

Laboratory testing for severe acute hepatitis of unknown aetiology in children

Interim guidance

17 June 2022



Key points

- This document provides interim guidance to countries on testing considerations and strategies for suspect cases of severe acute hepatitis of unknown aetiology in children.
- Diagnostics are grouped into three categories to support with prioritisation: 1) case definition diagnostics which are tests required to meet the current WHO case definition; 2) additional diagnostics to exclude other well-recognized causes of severe acute hepatitis in children; and 3) investigative diagnostics to identify a potential aetiology(ies).
- Prioritization should be given to routine collection of key specimens from as early after symptom onset as possible. Whole blood is the preferred specimen type but others including serum, plasma, throat swab, stool or urine are strongly encouraged. Liver biopsies should only be considered if clinically necessary and available. Specimens should be stored under the appropriate conditions.
- Assessments for other aetiological factors that are known to cause severe acute hepatitis in children, including other infectious agents, environmental exposures (toxins, medications), metabolic hereditary conditions, or autoimmune disorders should be considered in consultation with a paediatric hepatologist.
- A set of investigative diagnostics could be considered that include a variety of viruses, bacteria, parasites, and fungi. This exhaustive list should be adapted based on the country and region, particularly considering endemic infectious diseases.
- If laboratory capacity is limited, storage and referral to regional or global laboratories should be considered for the suggested investigative diagnostics.
- Any positive specimens should also be stored for further testing and/or investigation.

Background

Cases of severe acute hepatitis of unknown aetiology continue to be reported in children across multiple countries. The majority of reported cases are currently from the WHO European Region. Probable cases and cases pending classification have also been reported from the Region of the Americas, the Western Pacific Region, the South-East Asia Region, and the Eastern Mediterranean Region. Given the enhanced case finding activities, it is likely that more cases will be reported in the next weeks.

In some countries where the data from previous years are available, the number of cases exceeds the usual low background rates of severe acute hepatitis of unknown aetiology in children that would be observed in most countries. Five hepatitis viruses (A, B, C, E, and D where applicable) have typically caused the majority of hepatitis cases worldwide; however, the recent cases of severe acute hepatitis of unknown aetiology have all tested negative for these viruses. While the cause or causes are currently unknown, studies and investigations into potential known or unknown aetiologies are ongoing. These investigations include infectious (bacterial, fungal, parasitic, and virological) and non-infectious agents, environmental exposures such as toxins or certain medicines, autoimmune disorders, and metabolic hereditary conditions. To date, no clear risk factors or causes have been identified.

This document provides interim guidance to countries on testing considerations and strategies for suspect cases of severe acute hepatitis of unknown aetiology in children. It is primarily intended for clinical, programmatic, laboratory and diagnostic stakeholders across Member States and national public health authorities involved in the identification and investigation of cases of severe acute hepatitis in children.

WHO is closely monitoring developments related to this situation and will revise these recommendations as and when necessary.

Indications for testing

As the current aetiology(ies) is not yet known or characterised, the decision to test at present should be based on clinical assessment and the need to rule out potential known causes of severe acute hepatitis in children. Principles of Good Clinical Practice should prevail and guide clinicians and health care workers in supporting children presenting with signs and symptoms of severe acute hepatitis. WHO recommends that clinicians, epidemiologists and laboratory scientists consult the WHO case definition to determine which cases require reporting and potential further investigation. The case definitions are being regularly reviewed and assessed as new information becomes available (see Box 1).

Box 1. Case definition

The current WHO working case definitions are as follows:

Confirmed: N/A at present

Probable: A person presenting with an acute hepatitis (non hepA-E*) with serum transaminase >500 IU/L (AST or ALT), who is 16 years and younger, since October 2021

Epi-linked: A person presenting with an acute hepatitis (non hepA-E*) of any age who is a close contact of a probable case, since October 2021.

