titles**: **Signatures**:

# Annex IV

## Case definitions of viscerotropic and neurological adverse events following immunization against yellow fever, according to VAERS[1]

### Yellow fever vaccine associated viscerotropic disease

**Level 1. Viscerotropic disease**
Warning signs and symptoms during epidemiological surveillance

→

**Fever (temperature, > 38 °C; duration, > 24 hours) and one or more of the following signs and symptoms:**
- Nausea
- Vomiting
- Malaise (duration, > 72 hours)
- Myalgia (duration, > 24 hours)
- Arthralgia (duration, > 24 hours)
- Dyspnea

**Level 2. Viscerotropic disease**
Clinical signs and symptoms of disease

→

**Level 1 case definition with <u>at least one</u> of the following signs:**
- Jaundice (total bilirubin, ≥ 1.5 times above normal level)
- Liver dysfunction (elevation of AST and ALT, ≥ 3 times above normal level)
- Renal impairment (decrease in glomerular filtration rate, as measured by an elevation in BUN and creatinine of > 1.5 times normal levels and no history of renal disease)
- Tachycardia (heart rate, > 100 beats per minute) or bradycardia (heart rate, < 50 beats per minute)
- Rhabdomyolysis (CPK, > 5 times normal level)
- Respiratory distress (shortness of breath, ventilation or oxygenation impairment)
- Thrombocytopenia (platelet count, < 100 000/μL)
- Hypotension (systolic blood pressure, < 90 mm Hg for adults, or less than fifth percentile by age for children aged < 16 years; diastolic blood pressure, ≥ 15 mm Hg from lying to sitting, orthostatic syncope or orthostatic dizziness)
- Myocarditis (compatible abnormalities including ECG, ECHO, or changes in cardiac enzymes, or inflammation, by tissue biopsy)
- Disseminated intravascular coagulation (elevation of prothrombin time or activated partial thromboplastin time with fibrin split products)
- Haemorrhage

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, Blood urea nitrogen; CPK, creatine phosphokinase; ECG, electrocardiogram; ECHO, ultrasonic echo examination; Hg, mercury; VAERS, The Vaccine Adverse Event Reporting System.

[1] Unpublished case definitions for adverse events following yellow fever immunization from VAERS (Vaccine Adverse Events Reporting System, USA (http://vaers.hhs.gov/index).

### *Classification of viscerotropic serious adverse events as suspected, probable or confirmed vaccine reactions*

**1. Definition of suspected yellow fever vaccine-associated viscerotropic disease (YEL-AVD)**

The patient is defined as a SUSPECTED CASE if all the following elements are present:

Onset of symptoms occur with **1–10 days** following immunization with yellow fever vaccine, either given alone or in combination with other vaccines

**AND**

Level 2 viscerotropic disease with evidence of liver dysfunction

**AND**

No evidence of other diagnoses (differential diagnosis)

**2. Definition of probable yellow fever vaccine-associated viscerotropic disease (YEL-AVD)**

The patient is defined as a PROBABLE CASE if the SUSPECTED CASE definition applies (see above) and at least one of the following elements is present:

Histopathology consistent with yellow fever (e.g. liver midzonal necrosis, Councilman bodies)

**OR**

Isolation of yellow fever 17D[1] virus from blood (> 7 days after vaccination)

**OR**

Yellow fever 17D[1] virus concentration in serum on any day exceeds 3 log10 pfu/mL

**3. Definition of confirmed yellow fever vaccine-associated viscerotropic disease (YEL-AVD)**

The patient is defined as a CONFIRMED CASE if the PROBABLE CASE definition applies (see above) and at least one of the following elements is present:

Yellow fever specific antigen (17D[1] strain) in tissue demonstrated by immunohistochemistry (IHC)

