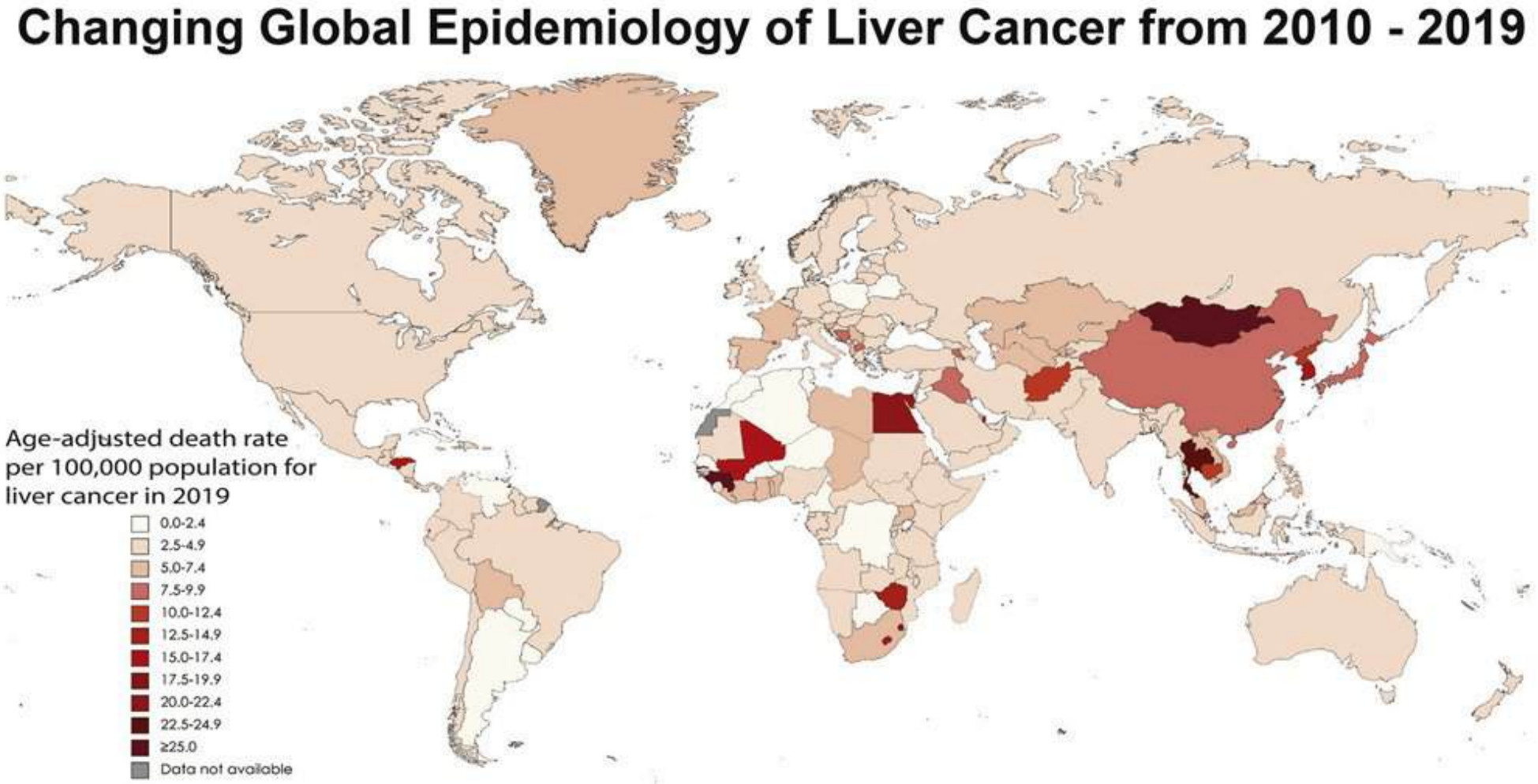
Screening and Diagnosis of Hepatitis Delta Virus

