Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Viral antigen nanoparticles for discriminated and quantitative detection of different subtypes of anti-virus immunoglobulins

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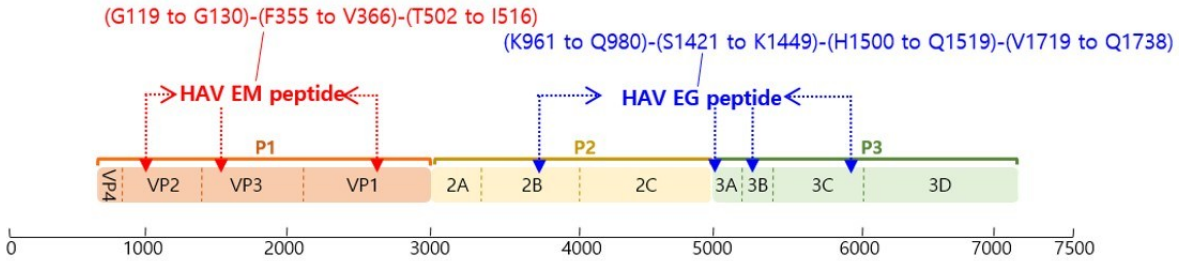
Figure S1



HAV genome sequence

MNMSKQGIFQ TVGSGLDHIL SLADIEEEQM IQSVDRTAVT GASYFTSVDQ SSVHTAEVGS HQI
EPLKTSV DKPGSKKTQG EKFFLIHSAD WLTTHALFHE 100 VAKLDVVKLL YNEQFAVQ **G119 L L**
RYHTYARF G130 IEIQVQINPT PFQQGGLICA MVPGDQSYGS IASLTVYPHG LLNCNINNVV RI
KVPFIYTR GAYHFKDPQY 200 PVWELTIRVW SELNIGTGTS AYTSLNVLAR FTDLELHGLT PLSTQ
MMRNE FRVSTTENVV NLSNYEDARA KMSFALDQED WKSDPSQG GGIKITHFTTWT SIPTLAA
QFP FNASDSVGQQ IKVIPVDPYF FQMTNTNPDQ KCITALASIC QMFC **F355 WRGDL VFDFQ**
V366 FPTK YHSGRLLFCF VPGNELIDVT GITLKQATTA 400 PCAVMDITGV QSTLRFRVPW ISDT
PYRVNR YTKSAHQKGE YTAIGKLIVY CYNRLTSPSN VASHVRVNVY LSAINLECFA PLYHAMDVT
T QVGDDSGGFS 500 T **T502 VSTEQNVP DPQVG I516** TTMR DLKGKANRGK MDVSGVQAP
V GAITTIEDPV LAKKVPETFP ELKPGESRHT SDHMSIYKFM GRSHFLCTFT FNSNNKEYTF 600 PI
TLSSTSNP PHGLPSTLRW FFNLFQLYRG PLDLTIIITG ATDVDGMAWF TPVGLAVDTP WVEKESA
LSI DYKTALGAVR FNTRRTGNIQ IRLPWYSYLY 700 AVSGALDGLG DKTDSTFGLV SIQIANYNH
S DEYLSFSCYL SVTEQSEFYF PRAPLNSNAM LSTESMMSRI AAGDLESSVD DPRSEEDRRF ESHIE
CRKPY KELRLEVGKS RLKYAQEELS NEVLPPPRKM KGLFSQAKIS LFYTEEHEIM KFSWRGVTAD T
RALRRFGFS MAAGRSVWTL EMDAGVLTGR LVRLNDEKWT 900 EMKDDKIVSL IEKFTSNKYW S
KVNFPHGML DLEEIAANSK DFPNMSETDL CFLLHWLNPK **K961 INLADRMLGLSGVQEIKE Q9**
80 GVGLIAECRT FLDSIAGTLK 1000 ---------- 1301 GCPMRLNMAS LEEKGRHFSS
PFIIATSNWS NPSPKTVYVK EAIDRRLHFK VEVKPASFFK NPHNDMLNVN LAKTNDAIKD MSCV
DLIMDG HNISLMDLLS 1400 SLVMTVEIRK QNMSEFMELW **S1421 QGVSDDDNDSAVAEFFQ**
SFPSGEPSNS K1449 L SSFFQSVTNH KWVAVGAAVG ILGVLVGGWF VYKHFSRREE EPIPAEGV
Y **H1500 GVTKPKQVIKLDADPVES Q1519** S TLEIAGLVRK NLVQFGVGEK NGCVRWVMNA L
GVKDDWLLV PSHAYKFEKD YEMMEFYFNR GGTYYSISAG NVVIQSLDVG 1600 FQDVVLMKVP
TIPKFRDITQ HFIKKGDVPR ALNRLATLVT TVNGTPMLIS EGPLKMEEKA TYVHKKNDGT TVDLTV
DQAW RGKGEGLPGM CGGALVSSNQ 1700 SIQNAILGIH VAGGNSIL **V1719 AKLVTQEMFQ**
NIDKKIES Q1738 RI MKVEFTQCSM NVVSKTLFRK SPIHHHIDKT MINFPAAMPF SKAEVDPMA
V MLSKYSLPIV 1800 ---------- 2201 EMIEYRLKSY DWWRMRFYDQ CFICDLS 2227

