# Hepatitis A Virus Testing and Resources

Starting in 2016 a multi-jurisdictional outbreak of hepatitis A virus (HAV) has been ongoing with several groups at highest risk, including persons using drugs, persons experiencing unstable housing or homelessness and men who have sex with men amongst others. As of July 2, 2021, over 41,000 cases have been reported from 35 states. Many state and local public health laboratories provide outbreak response support. This document details HAV resource information for our public health laboratories should their jurisdiction become involved now or as they engage in future preparedness.

For information on Hepatitis A Disease please visit the CDC website. Other useful resources include:

- 2015 STD Treatment Guidelines
- The ABCs of Hepatitis
- The Pink Book-Epidemiology and Prevention of Vaccine Preventable Diseases
- Hepatitis A serology
- Laboratory Markers of HAV (video, slide)

For information about the status of this outbreak, the CDC has created a specific site with relevant information that is regularly updated.

## Virus Biology and Diagnosis

Hepatitis A virus is a small, non-enveloped virus in the family *Picornaviridae* and the genus *Hepatovirus*. It is a relatively small, linear, positive stranded RNA virus with only 7500 nucleotides. There is only one serotype and six genotypes (I-VI).[1] Genotypes I, II and III infect humans and are further subdivided into subtypes A and B. HAV genotypes and subtypes have distinct geographic distribution. For further information about genotypes and the clinical implications, please refer to the CDC HAV FAQ.

Diagnosis of acute Hepatitis A infection is based on the combination of an appropriate clinical presentation and detection of anti-HAV IgM. Not all HAV IgM positive results indicate acute hepatitis A infection.[2,3] Therefore, repeat serologic testing or molecular testing to confirm presence of HAV RNA is suggested for clinical diagnosis. In the current outbreak, public health laboratories have reported challenges with discordance between the IgM positive samples and those that have detectable RNA. One public health laboratory has tentatively identified that vaccination status, storage time, and conditions and limit of detection could be impacting the discordance (IgM positive, no detectable RNA). CDC has reported that during the course of the current outbreak, 90-92% of IgM positive samples are RNA positive and can be amplified for sequencing, but each laboratory may have a different rate due to the patient population, initial IgM test performed and other items as outlined above such as vaccination status, storage time and conditions, and whether the test was performed in a person with a clinical presentation consistent with acute Hepatitis A infection.

Laboratory testing for acute hepatitis A is occurring mostly in commercial laboratories, although some public health laboratories may have or need to test for HAV IgM using a commercially available assay. The CDC does not routinely perform IgM testing and samples sent to the CDC need to have IgM results recorded before being sent. Please note that the CDC does not usually test specimens that have been collected more than 4 weeks after symptom onset, since the likelihood of recovering viral nucleic acid is very low. Specimen submission guidelines can be found here.

## Genotyping

Genotyping is used in the context of an outbreak situation to identify circulating strains and assess whether cases are linked (refer to CDC HAV FAQ for more details). The current CDC protocols for genotyping are based on the amplification and sequencing of the VP1-P2A junction region. When sequence variation within the VP1-P2A junction is used to define genotypes and subgenotypes, nucleotide sequence difference between genotypes is about 15 to 25%; within each sub-genotypes, they differ up to 7.5%.[4,5]

The conventional sequencing method, used for surveillance from 2007-2020, is a nested PCR followed by Sanger sequencing.[6] From 2007-2018, 2189 HAV IgM-positive samples were genotyped with I as the predominant genotype; ~0.5% were identified as genotype IIIA. In outbreak investigations (n=2495), genotype I was again the predominant genotype with 0.1% of genotype 3A also identified.

- A few public health laboratories involved in the current outbreak have evaluated real-time RT-PCR assays for HAV RNA detection. One method, a multiplex assay that distinguishes among several subgenotypes has been published. [7] Laboratories interested in learning more about this should contact Anne Gaynor.
- A control strain of HAV is available from BEI resources.

