Applied Materials Today 44 (2025) 102683

Contents lists available at ScienceDirect

Applied Materials Today

journal homepage: www.elsevier.com/locate/apmt

Development and validation of streamlined serodiagnosis of hepatitis delta virus

Arghun N. Dashdorj [a,b,1], Kyongman An [c,d,1], Nomin Ariungerel [b,e], Sunguck Han [c], Su Ah Kim [f], Tae-Hoon Kim [c], Minsup Chung [f], Odgerel Oidovsambuu [b,e], Byambasuren Ochirsum [b], Sanjaasuren Enkhtaivan [b], Saruul Enkhjargal [b,e], Purevjargal Bat-Ulzii [b], Andreas Bungert [a,b], N.D. Dashdorj Onom [a,b], Nara Bungert Dashdorj [a,b,*,**], Nam-Joon Cho [g,***]

[a] Onom Institute, Princeton, NJ 08540, United States
[b] The Liver Center, Onom Institute, Ulaanbaatar 14230, Mongolia
[c] Department of Research and Development, LUCA AICell Inc., Anyang 14055, Republic of Korea
[d] Department of Industrial AI Engineering, Graduate School of Management of Technology, Hoseo University, Asan, 31499, Republic of Korea
[e] Laboratory of Genetic Engineering, NUM, Ulaanbaatar 14201, Mongolia
[f] Department of Chemical Engineering, Hongik University, Mapo-gu, Seoul 04066, Republic of Korea
[g] School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue 639798, Singapore

ARTICLE INFO

Keywords:
Hepatitis delta virus
Anti-HDV Ab
Rapid diagnostic test
Lateral flow assay, Lipid coating

ABSTRACT

Hepatitis Delta virus (HDV), the most severe form of viral hepatitis in humans, exacerbates the liver disease caused by hepatitis B virus. Despite an estimated global prevalence of 12 – 20 million, HDV infections often go under-diagnosed due to insufficient training, testing, and funding. To address this challenge, we developed and validated anti-HDV antibody rapid diagnostics test (anti-HDV Ab RDT). The anti-HDV Ab RDT utilizes a defect-free bi-layer lipid membrane which enhances biofouling resistance, minimizing non-specific binding and significantly increasing the sensitivity of molecular detection. The total population of 1007 encompasses 548 HDV-RNA or anti-HDV Ab positives, 138 HDV-RNA negative but HBsAg positives, and 321 healthy participants. To increase the validity of the results, 702 samples were randomized prior to testing. For these samples, a blinded testing protocol was utilized. Results were recorded by photo at all minute marks. The anti-HDV Ab RDT demonstrated high performance with 547 true positives, 445 true negatives, 14 false positives, and 1 false negative sample, resulting in sensitivity of 99.8 % (95 % CI: 98.9–99.9 %), specificity of 96.9 % (95 % CI: 94.9–98.1 %), and an accuracy of 98.5 % when compared to reference tests. In conclusion, the anti-HDV Ab RDT emerges as an accessible, reliable tool for screening and diagnosis. Its high sensitivity and specificity make the anti-HDV Ab RDT an invaluable tool for both individual patient care and broader public health initiatives.

1. Introduction

Hepatitis D virus (HDV) represents a unique challenge in hepatology due to its dependency on hepatitis B virus (HBV) for replication and assembly [1–4]. Characterized by a circular, negative-sense single-stranded RNA genome, HDV requires the presence of HBV's envelope proteins to complete its lifecycle [3–7]. Consequently, HDV infections occur only as co-infections with HBV or as superinfections in individuals with pre-existing chronic HBV [2,3]. Despite being the most severe form of viral hepatitis in humans, HDV remains underdiagnosed and under-reported, with global prevalence estimates suggesting around 12 – 20 million infected people [4]. This figure likely underrepresents the true burden, as comprehensive screening for HDV is not widespread, especially in low-resource settings. Notably, Mongolia stands out as a

* Corresponding author at: Onom Institute, Princeton, NJ 08540, United States.
** Corresponding author at: The Liver Center, Onom Institute, Ulaanbaatar, 14230, Mongolia.
*** Corresponding author at: School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue 639798, Singapore.
E-mail addresses: nbd@onom.institute (N.B. Dashdorj), njcho@ntu.edu.sg (N.-J. Cho).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.apmt.2025.102683
Received 24 September 2024; Received in revised form 10 March 2025; Accepted 12 March 2025
Available online 22 March 2025

significant hotspot for both HBV and HDV, with prevalence rates among adults reported as 11.1 % and 6.5 %, respectively [8]. In fact, our earlier study showed that about 60 % of HBsAg-positive Mongols, approximately 125,000 – 150,000 people, are HDV-RNA positive or chronically infected with HDV [9]. Even Mongols living around the world are at a high risk of chronic HBV and HDV, as shown by a screening conducted among Mongols residing in Southern California [10]. The Liver Center in Ulaanbaatar, a center-of-excellence specializing in the diagnosis, treatment, and research of liver diseases, alone manages over 4000 HDV patients, highlighting the critical need for effective diagnostic tools in the country.

HDV exacerbates the clinical course of HBV infections, significantly increasing the risk of cirrhosis, acute liver failure, and hepatocellular carcinoma (HCC), thereby reducing life expectancy [2,11–13]. Accurate and timely diagnosis is thus paramount for effective patient management and controlling the spread of the virus. Current diagnostic approaches include serological assays for detecting HDV-specific antibodies and molecular techniques such as reverse transcription-polymerase chain reaction (RT-PCR) for quantifying HDV-RNA levels [14]. While RT-PCR is highly accurate, its dependence on advanced laboratory infrastructure and skilled personnel limits its use in resource-limited regions. Additionally, it only detects active HDV-RNA replication, meaning that patients with suppressed viral replication under treatment or in chronic stages of infection may yield false-negative results. Serological tests like ELISA, although commonly used, vary in sensitivity and specificity.

In response to these challenges, rapid diagnostic test (RDT) offers a valuable solution, offering quick, reliable, and easy-to-use diagnostics that do not require sophisticated equipment. Recent advancements in lateral flow assay (LFA) technology, which involves the migration of serum or plasma via capillary action across a test strip, interacting with antibodies labeled with colored particles [15,16], have enabled the development of RDTs capable of detecting anti-HDV antibodies (anti-HDV Ab) with high sensitivity and specificity. A pioneering study demonstrated that a RDT for anti-HDV Ab could achieve a sensitivity of 94.6 % and a specificity of 100 % in a cohort of 474 patients [17]. Despite these encouraging results, commercial RDTs for HDV are not widely available, creating a significant gap in the global diagnostic landscape.