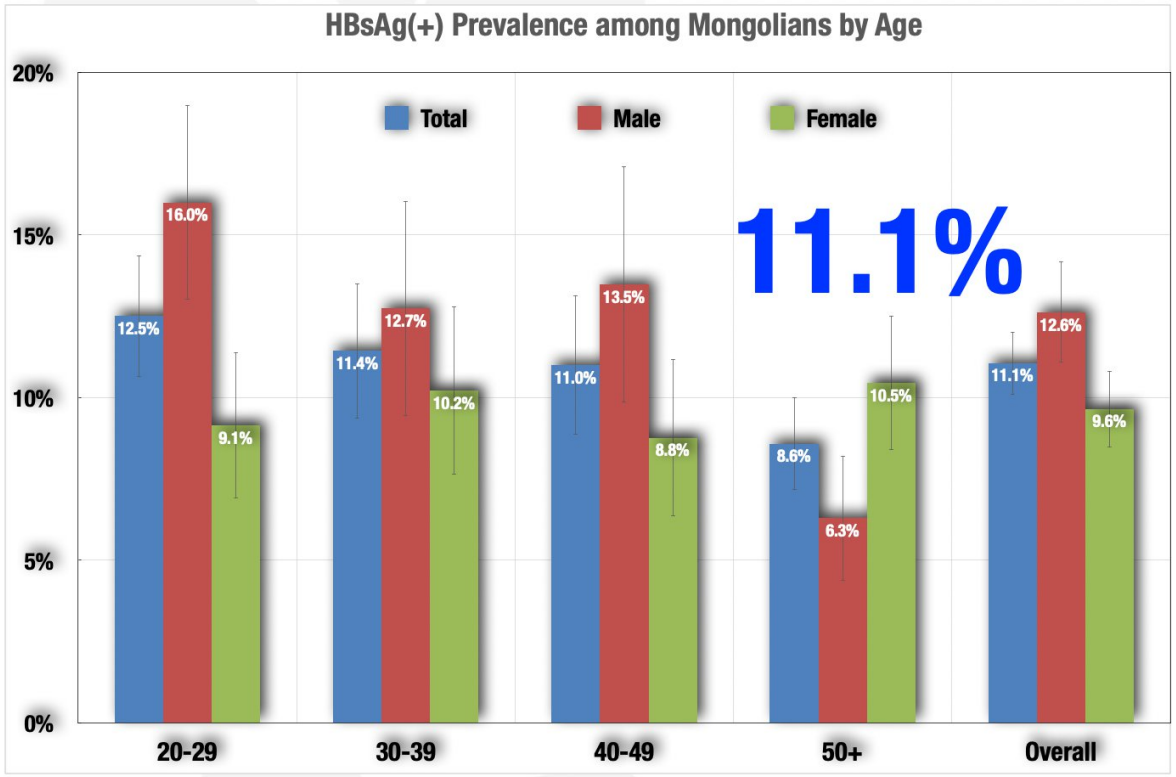
Naranjargal Dashdorj MD, Ph.D

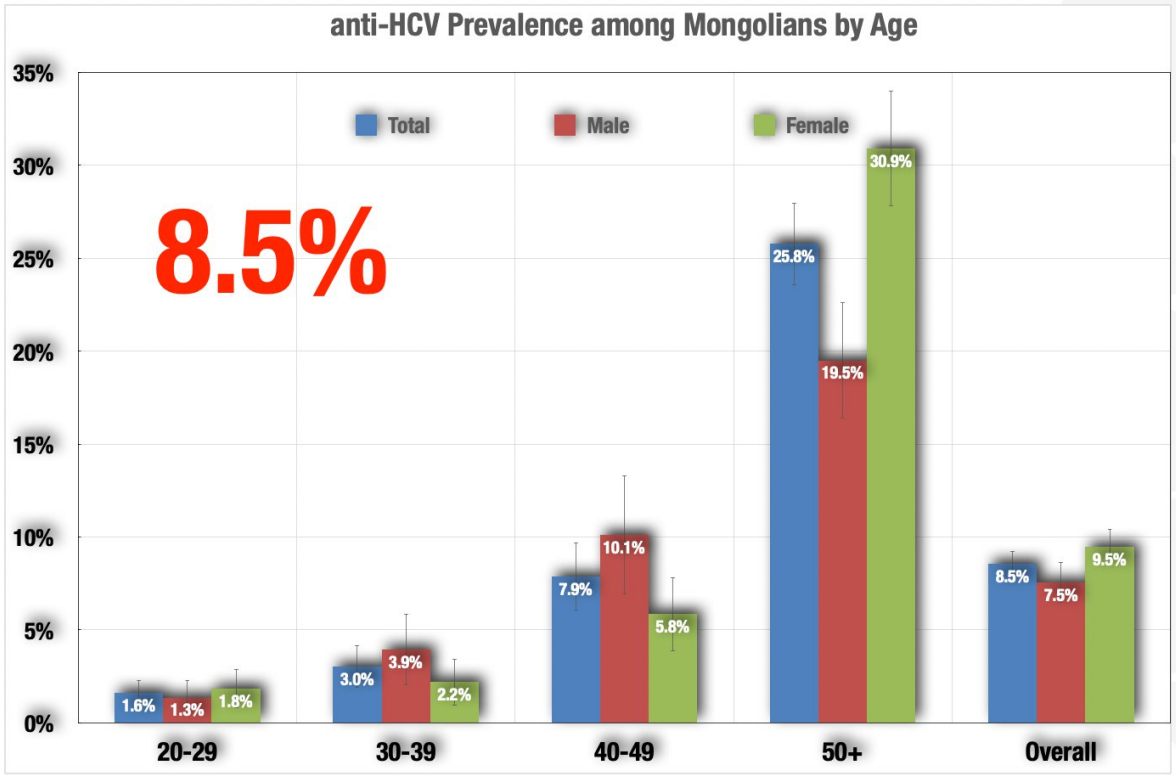
Liver disease and viral hepatitis burden in Mongolia



Daniel Q. Huang, el at. Cell Metabolism, Volume 34, Issue 7, 2022,

Liver disease and viral hepatitis burden in Mongolia



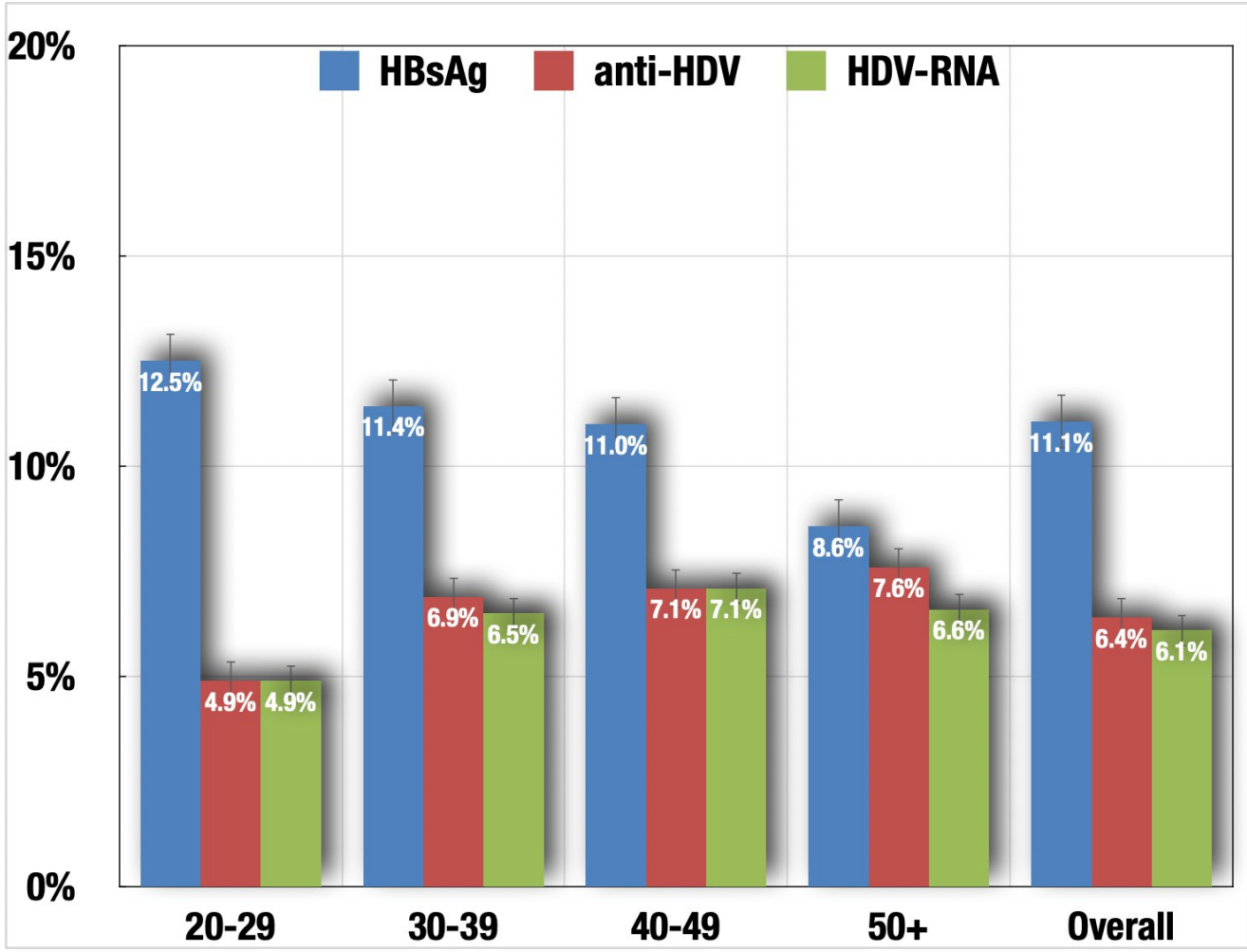


Dashtseren et al. Journal of Viral Hepatitis 2017 Feb 17.

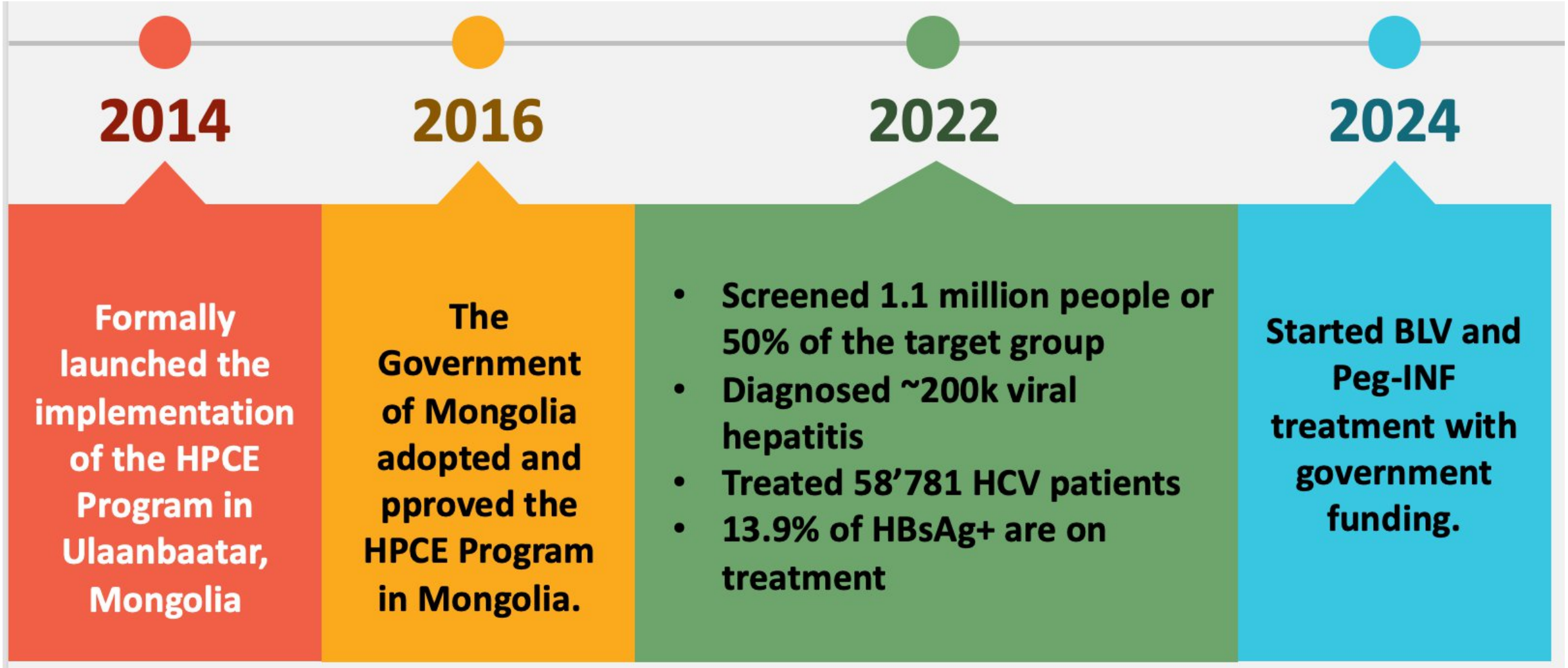
Liver disease and viral hepatitis burden in Mongolia