Figure S1. Sequences of HAV antigenic epitopes (EG and EM) that are used to detect anti-HAV IgG and IgM antibody markers in hepatitis A patient sera in this study. Colored (red or blue) sequences represent antigenic epitopes of viruses.

Figure S2

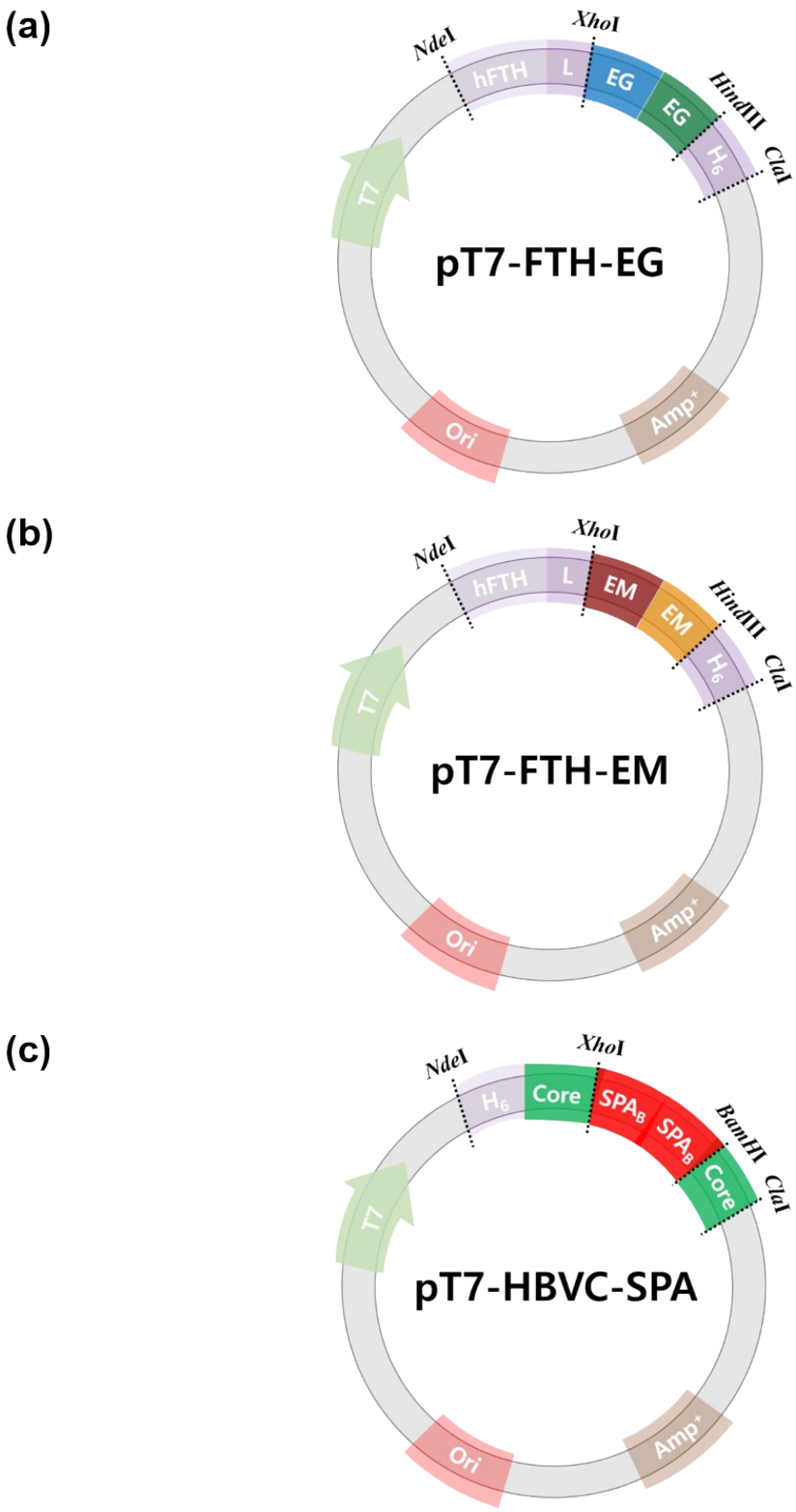


Figure S2. Schematic illustration of vector map of 3D probes that are used for hepatitis A diagnosis. **(a)** hFTH-$(EG)_2$-H_6, **(b)** hFTH-$(EM)_2$-H_6, **(c)** H_6-SPA_B-capsid.