In this study, we developed and conducted an independent validation of anti-HDV Ab RDT, designed to address the limitations of existing diagnostic methods. This novel RDT utilizes a recombinant small hepatitis Delta antigen (sHDVAg) as the target for antibody detection, ensuring broad applicability across HDV genotypes. In addition to the streamlined LFA platform, a defect-free bi-layer lipid coating technology was employed as an anti-fouling surface, effectively reducing non-specific binding events that often compromise assay sensitivity [18,19]. We used ELISA as a comparative serodiagnostic method due to its established clinical use, commercial availability, and reproducibility [9,17] compared to other available methods, including imaging-based and fluorescence-based techniques [20,21]. Altogether, our study presents a comprehensive evaluation of the anti-HDV Ab RDT, including its development, performance validation, and clinical utility. Through a robust analysis of over 1000 patient samples that include randomized cohort, we aim to establish the RDT's diagnostic accuracy, sensitivity, and specificity in detecting anti-HDV antibodies, as well as its potential role in integrating HDV screening into existing HBV diagnostic programs. The outcomes of this study not only fill a critical gap in HDV diagnostics but also provide essential insights for improving disease surveillance and management in endemic regions such as Mongolia.

2. Materials and methods

2.1. Development of recombinant sHDVAg and anti-HDV Ab monoclonal antibodies

The optimized antigen sequence selected for the RDT test line was chosen through a comprehensive sequence alignment and antigenicity comparison of small hepatitis delta virus antigen (sHDVAg) sequences across all recognized HDV genotypes. Ultimately, the sequence corresponding to GenBank ID ADI24879.1 was utilized (Fig. 1**A**). The recombinant sHDVAg protein was expressed using the pGEX-4T-1 vector (GE Healthcare, CAT# 28954549), which includes a Glutathione S-transferase (GST) tag, in an Escherichia coli bacterial expression system. Protein expression was induced with 0.5 mM isopropyl β-D-thiogalactoside (IPTG) (Sigma-Aldrich, CAT# I5502) when the optical density at 600 nm (OD600) reached 0.6, followed by incubation for 6 h at 37 °C. Post-induction, the cells were harvested by centrifugation at 15,000 × g for 15 min, and the supernatant was discarded. The resulting cell pellet was resuspended in cold phosphate-buffered saline (PBS) containing BugBuster Protein Extraction Reagent (Merck Millipore, CAT# 70584–4) and Complete Protease Inhibitor Cocktail (Roche, CAT# 11836170001). Cell lysis was performed via sonication, followed by an additional centrifugation step at 15,000 × g for 15 min to clear the lysate. The supernatant, containing the soluble fraction, was subjected to affinity chromatography using a GSTrap HP column (Cytiva, CAT# 17-5281-01) to purify the recombinant sHDVAg. Eluted protein fractions were pooled and subjected to buffer exchange using a dialysis bag (Sigma-Aldrich, CAT# D9402) and then concentrated to the required levels using Amicon Ultra-15 Centrifugal Filter Units (Merck Millipore, CAT# UFC901024) for subsequent use in the development of the anti-HDV Ab RDT kit. The purity of the eluted sHDVAg was confirmed by SDS-PAGE, followed by Coomassie blue staining, using a standard protein size marker to assess the molecular weight and purity (Fig. 1**B**).

Purified recombinant sHDVAg protein was used to immunize mice, eventually to generate a monoclonal antibody by GeneScript Biotech Korea. The purified antibodies were subsequently characterized for binding affinity and specificity using a customized protocol for ELISA. In detail, wells of a 96-well microtiter plate were coated with 1 μg/mL of the antigen in carbonate buffer and incubated at 37 °C for 1 hour. After washing with PBST, the wells were blocked with 2 % casein in PBS (Thermo Fisher Scientific, CAT# 37528) for 1 h at room temperature. Following another wash, serial dilutions of the primary antibodies were added and incubated for 1 h. After washing, a goat anti-mouse IgG Horseradish peroxidase conjugate (Thermo Fisher Scientific, CAT# 31430) at a 1:5000 dilution was added and incubated for 1 hour. The plate was developed with TMB Plus solution (Thermo Fisher Scientific, CAT# 34029) for 20 min, stopped with 1 M H2SO4, and absorbance was read at 450 nm.

Development and validation of the recombinant sHDVAg as depicted in Fig. 1 illustrates a comprehensive approach towards the creation of a pan-genotypic diagnostic tool for HDV. The gel reveals a predominant band corresponding to the expected molecular weight of sHDVAg, alongside minor bands that represent truncated isoforms in the total bacterial protein extract but sole single band of sHDVAg in the eluted protein from the GST-affinity chromatography, indicating a high level of purity in the purified antigen.

Immunogenicity and specificity of the sHDVAg were further validated using newly generated monoclonal antibodies, specifically 3G2 and 2D7. Antibodies exhibited a distinct, concentration-dependent binding to the antigen, with 3G2 demonstrating a higher binding affinity compared to 2D7 (Fig. 1**C**). Absorbance intensity, measured via ELISA [22], correlates with concentration of antibodies, confirming the robust interaction between the recombinant antigen and monoclonal antibodies. These findings validate the effectiveness of sHDVAg in binding to specific anti-HDV Ab, making it a viable component for the development of an RDT for anti-HDV Ab.

