

Identification and Characterization of Nucleic Acid Positive Sera from Indonesian and Chilean Patients with Hepatitis A Infections

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OBJECTIVES

- Identify RNA positive samples from Chilean and Indonesian patients showing clinical symptoms and manifestations of Hepatitis A.
- Perform IgM α -HAV ELISA, RNA extraction, RT-PCR, cloning, and nucleotide sequencing for nucleotide and amino acid variability studies.

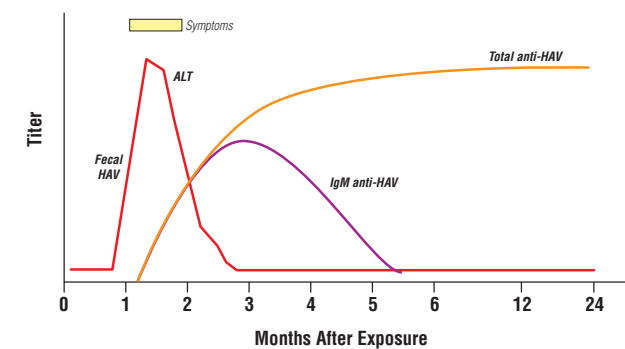
INTRODUCTION

- Hepatitis A is an enterically transmitted disease caused by the Hepatitis A Virus (HAV).
- HAV is a small, 27-nm icosahedral, non-enveloped, spherical virus of the genus Hepatovirus of the family Picornaviridae.
- The HAV genome consists of a single-stranded, linear, 7.5 kb RNA molecule encoding a polyprotein precursor of ~250 kD that is proteolytically processed to yield the structural proteins and enzymatic activities required for viral replication.
- Although HAV is usually acquired by the fecal-oral route, there are reports that HAV can be transmitted through the administration of pooled plasma derivatives.

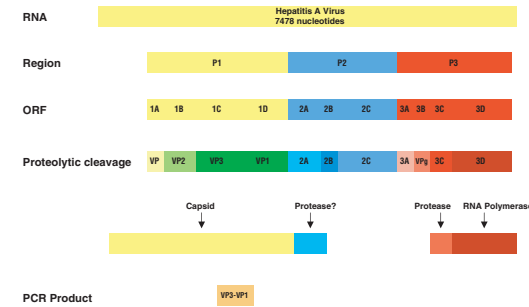
Symptoms Reported by Patients with Hepatitis A Infection

- Jaundice 40–80%
- Dark urine 68–94%
- Fever or chilliness 32–73%
- Fatigue/lassitude 52–91%
- Anorexia 42–90%
- Nausea or vomiting 26–87%
- Abdominal pain/discomfort 37–65%

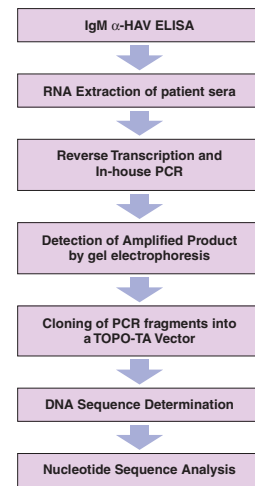
Natural History of HAV Infection



HAV Genome



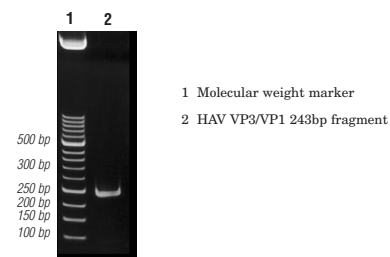
METHODS



PCR Amplification of HAV

Primer	Size	Sequence	Region	Product Size
2172	24	GCTCCTCTTTATCATGCTATGGAT	VP3/VP1	243 bp
2415	24	CAGGAAATGTCCTCAGGTACTTTCT	VP3/VP1	243 bp

HAV VP3/VP1 243 bp PCR Fragment



RESULTS

HAV Sample Data

Sample ID	IgM α -HAV ELISA (Signal/cutoff)	PCR	% NT Homology	% A.A. Identity
Indonesian Samples				
IND1	2.51	+	96.3	100
IND2*	2.24	+	94.2–96.3	100
IND3	0.43	+	94.2	100
IND4	2.38	+	96.7	100
IND6	2.83	+	95.9	98.8
IND7	2.14	+	95.9	98.8
IND8	2.33	+	95.9	98.8
IND9	2.27	+	94.9	98.8
IND10	1.54	+	93.8	98.8
IND11	2.06	+	96.3	100
IND12*	2.07	+	94.7	98.8–100
Chilean Samples				
SCL2	2.57	+	94.7	98.8
SCL3	2.48	+	94.7	98.8
SCL4	2.38	+	92.2	98.8
SCL7	2.04	+	94.7	98.8
SCL8*	2.39	+	90.5–90.9	97.5
SCL9	2.44	+	91.4	97.5
SCL10	2.12	+	91.4	97.5
SCL11	2.93	+	91.4	97.5
SCL12	2.36	+	94.7	98.8
SCL14	2.65	+	92.6	98.8
SCL15*	2.32	+	92.6–93.0	98.8
SCL16	2.22	+	91.4	97.5

(* Samples infected with multiple isolates.
% homology as compared to Cohen et al (1987)

Nucleotide Sequence Comparisons Between the 243 bp Fragments Amplified from INDONESIAN Sera and the Corresponding Region Reported by Cohen et al. (J. Virol. 61: 50–59, 1987)