**OR**

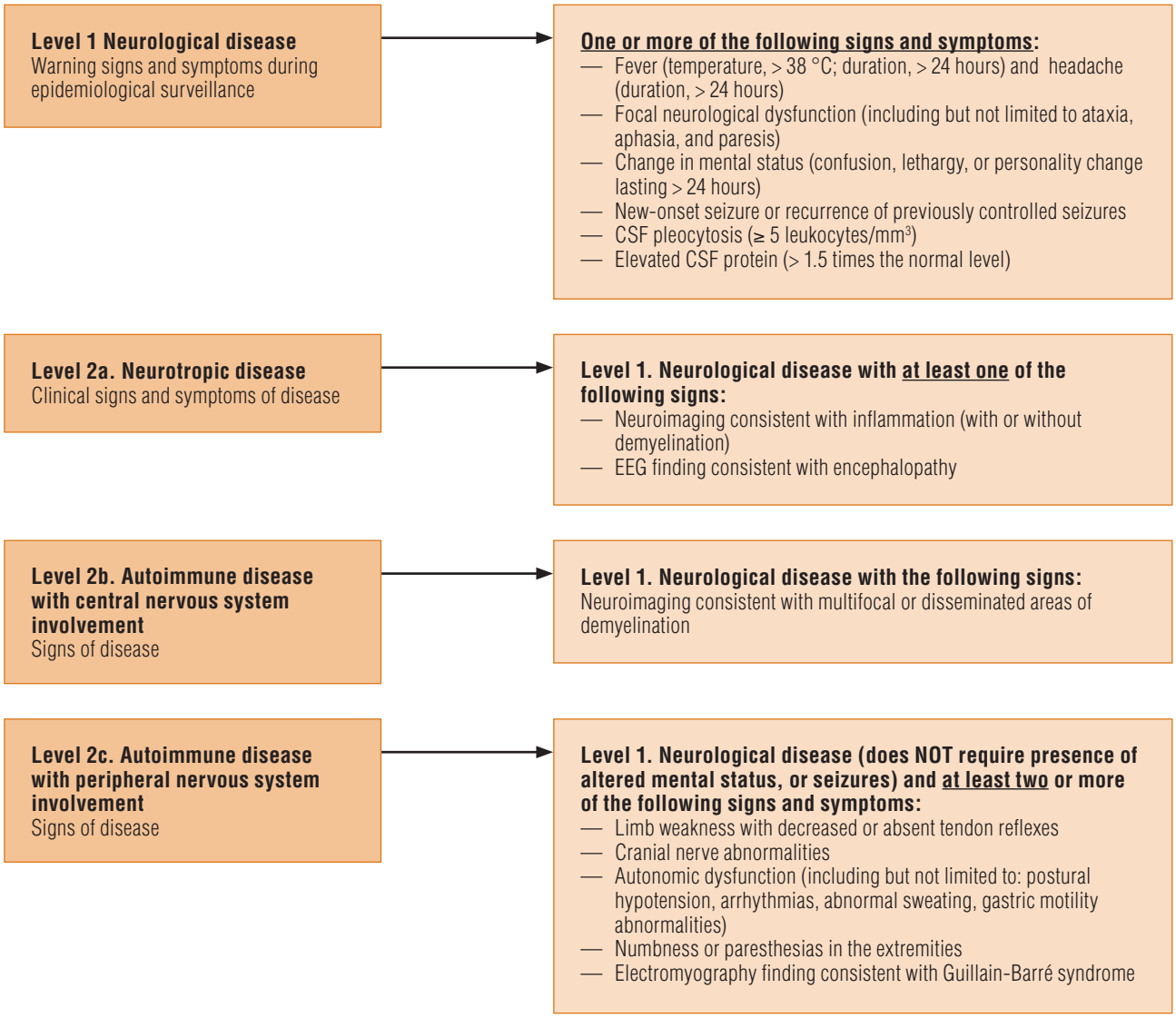
Isolation of yellow fever 17D[1] virus from tissue

**OR**

Amplification of yellow fever 17D[1] virus RNA from tissue

[1] Confirmed as 17D virus by monoclonal antibody analysis or nucleotide sequencing where the possibility of infection by wild-type or mutated 17D virus exists.

## Yellow fever vaccine-associated neurological disease




CSF, cerebrospinal fluid; EEG, electroencephalogram

### *Classification of neurological serious adverse events as suspected, probable or confirmed vaccine reactions*

**1. Definition of <u>suspected</u> yellow fever vaccine-associated neurological disease:**

The patient is defined as a SUSPECTED CASE if all the following elements are present:

> Onset of symptoms described above (levels 1 and 2a) within 1–30 days of vaccination with yellow fever vaccine, either given alone or in combination with other vaccines
>
> **AND**
>
> Patient fits <u>level 2a</u> case definition (neurotropic disease)
>
> **AND**
>
> No evidence of other diagnoses (differential diagnosis)

**2. Definition of <u>probable</u> yellow fever vaccine-associated neurological disease:**

The patient is defined as a PROBABLE CASE if the SUSPECTED CASE definition applies (see above) and at least one of the following elements is present:

> Isolation of vaccine-type yellow fever 17D[1] virus from blood (> 7 days after vaccination)
>
> **OR**
>
> Yellow fever 17D[1] virus concentration in serum on any day exceeds 3 log10 pfu/mL

**3. Definition of <u>confirmed</u> yellow fever vaccine-associated neurological disease:**

The patient is defined as a DEFINITE CASE if the SUSPECT CASE definition applies (see above) and at least one of the following elements is present:

> Detection in CSF of IgM-type antibodies specific to yellow fever
>
> **OR**
>
> Isolation of yellow fever 17D[1] vaccinal strain from CSF
>
> **OR**
>
> Amplification of 17D[1] viral (vaccinal) strain from CSF

[1] Confirmed as 17D virus by monoclonal antibody analysis or nucleotide sequencing where possibility of wild-type infection exist, inclusive of all 17D-derived vaccines.

**4. Definition of suspected neurotropic yellow fever vaccine-associated autoimmune disease with central nervous system involvement**

The patient is defined as a SUSPECTED CASE if all the following elements are present:

> Onset of symptoms described above (levels 1 and 2b) within 1–30 days of vaccination with yellow fever vaccine, either given alone or in combination with other vaccines
>
> **AND**
>
> Patient fits level 2b case definition (neurotropic disease)
>
> **AND**
>
> No evidence of other diagnoses (differential diagnosis)

**5. Definition of probable neurotropic yellow fever vaccine-associated autoimmune disease with central nervous system involvement:**

The patient is defined as a PROBABLE CASE if the SUSPECTED CASE definition applies (see above)

**AND**

> Yellow fever vaccine has been given alone (not in combination with other vaccines)

