Republic of the Philippines
Department of Health
OFFICE OF THE SECRETARY

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ADMINISTRATIVE ORDER
No. 2018-0006

SUBJECT: **Interim Guidelines for Specimen Collection, Initial Testing, Storage, Packaging and Transport for Confirmatory Testing of Cases from Surveillance of Adverse Events among Dengvaxia Vaccinees (AEDV), and Designation of Sub-National Laboratories and Partner Testing Laboratories**

I. BACKGROUND AND RATIONALE

In order to closely monitor recipients of the CYD-Tetravalent Dengue Vaccine (Dengvaxia), for the occurrence of possible cases of severe dengue infection, as well as other diseases in the longer term, the DOH established a system for the Surveillance of Adverse Events among Dengvaxia Vaccinees (AEDV Surveillance).

Confirmatory testing is an important part of the protocol for surveillance, diagnosis, and management of cases. To guarantee the quality of the results, it is necessary to ensure the appropriateness of the specimen needed for the test procedure, and maintain specimen integrity. In the past, several challenges in the collection, storage, and transport of specimens resulted from confusion regarding the protocol and information flow, resulting in hampered detection of the pathogens being tested.

The Research Institute for Tropical Medicine (RITM) designated as the National Reference Laboratory (NRL) for Dengue and other arboviruses, serves as the central laboratory for confirmatory testing. To augment the capacity of RITM for testing these cases, the San Lazaro Hospital (SLH) and Vicente Sotto Memorial Medical Center (VSMMC) have been identified as subnational reference laboratories (SNL). On the other hand, the University of the Philippines- National Institutes of Health (UP NIH) may serve as a partner testing laboratory for AEDV Surveillance.

These laboratories may perform Real-time Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR) and Dengue IgM and IgG Capture Enzyme-linked Immunosorbent Assay (ELISA). Plaque Neutralization Reduction Assay (PRNT), Cytokines, Immunohistochemistry (IHC), among other tests, shall be performed by RITM.

Hence, this administrative order is hereby provided as reference for all health agencies (DOH central offices, regional offices, health facilities, and other stakeholders) and their local counterparts.

II. OBJECTIVES

1. To guide health workers in the proper collection, initial testing, processing, storing, packaging, and transport of specimens from individuals who fulfill the case definition of the AEDV Surveillance, for laboratory confirmation in RITM, SNLs, and partner testing laboratories.
2. To guide health facilities, SNLs and partner testing laboratories tasked to augment the capacity of RITM, on the prescribed protocol for handling specimens for laboratory confirmation.

III. SCOPE AND COVERAGE

This issuance shall apply to health facilities and health professionals from both the public and private sectors catering to and managing individuals who have received at least one dose of Dengvaxia. This shall also include the DOH concerned offices and attached agencies, epidemiology and surveillance units, private and government health facilities and local government units.

These guidelines shall cover the handling of specimens from Dengvaxia vaccinees who meet the case definition for AEDV, as defined in this Order.

IV. DEFINITION OF TERMS

1. Rapid Diagnostic Test (RDT) – a collection of reagents and other materials for in-vitro diagnostics, intended to be used for the detection of either antigen or antibody from clinical samples, usually blood, within a shorter period.

2. Dengue NS1 RDT- an immunochromatography-based test to detect the Dengue virus non-structural protein 1 to suggest acute Dengue infection using either serum, plasma, or whole blood.

3. Dengue IgM/IgG RDT- an immunochromatography-based test to detect the Dengue antibodies isotype M and G to suggest either primary or secondary Dengue infection using either serum, plasma, or whole blood.

4. National Reference Laboratory (NRL) - considered as the highest level of laboratory in the country, mandated to provide laboratory confirmatory services, provide training, perform surveillance, do outbreak response, provide External Quality Assurance, perform kit evaluation, and conduct research.

5. Sub-National Laboratory (SNL)- a laboratory capable of performing real-time PCR testing, first established by the DOH during the pandemic Influenza AH1N1 (2009) to augment the response of RITM.

6. Partner Testing Laboratory- pertains to other laboratories not within the jurisdiction of the DOH, tasked to do confirmatory testing or other tests that may be appropriate (i.e., UP- NIH, US- Armed Forces Research Institute of Medical Sciences (AFRIMS), among others).