Figure S3

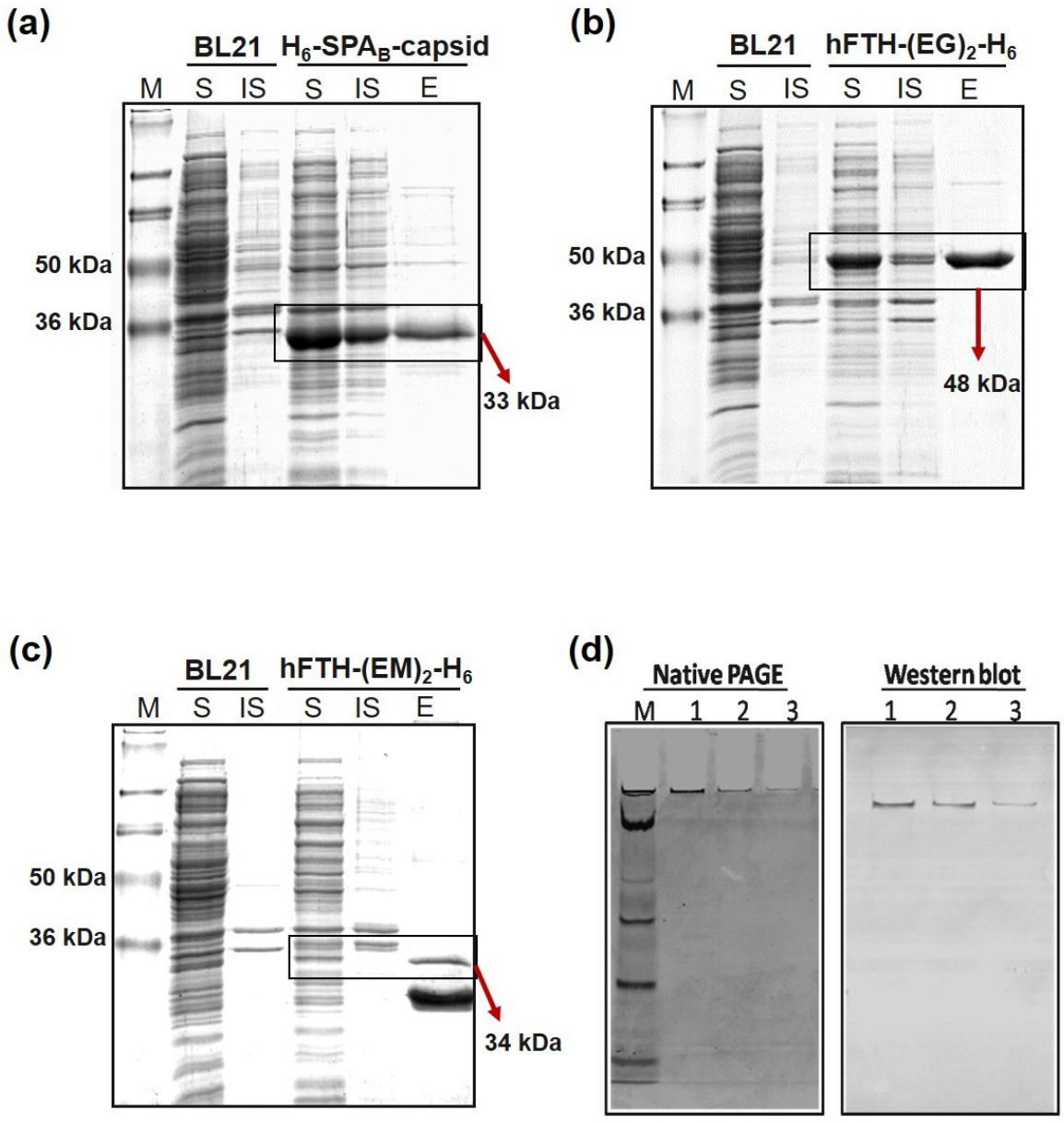


Figure S3. Results of SDS-PAGE, native PAGE and Western blot analyses of three types of HAV antigen nanoparticles to capture anti-HAV antibodies. **(a)** SDS-PAGE gel image of H_6-SPA_B-capsid, **(b)** hFTH-$(EG)_2$-H_6, **(c)** hFTH-$(EM)_2$-H_6. **(d)** native PAGE and Western blot image of 1) H_6-SPA_B-capsid, 2) hFTH-$(EG)_2$-H_6, 3) hFTH-$(EM)_2$-H_6, respectively. For Western blot, anti-His tag (H_6) antibody was used as primary antibody. (M: Seeblue protein marker, S and IS: soluble and insoluble fraction, E: purified and eluted protein, BL21: wild-type of *E.coli* strain BL21 (DE3)).