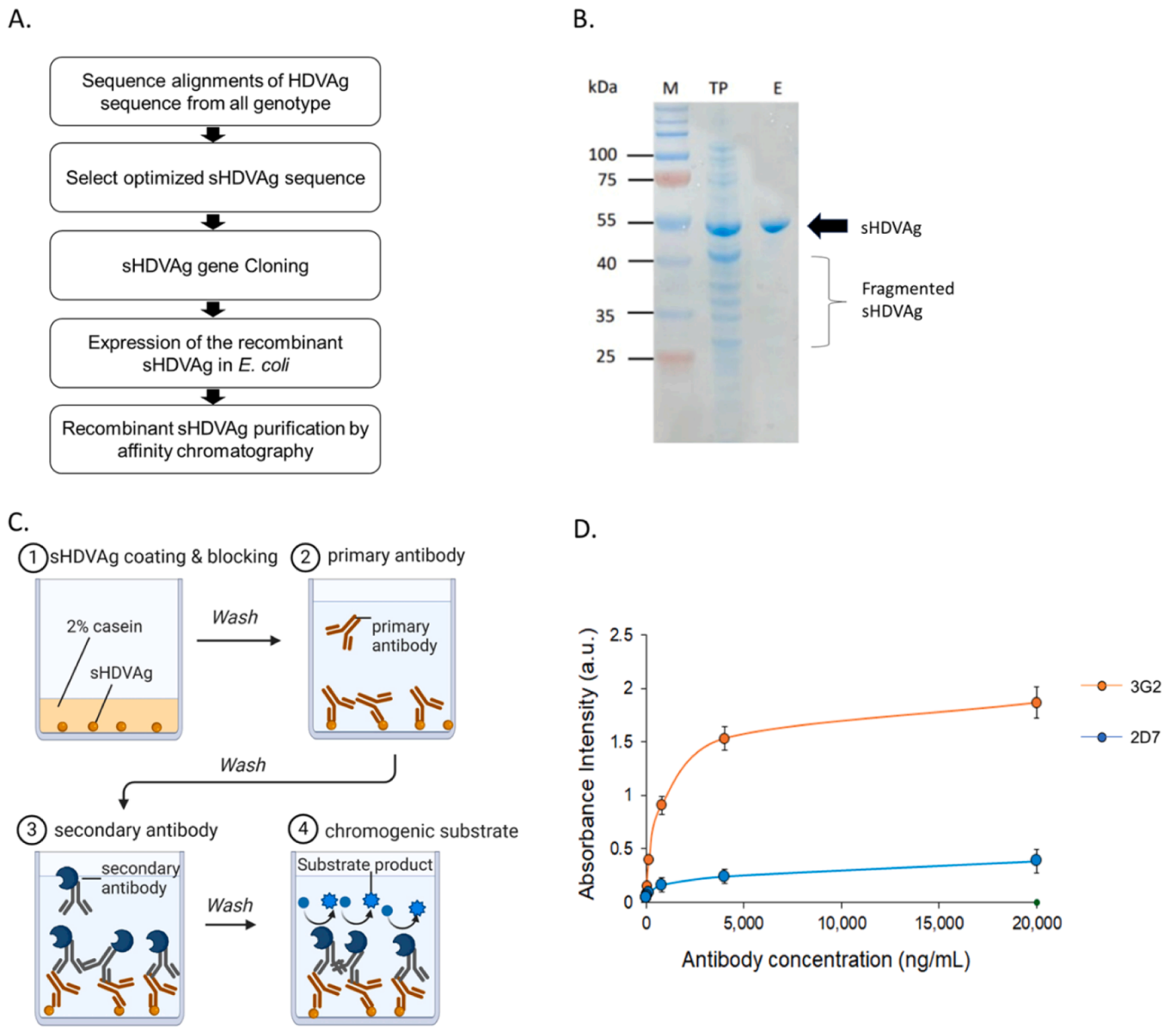

Fig. 1. Development of recombinant sHDVAg. (A) Schematic illustration for the process of recombinant sHDVAg preparation. (B) Coomassie staining image of purified proteins. M, TP, and E represent the size marker, total bacterial protein extract and eluted protein from the affinity chromatography. (C) A concise overview of the ELISA method to detect sHDVAg with its corresponding monoclonal antibodies, 3G2 or 2D7. (**D**) Validation of the purified antigen with newly generated anti-HDV Ab monoclonal antibodies (3G2, orange color; 2D7, blue color). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Development of anti-HDV Ab RDT

The anti-HDV Ab RDT was developed using recombinant sHDVAg by applying LFA principles. The schematic representation in Fig. 2**A** illustrates the LFA design for detecting anti-HDV Ab. The LFA consists of a sample pad, conjugate pad (Ahlstrom, CAT# 6615), reaction membrane (MDI Technologies), and absorbent pad. Conjugate pads were initially dried at 40 °C under low humidity conditions for 1.5 – 2 h. Post-drying, these pads were soaked in a solution containing gold-conjugated Protein A (Arista Biologicals, Inc., CAT# AGPTA-0101), sucrose (Sigma-Aldrich, CAT# S0389), trehalose dihydrate (Sigma-Aldrich, CAT# T0167) and a defect-free bi-layer lipid coating solution (LUCA AICell, One-Step blocker), which contains 1,2-dioleoylsn-glycero-3-phosphocholine (DOPC) phospholipid (Avanti Polar Lipids, 850375P)-based blocking solution [19]. Of note, unlike conventional methods that rely on BSA or other biomacromolecules, this approach integrates DOPC lipid molecules directly to form a stable, biofouling-resistant layer. The One-Step blocker is prepared by drying the DOPC in chloroform under a stream of nitrogen to form a thin film, which is further hydrated with PBS to a final concentration of 0.00063 nM. This ensures the elimination of non-specific interactions, thereby increasing the precision and sensitivity of molecular binding in the detection process [18,19,23]. The membrane was prepared by spotting it with a test line and a control line using a reel-to-reel dispenser (Shinhan FA, Korea). The test line was applied with 1.5 mg/mL recombinant sHDVAg and the control line with 1.0 mg/mL goat anti-chicken IgY (Arista Biologicals, Inc., CAT# ABGAC-0500) with an application rate of 0.8 μL/cm. For the detection of anti-HDV Ab, conjugate pads were treated with a mixture of gold-conjugated Protein A. The final assembly of the test strips involved aligning and attaching the absorbent pad, sample pad, conjugate pad, and membrane onto a backing card. These assembled components were then precisely cut into individual test strips. Each strip was encased in a