7. Case Definition for AEDV Surveillance- defined in AO 2018-0004, as an individual who received at least one dose of Dengvaxia vaccine, who became ill and was admitted to a health facility for any reason; or died for any reason.

V. GENERAL GUIDELINES

1. This guideline complements the interim guidelines for AEDV surveillance; dengue diagnosis, referral and management for Dengvaxia vaccinees; and investigation of reported deaths among Dengvaxia vaccinees.
2. All health facilities, either public or private, shall perform dengue rapid diagnostic testing (RDT), i.e. Dengue NS1 Ag RDT, and/or Dengue IgG/IgM RDT, as applicable, following AO 2016-0043 (Guidelines for the Nationwide Implementation of the Dengue Rapid Test).
3. All health facilities shall ensure the proper collection, storage, packing and transport of specimens from the referring institution to the next level laboratory, for confirmatory testing.
4. Referral of specimens shall follow the existing flow of notifiable diseases under the Philippine Integrated Disease Surveillance and Response (PIDSR) system of detection, notification, reporting and data management (collection, consolidation, analysis and interpretation) mechanisms following AO 2007-0036, unless otherwise stated in this Administrative Order.

VI. SPECIFIC GUIDELINES

1. Designation of SNLs and other laboratories to perform confirmatory testing for AEDV Surveillance

a. The following health facilities shall be designated as the testing laboratory, with the respective zoning of referral:

Testing Laboratory	Zoning of Referral
San Lazaro Hospital	Region 3
UP-National Institutes of Health	National Capital Region
Research Institute for Tropical Medicine	Region 4A
Vicente Sotto Memorial Medical Center	Region 7

b. Provided that, specimens sent in by regions that are not within the designated zoning of referral, shall still be accepted and processed at the testing laboratory.
c. Assessment, training, evaluation, and monitoring of the SNLs and partner testing laboratories shall be done by the NRL.

d. Standard testing algorithm and methods shall be provided by the NRL. On-site testing in the SNL and partner testing laboratory shall commence once all recommendations are met, and proficiency is attained.
e. Standard feedback mechanism and flow of reporting of results shall be established with the NRL and Epidemiology Bureau.

2. Specimen Collection and Initial Testing

a. Initial testing:
    i. Blood samples shall be collected from cases with fever of 1 to 5 days and tested for Dengue NS1-Ag RDT at the consulting/admitting health facility following AEDV surveillance.
    ii. Cases with more than 5 days of fever shall be tested at the consulting/admitting health facility using other diagnostic procedures, including a Dengue IgM/IgG RDT.

    **Note: Sensitivity and specificity of RDTs varies depending on the brand. Dengue NS1-Ag RDT has sensitivity of 49-59% and specificity of 93-99% while Dengue IgM/IgG RDT has sensitivity of 71-80% and specificity of 46-90%.*

b. Collection and Sending of Specimen for Confirmatory Testing
    i. Collect 5 to 6 ml of whole blood and process into serum. Place 3ml of serum into a cryovial labelled with the following: name of patient, age, sex, and date of collection.
    ii. Serum samples shall be sent to the designated health facility for Dengue RT-PCR and IgM/IgG ELISA testing
    iii. Additional biological samples may be collected from Dengvaxia vaccinees depending on the consideration of other reportable diseases under PIDSR.
    iv. For cases of Dengvaxia vaccinees with severe Dengue, the following specimens shall be collected: 1) acute phase serum, 2) convalescent-phase serum, and 3) whole blood in anticoagulant (Annex A).
    v. In cases of death, post-mortem samples may be collected and sent for confirmation and will be tested for: RT-PCR, IHC, routine histopathology and cytology, and other appropriate tests. (Annex A).

3. Specimen Storage

a. Specimens shall be stored at 2 to 8°C or at the body of refrigerator until arrangements for transport is made to maintain the quality.
b. Specimens should be sent within 48 hours after collection.