Figure S4

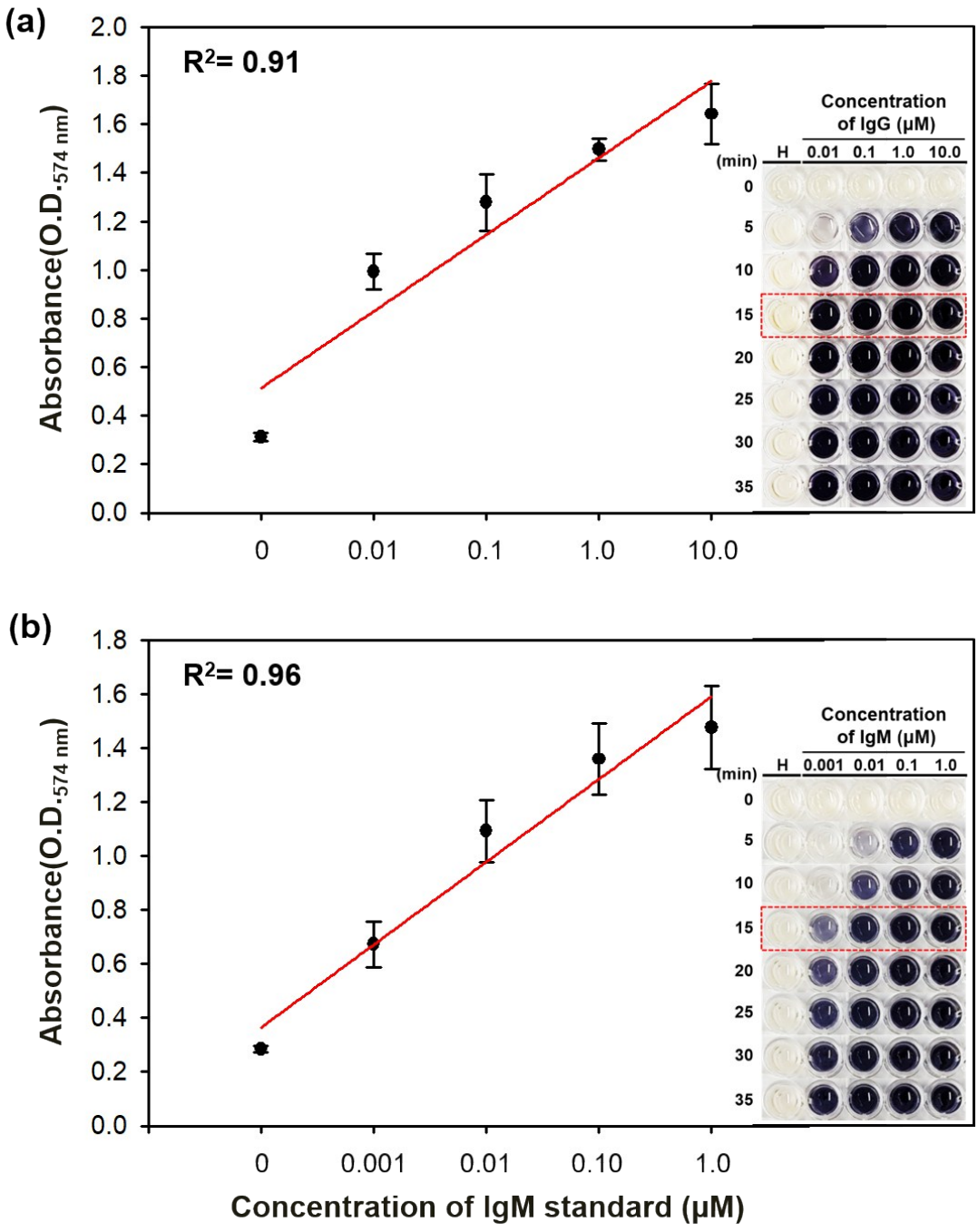


Figure S4. Quantitative analysis to confirm the reusability of anti-HAV IgG and IgM. (a) Re-quantitative analysis of anti-HAV IgG through vAgNP-based one-step-immunoassay using freeze-dried pre-assay solution 1 and standard IgG-spiked healthy sera. (b) Re-quantitative analysis of anti-HAV IgM through vAgNP-based one-step-immunoassay using freeze-dried pre-assay solution 2 standard IgM-spiked healthy sera. Each lyophilized pre-assay solution was stored for 3 months.