- Population of Mongolia ~3.5 million.
- ***anti-HCV prevalence*** - 11.1%
  - 150 thousand people
- ***HBsAg prevalence*** - 8.5%
  - 250 thousand (100 thousand mono HBV)
- ***anti-HDV prevalence*** - 60% of HBsAg+
  - 150 thousand people

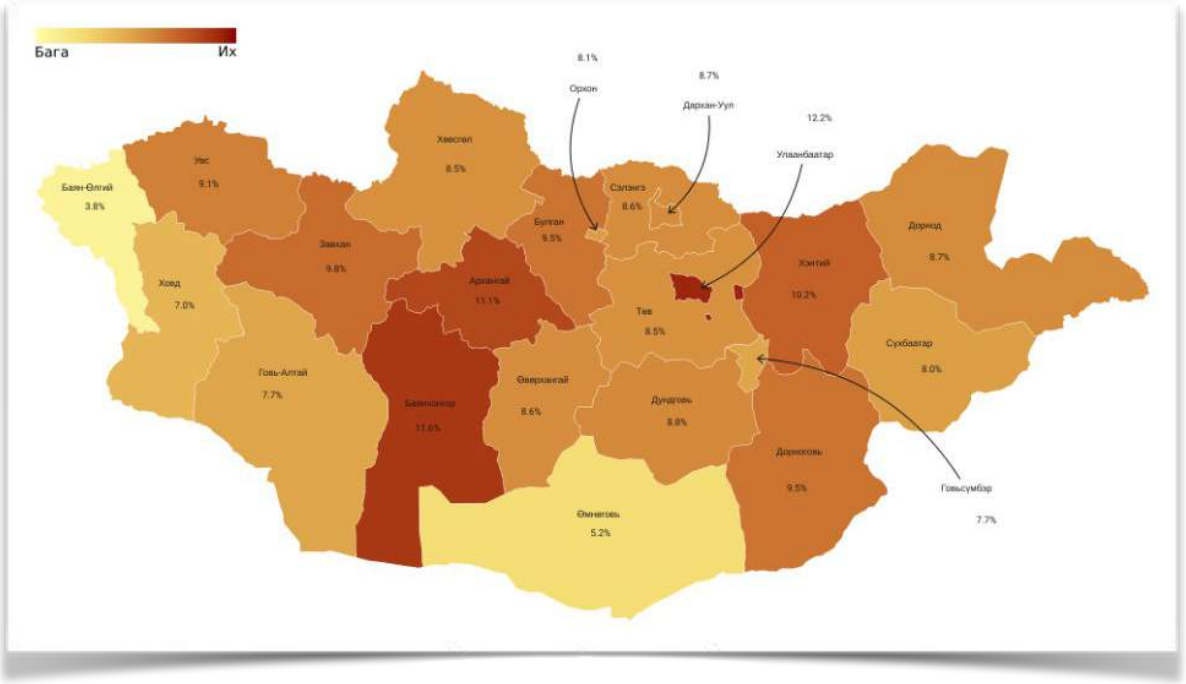
400 thousand people with viral hepatitis

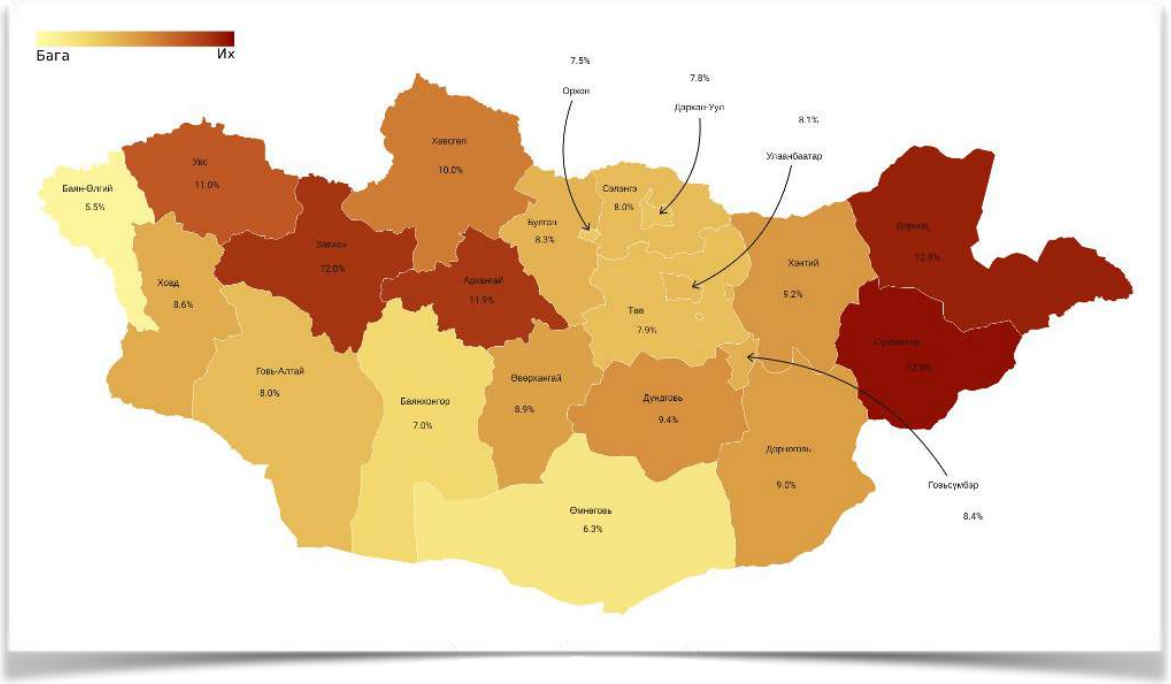


Hepatitis Prevention, Control and Elimination Program in Mongolia



Hepatitis Prevention, Control and Elimination Program in Mongolia





ANTI-HCV (+)

102,861 (9.8%)

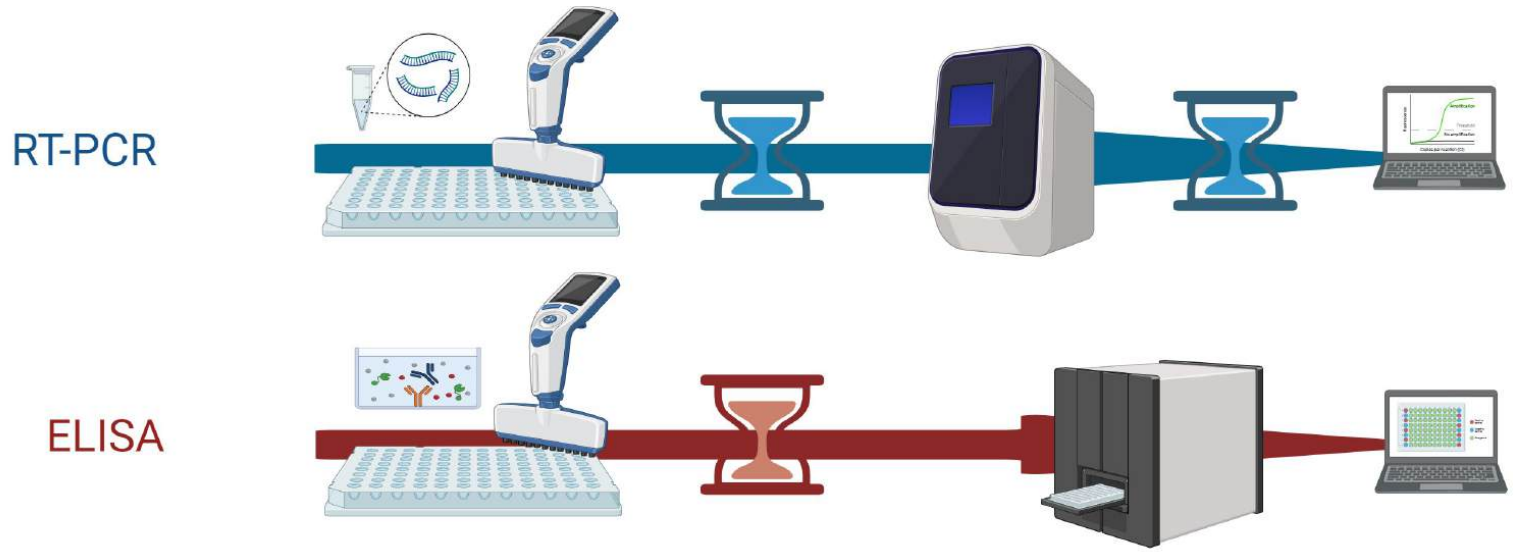
HBSAG (+)

