Operational Guidelines
for
Sentinel Surveillance of
Acute Encephalitis Syndrome (AES)

Ministry of Health
Government of Bhutan

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<td>Acute Encephalitis Syndrome</td>
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<td>AFP</td>
<td>Acute Flaccid Paralysis</td>
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<td>BAT</td>
<td>Bacterial Antigen Test</td>
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<td>CDC</td>
<td>Center for Disease Control</td>
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<td>CIF</td>
<td>Case Investigation Form</td>
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<td>CLSI</td>
<td>Clinical Laboratory Standard Institute</td>
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<td>Central Nervous System</td>
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<td>CSF</td>
<td>Cerebrospinal Fluid</td>
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<td>DGR</td>
<td>Dangerous Goods Regulations</td>
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<td>DHO</td>
<td>District Health Officer</td>
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<td>GRRH</td>
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<td>HI</td>
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<td>IATA</td>
<td>International Air Transport Association</td>
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<td>JDWNRH</td>
<td>Jigme Dorji Wangchuk National Referral Hospital</td>
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<td>JE</td>
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<td>JEV</td>
<td>Japanese Encephalitis Virus</td>
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<td>MT</td>
<td>Medical (laboratory) Technologist/Technician</td>
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<td>NIMHANS</td>
<td>National Institute of Mental Health and Neuro Science</td>
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<td>PHL</td>
<td>Public Health Laboratory</td>
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<td>PPE</td>
<td>Personal Protective Equipment</td>
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<td>Plaque Reduction Neutralization Tests</td>
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<td>RRT</td>
<td>Rapid Response Team</td>
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<td>SEARO</td>
<td>Southeast Asia Regional Office</td>
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<td>UN</td>
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<td>VDCP</td>
<td>Vector-Borne Disease Control Program</td>
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Introduction

Japanese encephalitis virus (JEV) is the leading cause of viral encephalitis in Asia. JEV virus is a Flavivirus, a genus of the family Flaviviridae. These viruses are transmitted by the bite from an infected arthropod. Human infections with these viruses are typically incidental, as humans are unable to replicate the virus to high enough titres to reinfect arthropods and thus continue the virus life cycle. South East Asia reports 300,000 - 500,000 JE cases to WHO annually and the death from this disease is estimated to be 10,000 – 15,000 per annum.

Most infections of humans are asymptomatic or result in a non-specific flu-like illness. Estimates of the ratio of symptomatic disease to asymptomatic infection vary between 1 in 25, and 1 in 1000. The JE case mortality of around 20-30% and about 30-50% of survivors are left with neurological sequelae following JE infection.

JE virus is transmitted naturally amongst wild and domestic birds and pigs by a species of *Culex* mosquitoes, the most important for human infection being *Culex tritaeniorhynchus* which breeds in pools of stagnant water such as paddy fields and marshy land. Although many animals can be infected with the virus, only those that develop high viraemia are important in the natural cycle. For maintaining and amplifying JE virus in the environment, birds may also be responsible for the spread to new geographical areas. Pigs are the most important natural host for transmission to humans since they are often kept close to humans. Pigs have prolonged and high viremia, and produce many offspring, providing a continuous source of new hosts.

Humans become infected with JE virus coincidentally when living or traveling in close proximity to animals and birds infected with JE or traveling to JE endemic area. Although most cases occur in rural areas, JE virus is also found on the edge of cities. Epidemiological studies have shown that after the monsoon rains, mosquitoes breed prolifically in large numbers invariably increasing the JE virus carriage and the infection rate of pigs, human infection. Serological surveys have shown that in rural Asia most of the populations are infected with JE virus during childhood or early adulthood.

JE is found across eastern and southern Asia including pacific. There are two epidemiological patterns recognized for JE. In northern areas large epidemics occur during the summer months, whereas in southern areas JE tends to be endemic, and cases occur sporadically throughout the year with a peak after the start of the rainy season. In endemic area, the disease primarily infects children, leaving approximately 70% of those who develop clinical illness either dead or neurologically disabled. Public health authorities have long concluded that vaccination is a necessary tool to control JE ever since the vaccine was available since 1945 and was recommended for routine use in endemic countries, but it has not reached to all countries in Asia mainly due to inadequate disease surveillance, limited vaccine supply, lack of guidance and program support for immunization combined with limited advocacy.

Geographically, the southern foothills of Bhutan fall under JE endemic area. The main vector for JE, *Culex* species is reported to be found in most malaria and dengue endemic area in the country (source VDCP). The southern part Bhutan is also a place where there is cross border migration of people. Moreover, human and animals (pig, horses)
dwell together in close proximity thereby creating an environment conducive for JE spread. Encephalitis cases are reported however due to inadequate disease surveillance in place, the true burden of encephalitis disease caused by JE and other agents is not yet known at the moment.

Acute Encephalitis Syndrome (AES) has also been documented to be caused by bacterial agents including *Haemophilus influenzae* (*H. influenza*), *Neisseria meningitidis* (*N. meningitidis*) and *Streptococcus pneumoniae* (*S. pneumoniae*) particularly in children.

With the strengthening of AFP surveillance in Bhutan and the integration of measles and maternal and neonatal tetanus with AFP surveillance, there is an opportunity to further broaden the scope of the system by incorporating the surveillance of Acute Encephalitis Syndrome. This would provide timely information of the JE burden in Bhutan and spearhead prevention and control measures. Since AES surveillance will also include testing for bacterial agents the system would provide information on future requirement and introduction of newer vaccines such as *Haemophilus influenzae* (*H. influenza*), *Neisseria meningitidis* (*N. meningitidis*) and *Streptococcus pneumoniae* (*S. pneumoniae*) in the country.

1. Surveillance of JE and AES in Bhutan
1.1 Objectives of JE/AES surveillance

- Assess the burden of Japanese Encephalitis.
- Assess the burden of other bacterial/viral agents causing AES encephalitis
- Ensure early detection and rapid response to JE/AES outbreak
- Obtain evidence to determine strategies for new vaccine introduction.

1.2 Synopsis of AES Surveillance Strategy for Bhutan

For surveillance purposes all cases of Acute Encephalitis Syndrome (AES) visiting the sentinel surveillance sites will be identified, notified and investigated. AES syndromic surveillance will be integrated with the AFP/ measles/ MNT surveillance. Case based data will be collected and laboratory confirmation of suspected cases will be done where feasible.