Figure S5

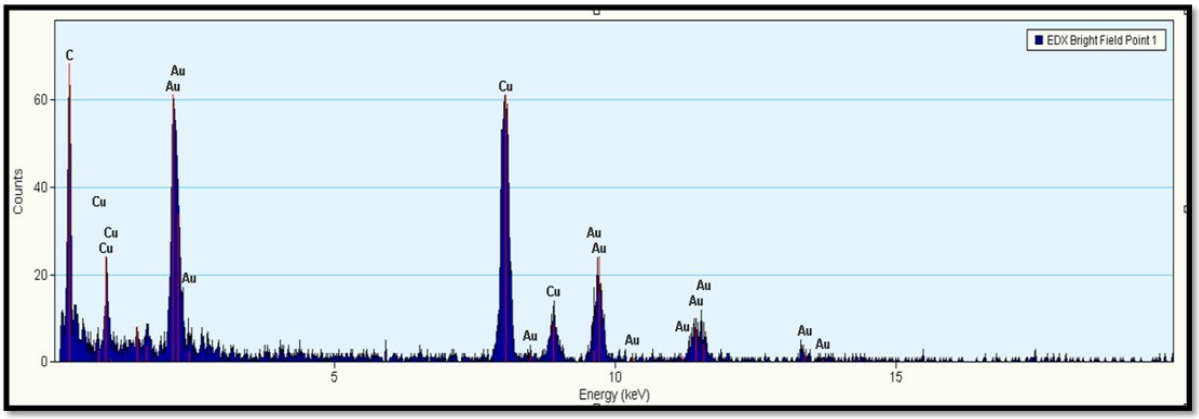
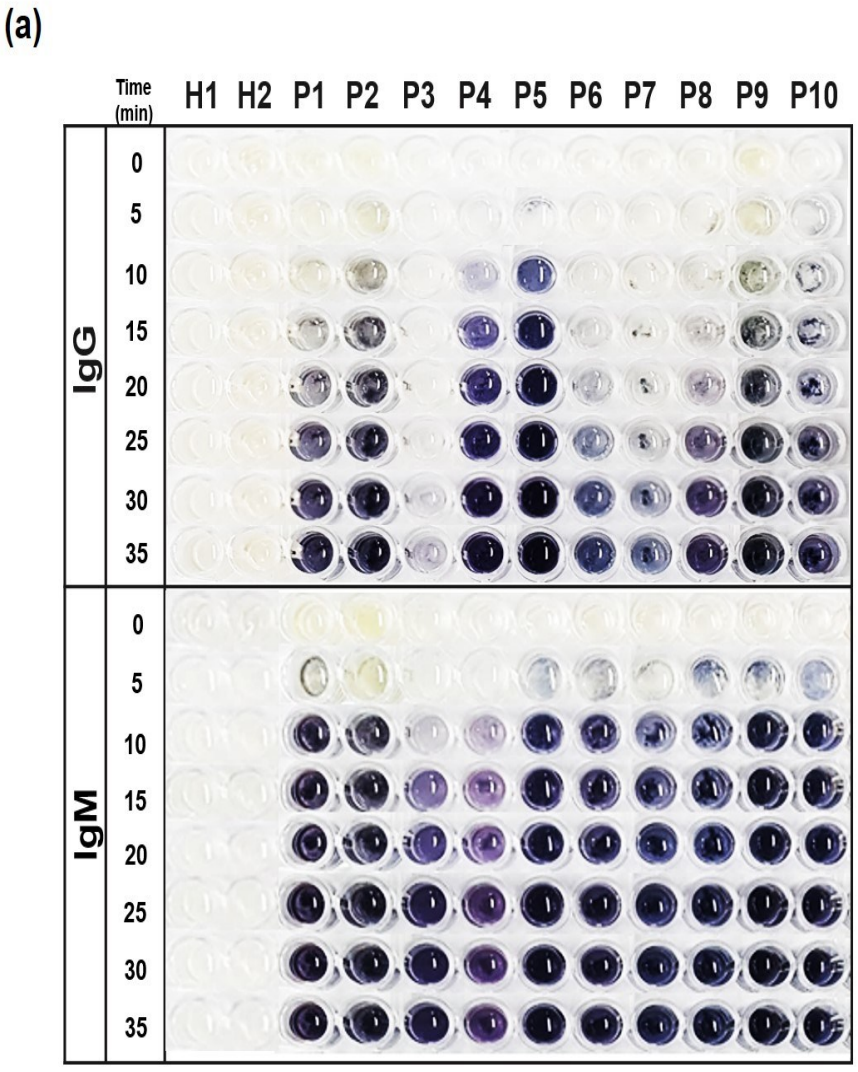