89,734 (8.1%)

Screening and Diagnosis of Hepatitis Delta

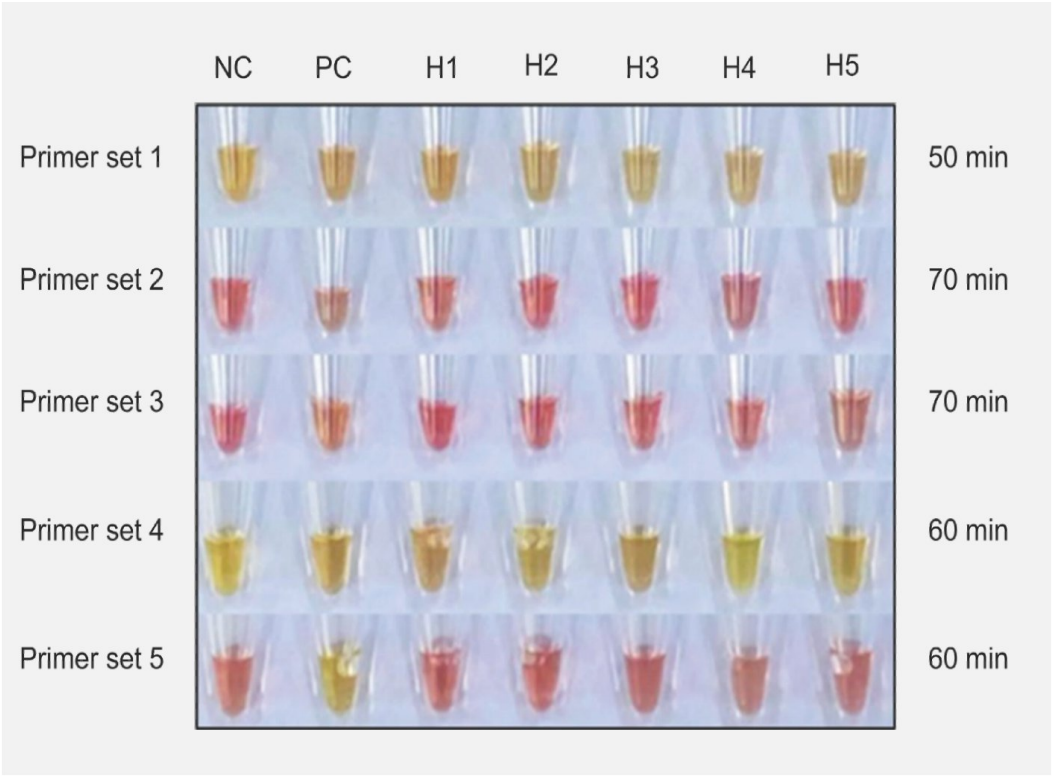
Challenges in HDV Screening and Diagnosis

- **Mongolian HBV guideline recommended to test all HBsAg positives for HDV infection since 2016.**
- **Current methods:** Serological assays (ELISA) and RT-PCR
- **ELISA:** Variable sensitivity/specificity and laboratory-dependent
- **RT-PCR:** Requires advanced lab
- **Need:** Rapid, accessible, and reliable diagnostic tool for point-of-care settings



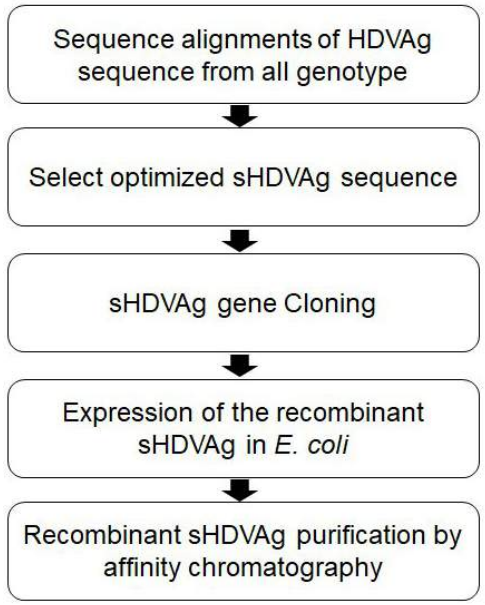
Objective

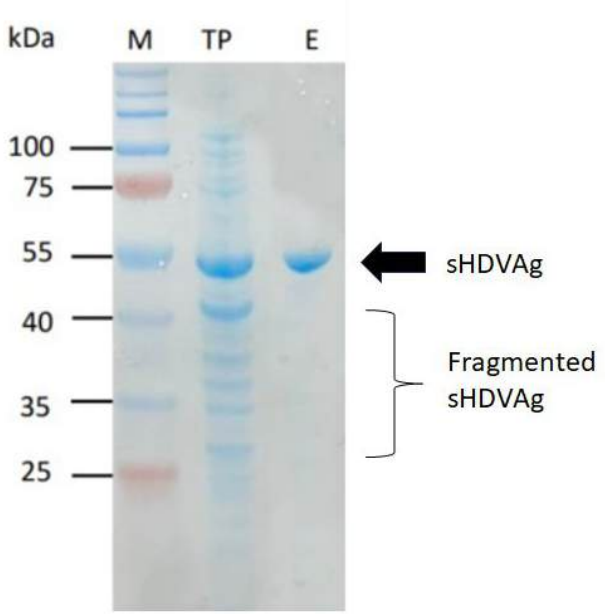
- Develop and validate anti-HDV antibody rapid diagnostics test (RDT)
- Develop and validate easy to use LAMP PCR

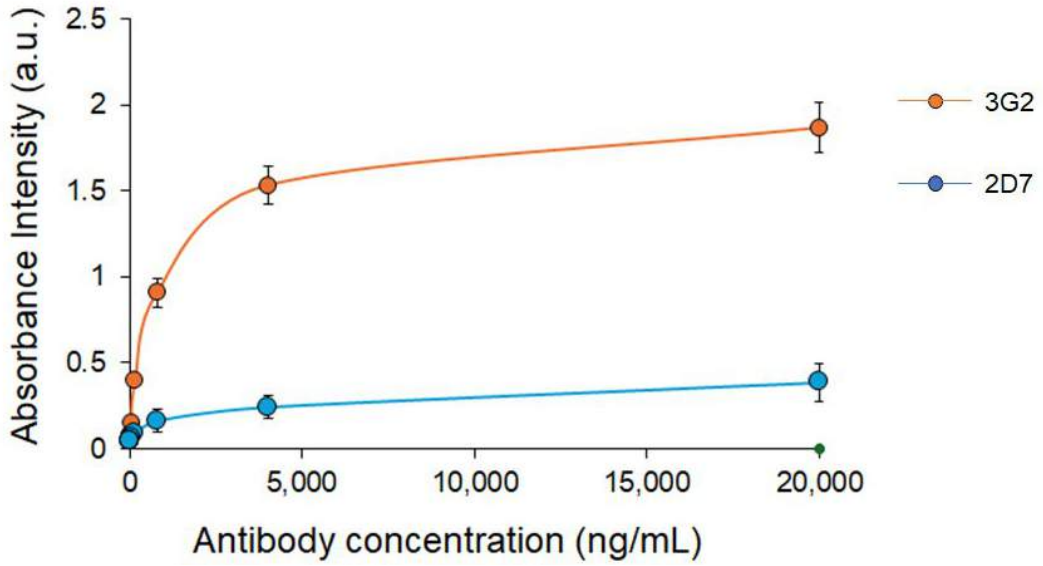


Development of Recombinant sHDVAg

- **Antigen:** Selected via sequence alignment (GenBank ID ADI24879.1), expressed in E. coli with GST tag.
- **Purification:** Affinity chromatography, confirmed by SDS-PAGE
- **Validation:** Monoclonal antibodies (3G2, 2D7) showed high binding affinity via ELISA
- **Purpose:** Pan-genotypic target for anti-HDV Antibody detection.