**If hepatitis A-E serology results are awaited but other criteria met, these can be reported and will be classified as “pending classification”. Case with other explanations for their clinical presentation are discarded.*

The current case definition indicates that the following diagnostic tests should be undertaken to identify probable cases of severe acute hepatitis of unknown aetiology in children:

- Assessment of severity: Aspartate transaminase (AST) and/or alanine transaminase (ALT)
- Hepatitis A virus
- Hepatitis B virus*
- Hepatitis C virus
- Hepatitis E virus

Current case definition would also require to exclude hepatitis cases caused by other known causes of acute hepatitis in children including environmental exposures (toxins, certain medications), metabolic hereditary conditions, or autoimmune disorders.

**Hepatitis D (or delta) virus testing is not required, as it is only relevant and undertaken in persons who are HBsAg positive to establish presence of co-infection.*

Recommendations for specimen collection, storage and transportation

It is recommended that a variety of specimens are collected from individuals meeting the case definition described above as soon as possible following symptom onset. Periodic testing should also be considered. As investigation into the aetiology(ies) of these hepatitis cases is ongoing, it is recommended that specimens are prioritized for collection (even without local testing capacity) for two purposes: 1) to meet the case definition; and 2) to support investigation of the aetiology(ies). Specimen referral to a national, regional or global laboratory could be considered when local capacity is limited.

Specimen collection and storage

Multiple specimens over time may be collected for investigative purposes. Specimens may be collected sequentially, including upon onset of symptoms, upon deterioration or changes in condition or presentation, prior to and following receipt of any treatments, and upon convalescence.

Whole blood is the preferred specimen type but others including serum, plasma, throat swab, stool or urine are strongly encouraged. It is not recommended to take a biopsy of liver tissue, but a sample may be collected in some cases if clinically indicated and available, e.g. from explant following transplantation. It is important to store samples appropriately for future testing and investigations. Any specimens collected for research purposes should take local and national ethical consideration and guidelines into account, including informed consent.

Information on collection and storage of specimens can be found in **Table 1**.

Table 1 Specimen collection and storage for diagnostic testing or storage for future investigation

Specimen type	Collection materials	Recommended temperature for storage and shipment
Whole blood ¹	Collection tube with EDTA	2-8 °C if ≤ 3 days –20 °C > 3 days
Serum	Serum-separator or dry tubes	2-8 °C if ≤ 7 days –20 °C > 7 days
Plasma	Collection tube with EDTA	2-8 °C if ≤ 3 days –20 °C > 3 days
Respiratory specimens ²	Dacron or polyester flocked swabs with virus transport medium (VTM)	2-8 °C if ≤ 3 days –20 °C > 3 days
Stool/rectal swab	Collection pot/dacron or polyester flocked swabs with Cary Blair transport media	2-8 °C if ≤ 3 days –20 °C > 3 days
Urine	Sterile container	2-8 °C if ≤ 3 days –20 °C > 3 days
Liver tissue ³	VTM, saline or 10% buffered formaldehyde (for histopathology, at room temperature)	2-8 °C if ≤ 3 days –20 °C > 3 days

¹ Whole blood is preferred but do not freeze whole blood for PCR

² May include throat swab, nasal swab, nasopharyngeal swab, sputum or bronchioalveolar lavage (BAL)

³ If clinically indicated or if tissue from liver explant is available

Specimens stored for future testing should be kept at –80 °C, where possible.

Any positive specimen should be stored for future testing and/or follow-up investigation.

Specimen shipment

Transport of specimens within national borders should comply with applicable national regulations. International transport of specimens should follow applicable international regulations as described in the [WHO guidance on regulations for the transport of infectious substances 2021 – 2022](#) (applicable as from 1 January 2021).

