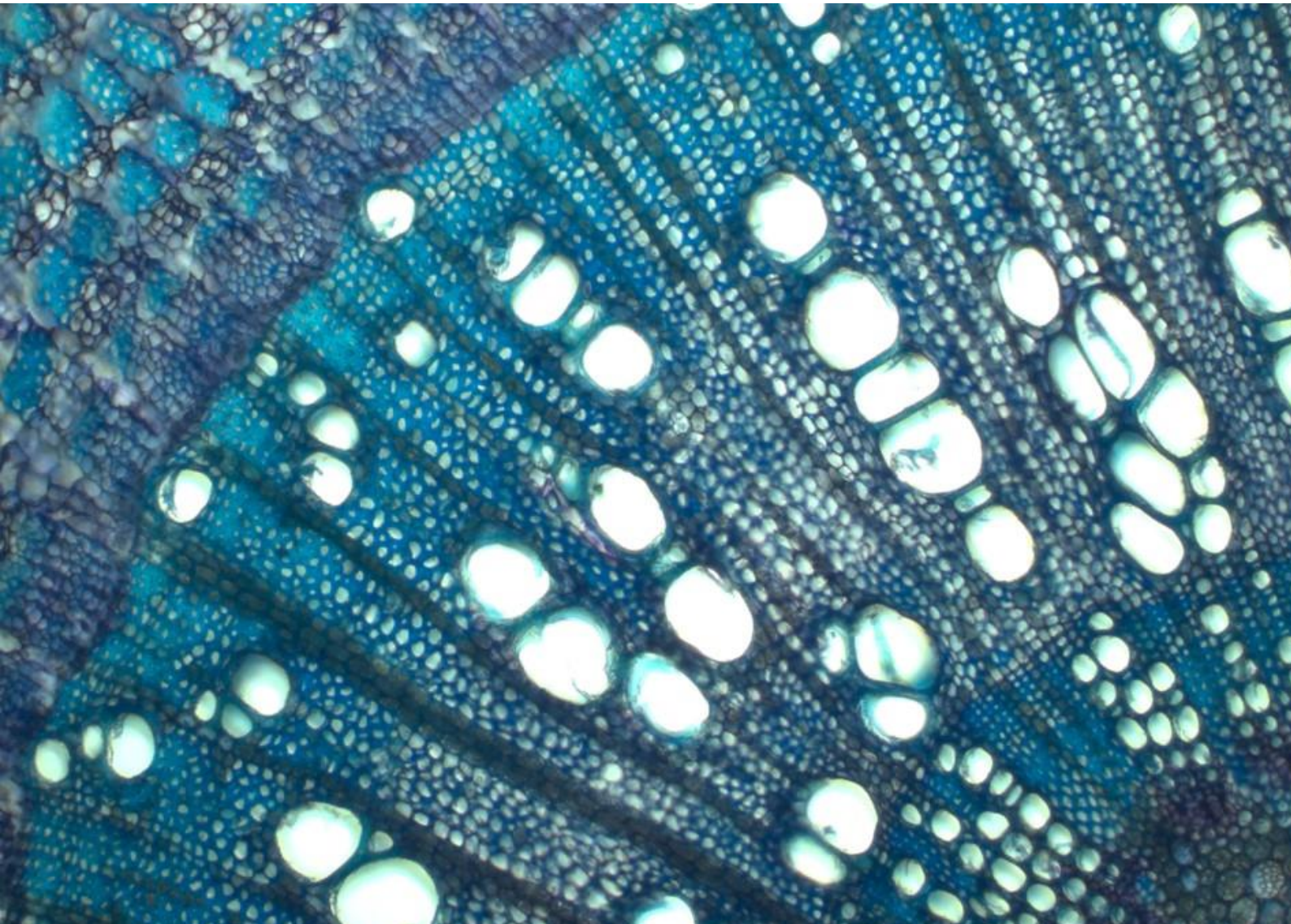
Australia's National
Science Agency

ACDP Japanese Encephalitis Virus Diagnostic Testing Factsheet

Version 3 – March 29th, 2022

An outbreak of Japanese Encephalitis, a mosquito-borne zoonotic viral disease, has recently been detected in Australia.

The CSIRO Australian Centre for Disease Preparedness is able to offer diagnostic testing for Japanese Encephalitis exclusion.