Initially, AES/JE surveillance in Bhutan will be confined to the following sentinel sites

- JDWNRH
- Gelephu Regional Referral hospital
- Mongar Regional Referral hospital
- Phuntsholing hospital
- Samdrup Jongkhar hospital

These sites are further categorized into:

A. SSSP: Sentinel Surveillance Site with Public Health laboratory at JDWNRH
B. SSSL: Sentinel Surveillance Sites with laboratory facilities at Gelephu Regional Referral hospital , Mongar Regional Referral and Phuntsholing hospital
C. SSS: Sentinel Surveillance Site without laboratory facility at Samdrup Jongkhar hospital

Monthly consolidated reporting of AES cases (using the AFP/ measles/ MNT system) with timely detection of outbreaks will take place in the sentinel sites and the districts where they are located. In outbreak situations laboratory confirmation will be done only for 5 to 10 cases. Later with the experience gained, surveillance will be expanded to other sites and districts.

Various activities pertaining to epidemiological surveillance i.e. collection, compilation, analysis and interpretation of data, follow-up action and feed back will be carried out in a systematic and
organized manner. Initially in the sentinel site’s Medical Officers specifically designated as Nodal Officers will assume the responsibility for the same, as the program evolves and expands the responsibility will be taken over by the district officers.

Epidemiological surveillance of JE would include

- Clinical surveillance and
- Laboratory based surveillance.

To carry out clinical surveillance of JE at the sentinel sites, a system will be established to identify, notify and investigate patients presenting with the signs and symptoms of encephalitis. All such cases will be investigated and the details documented in a standard case investigation form CIF (annex 3). For ease of analysis, a line list of these cases will be prepared on a standard format (annex 4) and submitted to the district and national health authorities.

To carry out laboratory surveillance, the laboratories in the sentinel sites will divide the CSF collected into two aliquots one of which will be used to conduct basic investigations such as TLC/ DLC, biochemistry and grams stain the other sample will be shipped to the PHL – Thimpu in cold chain for JE testing. The sentinel site laboratories will also separate serum from the blood specimens collected from all cases and ship the same to PHL – Thimpu for JE testing. Two serum specimens will be collected from each AES case, one at the time of admission (along with CSF) and another ten days later.

On a monthly basis, the PHL at Thimpu will send all CSF samples for confirmatory JE testing and detection of Bacterial pathogens and all JE positive and 10% representative JE negative serum samples to the Regional Reference Lab at NIMHANS Bangalore for confirmatory JE IgM testing and quality control.

The national program manager will initially coordinate all surveillance activities, collate, compile and analyze data and take action until the district program is able to manage the same.

2. AES definition and Case Classification

2.1 Clinical case definition for AES

Clinically, WHO defines a case of acute encephalitis syndrome as “a person of any age, at any time of year with the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) AND/OR new onset of seizures (excluding simple febrile seizures*). Other early clinical findings may include an increase in irritability, somnolence or abnormal behaviour greater than that seen with usual febrile illness”.

(*A simple febrile seizure is defined as a seizure that occurs in a child aged 6 months to less than 6 years old, whose only finding is fever and a single generalized convulsion lasting less than 15 minutes, and who recovers consciousness within 60 minutes of the seizure).
2.2 Case classification

AES case: A case that meets the clinical case definition for AES. AES cases are further classified in one of the following four ways.

- **Laboratory-confirmed JE**: A suspected case that has been laboratory-confirmed as JE.
- **Probable JE**: A suspected case that occurs in close geographical and temporal relationship to a laboratory-confirmed case of JE, in the context of an outbreak.
- **“Acute encephalitis syndrome” – other agent**: A suspected case in which diagnostic testing (e.g., bacteriological tests) is performed and an etiological agent other than JE virus is identified.
- **“Acute encephalitis syndrome” – unknown**: A suspected case in which no diagnostic testing is performed or in which testing was performed but no etiological agent was identified or in which the test results were indeterminate.

3. Process of AES surveillance

AES surveillance will be operationalised at three levels, the sentinel sites, the district, and the national level. The key person coordinating surveillance at the sentinel site will be the nodal officer, the district health officer will coordinate the activity at the district (currently this role will be shared by the nodal officer and the national program manager) and the national program manager will coordinate the activity for the country.

At the grassroots level, the two basic steps are required for AES surveillance at the sentinel surveillance site are

- Routine reporting
- AES case reporting

3.1 Routine reporting

The Nodal Officer at each sentinel sites should report the monthly report to the district before the 7th calendar day of the following month. The AES component has been added to the joint AFP/Measles/MNT form (annex 1). The Nodal Officer should visit all the wards/contacts that are likely to see AES cases, ensure that AES cases that have visited to the sentinel site are
incorporated in the routine report sent to the district. When AES cases are identified the case must be notified immediately.

3.2 AES Case Reporting

The following are the steps for reporting AES cases
1. Case detection and notification
2. Case investigation
3. Specimen collection and shipment
4. Processing of specimens in the laboratory
5. Data collation and reconciliation
6. Data analysis and interpretation
7. Initiating action

3.2.1 Case detection and notification:

Medical Officers (MOs), paediatricians, and other physicians, nurses who see patients with AES should inform the designated Nodal Officer of the sentinel site immediately upon presentation of an AES case. The nodal officer of an RU should immediately notify the district (DHO) of the case.

3.2.2 Case investigation:

Once a case of AES is reported, the Nodal Officer (DHO in future) must personally see the case to ascertain if the case meets the AES case definition. If the case is confirmed to be an AES case, a unique EPID number has to be assigned as follows
AES - BHU - DIS - YR - NUM

- AES denotes AES case code
- BHU indicates Bhutan,
- DIS indicates the district code,
- YR the year of rash onset eg 2011 and
- NUM denotes the serial number of the case detected in the district in that year.

Thus AES-BHU- CHU -11-001 will be the code assigned to the first AES case (001) investigated in Chukha (CHU) district in the year 2011 in Bhutan (BHU). The case was reported from Phuntsholing Hospital.
Using the case investigation form as a guide, the investigating officer (MO at present later the district focal person) should obtain the history and conduct a physical examination of the affected child. He should also coordinate the collection of specimens of CSF and serum and transport them to the identified laboratory. All details including, hospitalization, demographics, immunization, basic clinical features, specimens collected and dates of all events should be documented in the CIF.

If the case does not meet the case definition of AES, the Nodal Officer should discuss the findings with the reporting physician and record the case as not AES on the case investigation form. Documentation is required for all cases including such cases that are considered rejected”.

### 3.2.3 Specimen collection and transportation

Blood (serum) and cerebrospinal fluid (CSF) are the specimens to be collected for JE diagnosis. Blood samples should be collected from AES/ suspected JE cases within 4 days after the onset of illness for isolation of virus and at least 5 days after the onset of illness for detection of IgM antibodies. A second, convalescent samples should be collected at least 10-14 days after the first sample for serology.