Considerations for testing strategy

The current working case definition is exclusive, rather than inclusive, while the aetiology(ies) of this syndrome remains unknown. It requires that only two criteria are met for a case to be reported – the exclusion of viral hepatitis A, B, C, and E as underlying causes, and a measure of severity of hepatitis – AST or ALT testing. However, it is recognized that there are other well-recognized causes of severe acute hepatitis in children that should be prioritized as part of the ongoing clinical work-up of cases. Therefore, it is recommended that the initial diagnostic tests described below are prioritized to ensure that any possible case meets the case definition. Once a probable case is identified, an additional set of investigative diagnostics could be considered. The relevance and feasibility of the set of investigative tests will vary by region and country capacity, and as the investigations progress. Further, different tests may be available at different tiers of the national health system as well as in different countries and regions.

Due to the exhaustive and comprehensive nature of the investigative diagnostics and particularly where there are laboratory capacity limitations or resource constraints, facilities should prioritize collection and storage samples for future and/or referral testing.

- **Case definition diagnostics:** tests for pathogens described in the case definition, including hepatitis A, B, C and E, and liver function tests (AST/ALT).
- **Additional diagnostics:** to exclude other well-recognized causes of severe acute hepatitis in children, including environmental exposures (toxins, certain medications), metabolic hereditary conditions (such as Wilsons disease), or autoimmune disorders. Early consultation with a paediatric hepatologist is suggested to guide the diagnostic work-up for these conditions.

- **Investigative diagnostics:** tests, including comprehensive screening for viral and non-viral infections, toxicology, but also other special investigations such as metagenomics, that may support determination of the aetiology(ies) of the condition. (Table 3) There may be ethical considerations when carrying out testing for investigative or research purposes, rather than diagnostic/clinical purposes. National and regional contexts should be considered here, such as varying infectious disease pathogens.

Case definition diagnostics

As priority, specimens should be collected for the purpose of establishing that a case meets the probable case definition. The tests required for this are described in **Table 2** below, henceforth referred to as case definition diagnostics. These tests should be undertaken alongside testing for AST/ALT.

Table 2 Specimen collection and required testing for WHO case definition inclusion

Specimen type	Test	Pathogens
Whole blood or serum	Serology	Hepatitis A (IgM, IgG), B (Ag/Ab) ¹ , C (Ab), and E (IgM, IgG) viruses
	NAAT	Hepatitis A, C, and E viruses

¹ Hepatitis D virus should be tested only in the presence of a positive test for hepatitis B virus.

In bold is the primary relevant diagnostic required for each hepatitis virus.

NAAT: nucleic-acid amplification tests

In order to meet the case definition, viral hepatitis testing should be negative for each virus (A, B, C and E). In addition, as a measure of the severity of the acute hepatitis, AST/ALT tests should be carried out – with levels >500 IU/L to meet the case definition.

Investigative diagnostics

If the case definition diagnostic tests described above are negative, investigations can begin into differential, investigative diagnoses. At present, it is suggested to consider doing so in parallel, until further evidence is available. **Table 3** describes pathogens to test for by specimen type and laboratory technique. Additional specimen types and tests may be considered as investigations develop. Additional tests may be considered depending on pathogens that are endemic locally, such as *Plasmodium sp*, dengue virus, etc.

Table 3 Specimen collection and suggested investigative diagnostics

Specimen type	Test	Pathogens
Whole blood	NAAT	SARS-CoV-2, Adenovirus ¹ , enterovirus, CMV, EBV, HSV-1, HSV-2, HHV6, HHV7, VZV, parvovirus B19, <i>Leptospiriosis sp</i> ²
	Serology	CMV, EBV, SARS-CoV-2 (S&N proteins), VSV, HIV, adenovirus, parvovirus, rubella virus, Anti Streptolysin O titre, <i>Leptospiriosis sp</i> ²
	Culture	Standard culture for bacteria/fungi
	Toxicology	Local investigations according to medical history and geography
Throat swab (oro/nasopharyngeal)	NAAT	Respiratory virus panel (including adenovirus, bocavirus, enterovirus, influenza, parainfluenza, rhinovirus, RSV, SARS-CoV-2), <i>Mycoplasma</i> ³

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