Cohen nt #	Cohen nt	Ind-1-2	Ind-2-2	Ind-2-4	Ind-3-2	Ind-4-5	Ind-6-4	Ind-7-1	Ind-8-2	Ind-9-1	Ind-10-5	Ind-11-3	Ind-12-1	Ind-12-2
2198	T	C	C	C	C	C	C	C	C	C	C	C	C	—
2201	T	C	C	C	C	C	C	C	C	C	C	C	C	—
2207	A	G	G	G	G	G	G	G	G	G	G	G	G	G
2222	T	C	C	C	C	C	C	C	C	C	C	C	C	A
2231	T	—	—	—	—	—	—	—	—	—	—	—	C	—
2237	A	G	G	G	—	G	G	G	G	G	G	G	—	—
2246	T	—	—	—	—	—	—	—	—	—	—	—	C	—
2252	A	G	G	G	G	G	G	G	G	G	G	G	G	G
2264	A	—	T	—	T	—	—	—	—	—	—	T	T	—
2266	A	—	—	—	—	—	—	—	—	G	—	—	—	—
2279	T	—	C	—	C	—	—	—	—	C	—	G	C	—
2312	T	C	C	C	—	C	C	C	C	C	C	C	C	C
2315	C	T	T	T	T	T	T	T	T	T	T	T	T	T
2318	A	—	G	—	G	—	—	—	—	G	—	G	G	—
2321	G	—	—	—	A	—	—	—	—	—	—	—	A	—
2324	A	—	G	—	G	—	—	—	—	G	—	G	G	—
2357	A	—	—	—	G	G	G	G	G	—	—	—	—	—
2357	A	—	—	—	G	G	G	G	G	—	—	—	—	—
2363	C	T	T	T	T	T	T	T	T	T	T	T	T	T
2370	A	G	G	—	G	—	—	—	—	G	—	—	—	—
2384	T	—	—	—	C	—	—	—	—	C	—	C	—	—
2387	A	—	—	—	G	—	—	—	—	G	G	G	—	—

Nucleotide Sequence Comparisons Between the 243 bp Fragments Amplified from CHILEAN Sera and the Corresponding Region Reported by Cohen et al. (J. Virol. 61: 50–59, 1987)

Cohen nt #	Cohen nt	SCL-10	SCL-10	SCL-4-3	SCL-6	SCL-2	SCL-5	SCL-4	SCL-10-1	SCL-15	SCL-4	SCL-14-3	SCL-15-1	SCL-12	SCL-10-6
2198	T	C	C	—	C	C	C	—	—	C	—	—	—	—	—
2201	T	C	C	C	C	C	C	C	C	C	C	C	C	C	—
2207	A	G	G	G	G	G	G	G	G	G	G	G	G	G	G
2216	T	—	—	C	—	—	—	—	—	—	—	C	C	C	—
2222	T	C	C	A	C	A	A	A	A	A	C	A	A	A	A
2225	A	G	—	—	—	—	—	—	—	—	—	—	—	—	—
2237	A	G	G	—	G	—	—	—	—	—	G	—	—	—	—
2249	A	—	—	T	—	—	—	—	—	—	—	—	—	—	—
2252	A	—	G	G	G	—	G	—	—	—	G	G	G	G	—
2264	A	—	—	T	—	T	T	T	T	T	—	T	T	T	T
2273	A	—	—	—	G	G	G	G	G	G	—	—	—	G	—
2279	T	—	—	—	C	C	C	C	C	—	—	—	—	C	—
2288	C	T	T	—	T	T	T	T	T	T	—	—	—	T	—
2293	A	—	—	G	—	G	G	G	G	—	G	G	G	G	—
2294	A	G	G	G	G	G	G	G	G	G	G	G	G	G	G
2297	T	—	—	C	—	—	—	—	—	—	C	C	C	—	
2298	T	—	—	C	—	C	C	C	C	C	—	C	C	C	—
2300	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A
2306	A	—	—	G	—	G	G	G	G	G	—	G	G	G	G
2309	A	—	—	—	G	G	G	G	G	—	—	—	—	G	—
2312	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C
2315	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T
2318	A	—	—	—	T	T	T	T	T	—	—	—	—	T	—
2321	G	—	—	—	A	A	A	A	A	—	—	—	—	A	—
2324	A	—	—	G	—	G	G	G	G	—	G	G	G	G	—
2357	A	—	—	—	G	G	G	G	G	—	—	—	—	—	—
2357	A	—	—	—	G	G	G	G	G	—	—	—	—	—	—
2363	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T
2370	A	G	G	—	G	—	—	—	—	—	G	—	—	—	—
2384	T	—	—	—	C	—	—	—	—	—	C	—	C	—	—
2387	A	—	—	—	G	—	—	—	—	—	G	G	G	—	—

SUMMARY AND CONCLUSIONS

- Sera from eleven Indonesian patients and twelve Chilean patients with clinical symptoms and manifestations of Hepatitis A infections were investigated using anti-HAV IgM ELISA, RT-PCR, cloning, and DNA sequencing.
- The nucleotide sequence of a 243 bp VP3/VP1 fragment amplified by RT-PCR was determined for recombinant clones containing nucleotide sequences derived from the HAV infected samples.
- Compared with the corresponding sequences reported by Cohen (J. Virol. 61: 50-59, 1987), nucleotide homologies of 93.8%–96.7% and amino acid identities of 98.8%–100% for the Indonesian samples and nucleotide homologies of 90.5%–94.7% and amino acid identities of 97.5%–98.8% for the Chilean samples were obtained.
- The information generated from this study will help evaluate the HAV TMA (Transcription Mediated Amplification) test currently under development between Gen-Probe Incorporated and Chiron Corporation to make a safer plasma supply.