Patient information should be completely documented in the Case Investigation Form that must accompany the specimen when it is sent to the laboratory. It is also essential to label the vial with the patient’s name, Epid Number, date of collection and specimen type.

Following methods would be adopted for collection and transportation of Blood (serum) and CSF samples:
Serum Collection and Shipment

Specimen kit for blood collection:
- 5-ml vacutainer (non-heparinized) tube with a 23 gauge needle OR sterile disposable syringe and needle;
- Tourniquet
- Sterilizing swabs
- Serum storage vials
- Specimen labels
- Band-aid
- Zip-lock plastic bags
- Case Investigation Form
- Cold box with ice packs

Collection procedures
- Collect 5 ml of blood by venepuncture using aseptic precautions. If vacutainer is used, label the vacutainer tube. If syringe is used to collect blood, transfer the blood from the syringe to labeled sterile screw capped vials (label with the patient Epid Number, name, collection date and specimen number – for serum)
- The blood should be kept at room temperature until there is complete clot formation
- The clotted blood can be stored at 4 to 8° C for up to 24 hours before the serum is separated.
- Do not freeze whole blood

Steps in blood collection
- Label the tubes, including the unique patient identification number, using an indelible marker pen. Always check to ensure that the correct tubes are used for each patient.
- Place a tourniquet above the venepuncture site, palpate and locate the vein (Step 1).
- Disinfect the venepuncture site meticulously with 70% isopropyl alcohol (an alcohol swab) or 10% polyvidone iodine by swabbing the skin concentrically from the centre of the venepuncture site outwards (Step 2). Let the disinfectant evaporate. Do not re-palpate the vein
- If withdrawing blood with conventional disposable syringes, withdraw 3–5 ml of whole blood from adults and older children and 1ml from infants. Under asepsis, transfer the specimen to appropriate transport tubes. Secure caps tightly.
- If withdrawing blood with a vacuum system (e.g. Vacutainer®), withdraw the desired amount of blood directly into each transport tube (Step 3).
- Remove the tourniquet. Use a cotton swab to apply pressure to the venepuncture site until bleeding stops (Step 4) and apply a band-aid.
- Never recap used sharps. Discard directly into a suitable container (a proper sharps disposal container if available or a
container such as a coffee or other metal can which should be appropriately labeled before use).

- Recheck that the tubes used for sampling have been correctly labeled.
- After taking all the samples, complete the appropriate field data sheets or case investigation forms and the required laboratory request forms using the same identification numbers used on the tubes.

**Transportation of blood samples to the measles laboratory**

If vacutainer is used, the same can be shipped directly to the WHO accredited laboratory in cold chain

If syringe is used to collect the blood sample, there are two options available to ensure that the specimen reaches the WHO accredited measles laboratory in good condition

**Option 1- Transport of whole blood (less preferred)**

Transport whole clotted blood specimen to laboratory on ice, if it can reach the laboratory within 24 hours.

**Option 2- Transport of serum specimens (preferred)**

Separation of serum from blood can be done by 2 methods

- **Method 1**
  - After clotting, blood should be centrifuged at 1000g for 10 minutes.
  - Transfer the serum aseptically to labeled sterile screw capped vials

- **Method 2**
  - If centrifuge is not available, carefully remove the serum using a pipette, avoid extracting the red cells
  - Transfer the serum aseptically to labeled sterile screw capped vials.

Sterile serum can stored at 4-8°C for a maximum period of 7 days. In case a delay is anticipated, sera must be frozen at -20°C. Repeated freezing and thawing can have detrimental effects on the stability of IgM antibodies

**Precautions for shipment of specimens**

- Ship specimens to the laboratory as soon as possible without waiting for additional specimens
- Use Styrofoam box or Thermos flask.
- Place specimens in Zip-lock or plastic bags
- Place each specimen and laboratory request form (in a plastic bag taped to the inner surface of the top of a Styrofoam box) as shown.
- Place frozen ice packs on the bottom and along the sides of the box, put samples in the centre and place more ice packs on top
- Arrange for shipping
Cerebrospinal fluid (CSF) collection and shipment
CSF specimen would be collected in a sterile screw capped bottles under all aseptic precautions by a trained person. The containers should be properly labeled and transported at the earliest to the designated laboratory. All attempts would be made to collect CSF sample for confirmation of diagnosis.

Collection procedure
CSF is the fluid that bathes, cushions, and protects the brain and spinal cord. It flows through the skull and spine in the subarachnoid space, which is the area inside the arachnoid membrane. To obtain a specimen of cerebrospinal fluid the procedure is carried out by amedical officer. Lumbar puncture (spinal tap) is the most common means of collecting a specimen of CSF.

- The patient is positioned on his side with his knees curled up to his abdomen and with chin tucked in to his chest. (Occasionally this procedure is performed with the person sitting and bent forward).
- The skin is scrubbed, and a local anesthetic is injected over the lower spine. The spinal needle is inserted, usually between the 3rd and 4th lumbar vertebrae.
- Once the needle is properly positioned in the sub-arachnoid space, pressures can be measured and fluid can be collected for testing.
- After the sample is collected, the needle is removed, the area is cleaned, and a bandage is applied.
- The patient is asked to remain flat, or nearly flat, for 6 to 8 hours after the procedure.

Collection of CSF
Examination of CSF is an essential step in the diagnosis of any patient with evidence of meningeal irritation or infection of the central nervous system. Approximate 2-3 ml of CSF is collected and divided into two aliquots. One aliquot is sent to the sentinel site laboratory for physical, cytological, biochemical, and microscopic examination and the second aliquot is stored aseptically for serology, viral culture or bacteriological at the national public health laboratory at Thimpu. The following important precautions need to be taken for CSF collection and transportation:

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<th>Points</th>
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<td><strong>CSF is a precious specimen, handle it carefully and economically. It may not be possible to get a repeat specimen.</strong></td>
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<tr>
<td><strong>Collect CSF in a screw – capped sterile container (eg not in an injection vial with cotton plug).</strong></td>
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<tr>
<td><strong>Do not delay transport and laboratory investigations.</strong></td>
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- Perform physical inspection immediately after collection and indicate findings in the patient’s records.
- Store at 4°C, if delay in processing is inevitable.

Storage and transport of CSF sample
Place the specimens at +4°C as soon as possible after collection. Dispatch these at the earliest possible opportunity on wet ice in a large thermus or an ice-box to the designated laboratory (PHL Thimpu).