**6. Definition of suspected neurotropic yellow fever vaccine-associated autoimmune disease with peripheral nervous-system involvement:**

The patient is defined as a SUSPECTED CASE if all the following elements are present:

> Onset of symptoms described above (levels 1 and 2c) occurs within 1–30 days of vaccination
>
> **AND**
>
> Patient fits level 2c case definition autoimmune disease with central nervous system involvement
>
> **AND**
>
> No evidence of other diagnoses (differential diagnosis)

**7. Definition of probable neurotropic yellow fever vaccine-associated autoimmune disease with peripheral nervous-system involvement:**

The patient is defined as a PROBABLE CASE of autoimmune disease with peripheral nervous system involvement if the SUSPECTED CASE definition applies (see above)

**AND**

> Yellow fever vaccine has been given alone (not in combination with other vaccines)

# Annex V

## Detecting a serious adverse event following immunization against yellow fever (YF-AEFI)

### Yellow fever vaccine-associated viscerotropic disease

| CLINICAL SYNDROMES | SIGNS OR SYMPTOMS | LABORATORY FINDINGS | ONSET |
|---|---|---|---|
| Haemorrhage | Epistaxis, bleeding gums, purpura, petechiae, ecchymosis or other signs of spontaneous bleeding | Elevation of prothrombin time or activated partial thromboplastin time | 1–10 days |
| Hepatic insufficiency | Jaundice, abdominal pain, nausea, vomiting, diarrhoea, bleeding | Transaminases AST and ALT, ≥ 3 times normal levels<br>Total serum bilirubin, ≥ 1.5 times normal level | 1–10 days |
| Hypotension/shock | Cool extremities, weak/ absent pulses | Capillary refill time, > 3 seconds, systolic blood pressure, < 80; tachycardia | 1–10 days |
| Myocarditis | Haemodynamic instability | ECG abnormalities | 1–10 days |
| Renal insufficiency | Oliguria, anuria, haematuria, proteinuria | Serum creatinine, ≥ 1.5 times normal level<br>Oliguria, < 500 $cm^3$/24 hours<br>Urine analysis that reveals haematuria, proteinuria | 1–10 days |
| Respiratory insufficiency | Dyspnea, hypoxia | Abnormal chest X-ray | 1–10 days |
| Rhabdomyolysis | Myalgias, red/ brown urine due to myoglobinuria, | CPK, > 5 times normal<br>Hyperkalaemia and hyperphosphataemia result from the release of potassium and phosphorus from damaged muscle cells. Pigmented casts. Metabolic acidosis is common. | 1–10 days |
| Thrombocytopenia | Epistaxis, bleeding gums, purpura or other signs of spontaneous bleeding | Platelet count, < 100 000/mL<br>AND<br>confirmed by blood-smear examination<br>OR<br>the presence of clinical signs and symptoms of spontaneous bleeding | 1–10 days |

## Yellow fever vaccine-associated neurological disease

| NEUROLOGICAL SYNDROMES AND DEFINITIONS | SIGNS OR SYMPTOMS | LABORATORY FINDINGS | ONSET |
|---|---|---|---|
| Encephalitis – inflammation of the brain | Fever, altered mental status (encephalopathy) and/or focal neurological findings, focal weakness, cranial nerve palsies, seizures | CSF pleocytosis<br>CSF protein elevation<br>Clear CSF with negative Gram stain | Approximately 2–30 days |
| Meningitis – inflammation of the covering of the brain | Fever, headache, meningismus (nuchal rigidity, photo/phonophobia) | CSF pleocytosis<br>Clear CSF with negative Gram stain | Approximately 2–30 days |
| Anterior myelitis – inflammation of spinal cord motor neurons | Asymmetric limb weakness/paralysis, sensory loss generally absent | CSF pleocytosis, CSF protein elevation | Hours to days |
| Guillain-Barré syndrome – autoimmune disease of peripheral nerves | "Ascending" weakness – legs to arms, generally symmetric, hypo- or areflexia, ascending pain or dysesthesias, objective numbness generally absent | Cytoalbuminological dissociation – elevated CSF protein in absence of pleocytosis | Onset generally 1–4 weeks after vaccination |
| Acute disseminated encephalomyelitis – autoimmune CNS demyelinating process | Altered mental status, cranial nerve palsies, focal weakness, ataxia | CSF: pleocytosis (often less than acute encephalitis), elevated protein | 3–30 days |

CNS, central nervous system; CSF, cerebrospinal fluid.