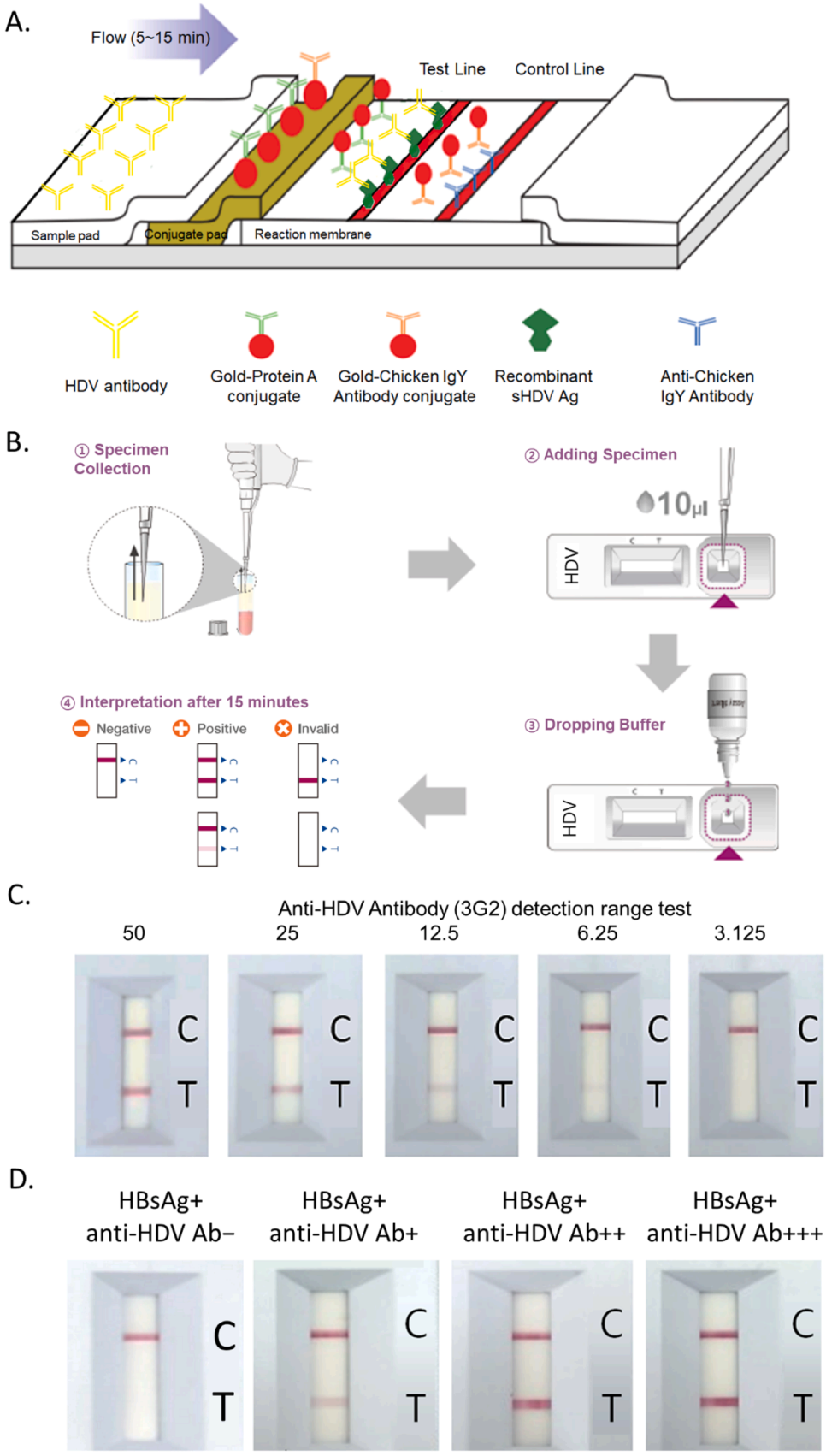

Fig. 2. Development of anti-HDV Ab RDT with recombinant sHDVAg. (A) Schematic representation of a lateral flow assay for detecting anti-HDV Ab in patient serum. (B) Testing protocol: Ten microliters of patient serum were used for the examination with the blocking buffer containing One-Step blocker. Results were interpreted after 15 min. (C) Anti-HDV Ab detection range test with newly generated anti-HDV monoclonal antibody, 3G2. (D) Validation of the anti-HDV Ab RDT using patient samples having different titers of anti-HDV Ab. C stands for control line and T stands for test line.

plastic cassette. The assembled test strips were packaged into aluminum pouches containing silica gel desiccants and were then sealed.

When a patient serum sample containing anti-HDV Ab antibodies is applied, these antibodies bind to a gold-conjugated goat anti-human IgG present in the conjugate pad, forming a complex that migrates by capillary action. A formation of a visible red test line indicates an existence of anti-HDV Ab in the serum, while the control line confirms the proper functioning of the assay. The detection range of the assay was validated using the anti-HDV Ab monoclonal antibody 3G2 (Fig. 2**C**). The assay detected antibodies in a concentration-dependent manner, with clear visibility of the test line down to the lowest tested concentration of 6.25 ng/mL although the captured image is faint. Still, these results indicate a high sensitivity of the RDT, capable of detecting even low levels of anti-HDV antibodies.

As a proof-of-concept study, samples with varying titers of anti-HDV antibodies were applied. The test successfully distinguished between HBsAg+/anti-HDV Ab− samples and HBsAg+/anti-HDV Ab+ samples with different anti-HDV Ab titers, as shown by the varying intensities of test lines (Fig. 2**D**).

2.3. Clinical study information

This study was approved by the Independent Ethics Committee at the Ministry of Health, Mongolia (N243). Serum preparation begins with blood collection using sterile equipment into a serum separator tube containing a clot activator. The blood is allowed to clot at room temperature (18–25 °C) for 30 – 60 min. After clotting, the sample is centrifuged at 1500 × g for 10 min at room temperature to separate the serum. The clear serum layer above the clot or gel barrier is carefully transferred to a clean, labeled container using a sterile pipette, avoiding contamination. The serum is frozen at −80 °C for long-term storage. All samples used for validation were randomly selected serum samples from the Blood Bank at the Liver Center. Additionally, all laboratory procedures including sample preparation, storage, and testing were performed at the Clinical and Molecular Diagnostics Laboratory of the Onom Institute. Informed consent forms were not obtained from participants as the study objectives were solely based on already available clinical samples.

2.4. Testing for anti-HDV Ab and HBsAg

Reference HBsAg and anti-HDV Ab tests were performed before the study on serum samples using the Sysmex HISCL-5000 (Chemiluminescence Enzyme Immunoassay) and Wantai HDV-IgG ELISA, respectively. Healthy patients were only tested for HBsAg and anti-HCV Ab.

