N-terminal of the protein, respectively. *E. coli* BL21-AI harbouring constructs were induced by addition of L-Arabinose. HCVcp was purified in both native and denatured condition by NI-NTA agarose and characterised by SDS-PAGE, Immunoblotting and SELDI-TOF mass spectrometry. Antigenic and immunogenic properties of HCVcp were evaluated with HCV-infected human and immunised mice sera by ELISA respectively. Ability of particulate formation of proteins was examined by immuno-gold electron microscopy.

Results: The yields of protein expression were 25 and 16 mg/L in denatured versus 7 and 4 mg/L in native purification for N- versus C-HCVcp, respectively. N-terminal fragmented products of 9 and 11 Kd, which were not due to proteolytic activity but apparently result of ribosomal release were identified. However, these fragmented products were not purified with C-HCVcp. Diagnostic properties of natively purified proteins were predominant and still better for C-HCVcp, However N-HCVcp reacted with C-HCVcp-Immunised mice sera in lower titers. Only natively purified proteins were capable of particulate formation and assembling to generate VLPs.

Conclusion: Purification in denatured/refolding condition may not result to proper conformation of HCVcp, thereby native purification may be undertaken for any kind of applications. C-HCVcp which can be purified as a homogenous product is predominant for diagnostic and pathogenic studies while N-HCVcp that is purified as both fragmented and complete products may be used for generation of antibodies because of better presentation of linear epitopes which are mostly located on the N-terminal of HCVcp.

P1882 Hepatitis C virus genotyping: correlation between real-time PCR and probe hybridisation assays

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Background: In the US, HCV is responsible of 3.1–4.8 million people chronically infected and of 8–10 thousand deaths per year. Genetic heterogeneity of virus may account for differences in clinical outcome and response to treatment. Factors influencing treatment outcome are HCV genotype, baseline viral load, liver fibrosis and inflammation. Patients infected with HCV genotype 1 tend to have reduced response rates in comparison to patients infected with genotypes 2 or 3.

Study design: A conventional HCV genotype method (line probe hybridisation, LiPA assay) was compared with a real time PCR genotyping assay (Abbott-Celera) targeting the 5'UTR and NS5B genomic regions. In the latter method, HCV genotype is obtained by comparison of cycle threshold values obtained in three PCR reactions each containing different primer/probe combinations. Probes are labeled with FAM, VIC, or NED. In reaction 1, the HCV genome is detected by FAM, genotype 1a by VIC, 1b by NED. In reaction 2, genotype 2a by FAM, 2b by VIC, 3 by NED. In reaction 3, genotype 4 by FAM, 5 by VIC, 6 by NED. Genotypes other than those mentioned above, are detected in reaction 1 (FAM) and give an indeterminate result. Sera of chronically-infected Italian patients were investigated.

		Real time PCR									
		1a	1b	2a	2b	3	4	5	1+4	indeterminate	Tot
LiPA	1	16	2				1				19
	1a	7									7
	1b	5	26							1	32
	2			3	1						4
	2a/2c			7							7
	3a					8					8
	4						2				2
	4a						1				1
	4c/4d						6				6
	5a							1			1
	1+4								1		1
	Tot	28	28	10	1	8	10	1	1	1	88

Figure 1. HCV genotyping: agreement between results of LiPA and real-time PCR assays.

Results: 88 samples were genotyped by real-time PCR and conventional LiPA. