OCTOBER 11-12, 2024 MILAN, ITALY
DeltaCure 3rd International Meeting
P9
Poster for you

Validation of Streamlined Serodiagnosis of Hepatitis Delta Virus

Arghun N. Dashdorj, Kyongman An, Nomin Ariungerel, Sunguck Han, Su Ah Kim, Tae-Hoon Kim, Minsup Chung, Odgerel Oidovsambuu, Byambasuren Ochirsum, Sanjaasuren Enkhtaivan, Saruul Enkhjargal, Purevjargal Bat-Ulzii, Andreas Bungert, ND Dashdorj Onom, Nara Bungert Dashdorj, Nam-Joon Cho

Introduction/Summary

- Hepatitis Delta Virus (HDV), the most severe form of viral hepatitis in humans, exacerbates the liver disease caused by the Hepatitis B Virus (HBV).
- Despite an estimated global prevalence of 12 million, HDV infections often go underdiagnosed due to limited resources for testing and training[1]. However, global estimates are vague due to the lack of widespread testing.
- Commercial RDTs for detection of anti-HDV are not available yet.
- Mongolia is a global hot spot for HDV with an estimate prevalence of HDV of 6.5% among adult population[2].
- The Liver Center in Ulaanbaatar, Mongolia is a non-profit clinic specializes in liver disease with over 4000 registered HDV patients.
- We have conducted the first independent validation study on the newly developed anti-HDV Ab RDT.

Study Design

- This study was approved by the Independent Ethics Committee at the Ministry of Health, Mongolia.
- The samples used for validation were randomly selected Group A (HBsAg+/anti-HDV Ab+), Group B (HBsAg+/anti-HDV Ab−), and Group C (HBsAg−/anti-HCV Ab−) samples.
- Samples were randomly selected from the blood bank at the Liver Center.
- Reference ELISA and HDV-RNA tests were performed prior to the study using the Sysmex HISCL-5000 and an in-house developed RT-PCR, respectively. Reference anti-HDV tests were conducted using the Wantai anti-HDV Ab CLEIA.
- Healthy volunteers had only been tested for HBsAg. HBsAg positives had been tested for anti-HDV. Only anti-HDV positives had also been tested for HDV-RNA.
- The diagnostic accuracy of the anti-HDV Ab RDT was evaluated by calculating its sensitivity and specificity compared to the standard results of RT-PCR and/or ELISA/CLEIA.
- 95% confidence intervals were calculated using the Wilson score interval method.

Methods

- The total population of 1007 encompasses 548 HDV-RNA or anti-HDV Ab positives (Group A), 138 HDV-RNA negative but HBsAg positives (Group B), and 321 healthy (Group C) participants.
- 702 samples were randomized prior to testing and 305 were not.
- For the randomized samples a blinded testing protocol was utilized
- The anti-HDV Ab RDT was performed according to the instructions provided by LUCA AICell.
- Results were examined by 3 people after 15 minutes, and by 1 person after 20 and 25 minutes.
- Results were recorded by photo at all minute marks (**Figure 1**).

Results for Non-randomized Cohort

- In the non-randomized cohort, 305 samples were included.
- Of these samples, 161 (52.8%) were male and 144 (47.2%) were female. The age range was between 5-78 and the median age was 44.
- The cohort contained 189 Group A, 46 Group B, and 70 Group C samples.
- The test results yielded 189 true positive, 115 true negative, one false positive, and no false negative results (**Table 1**).
- The anti-HDV Ab RDT demonstrated a sensitivity of 100% (95% CI: 98-100%), a specificity of 99.1% (95% CI: 95.3-99.9%), and an accuracy of 99.7%.

Table 1 **anti-HDV Ab RDT tests results for non-randomized samples**

	RT-PCR or ELISA Positive	RT-PCR or ELISA Negative	Total
anti-HDV Ab RDT Positive	189	1	190
anti-HDV Ab RDT Negative	0	115	115
Total	189	116	305

Results for Randomized Cohort

- The randomized cohort consisted of 702 samples.
- 377 (53.7%) females and 325 (46.3%) males with a median age of 48 and an age range between 20-78 were included.
- This cohort included 359 Group A, 92 Group B, and 251 Group C samples.
- After testing with the anti-HDV Ab RDT, there were 358 true positives, 330 true negatives, 13 false positives, and only one false negative (**Table 2**).
- The sensitivity, specificity, and accuracy of the RDT were 99.7% (95% CI: 98.9-99.9%), 96.2% (95% CI: 93.7-97.8%), and 98%, respectively.

Table 2 **anti-HDV Ab RDT tests results for randomzied samples**

	RT-PCR or ELISA Positive	RT-PCR or ELISA Negative	Total
anti-HDV Ab RDT Positive	358	13	371
anti-HDV Ab RDT Negative	1	330	331
Total	359	343	702

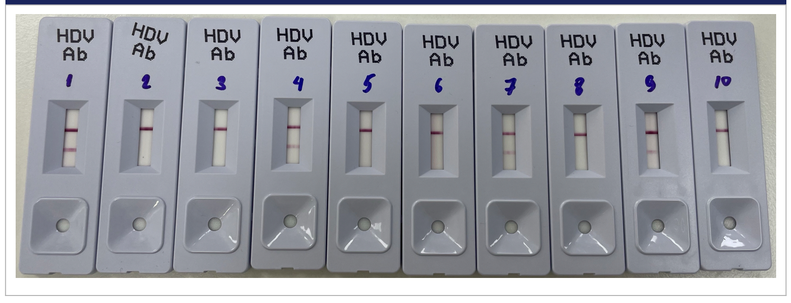
Results for Total Samples

- In total, the study included 1007 samples.
- 488 males (48.5%) and 519 females (51.5%), with an age range from 5 to 78 years and a median age of 47 were included.
- This cohort included 548 Group A, 138 Group B, and 321 Group C samples.
- After testing with the anti-HDV Ab RDT, there were 547 true positives, 445 true negatives, 1 false negative, and 14 false positives (**Table 3**).
- The anti-HDV Ab RDT exhibited an impressive sensitivity of 99.8% (95% CI: 98.9-99.9%), a specificity of 96.9% (95% CI: 94.9-98.1%), and an overall accuracy of 98.5%.
- No significant correlations were observed between the false results and variables such as age, gender, RT-PCR, or CLEIA results.
- The visual clarity of the test lines did not increase over 20 and 25 minutes.

Table 3 **anti-HDV Ab RDT tests results for total samples**

	RT-PCR or ELISA Positive	RT-PCR or ELISA Negative	Total
anti-HDV Ab RDT Positive	547	14	561
anti-HDV Ab RDT Negative	1	445	446
Total	548	459	1007

Figure 1 **The rapid test results for randomized samples after 15 min of incubation.**



Conclusion

- The anti-HDV Ab RDT represents a significant advancement in viral hepatitis diagnostics.
- Its high sensitivity, specificity, and ease of use make it an invaluable tool for both individual patient care and broader public health initiatives.
- By facilitating early and accurate diagnosis, the anti-HDV Ab RDT not only improves clinical outcomes for patients but also contributes to the global effort to control and eventually eliminate HDV.
- Further research is needed to validate the anti-HDV Ab RDT's performance across different HDV genotypes. Given the geographical variability in HDV genotype prevalence[3], a comprehensive validation is essential for the anti-HDV Ab RDT's global application.

Reference

1. Stockdale AJ, Kreuels B, Henrion MYR, et al. The global prevalence of hepatitis D virus infection: Systematic review and meta-analysis. J Hepatol 2020; 73: 523–32.
2. Chen X, Oidovsambuu O, Liu P, et al. A novel quantitative microarray antibody capture assay identifies an extremely high hepatitis delta virus prevalence among hepatitis B virus–infected mongolians. Hepatology 2017; 66. DOI:10.1002/hep.28957.
3. Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. Lancet 2011;378:73-85.