Over the course of the 2017-2020 HAV outbreak, CDC finalized the development of a next-generation sequencing based approach targeting the same VP1-P2A junction using a MiSeq platform and an online data analysis platform called *Global Hepatitis Outbreak Surveillance Technology* (GHOST) to help genotype and identify transmission links between cases. Ramachandran et. al. described the use of the technology along with the detection of previously uncommon HAV genotypes in the United States associated with the multistate HAV outbreaks between 2016-2019.[8]

- Jurisdictions that are experiencing outbreaks, but which are unable to perform testing themselves or at their state laboratory, may be able to submit samples to CDC for the next-generation sequencing protocol and GHOST analysis. The turnaround time is dependent on current volume at CDC. Please inquire upon shipping what the expected turnaround time will be from receipt of samples at the CDC Molecular Epidemiology Lab to returning results to your laboratory.
- State or local public laboratories with MiSeq access that are willing and able (ideally have had staff attend at least one GHOST Workshop or have demonstrated competency sequencing other pathogens on the MiSeq) can sequence the samples in-house and upload to the online GHOST portal. State or local laboratories performing testing are requested to send at least 10%

of their specimens (200ul) to CDC for quality control since the assay is relatively new.

- To obtain access to the GHOST protocols, GHOST user guide and access the portal please contact Suma Ramachandran (dcq6@cdc.gov) and copy Anne Gaynor (anne.gaynor@aphl.org) on your request.
  - Sequencing protocols available for review: Primer Preparation for NGS, NGS for HAV
- CDC is also able to provide a limited amount of controls and reagents (primers) to get laboratories started. When you request the protocols and other documents, the CDC will identify what other resources you may need to start.

## HAV Specimen Testing Requests

1. Read and follow instructions on the HAV Specimen Request Information including the "Dear Colleague Letter" from April 2019
2. If applicable review the Hepatitis Reference Laboratory Molecular/Serology Sample Handling and Shipping instructions

## References


1. Lemon SM, Ott JJ, Van Damme P, Shouval D. Type A viral hepatitis: A summary and update on the molecular virology, epidemiology, pathogenesis and prevention. J Hepatol. 2018;68: 167-84. Available at: https://doi.org/10.1016/j.jhep.2017.08.034
2. Alatoom A, Ansari Q, Cuthbert J. Multiple factors contribute to positive results for hepatitis A virus immunoglobulin M antibody. Arch Pathol Lab Med. 2013;137:90-95. Available at: https://doi.org/10.5858/arpa.2011-0693-oa
3. Landry ML. Immunoglobulin M for Acute Infection: True or False? Clin Vaccine Immunol. 2016;23(7):540-45. Available at: https://dx.doi.org/10.1128%2FCVI.00211-16
4. Robertson BH, Jansen RW, Khanna B, Totsuka A, Nainan OV, Siegl G, Widell A, Margolis HS, Isomura S, Ito K, et al. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. J Gen Virol. 119;73(Pt6):1365-77. Available at: https://doi.org/10.1099/0022-1317-73-6-1365
5. Vaughan G, Rossi LMG, Forbi JC, de Paula VS, Purdy MA, Xia G, Khudyakov Y. Hepatitis A virus: Host interactions, molecular epidemiology and evolution. Infect Genet Evol. 2014;21:227-43. Available at: https://doi.org/10.1016/j.meegid.2013.10.023
6. Nainan OV, Armstrong GL, Han XH, Williams I, Bell BP, Margolis HS. Hepatitis a molecular epidemiology in the United States, 1996-1997: sources of infection and implications of vaccination policy. J Infect Dis. 2005;191(6):957-63. Available at: https://doi.org/10.1086/427992
7. Probert WS, Gonzalez C, Espinosa A, Hacker JK. Molecular Genotyping of Hepatitis A Virus, California, USA, 2017–2018. Emerg Infect Dis. 2019:25(8):1594-96. Available at: https://doi.org/10.3201/eid2508.181489
8. Ramachandran S, Xia G, Dimitrova Z, Lin Y, Montgomery M, Augustine R, et al. Changing Molecular Epidemiology of Hepatitis A Virus Infection, United States, 1996–2019. Emerg Infect Dis. 2021;27(6):1742-1745. Available at: https://doi.org/10.3201/eid2706.203036

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