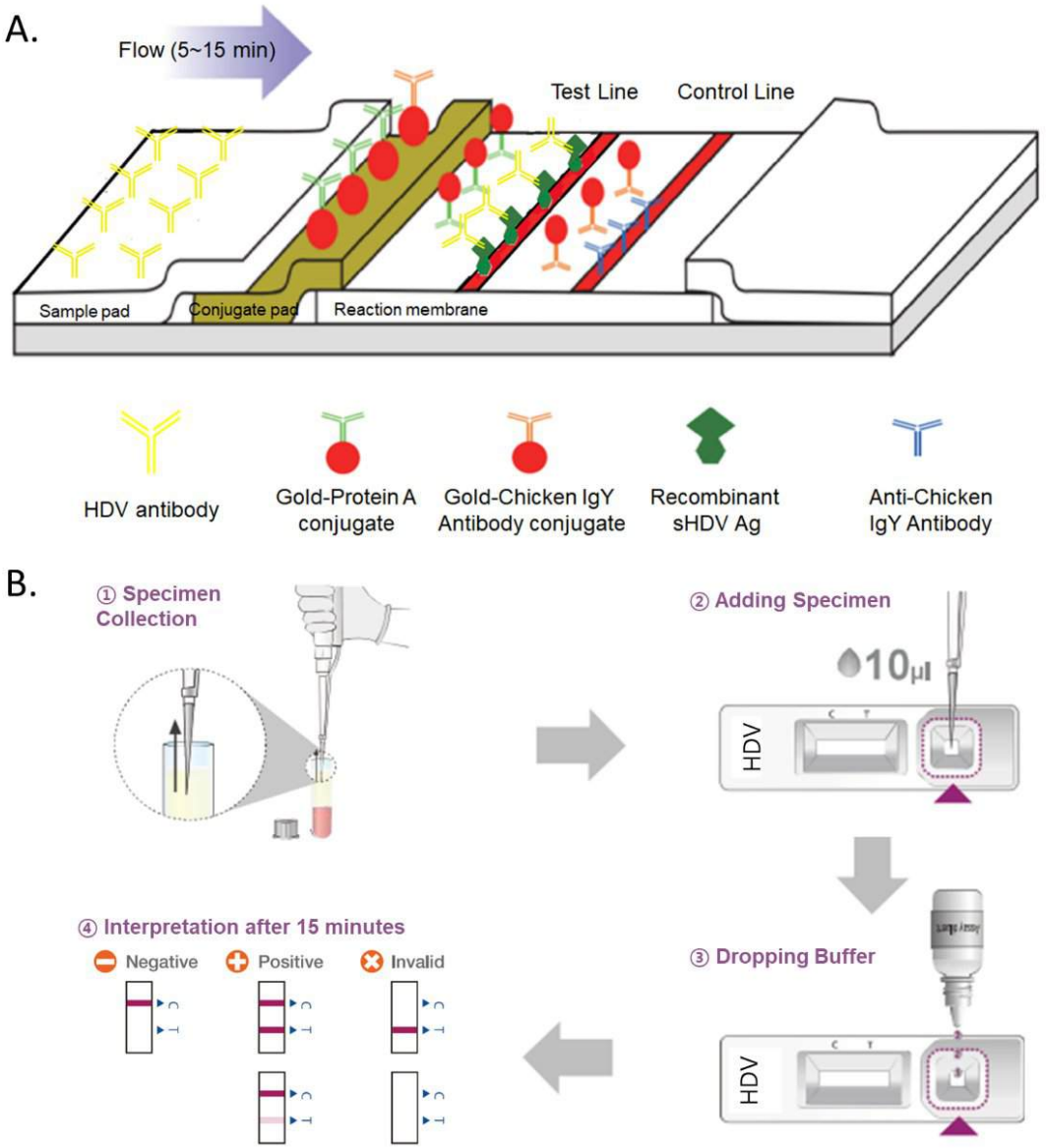






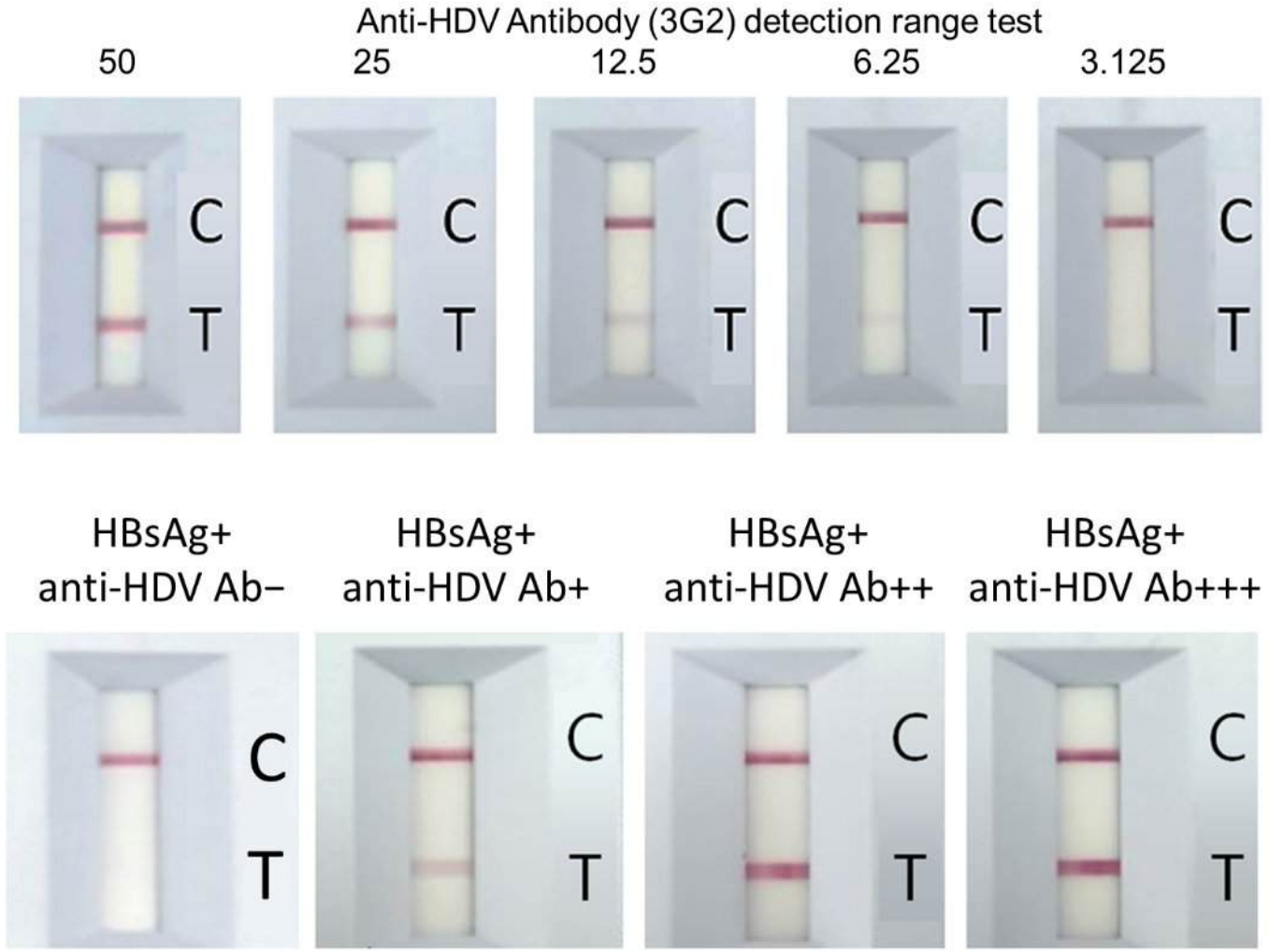
Anti-HDV Antibody RDT Design

- **Components:** Sample pad, conjugate pad, reaction membrane, absorbent pad.
- **Technology:** Gold-conjugated Protein A, DOPC lipid coating for biofouling resistance.
- **Mechanism:** Serum anti-HDV Ab binds gold conjugate, forms visible test line
- **Sample Volume:** 10 microliter
- **Testing Time:** 15 minutes