4. Specimen Packaging

a. Specimens shall be packed using the triple packaging system for shipment of infectious substances. The system consists of the following:
   i. Primary container- This is a screw-capped, durable receptacle. Ideally this shall be wrapped in absorbent material like cotton or gauze pad for fluid absorption in case of breakage.
   ii. Secondary container- This is a waterproof and durable receptacle to protect the primary container. Usually a re-sealable plastic bag can be used to serve this purpose.
   iii. Third container or outer shipping package- This is a durable insulated transport box or container which can protect the contents from physical damage during transit.

b. The cryovial placed inside the re-sealable plastic bag shall be placed inside the transport box with 6-8 pieces of frozen gel packs.

c. The case report form (CRF) shall be placed inside another re-sealable bag and placed inside the transport box.

5. Specimen Transport

a. Label the transport box with the shipper's address and the consignee's address.

b. Specimens shall be sent to:

Testing Laboratory	Shipment Address
San Lazaro Hospital	BARBARA V. SANTIAGO Central Laboratory Department San Lazaro Hospital Quiricada St., Sta. Cruz, Manila, 1113 Telephone Number: (02) 310-2005
UP-National Institutes of Health	DR. EDSEL SALVANA Institute of Molecular Biology and Biotechnology National Institutes of Health Building 623 Pedro Gil St, Ermita, Manila, 1000 Metro Manila Telephone Number: (02) 526-4349
Research Institute for Tropical Medicine	REX J. CENTENO Virology Department Research Institute for Tropical Medicine 9002 Research Drive, Filinvest Corporate City Compound Alabang, Muntinlupa City, 1781 Telefax Number: (02) 809-7120
Vicente Sotto Memorial Medical Center	DR. REYNETTE CHRISTINE LIGARAY Laboratory and Pathology Department Vicente Sotto Memorial Medical Center B. Rodriguez St, Sambag II, Cebu City, Cebu Telephone number: (032) 253 9891 Mobile number: 0998-9685879

c. The referring institution/facility is primarily responsible for the transport of samples to the proper laboratory, while Regional Epidemiology and Surveillance Unit (RESU) shall ensure that transport of samples is done.

Otherwise, there may also be cases wherein the RESU is delegated to be primarily responsible for the transport of such samples to the designated laboratory.

6. Referral and Reporting of Specimen

a. Surveillance Unit of referring institution/facility shall be informed by concerned laboratory staff before specimen collection procedure, for proper coordination and facilitation of reporting to the next higher surveillance unit and laboratory.
b. Weekly reporting of specimen collection shall be included in the weekly surveillance reporting following the PIDSR flow.
c. All information gathered from the specimens shall be kept secure and confidential. Measures to ensure data security and confidentiality shall be put in place by laboratories and surveillance units at all levels.

7. Feedback

a. Laboratory results shall be forwarded to the referring institution, with copies sent to the RESU, EB, and other appropriate offices of the DOH.
b. There shall be a regular and timely feedback of laboratory results within and between levels of the health delivery system.

VII. ROLES AND RESPONSIBILITIES

A. Department of Health (DOH) Central Office

1. Disease Prevention and Control Bureau (DPCB)

a. Oversee the procurement and distribution of Dengue NS1-Ag RDT supplies from the central to the peripheral health units. Ensure that the supplies are stored at room temperature, not exceeding 30°C;
b. Coordinate with RITM for the quality assurance system of the Dengue NS1-Ag RDT kits.

2. Epidemiology Bureau (EB)

a. Provide accurate, timely and complete data as basis for program use such as policy decisions, strategic directions and prioritization of resources;
b. Provide feedback mechanism for NRL, SNLs, and partner testing laboratories.

B. Research Institute for Tropical Medicine (RITM)

a. Provide technical assistance to the implementation of the RDT testing, RT-PCR, and ELISA.
b. Perform other confirmatory testing of samples as appropriate.
c. Establish and implement a Quality Assurance Program for the SNLs and partner testing laboratories.
d. Provide technical assistance to SNLs and partner testing laboratories such as, but not limited to: training, troubleshooting, procurement, facility enhancement, etc.