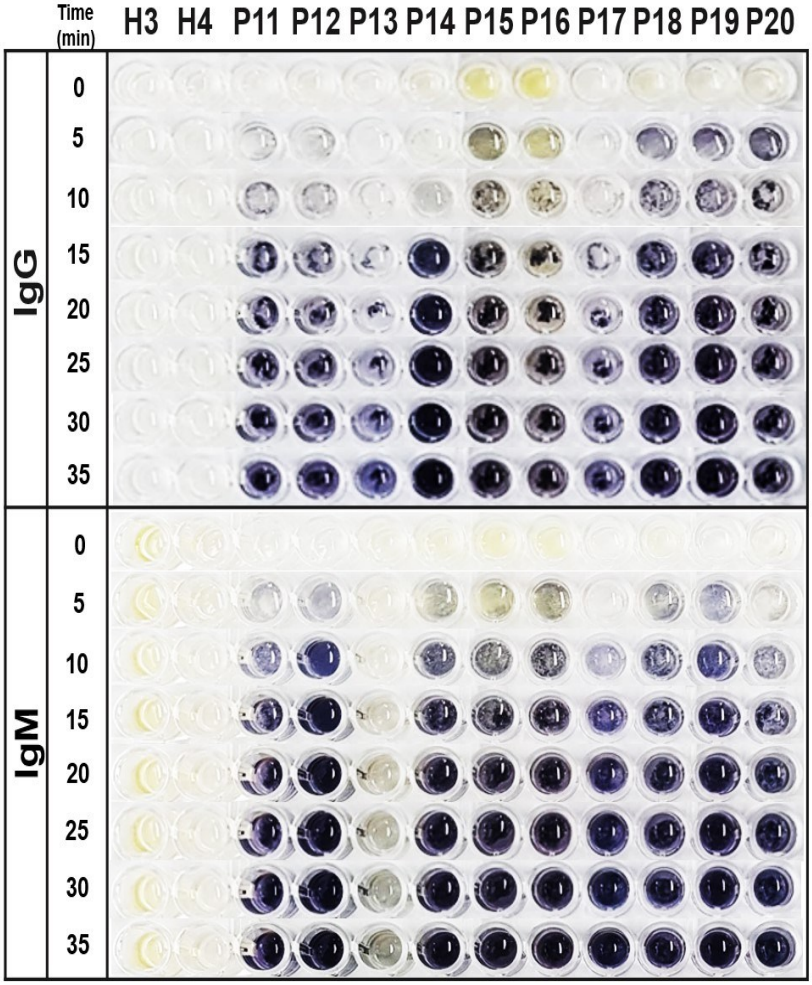


Figure S5. EDX spectroscopy analysis of the assay solution of hepatitis A patient serum.