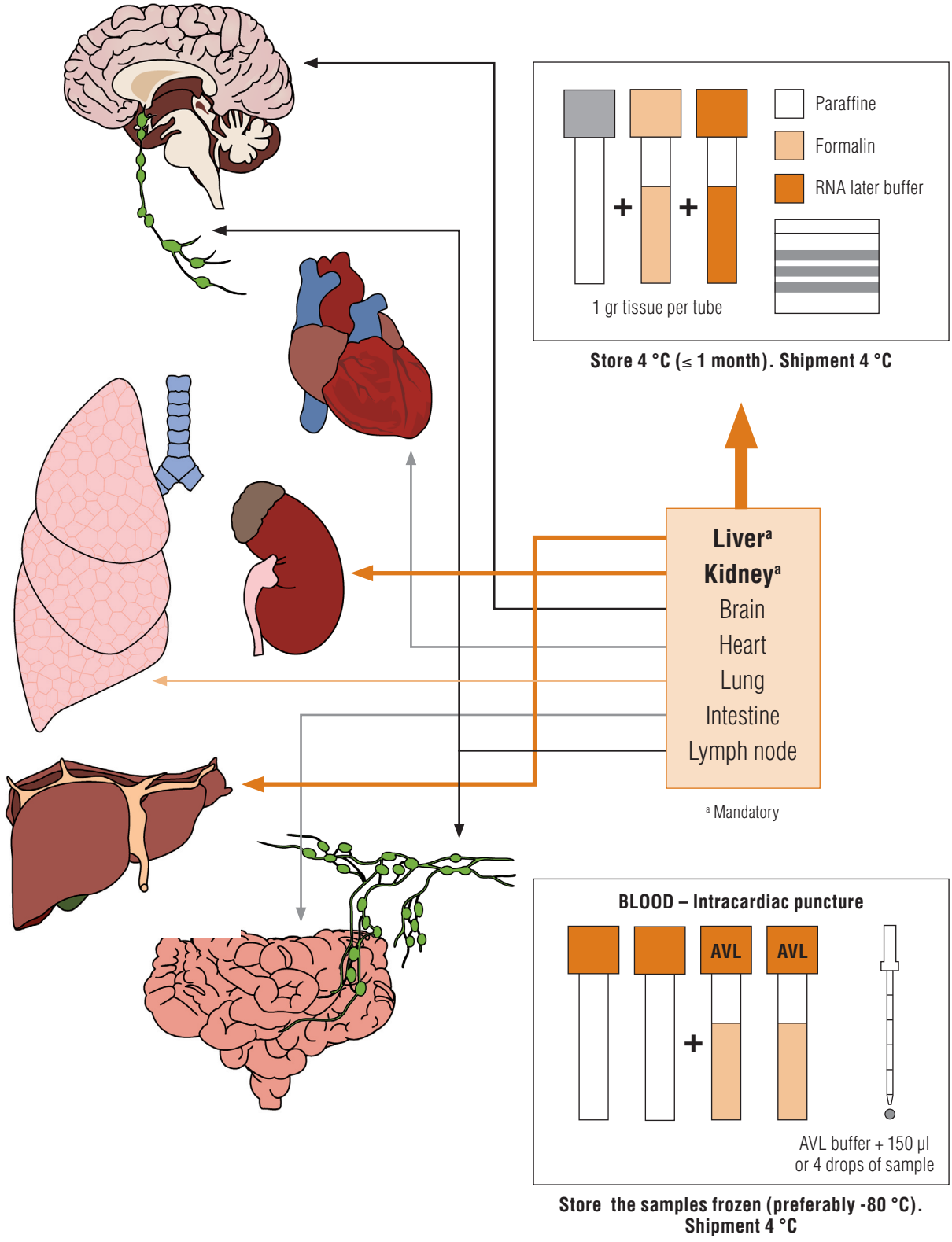
## Yellow fever vaccine-associated hypersensitivity reaction

| SEVERE HYPERSENSITIVITY REACTION (ALL) | SIGNS OR SYMPTOMS | ONSET |
|---|---|---|
| Anaphylactic shock/anaphylactoid | Cardiovascular collapse (e.g. altered consciousness, low blood pressure, weakness or absence of peripheral pulse, cold extremities may be accompanied by bronchospasm, laryngospasm, or laryngeal oedema or all of these symptoms with respiratory insufficiency that manifests immediately after the vaccination. | Immediate |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, Blood urea nitrogen; CPK, creatine phosphokinase; ECG, electrocardiogram

# Annex VI

## Autopsy specimens to be taken in the event of death after an adverse event following immunization against yellow fever (YF-AEFI)