C. Sub-National Laboratories and Partner Testing Laboratories

a. Perform confirmatory testing to AEDV Surveillance such as, but not limited to: RT-PCR and ELISA.
b. Provide accurate and timely feedback to the referring institution, NRL, EB, DPCB, and other stakeholders.
c. Store samples and molecular by-products in a safe and secure manner, for possible further testing by the NRL.
d. Send aliquots of clinical samples to RITM for quality assurance, further testing, and storage.
e. Participate in PT and other QA activities (site visit/audit) of the RITM National Reference Laboratories.
f. Monitor and keep an updated inventory of equipment, supplies, and reagents
g. Maintain proficiency of staff by undergoing training

D. Health facilities hosting the SNLs and Partner Testing Laboratories

a. Provide management support to the SNLs
b. Ensure that laboratories are operational in terms of personnel, infrastructure, and reagents and supplies.
c. Include support to the SNLs in annual hospital budget.

VIII. EFFECTIVITY

This Order shall take effect immediately.

FRANCISCO T. DUQUE III, MD, MSc
Secretary of Health

Annex A. Summary of Specimen Collection, Storage, and Transport

Sample Type	Timing of Collection	Quantity	Storage prior to transport	Transport
For vaccine recipients who are admitted and ill:				
Serum	Upon first contact with patient	3 ml	Refrigerator, 2 to 8°C	• Transport within 48 hours or 2 days after collection • Use the prescribed transport box with gel or ice packs
For vaccine recipients with severe Dengue:				
Serum- Acute Phase	Less than 5 days after onset of fever (< 5 days) Or upon first contact with the patient	3 ml	Refrigerator, 2 to 8°C	• Transport within 48 hours or 2 days after collection • Use the prescribed transport box with gel or ice packs
Serum- Convalescent Phase	More than 5 days from onset of fever (> 5 days) Or upon discharge	3 ml	Refrigerator, 2 to 8°C	• Transport within 48 hours or 2 days after collection • Use the prescribed transport box with gel or ice packs
Whole Blood- using Heparin as anticoagulant (Green Top)	Upon first contact with the patient	12 ml	Room Temperature	• Transport within 12 hours upon collection • Use the prescribed transport box with 1 gel or ice pack

For vaccine recipients who died:				
Formalin-fixed tissues (Embalmed remains): Post-mortem tissues from all major organs should be submitted for evaluation. Specimens should include: a. Heart (right ventricle, septum, and left ventricle). b. CNS (cerebral cortex, thalamus, basal ganglia, midbrain, pons, medulla, cerebellum, and spinal cord). Representative sections from the ff: a. Right & left lung b. Right & left kidney c. Spleen d. Liver e. Bone marrow f. Lymph nodes g. Any other organ showing significant gross pathology. h. Effusion: pleural and pericardial fluids For limited autopsy, liver should at least be included; collect multiple slabs of tissue.	Collect specimen, as soon as possible after death	At least three pieces from each organ, each measuring at least 5.0 x 5.0 x 1.0 cm Additional samples from the larger organs like the liver, kidneys, lungs and brain would be helpful. Place each specimen in a wide-mouthed container / jar, submerged in 10% NEUTRAL BUFFERED FORMALIN (Label should include: patient's name, specimen type, laterality, date and time collected) At least 5 to 10 mL fixed in 95% Ethanol (1:1 ratio)	Room temperature	• Transport immediately in a well-sealed container. • Store and ship at room temperature. • Specimens should not be frozen.
Fresh tissues: Post-mortem tissues from all major organs should be submitted for evaluation. Specimens should include: a. Heart (right ventricle, septum, and left ventricle)	Collect specimen FRESH, as soon as possible after death	At least three (3) pieces from each organ, each piece measuring at least 2.0 cm. All specimens (including effusion fluid) should be collected aseptically. Use a separate sterile	Frozen at -20°C prior to shipping; If unavailable, store at the refrigerator, 2 to 8 °C	• Transport immediately in a well-sealed container. • Use the prescribed transport box with gel or ice packs • Avoid repetitive freezing and

b. CNS (cerebral cortex, thalamus, basal ganglia, midbrain, pons, medulla, cerebellum, and spinal cord) Representative sections from the ff: a. right & left lung b. right & left kidney c. Spleen d. Liver e. Bone marrow f. Lymph nodes g. Any other organ showing significant gross pathology h. Effusion: pleural and pericardial fluids For limited autopsy, liver should at least be included; collect multiple slabs of tissue.		instrument for each collection site. Place each specimen in sterile wide-mouthed container / jar (Label should include patient's name, specimen type, laterality, date and time collected). At least 5 to 10 ml without any fixative.		thawing of specimens.