Criteria for rejection of CSF/ Serum samples
- Leakage of sample
- Haemolyzed sample
- Inadequate quantity
- Improper cold chain maintenance during transportation
- Improperly labeled sample
- Samples collected in improper containers
- Turbid serum sample (contaminated)

Specimen processing

3.2.4 Processing of specimens in the Laboratory
Specimens are processed at
- Sentinel site Laboratories
- Public Health Laboratory - Thimpu
**Processing in the sentinel site laboratory:**
The following tests will be done at all the sentinel surveillance site laboratories:
Serum: To be separated from clotted blood by centrifuging. The same will be labeled and shipped to the PHL Thimpu in cold chain
CSF:
Bio Chemistry:
Gram’s Stain
TLC/ DLC:
The laboratory diagnosis of *Haemophilus influenzae* (*H. influenza*), *Neisseria meningitidis* (*N. meningitidis*) and *Streptococcus pneumoniae* (*S. pneumoniae*) causing AES, will be performed if facility is available at the SSSL (in addition to standard gram staining, latex agglutination test and culture and identification protocol)

**Processing in the Public Health Laboratory Thimpu:**
Laboratory confirmation for JE virus infection is made by a presence of JE virus-specific IgM antibody in a single sample of CSF or serum samples detected by IgM-capture ELISA OR any one of the following:
- Detection of a four-fold or greater rise in JE virus-specific antibody (IgG) as measured by hemagglutination inhibition (HI) or plaque reduction neutralization tests (PRNT) in serum collected during the acute and convalescent-phase of illness. OR
- Detection of JE virus genome in CSF, serum, plasma, blood, or tissue by reverse transcriptase PCR or an equally sensitive and specific nucleic acid amplification test;
  OR
- Isolation of JE virus in CSF, serum, plasma, blood or tissue; OR
- Detection of JE virus antigens in tissue e.g. by immunofluorescence assay (IFA) or by immunohistochemistry.

**3.2.5 Data collation and reconciliation**
When clinically suspected AES cases are reported, it is important to probe from the patient or relatives about the occurrence of similar cases in the neighborhood. Travel history has to be documented. If the patient has traveled from another district, the details of the case (Case Investigation Form - annex 3) should be communicated to the district where the patient resides. The data obtained from the case investigation forms completed in sentinel site surveillance and the results from the laboratory should be collated and analyzed by the district.

**3.2.6 Data analysis and interpretation**
The district should prepare a linelist (annex 4) of all AES cases. Linelisting will enable easy data analysis, identify clustering of cases in time and space and take action. The analysis should include age distribution, vaccination status, geographic location and laboratory results. Currently, in Bhutan, the analysis will be done at the national level until the district capacity is built up.

An AES outbreak should be suspected if there are more reported suspected AES cases from an area in a particular period compared to the same period in previous years. Case mapping is important to identify clusters and the detection of suspected outbreaks. A site visit by the district health officer is warranted if this happens.

**3.2.7 Initiating action**
As soon as the outbreak is suspected, the risk of a large outbreak with high morbidity and mortality must be assessed. Data should be analyzed to identify the cause of the AES outbreak.
This evaluation is needed to determine susceptibility and potential spread in both affected and neighboring areas as well as the appropriate vaccination response (if the cause of the AES outbreak is vaccine preventable) to control the outbreak. To evaluate the risk of further transmission, morbidity and mortality, the following factors should be taken into consideration:

- Population characteristics such as size, density, movement, and setting
- Mortality rates
- Period of the year such as seasonal outbreaks or holidays, festivals and social events that would increase opportunities for spread
- Cases reported and comparison with previous years
- Access to health services
- Sensitivity of the surveillance system

Following a visit to the suspected outbreak locality by the district medical officer, if an outbreak is identified, it is essential that house-to-house survey is conducted in the affected area and a line list with basic epidemiologic data is completed and specimens collected from at least 5 to 10 suspected cases and sent to the PHL for testing. The AES linelist (annex 4) could be modified and used for outbreak investigation.

It is necessary to enhance social mobilization activities to inform the affected communities about the suspected outbreak. If the AES outbreak is vaccine preventable, the data analysed will provide information to vaccinate specific age-group of previously unvaccinated persons. An AES outbreak due to a vaccine preventable disease provides an opportunity to identify program weaknesses in routine immunization and a chance to correct them.

4. Operationalizing AES Surveillance in Bhutan

The purpose of JE and AES surveillance is to estimate disease burden and understand the disease pattern in terms of its influence on morbidity and mortality. The incidence of JE and AES will form the basis of any future planning for prevention and control of JE or other agents identified as responsible for AES. JE and AES surveillance would thus facilitate generation of authentic and valid information on epidemiological, clinical, laboratory and entomological parameters on regular basis. This surveillance will be carried out through sentinel sites in the beginning. Later the program will be expanded to other parts of the country to other health institutions as well. In the first phase, epidemiological surveillance for AES /JE will be conducted in the five sentinel surveillance sites and the districts where they are located. These units will report all AES and JE cases based on standard case definition. The sites are

- JDWNRH
- Gelephu Regional Referral hospital
- Mongar Regional Referral hospital
- Phuntsholing hospital
- Samdrup Jongkhar

These sites are further categorized into

- SSSP: Sentinel Surveillance Site with Public Health laboratory at JDWNRH
- SSSL: Sentinel Surveillance Sites with laboratory facilities at Gelephu Regional Referral hospital, Mongar Regional Referral and Phuntsholing hospital
- SSS: Sentinel Surveillance Site without laboratory facility at Samdrup Jongkhar hospital
4.1 Roles and Responsibilities

All sentinel sites would have a designated nodal officer for coordination of JE/AES surveillance activities. The Medical Officers (MOs), pediatricians, and other physicians, nurses who see patients with AES should inform the designated Nodal Officer immediately upon presentation of the AES case. The case should be further subjected to laboratory investigations for JE/ AES. The nodal officer should immediately notify the District Health Officer (DHO). The case should be investigated using the standard case investigation form (CIF annex 3). CSF and serum specimens should be collected immediately and sent to the designated laboratory for testing. A second serum sample should be collected after 10 days or at the time of discharge for testing.

4.1.1 Role of Sentinel Surveillance Sites with Public Health Laboratory (SSSP)

The JDWNRH has been designated as the Sentinel Surveillance Site (SSSP) with public health laboratory. This is because JDWNRH is the tertiary care hospital with a public health laboratory which has the capacity to diagnose JE, and perform tests to detect other agents such as Haemophilus influenzae (H. influenza), Neisseria meningitidis (N. meningitidis) and Streptococcus pneumoniae (S. pneumoniae).