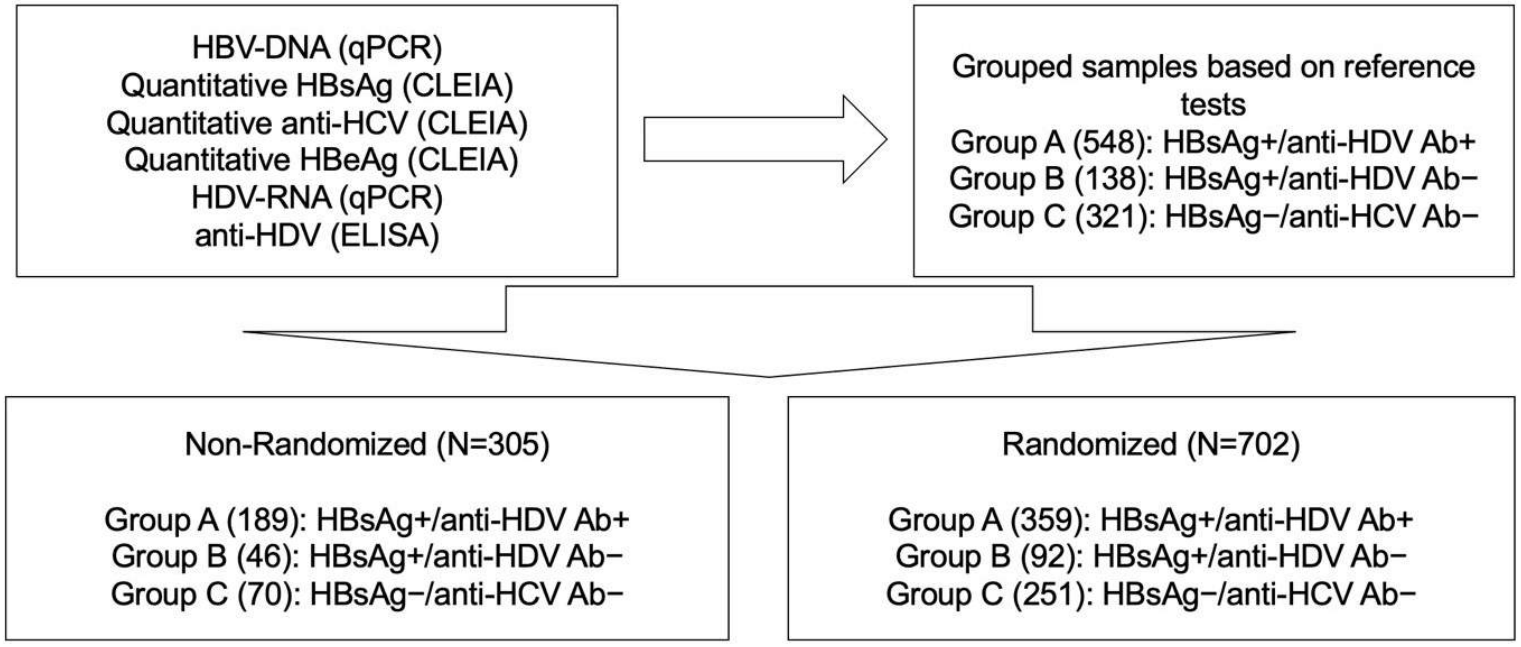
Anti-HDV Antibody RDT Sensitivity

- **Sensitivity:** Detects down to 6.25 ng/mL



Clinical Study Design

- **Sample Size:** 1007 serum samples (548 anti-HDV Ab+, 138 HBsAg+/anti-HDV Ab–, 321 healthy).
- **Cohorts:** Non-randomized (**305**) and randomized (**702**) for validation.
- **Testing:** Blinded protocol, results recorded at 15, 20, 25 minutes.
- **Reference Tests:** RT-PCR (HDV-RNA), ELISA/CLEIA (anti-HDV Ab, HBsAg).



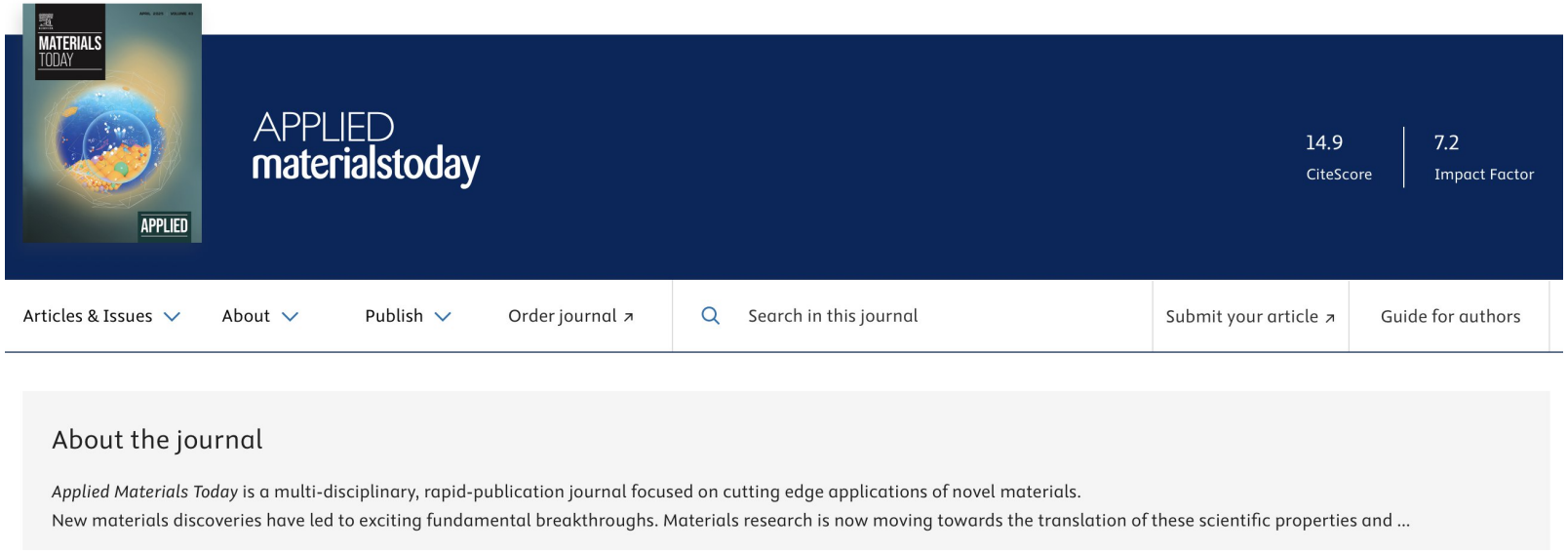
Validation Results

- True Positives: 547, True Negatives: 445, False Positives: 14, False Negatives: 1
- Consistent performance across randomized and non-randomized 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%**

Strengths

High sensitivity/specificity, rapid (15 min), no advanced equipment needed.