The HISCL-5000 fully automated immunoassay system (Sysmex Corporation, Japan) was used to test for HBsAg. Levels of HBsAg were expressed in international units per milliliter (IU/mL). Values of HBsAgv < 0.03 IU/mL were considered negative, and values of ≥ 0.03 IU/mL were considered positive. According to the manufacturer's instructions, levels of HBsAg 0.03 IU/mL through 5 IU/mL were rerun on the HISCL-5000 in duplicate to confirm the test results. HBsAg positive samples were tested for anti-HDV Ab. An HDV-IgG ELISA (Wantai, PRC) was used to detect anti-HDV Ab. The Wantai HDV-IgG kit was used due to its high levels of sensitivity and specificity, was readily available and used in daily clinical practice.

2.5. Testing for HDV-RNA

Reference RT-PCR tests were performed before the study. An in-house developed RT-PCR was used to test for HDV-RNA. RT-PCRs were used for quantitative measurements of HDV-RNA, which has a reported lower limit of quantification (LLOQ) of 50 IU/mL. GeneXpert (Cepheid, CA, USA) was used for quantitative measurements of HBV-DNA (LLOQ of 6 IU/mL).

2.6. Testing with the anti-HDV Ab RDT

The randomized samples were mixed and given a random number between 0 and 705. The tests were then conducted in groups of 10.

As introduced in the Fig. 2**B**, 10 μl of serum was collected using a micropipette. Holding the micropipette perpendicular to the testing device, collected serum was added to specimen well. Then the buffer bottle was held perpendicular to the testing device and without contact 3 drops (100 ~ 120 μl) were added into the specimen well. The results were then examined by 3 people after 15 min, and by 1 person after 20 and 25 min. Results were then recorded by photo at all minute marks. The test conductor and examiners did not know the patients' clinical information or reference test results.

3 samples from the randomized cohort were duplicates, so they were removed. For 16 samples the examiners had a difference in votes for the result and 9 samples had extremely faint test lines. These samples were thus retested.

2.7. Statistical analysis

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.

$$Sensitivity = \frac{\text{True Positives (TP)}}{\text{True Positives (TP)} + \text{False Negatives (FN)}} \text{ X } 100\ \%$$

$$Specificity = \frac{\text{True Negatives (TN)}}{\text{True Negatives (TN)} + \text{False Positives (FP)}} \text{ X } 100\ \%$$

$$Accuracy = \frac{\text{True Positives (TP)} + \text{True Negatives (TN)}}{\text{Total Samples (TP + TN + FP + FN)}} \text{ X } 100\ \%$$

95 % confidence intervals (CI) were calculated using the Wilson score interval method.

3. Results and discussion

3.1. Experimental design

The study utilized 1007 samples, included and categorized based on HBV and HDV infection statuses. Samples were grouped into three distinct groups: Group A (HBsAg+/anti-HDV Ab+), Group B (HBsAg+/anti-HDV Ab−), and Group C (HBsAg−/anti-HCV Ab−). This classification enabled a thorough evaluation of sensitivity and specificity of the anti-HDV Ab RDT across different patient profiles. To eliminate potential biases, the study incorporated both randomized and non-randomized cohorts (Fig. 3).

3.2. Validation of the anti-HDV Ab RDT

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 (Table 1**A**). This cohort included 235 HBV positive samples, confirmed by RT-PCR or HBsAg CLEIA. Within this group, 189 samples were either anti-HDV Ab or HDV-RNA positive and were thus categorized as Group A (Table 1**B**). Of these samples, 186 were HDV-RNA positive by RT-PCR, one was HDV-RNA negative, and 2 had unknown HDV-RNA status but were anti-HDV Ab positive. Furthermore, the cohort contained 46 HBsAg positive but anti-HDV Ab negative (Group B) and 70 HBsAg and anti-HCV Ab negative (Group C) samples (Table 1**B**). The test results yielded 189 true positive and 115 true negative results (Table 1**C**). Only one false positive result was observed, and no false negatives were detected. 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 overall accuracy of

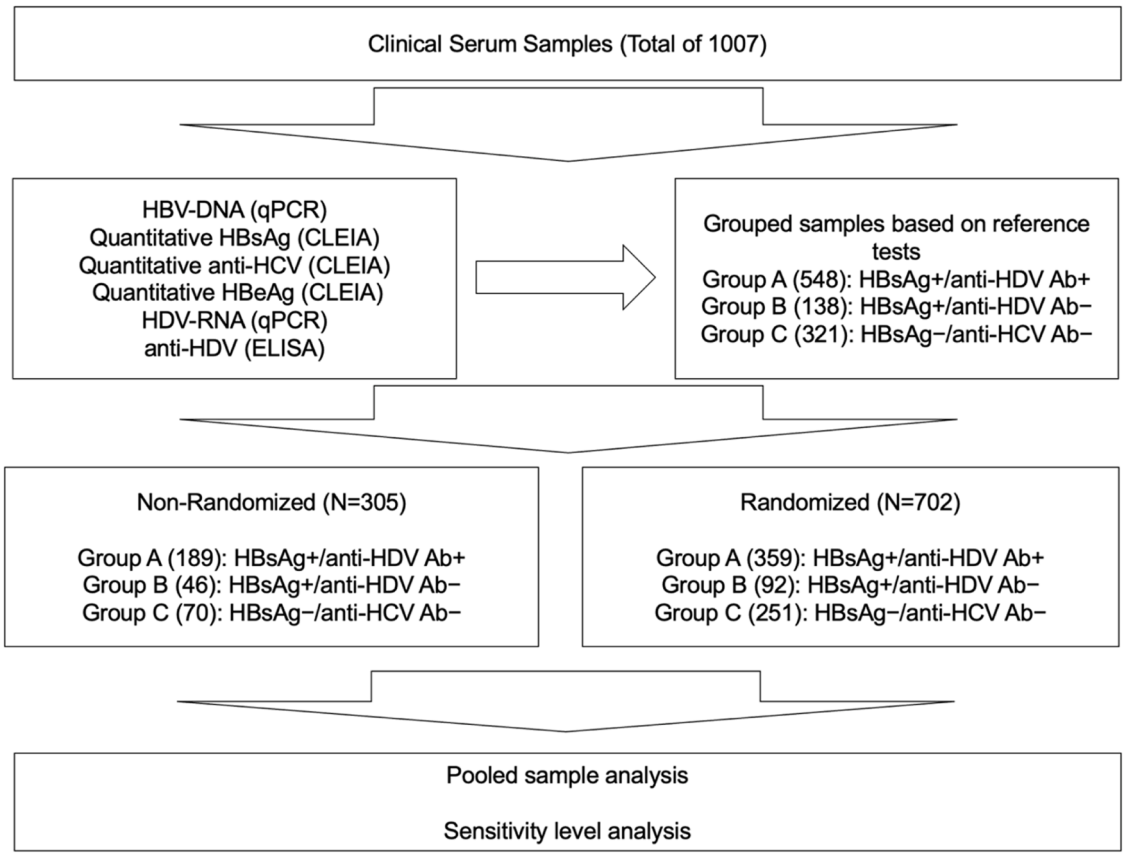

Fig. 3. Schematic illustration of the strategic planning for the validation of the anti-HDV Ab RDT.

Table 1
(A) Demographics for non-randomized cohort (Total N = 305). (B) Categorization of non-randomized samples based on their infection history of hepatitis B/D virus. (C) Analysis of anti-HDV Ab RDT results for the non-randomized cohort.

A. Age Group			
	N (%)	**Male**	**Female**
≤20	4 (1.3 %)	3	1
21–30	12 (3.9 %)	7	5
31–40	105 (34.4 %)	63	42
41–50	94 (30.8 %)	58	36
51–60	61 (20 %)	22	39
61–70	22 (7.2 %)	6	16
70+	7 (2.3 %)	2	5
Total	305	161	144

B. Hepatitis B/D Viral Infection			
	Total N = 305		
HBV infection (RT-PCR or HBsAg)	HBsAg+ and HBV-DNA+ (N = 235)	HBsAg− and HBV-DNA− (N = 70)	
HDV infection	Group A HDV-RNA+ (N = 189)	Group B HDV-RNA− (N = 46)	Group C HDV-RNA− (N = 70)

C. Anti-HDV Ab RDT Results			
	Anti-HDV Ab Positive	Anti-HDV Ab Negative (B + C)	Total
Positive	189	1	190
Negative	0	115	115
Total	189	116	305
Accuracy	99.7 %		

Table 2
(A) Demographics for randomized cohort (Total N = 702). (B) Categorization of randomized samples based on their infection history of hepatitis B/D virus. (C) Analysis of anti-HDV Ab RDT results for the randomized cohort.

A. Age Group			
	N (%)	**Male**	**Female**
≤20	3 (0.4 %)	3	0
21–30	28 (4.1 %)	13	15
31–40	155 (22.0 %)	84	71
41–50	219 (31.2 %)	96	123
51–60	179 (25.5 %)	80	99
61–70	106 (15.1 %)	43	63
70+	12 (1.7 %)	7	5
Total	702	326	376

B. Hepatitis B/D Viral Infection			
	Total N = 702		
HBV infection (RT-PCR or HBsAg)	HBsAg+ and HBV-DNA+ (N = 451)	HBsAg− and HBV-DNA− (N = 251)	
HDV infection	Group A HDV-RNA+ (N = 359)	Group B HDV-RNA− (N = 92)	Group C HDV-RNA− (N = 251)

C. Anti-HDV Ab RDT Results			
	Anti-HDV Ab Positive	Anti-HDV Ab Negative (B + C)	Total
Positive	358	13	371
Negative	1	330	331
Total	359	343	702
Accuracy	98 %		

99.7 % (Table 4).

The randomized cohort consisted of 702 serum 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 (Table 2**A**). This cohort included 359 anti-HDV Ab or HDV-RNA positive (Group A), 92 HBsAg positive but anti-HDV Ab negative (Group B), and 251 HBsAg and anti-HCV Ab negative (Group C) samples. The distribution of samples is further broken down based on the results of HDV-RNA and anti-HDV Ab test results (Table 2**B**). Of the Group A samples, 313 were confirmed HDV-RNA positive by RT-PCR, 41 were HDV-RNA negative, and 5 samples had an unknown HDV-RNA status. All 359 samples in Group A tested positive for anti-HDV Ab using ELISA. 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**C**). 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 4).

In total, there are 488 males (48.5 %) and 519 females (51.5 %), with an age range from 5 to 78 years and a median age of 47 (Table 3**A**). In total, this cohort included 548 anti-HDV Ab or HDV-RNA positive (Group A), 138 HBsAg positive but anti-HDV Ab negative (Group B), and 321 HBsAg and anti-HCV Ab negative (Group C) samples (Table 3**B**). 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**C**). The anti-HDV Ab RDT exhibited an impressive sensitivity of 99.8 % (95 % CI: 98.9–99.9 %) and a specificity of 96.9 % (95 % CI: 94.9–98.1 %). The overall accuracy of the anti-HDV Ab RDT was calculated to be 98.5 % (Table 4). These metrics highlight the high efficacy of the anti-HDV Ab RDT in accurately detecting anti-HDV Ab. No significant correlations were observed between the false results and variables such as age, gender, RT-PCR, or CLEIA results. Moreover, the visual clarity of the test lines did not increase over 20 and 25 min.

3.3. Discussion

In this comprehensive study, we developed and assessed the efficacy of the anti-HDV Ab RDT, highlighting its impressive diagnostic performance in identifying anti-HDV Ab (sensitivity 99.8 %, specificity 96.9 %, and accuracy 98.5 %) across a robust validation cohort of 1007 patient samples. The anti-HDV Ab RDT provides a reliable, streamlined method for screening and diagnosis HDV, significantly improving early detection and management, especially in resource-limited settings. The anti-HDV Ab RDT's main advantage lies in its simplicity and quick results, without requiring advanced lab infrastructure. Unlike ELISA and RT-PCR, which need specialized equipment and expertise, the anti-HDV Ab RDT uses LFA technology, making it ideal for point-of-care testing. This is crucial in low- and middle-income countries (LMICs) where HDV prevalence is high and lab resources are scarce, offering a solution to improve HDV detection rates and address gaps in global hepatitis diagnostics. However, the anti-HDV Ab RDT did show a slightly lower specificity compared to gold-standard methods, which may be attributed to the visual interpretation of the test lines. This underscores the importance of further refining visual detection methods. The possibility of false positives could still be managed in clinical settings through confirmatory tests using molecular assays like RT-PCR, especially in cases where HDV diagnosis carries significant clinical implications.

Introduction of the anti-HDV Ab RDT holds significant promise for expanding HDV screening, especially in regions with limited access to comprehensive diagnostic facilities. This test can be seamlessly integrated into existing HBV screening programs, providing dual benefits by simultaneously detecting HBV and HDV superinfections. Furthermore, the development of a duplex test for both HBsAg and anti-HDV Ab would enable simultaneous screening for HBV and HDV infections. This would be particularly beneficial in endemic regions, facilitating the differentiation between HBV mono-infection and HBV/HDV superinfection.

New treatments and drugs for HDV are continuously emerging, offering hope for better disease management and control [24]. The anti-HDV Ab RDT can complement these therapeutic advancements by facilitating early diagnosis and monitoring, thereby contributing to the global effort to control and potentially eliminate HDV. However, further research is necessary to validate the anti-HDV Ab RDT's performance across different HDV genotypes, as HDV genotype prevalence varies significantly by region [4]. Comprehensive validation is crucial to ensure the anti-HDV Ab RDT's global applicability. Another key challenge lies in making the anti-HDV Ab RDT affordable and accessible, especially in LMICs where HDV burden is the greatest. Efforts to reduce production costs and provide subsidies in these regions will be essential for maximizing the anti-HDV Ab RDT's public health impact.

Table 3
(A) Demographics for total samples (Total N = 1007). (B) Categorization of total samples based on their infection history of hepatitis B/D virus. (C) Analysis of anti-HDV Ab RDT results for total samples.

A. Age Group

	N (%)	Male	Female
≤20	7 (0.7 %)	6	1
21–30	40 (4 %)	20	20
31–40	260 (25.8 %)	147	113
41–50	313 (31.1 %)	154	159
51–60	240 (23.8 %)	102	138
61–70	128 (12.7 %)	49	79
70 +	19 (1.8 %)	9	10
Total	1007	487	520

B. Hepatitis B/D Viral Infection

	Total N = 1007		
HBV infection (RT-PCR or HBsAg)	HBsAg+ and HBV-DNA+ (N = 686)	HBsAg− and HBV-DNA− (N = 321)	
HDV infection	Group A HDV-RNA+ (N = 548)	Group B HDV-RNA− (N = 138)	Group C HDV-RNA− (N = 321)

C. Anti-HDV Ab RDT Results

	Anti-HDV Ab Positive	Anti-HDV Ab Negative (B + C)	Total
Positive	547	14	561
Negative	1	445	446
Total	548	459	1007
Accuracy	98.5 %		

Table 4
Sensitivity, specificity, and accuracy of anti-HDV Ab RDT for all cohorts.

Cohort	Sensitivity	Specificity	Accuracy
Non-randomized	100 % (95 % CI: 98–100 %)	99.1 % (95 % CI: 95.3–99.9 %)	99.7 %
Randomized	99.7 % (95 % CI: 98.4–99.9 %)	96.2 % (95 % CI: 93.7–97.8 %)	98 %
Total	99.8 % (95 % CI: 98.9–99.9 %)	96.9 % (95 % CI: 94.9–98.1 %)	98.5 %

4. Conclusion

In conclusion, the anti-HDV Ab RDT represents a significant advancement in viral hepatitis diagnostics. The anti-HDV Ab RDT's 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.

Credit authorship contribution statement

Arghun N. Dashdorj: Conceptualization, Methodology, Investigation, Writing – original draft, Writing- review & editing. **Kyongman An**: Conceptualization, Methodology, Investigation, Writing – original draft, Writing- review & editing. **Nomin Ariungerel**: Methodology, Investigation. **Sunguck Han**: Methodology, Investigation. **Su Ah Kim**: Methodology, Investigation. **Tae-Hoon Kim**: Methodology, Investigation. **Minsup Chung**: Methodology, Investigation. **Odgerel Oidovsambuu**: Investigation. **Byambasuren Ochirsum**: Methodology, Investigation. **Sanjaasuren Enkhtaivan**: Methodology, Investigation. **Saruul Enkhjargal**: Methodology, Investigation. **Purevjargal Bat-Ulzii**: Methodology, Investigation. **Andreas Bungert**: Writing- review & editing. **ND Dashdorj Onom**: Conceptualization, Writing- review & editing, Supervision. **Nara Bungert Dashdorj**: Conceptualization, Writing- review & editing, Supervision. **Nam-Joon Cho**: Conceptualization, Writing- review & editing, Supervision.

CRediT authorship contribution statement

Arghun N. Dashdorj: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Kyongman An:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Nomin Ariungerel:** Methodology, Investigation. **Sunguck Han:** Methodology, Investigation. **Su Ah Kim:** Methodology, Investigation. **Tae-Hoon Kim:** Methodology, Investigation. **Minsup Chung:** Methodology, Investigation. **Odgerel Oidovsambuu:** Investigation. **Byambasuren Ochirsum:** Methodology, Investigation. **Sanjaasuren Enkhtaivan:** Methodology, Investigation. **Saruul Enkhjargal:** Methodology, Investigation. **Purevjargal Bat-Ulzii:** Methodology, Investigation. **Andreas Bungert:** Writing – review & editing. **N.D. Dashdorj Onom:** Writing – review & editing, Supervision, Conceptualization. **Nara Bungert Dashdorj:** Writing – review & editing, Supervision, Conceptualization. **Nam-Joon Cho:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Nam Joon Cho reports financial support was provided by LUCA AICELL Inc. Nam Joon Cho reports a relationship with LUCA AICELL INC that includes: equity or stocks. Han Sunguck, An Kyongman, Kim Taehoon has patent pending to LUCA AICELL. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded and supported by the Onom Foundation, an operating foundation dedicated to the global eradication of viral hepatitis, and LUCA AICell, a biotechnology company that also contributed essential manufacturing resources. Additionally, this research received support from the Research Collaboration Agreement (RCA), utilizing lipid technology described in this manuscript, developed in collaboration with Nanyang Technological University and LUCA AICell.

Data availability

Data will be made available on request.