Role of the nodal officer of this site would be to

- Routine monthly reporting to district (AFP/ measles/ MNT AES form annex 1)
- Case detection and notification
- Case investigation (Complete CIF annex 3)
- Specimen (CSF and serum) collection
- Documentation and specimen shipment to PHL laboratory at JDWNRH in cold chain
- Complete and maintain linelist (annex 4)

Role of the clinical microbiology laboratory at the JDWNRH will be the same as that of other labs in SSSL and is outlined in section 4.1.2

Role of the Public Health Laboratory at JDWNRH

- Sample receipt:
  - From surveillance hospital site
- Testing and reporting within 7 days of receipt of samples in the Laboratory
  - Serum: JE IgM
  - CSF: JE IgM and Latex agglutination test and/or PCR
- Storage:
  - CSF and serum in -20 freezers
- Shipment to reference Laboratory: 1st week of every month in Cold chain
  - All CSF samples for confirmatory JE testing and detection of Bacterial pathogens
  - All JE positive and 10% representative JE negative serum samples for confirmatory JE IgM and QC
- Report:
  - on completion of test: To the sentinel site, District program officer and National
Program officer

- Monthly consolidated report by 10th of the following month along with the Laboratory line list to District and National Program officers

### 4.1.2 Role of Sentinel Surveillance Sites with Laboratory (SSSL)

The Gelephu Regional Referral hospital, the Mongar Regional Referral hospital and the Phuntsholing hospital have been designated as the Sentinel Surveillance Sites with laboratory (SSSL). This is because these are referral hospitals with laboratory capacity to perform basic tests for CSF such as counts, biochemistry and gram stain. In addition they would aliquot the samples and send them in cold chain to the national laboratory to detect JE and other agents such as Haemophilus influenzae (H. influenza), Neisseria meningitidis (N. meningitidis) and Streptococcus pneumoniae (S. pneumoniae).

Role of the nodal officer of this site would be to

- Routine monthly reporting to district (AFP/ measles/ MNT AES form annex 1)
- Case detection and notification
- Case investigation (Complete CIF annex 3)
- Specimen (CSF and serum) collection
- Documentation and specimen shipment to PHL laboratory at JDWNRH in cold chain
- Complete and maintain linelist (annex 4)

Role of the laboratories at Gelephu Regional Referral hospital, Mongar Regional Referral hospital, the Phuntsholing hospital and the clinical microbiology lab at JDWNRH

- Provide disposable or sterilized vials for sample collection to the hospital sentinel site
- Sample receipt:
  - From surveillance hospital site
- Separate serum, Aliquot CSF:
  - As per recommendation
- CSF Testing if possible:
  - TLC & DLC, Bio chemical tests, Grams stain, latex agglutination test and Culture
- Storage:
  - CSF and serum in -20 freezers
- Shipment to National PH Laboratory:
  - CSF and serum samples in Cold Chain
- Documentation and weekly report to the District Program officer

### 4.1.3 Role of Sentinel Surveillance Sites without Laboratory (SSS)

The Samdrup Jongkhar Hospital has been designated as a Sentinel Surveillance Site without laboratory (SSS). This is because this is a large hospital catering to high risk populations. Since this hospital does not have the facility to do the laboratory tests, serum samples from this site will be shipped to the PHL at JDWNRH for processing. CSF samples will not be collected.

Role of the nodal officer of at this site
• Routine monthly reporting to district (AFP/measles/MNT AES form annex 1)
• Case detection and notification
• Case investigation (Complete CIF annex 3)
• Specimen (serum) collection including separation of serum from the blood
• Specimen shipment to the site laboratory for processing
• Complete and maintain linelist (annex 4)

4.2 Records and Reports:

4.2.1 At the Sentinel Sites

All sentinel sites (SSSP, SSSL and SSS) would be required to maintain documentation of the AES cases being treated by various doctors of the unit and in various specialty departments & wards etc. Two kinds of reports are to be maintained by the sites

• Monthly report (including “Zero” report) in the AFP/measles/MNT and AES form (annex 1)
• Case Investigation Form (CIF annex 3) and
• Linelist (annex 4)

Monthly report should be sent to the district and the national level in the AFP/measles/MNT form modified to include AES. Along with the monthly report if cases of AES are reported, the monthly report should be accompanied by the CIF and linelist.

4.2.2 At the district

The sentinel sites should send a copy of the following forms and the district should maintain a record of

• Monthly report (including “Zero” report) in the AFP/measles/MNT and AES form (annex 2)
• Case Investigation Form (CIF annex 3) and
• Linelist (annex 4)

District Plan for the future:
The district should take up full ownership of the data when there is a surveillance focal person designated. When such a person is appointed,
• All data from the sentinel site should be routed through the district to the national level.
• Coordinate the specimen collection and ship samples to the national level in cold chain
• Merge the laboratory data with the field data
• Complete the case classification and thus the CIF
• Maintain all data, analyze (Time place person) the same, identify disease patterns and take local action
• Coordinate referral of some complex cases to the national level

4.2.3 At the National Level
Until a district surveillance focal person is appointed, the national level (Senior Program Officer - VPDP) will take up the full responsibility for the following
• Collating Monthly reports from all sentinel surveillance sites
  o Including “Zero” report in the AFP/ measles/ MNT and AES form (annex 1 and 2)
  o Case Investigation Form (CIF) and Linelist
• Merge the Laboratory data with the field data
• Complete the case classification and thus the CIF
• Maintain all data, analyze (Time place person) the same, identify disease patterns
• Take local action
• Develop national guidelines
• Ensure availability of supplies at all levels (for the Laboratory and the sentinel sites)
• Conduct special studies based on program requirements

4.3 National Plan for the future
Once the district level surveillance focal persons are in place the role of the national level will be the following
• Ensure availability of logistics at all levels
• Data collection and processing
• Trainings
• Feedback

5. AES Laboratory network

5.1 Public Health Laboratory - Thimpu
PHL will function as a national reference Laboratory for JE and test specimens from suspected cases by IgM ELISA both in serum and CSF. However, PHL will also collaborate with Microbiology unit, clinical Laboratory, JDWNRH for bacteriological confirmation of common bacterial agent causing AES in CSF samples. PHL will send representative number of IgM positive JE sera periodically to WHO Regional Reference Laboratories for reconfirmation and JE negative samples for further analysis. Besides PHL, GRRH laboratory will also test samples by IgM ELISA under AES/JE laboratory network and report directly to PHL. GRRH Laboratory will send all JE IgM positive samples and 10% of representative JE negative samples to PHL for confirmation and PHL will forward the samples to regional reference Laboratory.

