Hepatitis A Virus
Testing and Resources

As of March 2017, there has been a multi-jurisdictional outbreak of hepatitis A virus (HAV) among persons who are homeless and persons who abuse drugs. As of January 19, 2020, over 30,000 cases have been reported from 32 states. Several of our state and local public laboratories have been assisting states in the outbreak response. APHL has compiled information and resources that we would like to share with our public health laboratories to ensure everyone has access to the information and points of contact 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 to house relevant information that is updated every Monday.

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 distinctive geographic distribution. For further information about genotypes and the clinical implications, please refer to the CDC HAV QA.

Diagnosis of acute Hepatitis A virus is based on detection of IgM antibody. There are several commercially available HAV IgM Assays. No repeat serologic testing or molecular testing to confirm RNA is needed for the clinical diagnosis. In the current 2017-2020 outbreak public health laboratories have reported challenges with discordance between the IgM positive samples and those that have 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.

Most of the clinical diagnosis of HAV is occurring outside of the public health laboratories, although some public health laboratories may have or need to test for HAV using a commercially available IgM assay. The CDC does not routinely perform IgM testing. Samples sent to the CDC would need to have IgM results recorded before being sent to CDC. Please note that the CDC does not usually test specimens that have been collected more than 4 weeks after symptom onset as 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 gain a better understanding of the types of viruses that are circulating and 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%.\(^2\)\(^3\)

The conventional sequencing method is a nested PCR followed by Sanger sequencing.\(^4\) The method has been used for surveillance from 2007-2020. From 2007-2018, 2189 HAV isolates were genotyped with 1A/1B as the predominant genotype and ~0.5% identified as genotype 3A. In outbreak investigations (n=2495) genotype 1A/1B 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.\(^5\) 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 has 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.

- Jurisdictions that are experiencing outbreaks, but which are unable to perform testing themselves or at their state laboratory, can 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.

Shipping

1. Read and refer to CDC SOP for: Molecular/Serology Sample Handling and Shipping Instructions
2. Request HRL Shipping Manifest from hepaoutbreaklab@cdc.gov
3. Complete HRL Shipping Manifest, include a copy in the shipment and email a copy to Ryan Augustine (mvv7@cdc.gov) and (hepaoutbreaklab@cdc.gov)

4. Prior to shipment, please email hepaoutbreaklab@cdc.gov and request approval for specimen testing. Please provide a brief description and which criteria the specimen meets.

5. Ship only on Monday, Tuesday or Wednesday to CDC.
   a. Hepatitis Reference Laboratory
      Attn: Saleem Kamili
      MailStop A33
      Building 18, Room 3-218
      Centers for Disease Control
      1600 Clifton Road NE
      Atlanta, GA 30333

References


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