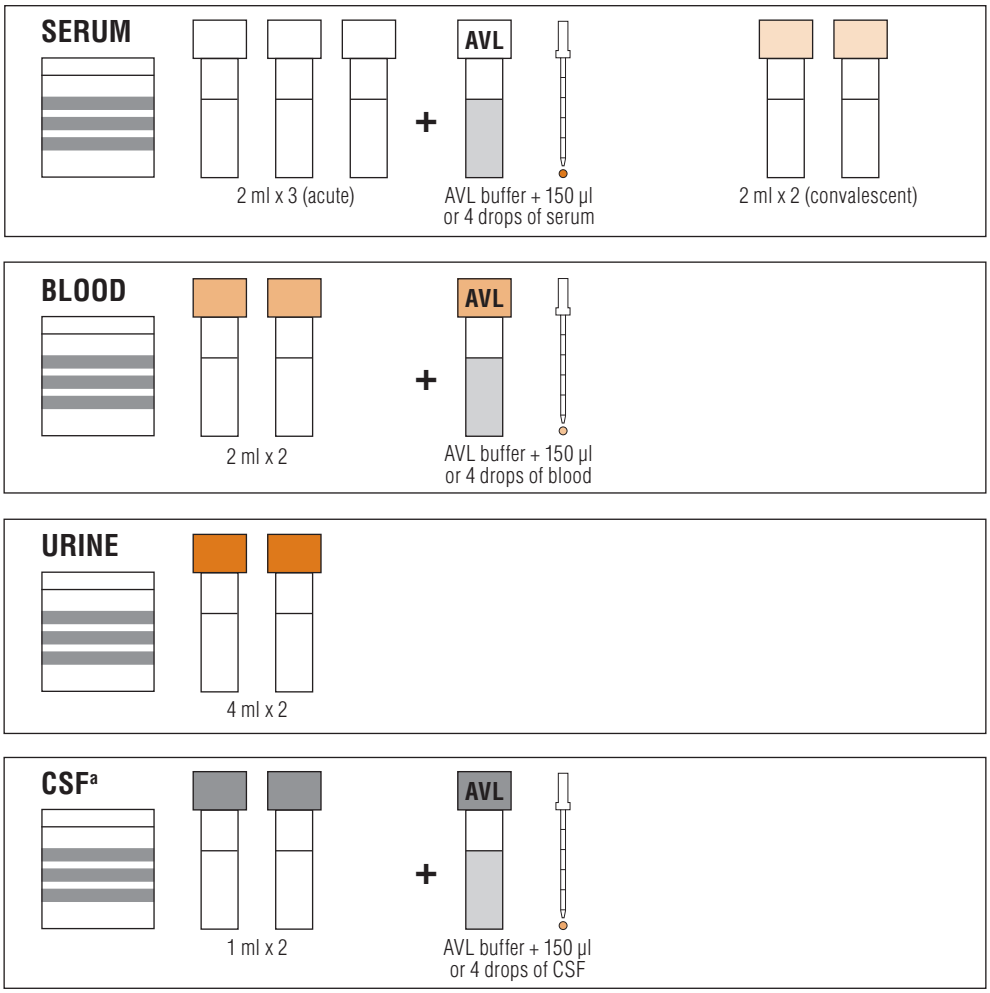



**Store 4 °C (≤ 1 month). Shipment 4 °C**

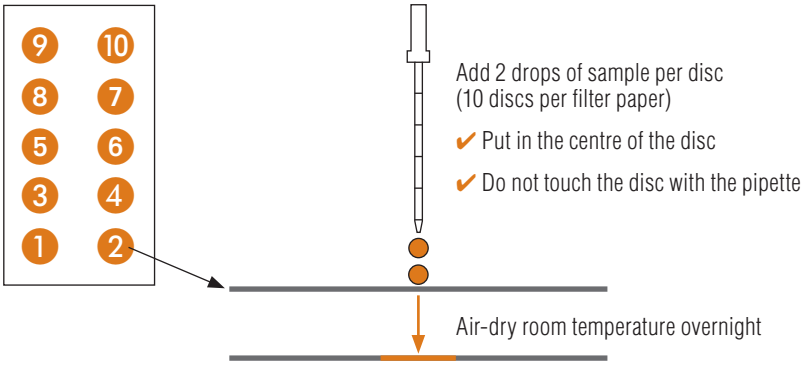
**Store the samples frozen (preferably -80 °C). Shipment 4 °C**

# Annex VII

## Specimens to be taken for diagnosis of a suspected adverse event following immunization against yellow fever (YF-AEFI)




### Filter paper preparation of CSF or serum samples




AVL, lysis buffer for viral nucleic acid purification; CSF, cerebrospinal fluid.

[a] only in suspected neurotropic adverse events.

# Annex VIII

## Detection of a serious adverse event following immunization against yellow fever (YF-AEFI): warning signs

| ADVERSE EVENT | WARNING SIGNS | LABORATORY/CLINICAL ASSESSMENT |
|---|---|---|
| Encephalitis | Convulsions<br>Mental status change: confusion, lethargy or personality change | CSF cytology, bacteriology, proteins<br>The clinical warning signs are convulsions or deterioration of the state of consciousness |
| Guillain-Barré syndrome | Ascending flaccid paralyses | CSF cytology and bacteriology<br>In case of Guillain-Barré syndrome, the first signs will be walking impairment (duck gait) followed by an ascending paresis |
| Paresis of cranial nerves | Signs of neurological deficiency | The clinical test will show a paresis of one of the XII cranial nerves, smell impairment, acute visual loss, facial palsy, swallowing disorders, diplopia, hearing impairment, etc. |
| Hepatic insufficiency | Jaundice (with or without encephalopathy) | AST and ALT, > 1000 IU/L<br>Bilirubin (> 1.5 times), alkaline phosphatase, gamma glutamyltransferase<br>Clinical manifestations are the same as a jaundice or digestive symptoms (nausea and vomiting) |
| Rhabdomyolysis | Dark urine: brown-red | Myoglobinuria (brown-red or dark urine), CPK (> 5 times), hypokalaemia |
| Renal insufficiency | Oligoanuria (urine volume < 500 $cm^3$) | Creatininemia level; 3–12 mg/dL<br>Proteinuria<br>Oliguria, < 500 $cm^3$<br>Urine test that shows a haematuria, cylindruria (clinically the patient consults for haematuria or oliguria) |
| Thrombocytopenia | Epistaxis, ulorrhagia, bruises or other haemorrhagic signs (platelets, < 50 000/mL) | Platelets level<br>Haemogram: leukocytosis can be found (neutrophilia with a shift to the left).[a] Leukopenia can also be seen (with lymphocytosis + eosinophilia).<br>Clinically the patient consults for epistaxis, ulorrhagia or other haemorrhagic signs |
| Septicaemia (bacteriaemia) | Temperature, < 36 °C or > 38.3 °C; pulse rate, > 90 beats/minute; tachycardia, polypnoea or hypocapnia, leukocytosis | At least three haemocultures, when shivering or fever occur |
| Death | | Investigation on viral attack of organs (viral antigen in tissues)<br>Detection and culture of virus (in tissues and blood)<br>Genome sequencing |

ALT, alanine aminotransferase (also known as serum glutamic-pyruvic transaminase, SPGT); AST, aspartate aminotransferase (also known as serum glutamic-oxaloacetic transaminase, SGOT); CPK, creatine phosphokinase; CSF, cerebrospinal fluid.