5.2 Regional Reference Laboratory – NIMHANS, Bangalore, India
The Neurovirology department of National Institute of Mental Health and Neuro Science (NIMHANS) in Bangalore, India is identified as the WHO Regional Reference Laboratory for AES in the South East Asia region. NIMHANS will provide confirmation of the results and provide technical support as required by the program.

5.3 Global Specialized Laboratory, Centers for Disease Control, Atlanta, USA
CDC, Atlanta, USA is the global specialized Laboratory for JE other flavivirus. CDC will play a major role in providing molecular analysis of JE virus genome and interpreting molecular epidemiological data for regional and PHL.

6. JE outbreak and rapid response

6.1 JE Outbreak
JE outbreak is defined as an ‘abnormal increase’ in suspected JE cases as compared with normal transmission periods. However, there is no single definition of JE outbreak as in areas where JE endemicity is low. Therefore, every case may warrant an investigation. An ‘abnormal increase’ should be defined as an increase above and beyond the normal range of seasonal variation of reported cases which can be only determined if prevalence is known. To confirm an outbreak, at least 5-10 samples from suspected JE cases should to be collected and confirmed by PHL. Once confirmed, all suspect cases should be only line listed and surveillance must be kept for minimum one month from the day last case is reported.

The suspected outbreak of JE should be investigated by a rapid response team (RRT) from the national level to verify the outbreak. However, preliminary outbreak of JE should be investigated at the local level by a district rapid response team. The RRT from national level will only come to site if district cannot conduct outbreak investigation or an outbreak has major programmatic implications. The composition and responsibilities of the RRT are as follows:
6.2 Rapid Response Team (RRT)
- Epidemiologist
- Microbiologist
- Pediatrician
- Entomologist
- Veterinarian

6.2.1 Responsibilities of RRT at national level
- Provide technical expertise for any outbreak and provide all technical assistance to the RRT at district level.
- Visit outbreak sites to investigate outbreak at the request of district RRT.
- Provide all logistic support including drugs, PPE and other necessary supplies
- Recommend appropriate interventions to be undertaken to DoPH/ MoH based on findings and/ or outbreak reports submitted by district RRT to prevent future outbreaks.

6.2.2 Rapid response team (RRT) at district level
- District medical officer (DMO)
- District health officer (DHO)
- District Malaria Supervisor (DMS)
- Medical Technologist/ Technician(MT)

6.2.3 Responsibilities of RRT in district
- In suspected outbreak of JE, the RRT team from district should immediately visit the affected site.
- Ascertain cases based on clinical case definition and send serum sample from 5 – 10 cases to the PHL for laboratory confirmation.
- Inform the National RRT for any assistance if required.
- Inform the local authority about the situation and possible risks.
- Ensure logistics support including drugs, PPE and other necessary supplies (annex)
- Immediately implement intervention measures to contain the outbreak.

6.3 Rapid Response to JE outbreak
Once an outbreak is believed to have occurred, the RRT in district should immediately conduct a rapid investigation and implement appropriate intervention measures. A national Rapid Response Team may visit the site to conduct outbreak investigation if district lacks capacity and request national RRT. A detailed outbreak investigation will be conducted to establish outbreak epidemiology, etiology, and recommend both short and long term interventions measures to responding to the outbreak and prevent future outbreaks. An outbreak response should include the following steps/actions:
- Investigations to confirm the outbreak
  - Establish that the ‘suspect cases’ fit the case definition of JE by obtaining information regarding sign and symptoms of disease, onset of illness, place of residence, recent travel history and vector prevalence, etc. The cases must be line listed.
  - Conduct a rapid search for additional cases that may have occurred in the locality but not reported to the health facilities.
  - Deaths, if any, should be determined that have not been reported or for which no cause has been known.
- Information, Education and Communication
• IEC campaign should be planned once outbreak is confirmed and launched for general public to ensure all suspected JE cases are taken to the nearest health facility for checkup and supportive care.
• IEC must provide the information to the community regarding the diseases and its possible threats.
• In case of confirmed JE outbreak, community must be encouraged to isolate pigs and horses from human settlements
• Encourage the use of insecticide treated bed mosquito net.
• IEC should be communicated though local media if available till outbreak is contained.

6.4 Data collection and reporting
During the outbreak, all suspected cases must be investigated and data should be collected using line list form (annex 4). The form may be modified to capture essential data. Data collected should have detail demographic information, address of places, and date of onset of illness and other vital information. After an outbreak, comprehensive report must be prepared by RRT and submit to Department of Public Health and relevant programs for documentation and future reference. The report should be prepared in standard format provided (annex 5)

7. Entomology Surveillance for JE
Japanese Encephalitis (JE) is a disease principally of rural agricultural areas, particularly in rice cultivation areas, where vector mosquitoes proliferate in close association with pigs, wading birds and ducks, the principal amplifying hosts. Vector mosquito is able to transmit the JE virus to a new host usually the pig after infected bite of a host with an incubation period of 14 days.

7.1 Vector Mosquitoes of Japanese Encephalitis
The vector of JE is from Culex vishnui group consisting of Cx. tritaeniorhynchus, Cx. vishnui and Cx. pseudovishnui. Female mosquitoes get infected after feeding on a vertebrate host harboring JE virus and after 9-12 days of extrinsic incubation period, they can transmit the virus to other hosts.

Cx. vishnui subgroup of mosquitoes are very common, widespread and breed in water with luxuriant vegetation, mainly in paddy fields and their abundance may be related to their breeding in rice fields, fish ponds, shallow ditches, pools etc. paddy fields are the favorable breeding places during the rainy season and irrigation channels bordering the paddy fields support breeding during the non-monsoon months. Rain water collections in low lying areas with aquatic vegetation/submerge grasses support the breeding during post monsoon months. However, permanent water collection in ponds, ditches etc with aquatic vegetation such as water hyacinth, elephant grass, etc. provide favorable breeding places during all months. In view of the breeding habitats of the vector mosquitoes, JE is usually associated with rural areas with paddy cultivation.

Cx. tritaeniorhynchus, the principal vector of JE has been reported to be exophilic and zoophilic and both exophilic and endophagic. They prefer to feed on cattle and also feed on pig. Cattle such as cows may reduce risk by diverting vector mosquitoes (zoo prophylaxis).
For planning vector control measures, the bionomics of vector mosquitoes in the area needs to be studied.