Agent:

Japanese encephalitis (JE) virus is enveloped and has a single-stranded RNA genome of approximately 11kb. It is a member of the *Flavivirus* genus, family *Flaviviridae*.

Species Affected:

Humans, horses and donkeys are considered dead-end hosts but can develop severe encephalitis following infection. Infection of pregnant sows or gilts can lead to reproductive failure with abortions and stillbirths, or piglets born with wasting syndrome or hind limb tremor. Infection of boars has been associated with aspermia. Encephalitis in piglets has also been observed following infection. Infected pigs are known to shed JE virus in oro-nasal secretions and non-vector transmission between pigs has been shown experimentally to occur via this route. Experimental infection of chicks and ducklings can result in stunted growth or encephalitis. Please note, explosive outbreaks of arboviruses can also lead to rare or previously unrecognised forms of disease, and may affect new species.

Laboratory Tests

Specimens required

Please note: Any potentially infected materials must be handled appropriately to prevent the risk of human infection.

As Hendra virus infection is a differential diagnosis in cases of neurological disease in horses, please consult with the relevant jurisdictional guidelines prior to commencing sample collection.

For Japanese encephalitis, there is a possibility that humans may be infected by direct contact of infectious material with broken skin or mucous membranes, accidental parenteral inoculation or aerosol. Diagnosticians collecting samples should also take the appropriate precautions. The OIE recommends that at-risk field veterinarians and laboratory workers should be vaccinated (OIE Terrestrial Manual 2016).

Post-mortem

Post-mortem specimens should be collected from animals with neurological signs killed in the acute stage of the disease or from recently dead animals.

If it is safe to do so, the brain should be removed aseptically, and brain tissue specimens (including cortex, midbrain and brainstem) and cerebrospinal fluid (CSF) collected into sterile containers. For pigs, a full range of tissues (including brain, aborted fetuses, placenta from fetuses, tonsils, spleen, liver, kidney, lung, heart) should be collected into both sterile containers for PCR and neutral buffered formalin for histopathology to rule out other diseases in the differential diagnosis list. For horses, additional samples (other than CNS samples) can be collected into neutral buffered formalin only.

For **aborted fetuses and neonates**, JEV can be detected from a range of tissues and fluids, but is found most frequently or in highest levels in the brain/CNS, placenta, lung, heart, and abdominal and thoracic fluid. Other relevant specimens include tonsils, liver, and kidney. These tissue samples should be collected into sterile

containers. **Please note: While ACDP values receiving whole carcasses for necropsy we do not have the capacity for high throughput. Where possible, please avoid submitting large batches of carcasses to ACDP for JE testing.**

Ante-mortem

From animals in the acute stage of disease, collect whole blood (EDTA)* and serum samples for virus detection and serology. For serum, collect at least 7–10 mL of blood from animals in the acute phase and convalescent stage of the disease, and from 'in-contact' animals. Collect paired serum samples 2–4 weeks apart.

Ideally, serum should be separated from the clot before shipment, to prevent haemolysis. Both clot and serum should be submitted.

Cerebrospinal fluid (CSF) should be collected from animals presenting with neurological signs. In horses, if possible and practical, a minimum of 5 mL of CSF can be collected into suitable sterile containers from the lumbosacral space in standing animals or from the atlanto-occipital space in recumbent horses.

Oral-nasal swabs and semen may be considered as an additional sample for agent detection in pigs.

* Please Note: The current Japanese encephalitis AUSVETPLAN Response Strategy recommends the collection of heparin blood samples for Japanese ecephalitis diagnosis. ACDP's current recommendation is that EDTA blood, rather than Lithium heparin blood, is the preferred sample type.

JE Virus detection and characterisation

Virus detection

Definitive diagnosis of JE can be made based on virus detection. The primary method of agent detection for JE virus is by real-time reverse transcription (RT-)PCR in post-mortem tissues (particularly CNS tissue or fetal tissues) or blood and CSF collected from acute cases.

Due to the short viraemia associated with JEV infection (4-5 days) there is only a short diagnostic window for virus detection by PCR in blood and serum samples. The presence of virus is also transient in the CSF and is rarely isolated or detected by PCR. Negative PCR results obtained from blood, serum and CSF samples must be interpreted in this context. Oral-nasal swabs may also be tested; however use of this sample for agent detection has not been well validated.

Histopathology is also available for JE virus investigation. Virus isolation may be performed, however please note that the positive isolation rate from diseased or dead animals is usually very low. This may be due to the brevity of the viraemic period or low levels of infectious virus present in specimens resulting from instability under certain environmental conditions, or the presence of antibody in infected animals.