[a] A "shift to the left" refers to the presence of increased proportions of younger, less well-differentiated neutrophils and neutrophil-precursor cells in the blood.

# Annex IX

## Differential diagnosis of adverse events involving viscerotropic and neurological disease following immunization against yellow fever (YF-AEFI)

### Viscerotropic disease

#### *GENERAL SEVERE FEBRILE ILLNESS*

- ☐ Yellow fever
- ☐ Severe malaria
- ☐ Bacterial sepsis
- ☐ Rickettsia/Borrelia
- ☐ Dengue
- ☐ HIV

**A. With jaundice**

- ☐ Hepatitis A, B, C, D, E
- ☐ CMV
- ☐ EBV
- ☐ Leptospirosis
- ☐ Liver pathology
  - — Abscess
  - — Cholangitis

**B. With haemorrhage**

- ☐ Yellow fever
- ☐ Dengue
- ☐ Rift Valley
- ☐ Other viral haemorrhagic fevers Lassa/Ebola/Marburg/Crimean-Congo
- ☐ Meningococcaemia
- ☐ Plague

### Neurological disease

#### *MENINGOENCEPHALITIS*

- ☐ Cerebral malaria
- ☐ Pyogenic bacteria
  - — *Meningococcus*
  - — *S. pneumoniae*
- ☐ Other diagnosis

**A. Bacterial**

- ☐ Syphilis
- ☐ Tuberculosis

**B. Viral**

- ☐ Yellow fever
- ☐ HSV1, HSV2
- ☐ Varicella Zoster virus
- ☐ Enterovirus
- ☐ Arboviruses
  - — West Nile
  - — Semliki Forest
- ☐ HIV
- ☐ Rabies

#### *ACUTE FLACCID PARALYSIS*

**A. Viral**

- ☐ Yellow fever
- ☐ Poliovirus
- ☐ Enterovirus
- ☐ Varicella Zoster
- ☐ West Nile
- ☐ Other Flaviviruses

**B. Other**

- ☐ Snake bite
- ☐ Botulism
- ☐ Toxins
- ☐ Nerve injury

# Annex X

## Reference laboratory: sample collection, storage and transport and yellow fever specific investigations

### 1. Type of sample and storage for transport to a WHO reference laboratory

***Level 1. Samples for specific virological/serological studies to be taken when patient becomes acutely ill***

| SPECIMEN | QUANTITY | COLLECTION TUBES | STORAGE AND TRANSPORT |
|---|---|---|---|
| Whole blood (only in case of special research) | Minimum 1 x 5 mL and preferably 15 mL (shall be adapted in case of small children) | Sodium citrate | Ship on wet ice (do not freeze); at reference laboratory, separate peripheral blood mononuclear cells and store serum at –70 °C or liquid nitrogen, do NOT store at –20 °C |
| Serum | Minimum 5 mL, preferably 10 mL; blood drawn and serum separated aseptically; serum placed in multiple aliquots in cryovials, approx. 1 mL/vial (volume shall be adapted in case of small children) | Dry | Ship on wet ice or frozen on dry ice; store in laboratory at –70 °C or liquid nitrogen, do NOT store at –20 °C |
| Urine | Minimum 5 mL; place in screw cap tube | Dry | Same as serum |
| Saliva | Minimum 0.2 mL; place in cryovial | Dry | Same as serum |
| Stools | Minimum 1 g, place in cryovial or screw cap tube | Dry | Same as serum |
| Cerebrospinal fluid | 5 mL, and in case of children 3 mL; place in multiple aliquots of 0.5 mL in cryovials | Dry | Same as serum |
| Other, e.g. pleural/ peritoneal fluid | Minimum 200 µL; place in cryovials | Dry | Same as serum |

NOTE: Label each specimen vial/tube clearly marked with: patient name, identification number (if any), age/sex, and date and time collected. Use pencil or waterproof ink. Ensure that proper waterproof labels are used that are compatible with the storage temperatures.

***Level 2. Samples to be taken during the convalescent phase***

| SPECIMEN | QUANTITY | TUBES | STORAGE AND TRANSPORT |
|---|---|---|---|
| Whole blood (only in case of special research) | Minimum 1 x 5 mL and preferably 15 mL (shall be adapted in case of small children) | Sodium citrate | Ship on wet ice (do not freeze); at reference laboratory, separate peripheral blood mononuclear cells and store serum at –70 °C or liquid nitrogen, do NOT store at –20 °C |
| Serum | Minimum 5 mL, preferably 10 mL; blood drawn and serum separated aseptically; serum placed in multiple aliquots in cryovials, approx. 1 mL/vial (volume shall be adapted in case of small children) | Dry | Ship on wet ice or frozen on dry ice; store in laboratory at –70 °C or liquid nitrogen, do NOT store at –20 °C |

NOTE: Label each specimen vial/tube clearly marked with: patient name, identification number (if any), age/sex, and date and time collected. Use pencil or waterproof ink. Ensure that proper waterproof labels are used that are compatible with the storage temperatures.