Accepted for publication on March 13, 2025

Implications

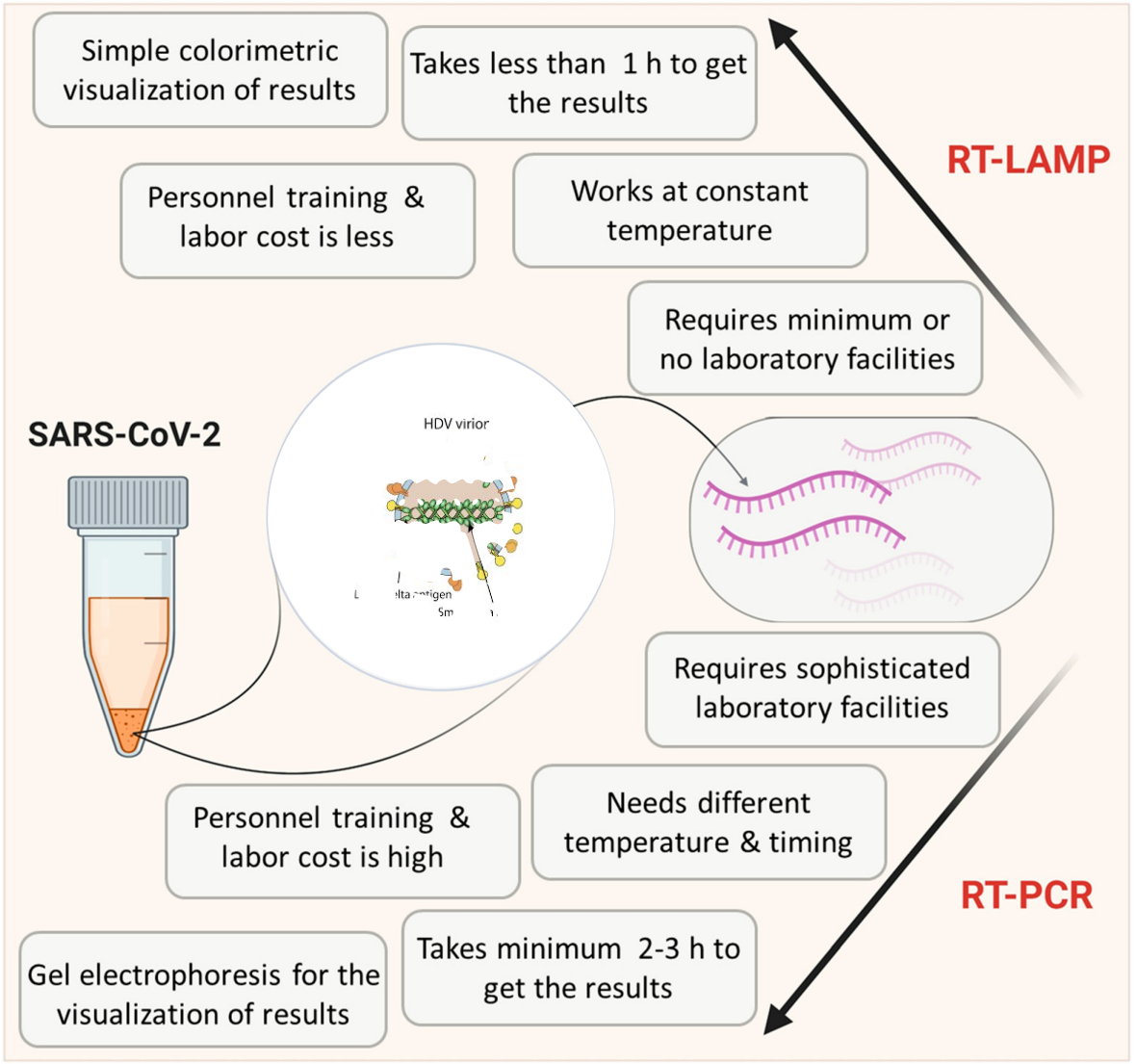
- Ideal for point-of-care in low-resource settings
- Integrates with HBV screening for dual detection
- Advantage over RT-PCR/ELISA: Accessibility and speed

Future Directions

Validate across HDV genotypes globally.

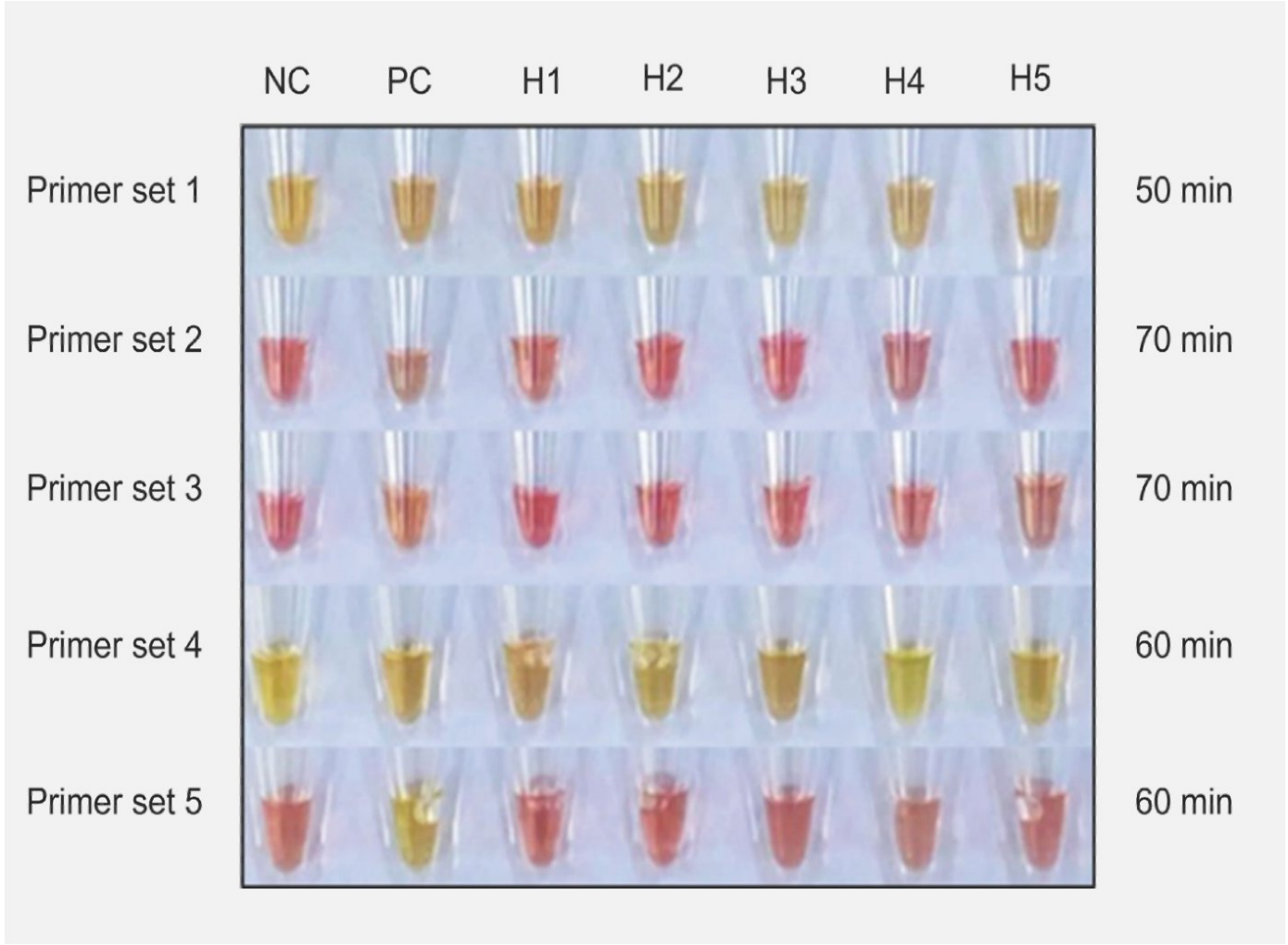
Development of LAMP PCR for HDV-RNA detection

- **Primer Set:** Different primer sets for the HDV-LAMP assay were designed using NEB-LAMP online tool on a high homology region of HDV genotype-1 sequence.
- **Assay:** The assay was performing using WarmStart Colorimetric and Fluorescent LAMP2 X Master Mix (DNA & RNA) kits.
- **Testing:** Serum samples from 20 HDV-infected patients and 20 healthy people (a total of 40 people). Viral RNA was isolated from the participants' blood serum to compare primer sets and assess assay specificity. Serially diluted recombinant construct containing HDV genomic sequence were used for the analysis of the limit of detection (LOD) for colorimetric and fluorescent assays.
- **Validation:** The amplified products were confirmed by gel electrophoresis and dissociation curve analysis. The overall HDV-LAMP assay was characterized for sensitivity, specificity, optimal reaction temperature, and time-point of detection.