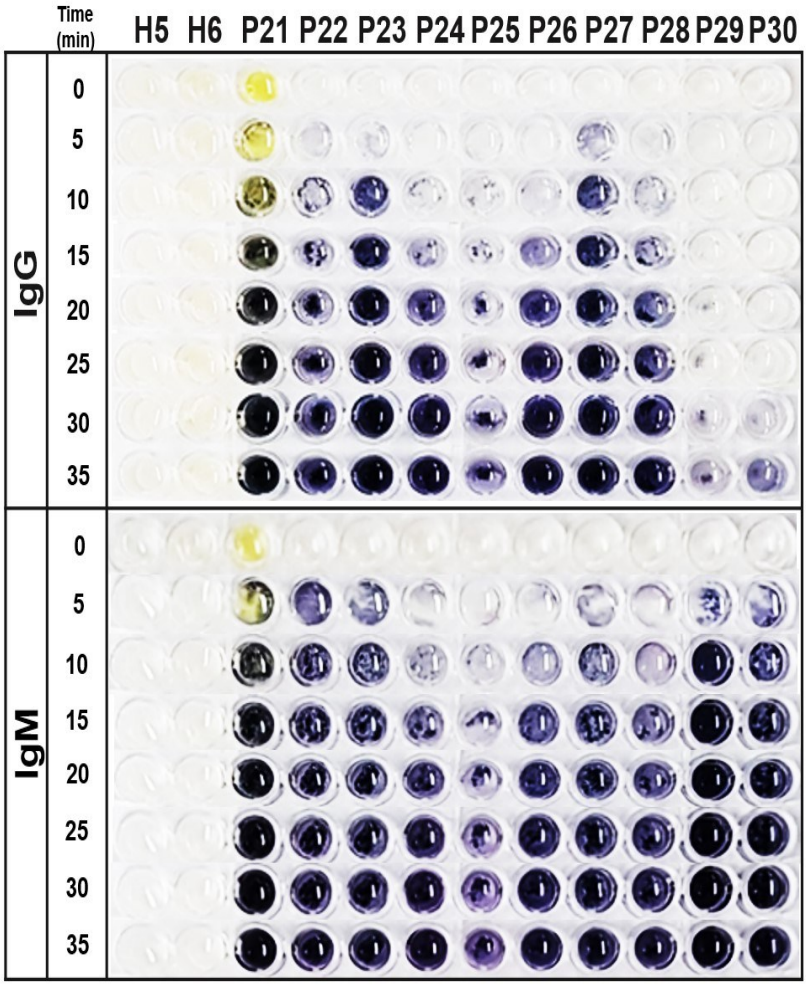
Figure S6


(a)
Time (min)
H1 H2 P1 P2 P3 P4 P5 P6 P7 P8 P9 P10
IgG
IgM
0
5
10
15
20
25
30
35

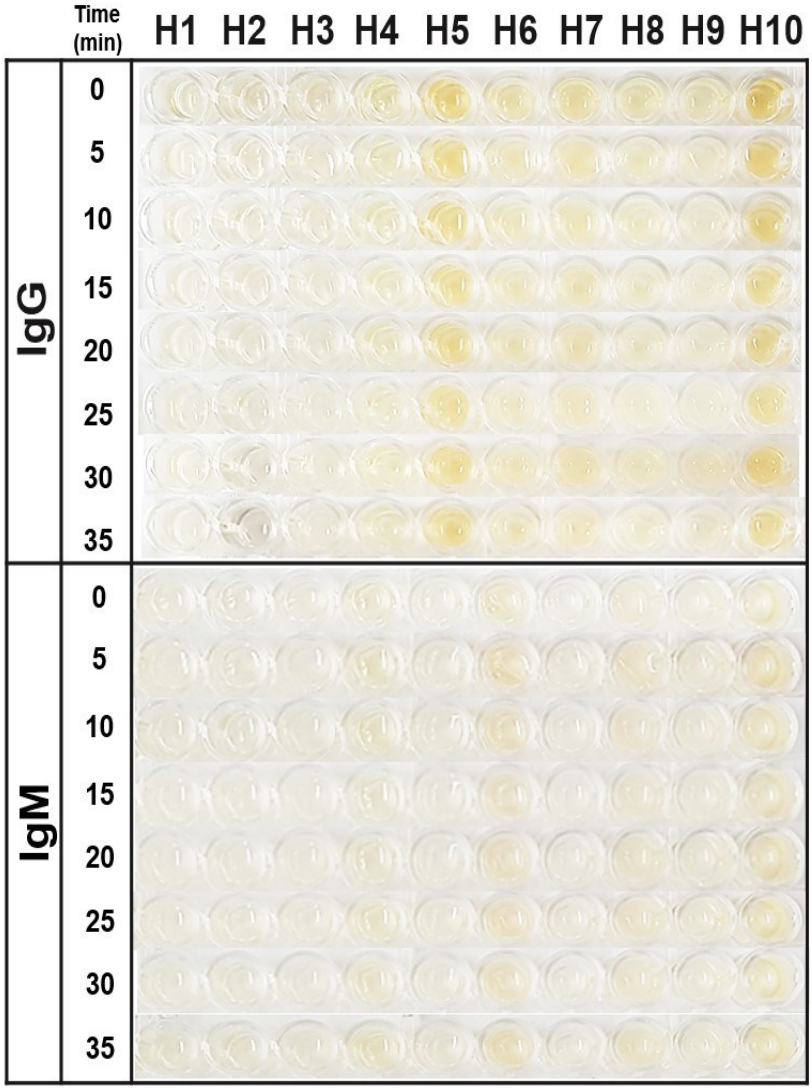
(a)