### *Level 3. Samples to be taken post-mortem*

| SPECIMEN | QUANTITY | TUBES | STORAGE & TRANSPORT |
|---|---|---|---|
| Whole blood[a] (only in case of special research) | Minimum 1 x 5 mL and preferably 15 mL (shall be adapted in case of small children) | Sodium citrate | Ship on wet ice (do not freeze); at reference laboratory, separate peripheral blood mononuclear cells and store serum at –70 °C or liquid nitrogen, do NOT store at –20 °C |
| Serum[a] | Minimum 5 mL, preferably 10 mL; blood drawn and serum separated aseptically; serum placed in multiple aliquots in cryovials, approx. 1 mL/vial (volume shall be adapted in case of small children) | Dry | Ship on wet ice or frozen on dry ice; store in laboratory at –70 °C or liquid nitrogen, do NOT store at –20 °C |
| Cerebrospinal fluid | 5 mL, and in case of children 3 mL; place in multiple aliquots of 0.5 mL in cryovials | Dry | Same as serum |
| Autopsy tissues | 1–2 g of the following tissues: liver, kidney, heart, spleen, lung, thymus, adrenal gland, brain, small and large intestine, mesenteric lymph node | Formalin-fixed; tissues may be grouped in same jar | Ambient temperature |
| | **AND** 1–2 g of same tissues as above | Cryovials (no preservative) and separate per tissue | Ship on wet ice or frozen on dry ice; store in laboratory at –70 °C or liquid nitrogen, do NOT store at – 20 °C |

[a] Blood may be obtained post mortem by direct cardiac puncture.
NOTE: Label each specimen vial/tube clearly marked with: patient name, identification number (if any), age/sex, and date and time collected. Use pencil or waterproof ink. Ensure that proper waterproof labels are used that are compatible with the storage temperatures.

## 2. Yellow fever specific investigations

### *Level 1. Analyses on samples when patient becomes acutely ill*

| SPECIMEN | DIAGNOSIS OF YELLOW FEVER | COMPLEMENTARY RESEARCH ON YELLOW FEVER |
|---|---|---|
| | Investigations in international reference laboratory | *Investigations in secondary international reference laboratories* |
| Whole blood | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever isolation (quantitative titration if virus isolated & sequencing) | — *Yellow fever immunohistochemistry*<br>— *Electron microscopy*<br>— *Host-specific genetics*[a]<br>— *T-cells*[b] |
| Serum | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever virus isolation (quantitative titration and sequencing in virus isolated)<br>— Antibody IgM ELISA and/or IFA,<br>— Antibody IgG ELISA and/or IFA,<br>— Neutralizing antibodies, CF, HI, IgE<br>— Exclusion of HIV[a] | — *Cytokines*<br>— *Storage* |
| Urine | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever virus isolation (quantitative titration and sequencing in virus isolated) | |
| Saliva | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever antibodies | |
| Stools | — Yellow fever RT-PCR and quantitative PCR | — *Electron microscopy* |
| Cerebrospinal fluid | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever virus isolation (quantitative titration and sequencing in virus isolated)<br>— Yellow fever IgM and neutralizing antibodies | |
| Other, e.g. pleural/ peritoneal fluid | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever virus isolation (quantitative titration and sequencing in virus isolated) | |

CF, complement-fixing; HI, haemagglutination inhibition assay; HIV, human immunodeficiency virus; IFA, immunofluorescence assay; Ig, immunoglobulin; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction
[a] Requires informed consent.
[b] T-cell studies require special handling of blood samples, separation and freezing of cells.

### *Level 2. Analyses on samples taken during the convalescent phase*

| SPECIMEN | DIAGNOSIS OF YELLOW FEVER | COMPLEMENTARY RESEARCH ON YELLOW FEVER |
|---|---|---|
| | Investigations in WHO international reference laboratory | *Investigations in secondary international reference laboratories* |
| Whole blood | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever virus isolation (quantitative and sequencing) | — *Yellow fever immunohistochemistry*<br>— *Electron microscopy*<br>— *Host-specific genetics*[a]<br>— *T-cells*[b] |
| Serum | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever virus isolation (quantitative and sequencing)<br>— Antibody IgM ELISA and/or IFA<br>— Antibody IgG ELISA and/or IFA<br>— Neutralizing antibodies, CF, HI, IgE<br>— Exclusion of HIV | — *Cytokines*<br>— *Storage* |

CF, complement-fixing; HI, haemagglutination inhibition assay; HIV, human immunodeficiency virus; IFA, immunofluorescence assay ; Ig, immunoglobulin; PCR, polymerase chain reaction; RT-PCR

[a] Requires informed consent.

[b] T-cell studies require special handling of blood samples, separation and freezing of cells.