Agent characterisation

Additional JE virus characterisation can be performed in positive cases. Conventional PCR assays and capillary sequencing is available for additional confirmation and genotyping of positive results, whilst Next-generation sequencing can be performed on isolates and clinical samples with high viral loads to obtain whole genome sequences. Additional characterisation will be performed on selected cases only.

Serology

Serological tests can be used in conjunction with virus detection methods for diagnosis of JE, and are also important to determine the prevalence of infection in an animal population and the geographical distribution of the virus.

Interpretation of serological tests is complicated by the antigenic relatedness of flaviviruses, particularly those belonging to the same serogroup. Within the JE serogroup, Australia has five endemic viruses: Murray Valley encephalitis (MVE), Kunjin (KUN; a subtype of West Nile virus), Alfuy, Kokobera and Stratford. Of these, MVE and KUN viruses have been associated with outbreaks of encephalitis in humans and horses. Serological test results need to be performed and interpreted in this context, and testing for JE, MVE and KUN viruses should ideally be performed in parallel to assess the presence of cross-reactive antibody.

Interpretation should also take into account the age of the animal and potential for prior exposure to related flaviviruses. Some horses may have also received JE vaccination for travel or prior to importation.

Serological tests to detect IgM and IgG antibodies to flaviviruses include competitive (C-)ELISAs, IgM capture (MAC-)ELISAs, and virus neutralisation (VN) tests. To resolve cross-reactive antibodies, the more specific VN tests are used. Of the different VN test formats that may be employed, the plaque reduction neutralisation test (PRNT) is considered the most specific.

A diagnosis of recent infection can be based on the demonstration of seroconversion between paired serum samples collected in the acute or convalescent phases, or a significant rise in antibody levels (fourfold or greater) using the neutralisation test. Where available, IgM assays may also be used to assess evidence of recent infection; however cross reactions with related flaviviruses can still occur and should be considered when interpreting results from IgM assays.

Prioritisation and Charging of Exclusion and Surveillance testing

As the JEV response moves forward, it is important to distinguish diagnostic / disease exclusion testing from surveillance and non-JEV related testing.

Disease exclusion

Testing for JEV on properties not previously diagnosed as part of the response forms the highest priority testing at ACDP in this response. These cases are treated as is usual for EAD exclusion submissions, with no charge to the submitter. Note that testing for differential diagnoses identified upon submission will be cancelled in the event of a JEV positive diagnosis, unless specifically requested otherwise.

Surveillance

In line with the national surveillance plan, a number of categories of surveillance samples have been identified. These include (but are not limited to):

- Testing of historic material
- Wider testing carried out on properties already confirmed as JEV positive
- Opportunistic testing to delineate historic or geographical extent of disease

This work will be carried out at a lower priority than diagnostic work, but higher than that of other routine work. This work is done on a fee-for service basis.

Other testing

Testing for JEV for purposes unrelated to the outbreak, or testing unrelated to JEV that is requested along with JEV testing, does not form part of the outbreak response and will be done on a fee-for-service basis, and at routine priorities. Examples include:

- Testing of boars for JEV to enable movement of semen for commercial purposes
- Testing of any animal for JEV for international travel
- Testing for endemic agents such as Ross River virus alongside JEV.

Table of Laboratory Tests currently available at CSIRO-ACDP for the diagnosis of JE

Test	Specimen Required	Test detects	Time taken to obtain result[3]	Cost (where applicable[4], AUD$, Ex GST)
Agent Detection				
Real time RT-qPCR	**Pigs** • Aborted fetal or neonatal piglet post-mortem samples (including fetal brain/CNS tissue, lung, heart, abdominal and thoracic fluid and placenta) • Fresh brain - Remove the brain aseptically and collect a range of brain tissue samples (including cortex, midbrain and brainstem) • Spinal cord • Tonsils, spleen, liver, kidney, lung, heart • Antemortem - EDTA blood or serum, semen, oro-nasal swabs (note limitations discussed above) **Horses** • Fresh brain - Remove the brain aseptically and collect a range of brain tissue samples (including cortex, midbrain and brainstem) • Spinal cord • Cerebrospinal fluid (CSF) • Antemortem - EDTA blood or serum (note limitations discussed above)	Viral RNA	4-6 hours	$415 + $55 per additional sample
Histopathology	Formalin-fixed tissues (especially CNS and aborted fetuses)	Microscopic changes	2 days	$146 per sample (+$146 per gross necropsy)
Immunohistochemistry	Formalin-fixed tissues (especially CNS and aborted fetuses)	Viral antigens in tissue	3 days	$123 per sample