Time (min)
H3 H4 P11 P12 P13 P14 P15 P16 P17 P18 P19 P20
IgG
0
5
10
15
20
25
30
35
IgM
0
5
10
15
20
25
30
35

(a)


Time (min)
H5 H6 P21 P22 P23 P24 P25 P26 P27 P28 P29 P30
IgG
IgM
0
5
10
15
20
25
30
35

(b)


Time (min)
H1 H2 H3 H4 H5 H6 H7 H8 H9 H10
IgG
0
5
10
15
20
25
30
35
IgM
0
5
10
15
20
25
30
35

(b)

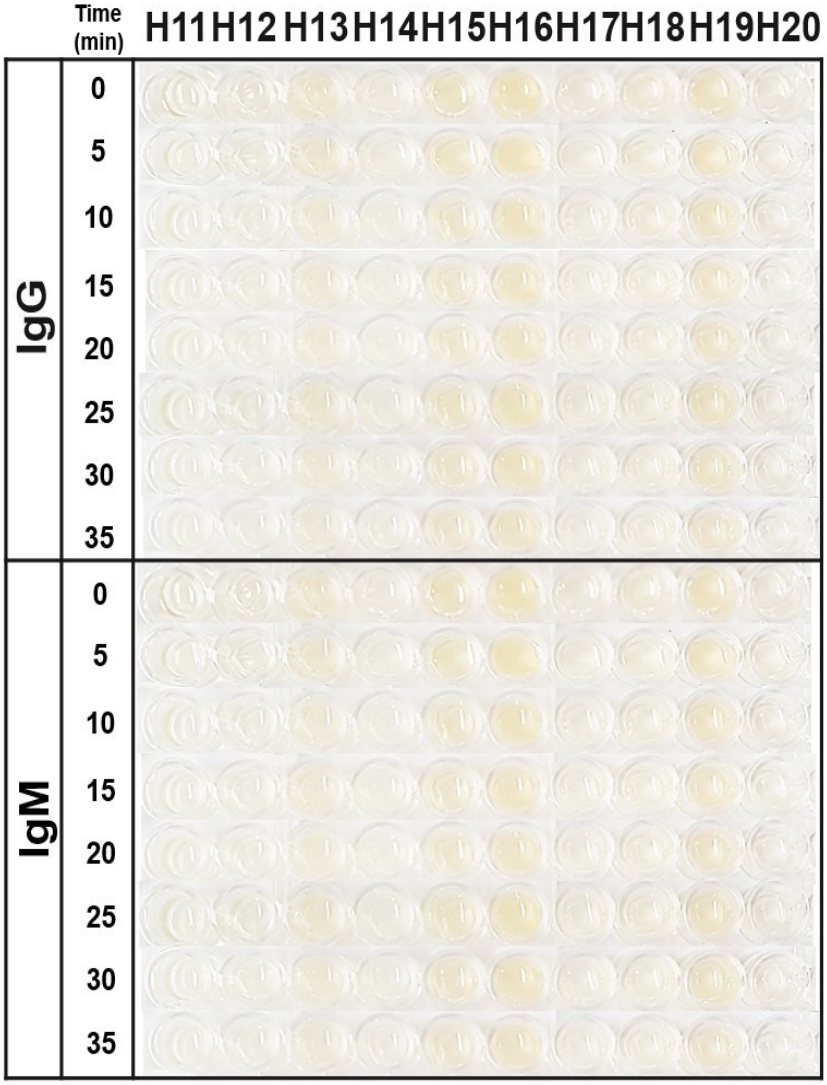


Figure S6. Photographic images of 96-well plates where one step-immunoassay were performed for diagnosis of hepatitis A. **(a)** Test results of 30 hepatitis A patient sera about anti-HAV IgG and IgM antibodies, respectively (P1 to P30) (time-course images for 35 min). **(b)** Test results of 20 healthy control sera about anti-HAV IgG and IgM antibodies, respectively (N1 to N20).