### *Level 3. Analyses on samples to be taken post mortem*

| SPECIMEN | DIAGNOSIS OF YELLOW FEVER | COMPLEMENTARY RESEARCH ON YELLOW FEVER |
|---|---|---|
| | Investigations in WHO international reference laboratory | *Investigations in secondary international reference laboratories* |
| Tissues | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever isolation (quantitative and sequencing) | — *Yellow fever immunohistochemistry*<br>— *Histopathology*<br>— *Electron microscopy*<br>— *Host-specific genetics*[a] |
| Whole blood | | — *Host-specific genetics*[a] |
| Serum | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever virus isolation (quantitative and sequencing)<br>— Antibody IgM ELISA and/or IFA,<br>— Antibody IgG ELISA and/or IFA,<br>— Neutralizing antibodies, CF, HI, IgE<br>— Exclusion of HIV[a] | — *Cytokines*<br>— *Storage* |
| Cerebrospinal fluid | — Yellow fever RT-PCR & quantitative PCR<br>— Yellow fever virus isolation (quantitative and sequencing)<br>— Yellow fever IgM and neutralizing antibodies | |
| Other, e.g. pleural/ peritoneal fluid[b] | — Yellow fever RT-PCR and quantitative PCR<br>— Yellow fever isolation (quantitative and sequencing) | |

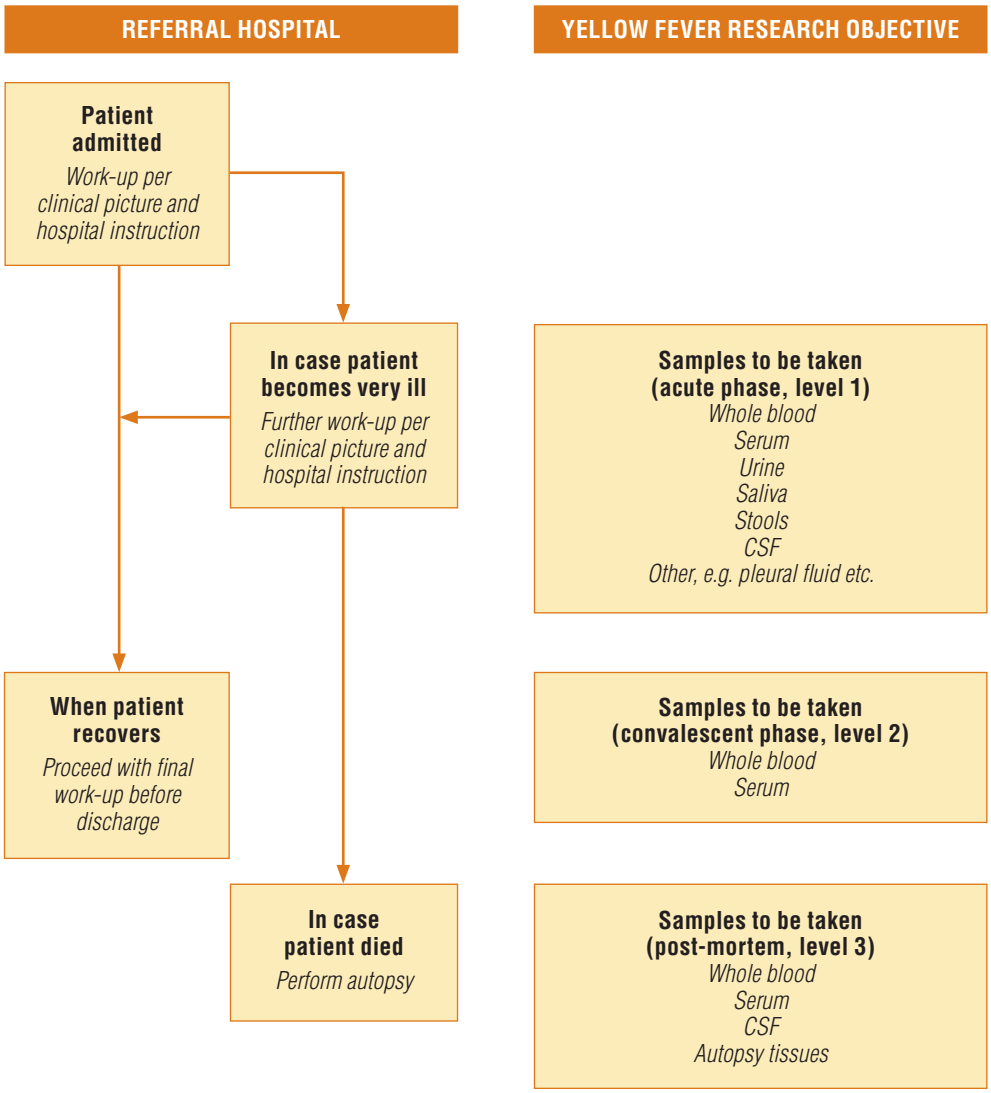
CF, complement-fixing; HI, haemagglutination inhibition assay; HIV, human immunodeficiency virus; IFA, immunofluorescence assay; Ig, immunoglobulin; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction

[a] Requires informed consent.

[b] Urine sample could be obtained through vesical puncture

# Annex XI

## Patient admission flowchart: serious adverse event following immunization (AEFI) against yellow fever identified as a possible vaccine reaction

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