Agent characterisation				
Virus isolation and Identification[1]	As for Real Time RT-qPCR	Infectious virus	2-3 weeks	$200 per sample
PCR and capillary sequencing	As for Real Time RT-qPCR	Viral RNA and partial NS5 gene	2-3 days	$378 + $49 per additional sample
Next Generation sequencing	As for Real Time RT-qPCR	Viral RNA – partial to whole genome	2-7 days	$378 + $49 per additional sample
Serology				
Flavivirus C-ELISA	Serum	Group-reactive antibodies to flaviviruses	1-3 days	$32 per sample
JEV, MVEV and KUNV specific C-ELISAs, as a panel (pigs only)[2]	Serum	Antibody specific to each flavivirus [2]	1 day	$32 per sample
Plaque reduction neutralisation test (PRNT)	Serum	Neutralising antibody to specific flaviviruses (JEV, MVEV, KUNV)	1-3 weeks	$196 per sample
IgM ELISA[2]	Serum, CSF	JEV IgM antibody	1 day	$32 per sample

C-ELISA = competitive enzyme-linked immunosorbent assay; CNS = central nervous system; CSF = cerebrospinal fluid; MVEV = Murray Valley encephalitis virus, KUNV = Kunjin virus;

[1] Isolates subjected to ELISA tests to identify the virus as a flavivirus, and serum neutralisation or PCR to confirm the virus as JEV

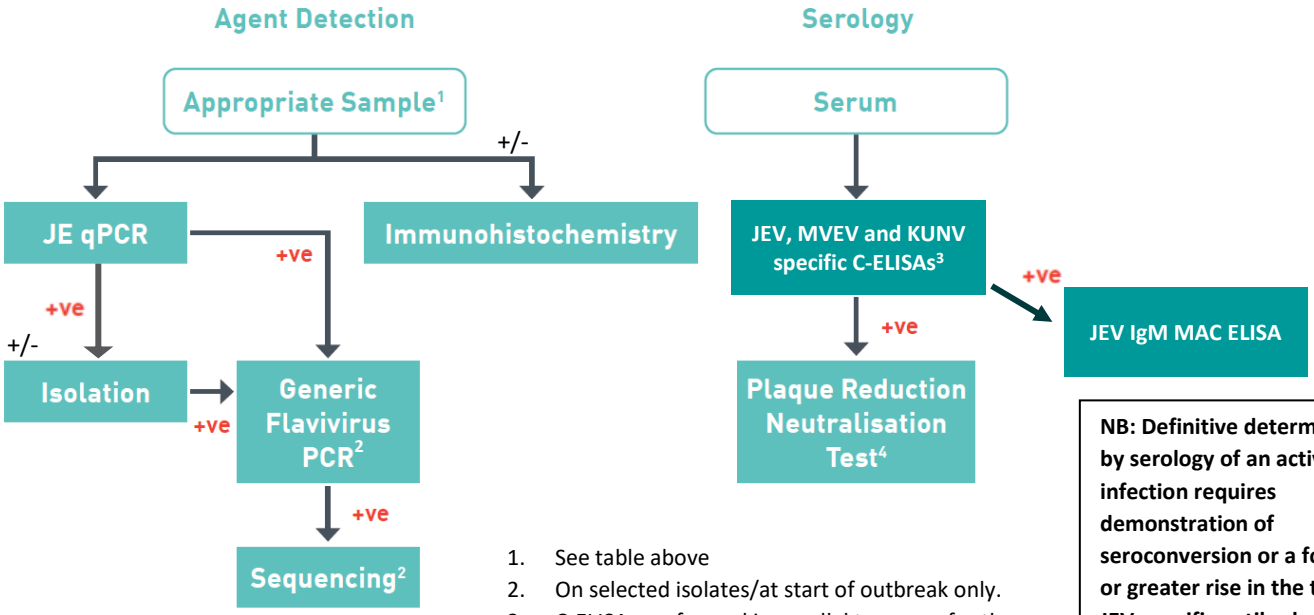
[2] Results should be interpreted with respect to potential cross-reactivity with other flaviviruses tested.