7.2 Objective of Entomological surveillance
1. To identify the JE vector mosquitoes in an area
2. To monitor JE vector abundance in JE endemic areas
3. To detect JE virus in vector mosquitoes
4. To suggest appropriate vector control

7.3 Procedure
Entomologist and insect collectors at VDCP will be responsible for entomological surveillance and trainings of the Medical technicians in JE endemic areas. VDCP will identify index villages in the country for entomological surveillance.

7.4 Choice of index villages
- At least 3 chiwogs in which JE has occurred in the recent past (past five years)
- At least 2 villages which remained unaffected till date would be monitored in each affected Dzongkhag.
- Sampling would be carried out on fortnightly basis
- Surveillance would be carried out round the year to know the JE vector density, their resting behavior and detection/isolation of JE virus from vector mosquitoes.

Following entomological investigations will be carried out:

7.4.1 Larval surveys
Larval density & mapping of breeding sites survey should be carried out by the entomological team periodically. All potential breeding sites will be surveyed and will be reported on the standard performa. All permanent breeding sites of JE vectors would be identified (mapped) and provided to Dzongkhag Health Officer/Dzongkhag Malaria Supervisor for implementation of control measures.

7.4.2 Adult surveys
Indoor/Outdoor resting collection and the Dusk collection should be carried out from fixed as well as random sites in indoor sites such as human dwelling/cattle sheds/mixed dwelling and outdoor situations such as bushes, plantations, standing crops, etc. by hand catch method using suction tubes. PerMan Hour Density (PMHD) will be monitored and reported in standard prescribed format AESF-7. This collection would be carried out in the index villages only.

Cx. tritaeniorhynchus predominantly rests outdoor on agricultural crops and wild vegetation, depending on local situation, where they can also be monitored by BPD Hop Cage method. The density of mosquito may be estimated as average number of mosquitoes collected per 10 Hop Cages. The larger the area covered by hopping, the better representation of the mosquito density.

\[
\text{Mosquito density (Per 10 HC) = } \frac{\text{Total number of mosquitoes collected} \times 10}{\text{Total number of hops made on vegetation}}
\]

Hourly collection during night using different baits would be done in selected entomological units with trained teams in JE endemic areas particularly where pig population would be less or negligible. This study will be used to find out other susceptible animal reservoirs in areas other than pigs. Viral isolation in those animals should be also done. Hourly collection report will be monitored and reported in standard prescribed format.
Monthly Integrated AFP, Measles and MNT Report from sentinel site

(To be sent to the district/National Level before the 7th calendar day of every month for the previous month)

Reporting for the month of ..................................Year ...........................................

Name of the reporting sentinel site.................................................................

Number of reporting sites...........................................................................

Number of reporting sites included in this report (including your hospital)........

Name of those reporting sites not included in this report:.....................
a) ........................
b) ........................
c) ........................

Section I: AFP Zero Report

Number of Acute Flaccid Paralysis cases reported in this month.........................
(Note case ID below; attach forms on any case; if no cases to report, indicate "Zero")

ID No for the case (if reported)  Name of reporting site
.......................................................... ..........................................................

Section II: Measles Case Report

Number of Measles suspects .................................................................
(Attach forms on any case; if no cases to report, indicate "Zero")

Number of Measles cases reported for this month........................................
(Attach forms on any case; if no cases to report, indicate "Zero")

Section III: Maternal and Neonatal Tetanus Case/Suspect Report

Number of maternal deaths reported ........................................................
(Attach forms on any case; if no cases to report, indicate "Zero")

Number of maternal tetanus (suspect) reported in this month........................
(Attach forms on any case; if no cases to report, indicate "Zero")

Number of neonatal death reported in this month......................................
(Attach forms on any case; if no cases to report, indicate "Zero")

Number of neonatal tetanus (suspect) reported in this month......................
(Attach forms on any case; if no cases to report, indicate "Zero")

Section IV: AES Report

ONLY FOR JDWNRH/ Gelephu RR hospital / Mongar RR hospital/
Phuntsholing hospital / Samdrup Jongkhar Hospital (Please circle)

Number of New Acute Encephalitis Syndrome cases reported in this month........
(Note case ID below; attach forms on any case; if no cases to report, indicate "Zero")

Epid No for the case (if reported)  Name of reporting site
.......................................................... ..........................................................

Name of the reporting officer......................................................Designation..........................
Signature..........................................................Date..........................

Annex 1
Reporting for the month of ………………………………Year …………………………….
Name of the reporting Dzongkhag………………………………………………………….
Number of reporting sites…………………………………………………………………….
Number of reporting sites included in this report (including your hospital)……………….
Name of those reporting sites not included in this report:………………
a) ……………
b) ……………
c) ……………

Section I: AFP Zero Report
Number of Acute Flaccid Paralysis cases reported in this month……………………………..
(Note case ID below; attach forms on any case; if no cases to report, indicate "Zero")

ID No for the case (if reported) Name of reporting site
…………………………………  ………………………………………..

Section II: Measles Case Report
Number of Measles suspects…………………………………………………………………….
(Attach forms on any case; if no cases to report, indicate "Zero")
Number of Measles cases reported for this month……………………………………………….
(Attach forms on any case; if no cases to report, indicate "Zero")

Section III: Maternal and Neonatal Tetanus Case/Suspect Report
Number of maternal deaths reported………………………………………………………….
(Attach forms on any case; if no cases to report, indicate "Zero")
Number of maternal tetanus (suspect) reported in this month……………………………..
(Attach forms on any case; if no cases to report, indicate "Zero")
Number of neonatal death reported in this month……………………………………………
(Attach forms on any case; if no cases to report, indicate "Zero")
Number of neonatal tetanus (suspect) reported in this month……………………………..
(Attach forms on any case; if no cases to report, indicate "Zero")

Section IV: AES Report
ONLY FOR JDWNRH/ Gelephu RR hospital / Mongar RR hospital/ Phuntsholing hospital / Samdrup Jongkhar Hospital (Please circle)
Number of New Acute Encephalitis Syndrome cases reported in this month……………….
(Note case ID below; attach forms on any case; if no cases to report, indicate "Zero")
EPID No for the case (if reported) Name of reporting site
…………………………………  ………………………………………..
Name of the reporting officer………………………………Designation………………………
Signature…………………………………………Date…………………………
**ACUTE ENCEPHALITIC SYNDROME/ SUSPECTED JE CASE INVESTIGATION FORM and LAB REQUEST FORM**

