May 15, 2019

Dear Colleagues:

During the past three years, the United States has been experiencing widespread outbreaks of hepatitis A virus (HAV) infections among various at-risk populations. The Centers for Disease Control and Prevention, Division of Viral Hepatitis Laboratory has been conducting genetic characterization of circulating hepatitis A virus strains from clinical samples received from state health departments using in-house laboratory developed molecular methodologies. This letter is issued to inform our public health laboratory partners about an update in our testing methodologies and a new algorithm for testing specimens received in our laboratory. Background information is included to explain the rationale for the changes.

The standard HAV genetic assay, developed in our laboratory and based on amplification of the VP1-P2B genomic region of HAV, has been successfully used for investigating more than 90 hepatitis A outbreaks over the last several decades, leading to the identification of more than 11,000 unique HAV strains in the United States. The same assay applied to the current multiple state hepatitis A outbreak investigations detected HAV RNA in about 95% of samples that were IgM anti-HAV positive. Analysis of sequences obtained from these HAV RNA positive samples (a total of 4,245 samples from all states as of May 10, 2019) identified HAV genotype IB in 86.6% samples (n=3,677). Other genotypes identified included IA in 11.5% (n=487) and IIIA in 1.9% (n=81) of samples.

While the overall viremic rate of about 95% was observed in all samples across all states, we noticed an unusually low rate of HAV RNA positivity (~50%) in a sample set (n=36) received from MA using the VP1-P2B standard assay. Sequencing of the RNA positive samples revealed HAV genotype IIIA, indicating the presence of this genotype for the first time in this outbreak investigation. Due to the observed decreased amplification rate, another PCR methodology, that amplifies the 5’UTR region of the HAV genome, was applied to 14 of the 18 originally negative samples from MA, and 13 were found to be subsequently positive for HAV RNA.

When the low positivity rate for the original sample set was reported to MA, they requested our lab return the original sample set, along with additional submitted, but untested samples. The samples were returned to MA on December 13, 2018. MA conducted additional testing on their samples and based on their input, as well as the results of our 5’UTR testing, we optimized our standard VP1-P2B assay using PCR primers matching genotype IIIA. We have applied this modified protocol to stored RNA extracts
from 8 of the original 18 negative samples from MA and subsequently detected HAV genotype IIIA in all of them, further substantiating the performance of the modified assay.

Based on the lessons learned during this outbreak investigation and identification of an otherwise rare HAV genotype IIIA in MA and NH, CDC’s Hepatitis Laboratory started using the following algorithm effective 04/08/2019:

- All specimens are tested using the standard VP1-P2B assay.
  - Positive samples are sequenced and the results of the analysis are reported.
  - Negative specimens are retested using the modified Vp1-P2B assay.
    - Positive samples are sequenced and the results of the analysis are reported. Samples that test negative by both assays are reported as such.

Please see below the schema of the updated testing algorithm. Additionally, all specimens tested as part of this outbreak, that previously tested negative for HAV RNA, are being retested utilizing the updated algorithm, provided enough specimen remains.

Please do not hesitate to contact us with any questions.

Sincerely,

Carolyn Wester, MD, MPH     Saleem Kamili, PhD
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Division of Viral Hepatitis, CDC

Enclosures
Amplification of VP1-P2B region using universal primers

HAV RNA detected
  - Sequencing
    - HAV Genotype reported

HAV RNA not detected
  - Specimen reflexed to modified VP1-2B amplification protocol optimized to detect genotype IIIA
    - HAV RNA detected
      - Sequencing
        - HAV Genotype reported
    - HAV RNA not detected
      - Reported as HAV RNA negative

A = testing algorithm effective 4/8/2019