[3] Due to the large volume of samples being received, ACDP has instituted a triage-based approach to exclusion testing (see above). As such, diagnostic reporting timelines may be affected. For further information, please contact the laboratory.

[4] Charges do not apply for disease exclusion testing. See 'Prioritisation and Charging of Exclusion and Surveillance testing' above.

CSIRO-ACDP Diagnostic Algorithms by Species

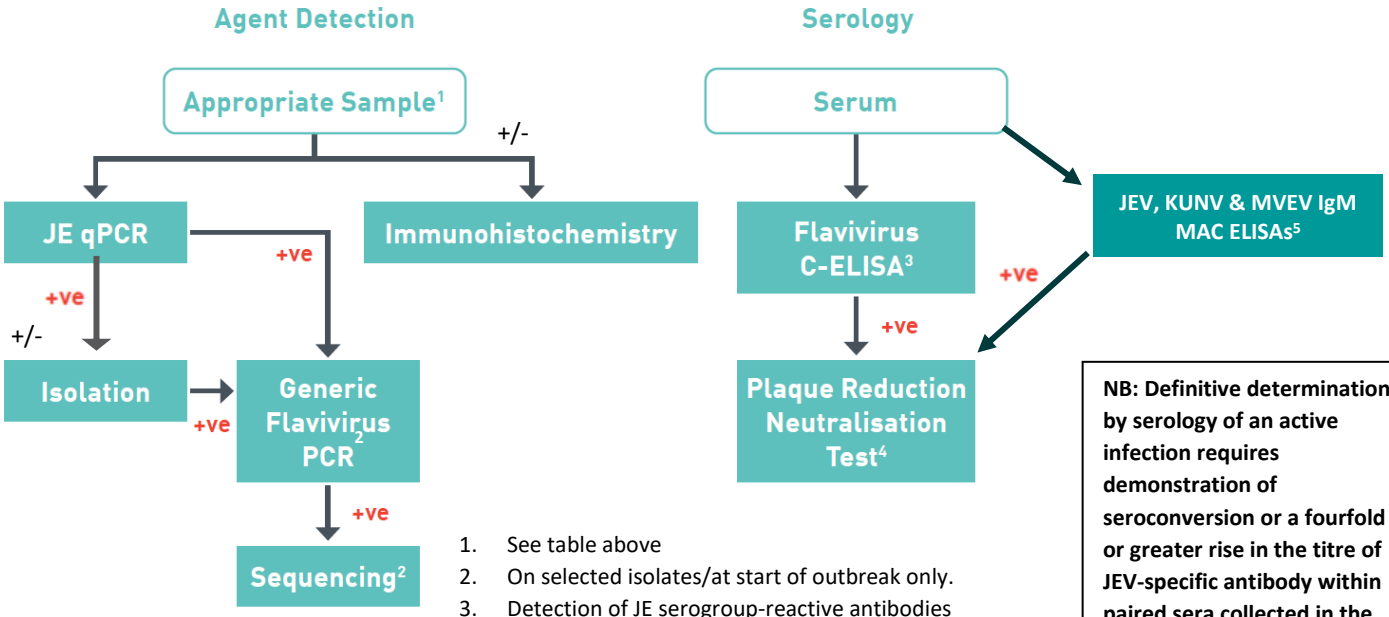
Pigs



1. See table above
2. On selected isolates/at start of outbreak only.
3. C-ELISAs performed in parallel to assess for the presence of cross-reactive antibody
4. Result specific for JEV, KUNV and MVEV. Requires paried sera to dectect recent infection.

NB: Definitive determination by serology of an active infection requires demonstration of seroconversion or a fourfold or greater rise in the titre of JEV-specific antibody within paired sera collected in the acute and convalescent phases, and taken at least 14 days apart.

Horses



1. See table above
2. On selected isolates/at start of outbreak only.
3. Detection of JE serogroup-reactive antibodies
4. Result specific for JEV, KUNV and MVEV. Requires paried sera to dectect recent infection.
5. IgM MAC ELISAs performed in parallel to assess for the presence of cross-reactive antibody.

NB: Definitive determination by serology of an active infection requires demonstration of seroconversion or a fourfold or greater rise in the titre of JEV-specific antibody within paired sera collected in the acute and convalescent phases, and taken at least 14 days apart.

Contact us

ACDP Accessions
+61 3 5227 5414
acdp-accessions@csiro.au