**Annex 3**

<table>
<thead>
<tr>
<th><strong>Reporting information</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital:</td>
<td>Date Case admitted: _____ / _____ / _____</td>
</tr>
<tr>
<td>Date Case Reported: _____ / _____ / _____</td>
<td>Notified by: ________________________________</td>
</tr>
<tr>
<td>Date Case Investigated: _____ / _____ / _____</td>
<td>Investigated by: ________________________________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Patient information</strong></th>
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<tbody>
<tr>
<td>Patient's Name:</td>
<td>Sex: _____</td>
</tr>
<tr>
<td>Date of birth: _____ / _____ / _____</td>
<td>Age: years _____ months _____</td>
</tr>
<tr>
<td>Father's Name:</td>
<td></td>
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<tr>
<td>Present Address:</td>
<td>Landmark: ________________________________</td>
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<tr>
<td></td>
<td>Phone no: ________________________________</td>
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<tr>
<td>District:</td>
<td>Country: _____ Setting: Urban / Rural</td>
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<table>
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<tr>
<th><strong>Travel history one month prior to onset</strong></th>
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</thead>
<tbody>
<tr>
<td>Travel? Yes / No</td>
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</tr>
<tr>
<td>Dates of travel</td>
<td>Date from:</td>
</tr>
<tr>
<td></td>
<td>Date to:</td>
</tr>
<tr>
<td>Place of Visit</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>District and Province</td>
<td>Is this a JE / Dengue endemic area? Yes / No</td>
</tr>
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<table>
<thead>
<tr>
<th><strong>Immunization history</strong></th>
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</tr>
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<tbody>
<tr>
<td>Most recent vaccine taken: (specify type)</td>
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<tr>
<td>Date: _____ / _____</td>
<td>Dose Number: 1 / 2 / 3 / 4 / 5 / B</td>
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<tr>
<td>Other vaccines given earlier</td>
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</tr>
<tr>
<td>Date: _____ / _____</td>
<td>Dose Number: 1 / 2 / 3 / 4 / 5 / B</td>
</tr>
<tr>
<td>Date: _____ / _____</td>
<td>Dose Number: 1 / 2 / 3 / 4 / 5 / B</td>
</tr>
<tr>
<td>Date: _____ / _____</td>
<td>Dose Number: 1 / 2 / 3 / 4 / 5 / B</td>
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<table>
<thead>
<tr>
<th><strong>Signs and Symptoms</strong></th>
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<tr>
<td>Date of onset of first symptom: _____ / _____ / _____</td>
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<tr>
<td>Fever: Yes / No / Unknown</td>
<td>Change in mental status: Yes / No / Unknown</td>
</tr>
<tr>
<td>Seizure: Yes / No / Unknown</td>
<td>Paralysis: Yes / No / Unknown</td>
</tr>
<tr>
<td>Headache: Yes / No / Unknown</td>
<td>Neck rigidity: Yes / No / Unknown</td>
</tr>
<tr>
<td>Unconsciousness: Yes / No / Unknown</td>
<td></td>
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<tr>
<td>Any other, specify:</td>
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</table>

<table>
<thead>
<tr>
<th><strong>Sample collection and shipment (Coordinated by District)</strong></th>
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<tbody>
<tr>
<td>Date Collected</td>
<td>Date Sent to lab</td>
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<tr>
<td>Serum 1</td>
<td>Name of Laboratory</td>
</tr>
<tr>
<td>Serum 2</td>
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</tr>
<tr>
<td>CSF</td>
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<table>
<thead>
<tr>
<th><strong>For use by the receiving laboratory:</strong></th>
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</thead>
<tbody>
<tr>
<td>Name of laboratory:</td>
<td></td>
</tr>
<tr>
<td>Name of person receiving the specimen:</td>
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</table>

<table>
<thead>
<tr>
<th><strong>Date</strong></th>
<th><strong>Condition</strong>*</th>
<th><strong>Processed?</strong></th>
<th><strong>Test performed</strong></th>
<th><strong>Laboratory Result</strong></th>
<th><strong>Date Result sent to program</strong></th>
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</thead>
<tbody>
<tr>
<td>CSF</td>
<td></td>
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<tr>
<td>CSF (JE)</td>
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<tr>
<td>Serum 1</td>
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<tr>
<td>Serum 2</td>
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| **Remarks** |  |
|----------------|  |
| **Status at end of hospitalization and Final Classification (By District)** |  |
| Outcome: Alive / Dead / Unknown | Date of discharge: _____ / _____ / _____ |
| If alive, status of recovery: Recovered completely / Recovered with disability |  |
| If died, date of death: _____ / _____ / _____ |  |
| Final classification: Laboratory confirmed _____ / Probable _____ / AES unknown / AES other agent (specify _____) |  |
| Clinical diagnosis: |  |
| (Name & Signature) | Designation |
### ACUTE ENCEPHALITIS SYNDROME LINE LIST

**Report Date:** __/__/____

**Period of report:** From: ____________(date); to: ____________(date)

**Reported by:** __________________________(Name)

**Designation:**

<table>
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<tr>
<th>Case EPID Number</th>
<th>Name</th>
<th>Date of Admission</th>
<th>Date of Investigation</th>
<th>District of residence (Code)</th>
<th>Sex (M/F)</th>
<th>Date of Birth</th>
<th>Place of residence (City)</th>
<th>Date of recent vaccine</th>
<th>Date of onset of first symptom</th>
<th>Fever?</th>
<th>Altered sensorium?</th>
<th>Date of investigation</th>
<th>Serum</th>
<th>CSF</th>
<th>Final case Classification</th>
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*Daily report during epidemic/outbreak, Weekly report in transmission season and monthly report every month*

(Date & Signature)  
Designation

**Annex 4**
OUTBREAK INVESTIGATION REPORT

General information
District: ............................................................
Area: ............................................................
Village/Ward: ............................................................
Population (Rural/Urban): ............................................................

Background information
Person reporting the outbreak: ............................................................
Date of report: ............................................................
Date when investigations started: ............................................................
Person(s) investigating the outbreak: ............................................................

Details of investigation
Describe how cases were found - may include a) house to house search in the affected area; (b) visiting places adjacent to the affected area; (c) conducting record reviews at local hospitals; (d) requesting health workers to report similar cases in their areas etc.:

________________________________________________________________________

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Descriptive epidemiology
Cases by time, place and person (attach summary tables and relevant graphs and maps)
Age specific attack rates and mortality rates

High risk age groups and geographical areas
Vaccination status of cases, unaffected population

Prevalence and density of JE vectors

Prevalence of reservoirs specially Pigs and Horses

Description of control measures
________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________
Description of measures for follow-up visits


Brief description of problem encountered


Factors which contributed to the outbreak


Conclusions and recommendations


Date

(Name and designation)