References

[1] A. Kos, R. Dijkema, A.C. Arnberg, P.H. van der Meide, H. Schellekens, The hepatitis delta (delta) virus possesses a circular RNA, Nature 323 (6088) (1986) 558–560.
[2] M. Rizzetto, Hepatitis D: thirty years after, J. Hepatol. 50 (5) (2009) 1043–1050.
[3] F. Negro, Hepatitis D virus coinfection and superinfection, Cold. Spring. Harb. Perspect. Med. 4 (11) (2014) a021550.
[4] S.A. Hughes, H. Wedemeyer, P.M. Harrison, Hepatitis delta virus, Lancet 378 (9785) (2011) 73–85.
[5] J.M. Taylor, Structure and replication of hepatitis delta virus RNA, Curr. Top. Microbiol. Immunol. 307 (2006) 1–23.
[6] C. Sureau, F. Negro, The hepatitis delta virus: replication and pathogenesis, J. Hepatol. 64 (1 Suppl) (2016) S102–S116.
[7] B. Pearlman, Hepatitis delta infection: a clinical review, Semin. Liver. Dis. 43 (3) (2023) 293–304.
[8] B. Dashtseren, A. Bungert, P. Bat-Ulzii, M. Enkhbat, O. Lkhagva-Ochir, G. Jargalsaikhan, A. Enkhbat, O. Oidovsambuu, J. Klemen, N. Dashdorj, N. Dashdorj, Z. Genden, D. Yagaanbuyant, Endemic prevalence of hepatitis B and C in Mongolia: a nationwide survey amongst Mongolian adults, J. Viral. Hepat. 24 (9) (2017) 759–767.
[9] X. Chen, O. Oidovsambuu, P. Liu, R. Grosely, M. Elazar, V.D. Winn, B. Fram, Z. Boa, H. Dai, B. Dashtseren, D. Yagaanbuyant, Z. Genden, N. Dashdorj, A. Bungert, N. Dashdorj, J.S. Glenn, A novel quantitative microarray antibody capture assay identifies an extremely high hepatitis delta virus prevalence among hepatitis B virus-infected mongolians, Hepatology 66 (6) (2017) 1739–1749.
[10] T.L. Fong, B.T. Lee, M. Chang, K. Nasanbayar, E. Tsogtoo, D. Boldbaatar, E. D. Dashdorj, N.E. Clifford, A.N. Dashdorj, B.R. Bang, T. Chida, C. Lim, M. Sugiyama, M. Mizokami, N.J. Dashdorj, P. Liu, J.S. Glenn, N.D. Dashdorj, T. Saito, High prevalence of chronic viral Hepatitis and liver fibrosis among mongols in Southern California, Dig. Dis. Sci. 66 (8) (2021) 2833–2839.
[11] G. Fattovich, G. Giustina, E. Christensen, M. Pantalena, I. Zagni, G. Realdi, S. W. Schalm, Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European concerted action on viral hepatitis (Eurohep), Gut 46 (3) (2000) 420–426.
[12] G. Fattovich, S. Boscaro, F. Noventa, E. Pornaro, D. Stenico, A. Alberti, A. Ruol, G. Realdi, Influence of hepatitis delta virus infection on progression to cirrhosis in chronic hepatitis type B, J. Infect. Dis. 155 (5) (1987) 931–935.
[13] R. Romeo, E. Del Ninno, M. Rumi, A. Russo, A. Sangiovanni, R. de Franchis, G. Ronchi, M. Colombo, A 28-year study of the course of hepatitis Delta infection: a risk factor for cirrhosis and hepatocellular carcinoma, Gastroenterology 136 (5) (2009) 1629–1638.
[14] C. Pan, R. Gish, I.M. Jacobson, K.Q. Hu, H. Wedemeyer, P. Martin, Diagnosis and management of hepatitis Delta virus infection, Dig. Dis. Sci. 68 (8) (2023) 3237–3248.
[15] B. O'Farrell, Evolution in lateral flow–Based immunoassay systems, in: R. Wong, H. Tse (Eds.), Lateral Flow Immunoassay, Humana Press, Totowa, NJ, 2009, pp. 1–33.
[16] C. Parolo, A. de la Escosura-Muniz, A. Merkoci, Enhanced lateral flow immunoassay using gold nanoparticles loaded with enzymes, Biosens. Bioelectron. 40 (1) (2013) 412–416.
[17] F.A. Lempp, I. Roggenbach, S. Nkongolo, V. Sakin, F. Schlund, P. Schnitzler, H. Wedemeyer, F.L. Gal, E. Gordien, C. Yurdaydin, S. Urban, A rapid point-of-care test for the serodiagnosis of Hepatitis Delta Virus infection, Viruses 13 (12) (2021).
[18] B.K. Yoon, T.N. Sut, K.Y. Yoo, S.H. Lee, Y. Hwang, J.A. Jackman, N.J. Cho, Lipid bilayer coatings for rapid enzyme-linked immunosorbent assay, Appl. Mater. Today 24 (2021) 101128.
[19] K. An, H. Tae, J. Shin, J. Bae, S. Han, G. Choi, T.-H. Kim, N.-J. Cho, Revolutionizing ELISA: a one-step blocking approach using lipid bilayer coatings, Appl. Mater. Today 40 (2024).
[20] Y. Zeng, K.M. Koo, M. Trau, A.G. Shen, J.M. Hu, Watching SERS glow for multiplex biomolecular analysis in the clinic: a review, Appl. Mater. Today 15 (2019) 431–444.
[21] L.S. Chen, L.Y. Sun, L. Ye, Y.P. Wang, Fluorescent sensor array for selective recognition of biothiols, Opt. Mater. 106 (2020).
[22] S.D. Gan, K.R. Patel, Enzyme immunoassay and enzyme-linked immunosorbent assay, J. Invest. Dermatol. 133 (9) (2013) e12.
[23] J.A. Jackman, N.J. Cho, Supported lipid bilayer formation: beyond vesicle fusion, Langmuir 36 (6) (2020) 1387–1400.
[24] A.A. Roca Suarez, E. Batbold, B. Bartosch, N. Dashdorj, B. Testoni, F. Zoulim, Emerging anti-HDV drugs and HBV cure strategies with anti-HDV activity, Liver. Int. 43 (Suppl 1) (2023) 87–95.