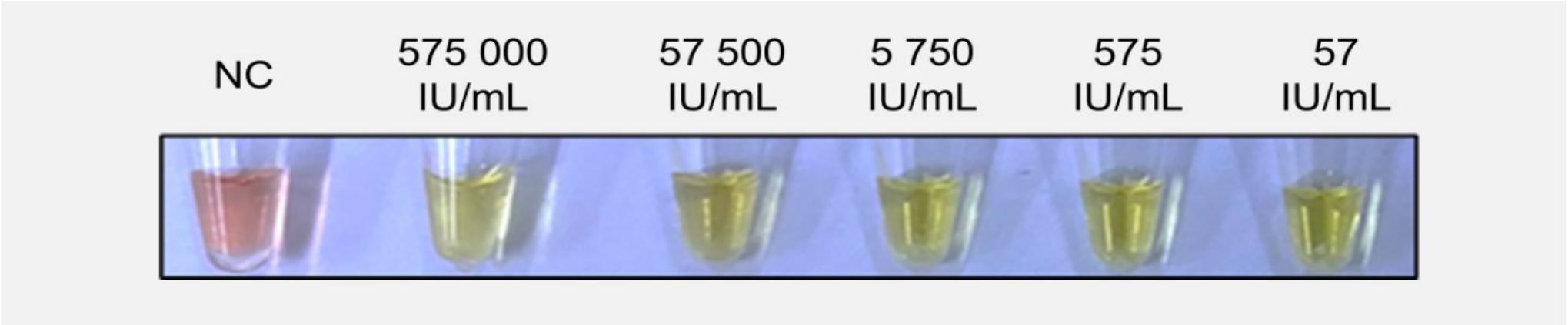


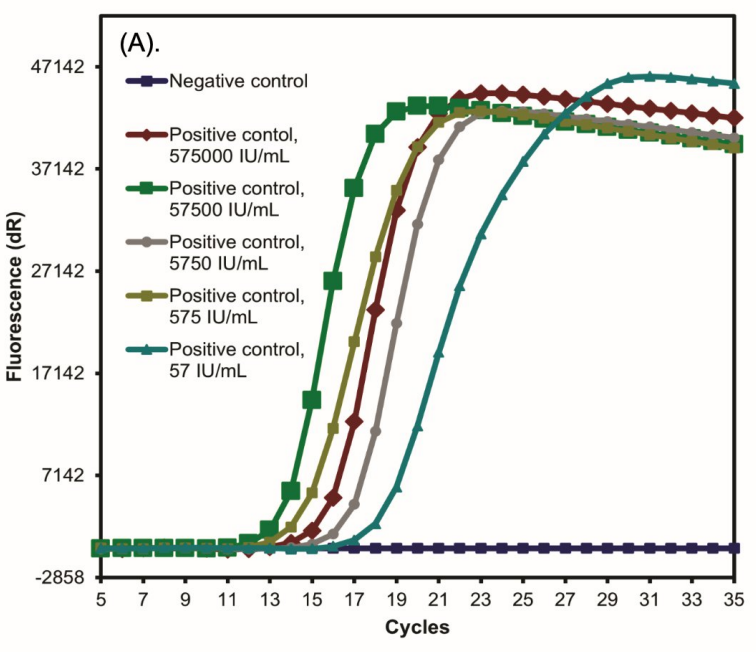
Picture adapted from Augustine R Biology 2020, 9, 182.

Diagnosis of HDV: Loop-Mediated Isothermal Amplification PCR, LAMP PCR



Diagnosis of HDV: Loop-Mediated Isothermal Amplification PCR, LAMP PCR



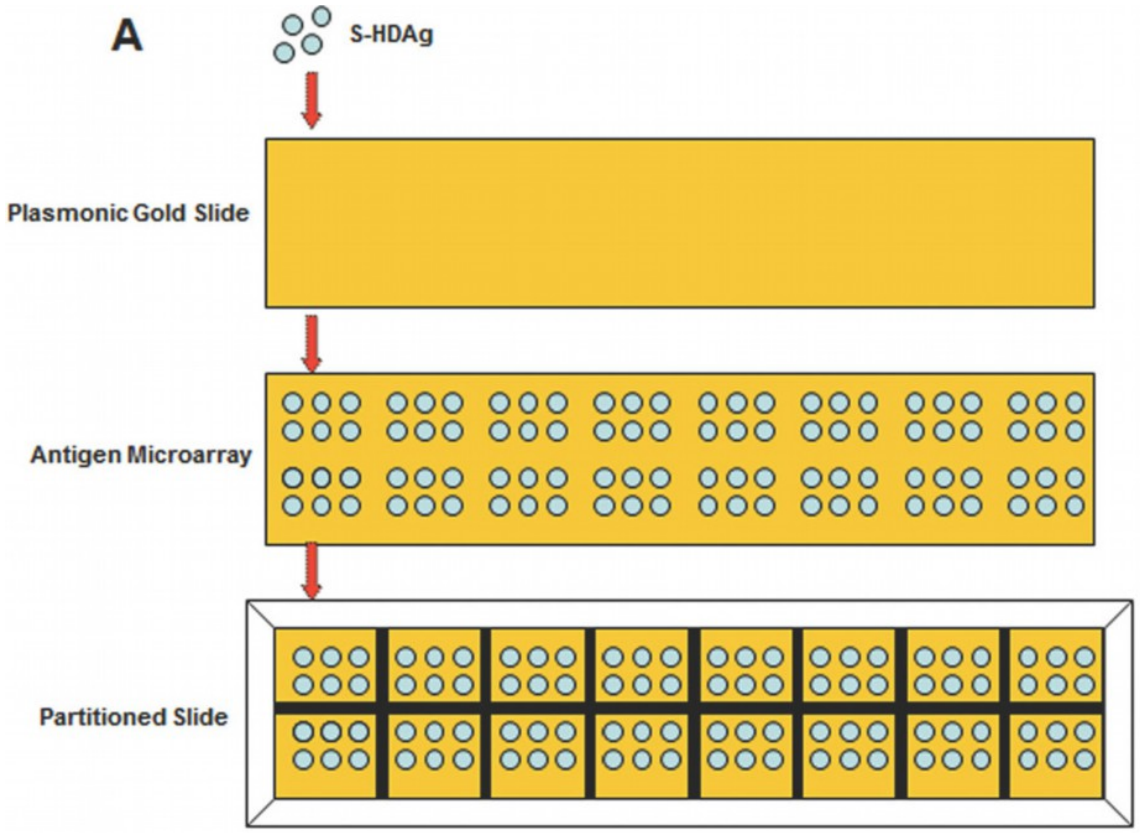


(A).	Positive	Negative	Total
Positive	17 (TP)	3 (FP)	20
Negative	0 (FN)	20 (TN)	20
Total	17	23	40

(B).	Positive	Negative	Total
Positive	18 (TP)	2 (FP)	20
Negative	0 (FN)	20 (TN)	20
Total	20	22	40

EnkhjargalS. et al, Poster AASLD 2024

One step test: Quantitative microarray antibody capture (Q-MAC) assay



Age group	N	HBsAg-positive	anti-HDV positive by ELISA	anti-HDV positive by Q-MAC	HDV-RNA
20-29	314	39	19	15	15
30-39	239	27	17	16	15
40-49	226	24	18	15	15
>50	379	33	29	29	25
TOTAL	1158	123 (10.6%)	83 (67.47%)	75 (60.9%)	70 (56.9%)

Oidovsambuu, el at. Hepatology, Vol 66, No 6, 2017

Summary & Conclusion

- Anti-HDV Antibody RDT: A breakthrough in HDV diagnostics with 99.8% Sensitivity and 96.9% Specificity
- Impact: Enhances early diagnosis, supports public health initiatives to control HDV.
- LAMP PCR: We have demonstrated the use of HDV-LAMP assay. It is a rapid, user-friendly, and very sensitive diagnostic assay for detecting HDV-RNA in blood samples.
- Call to Action: Further research and deployment to eliminate HDV globally.

Thank you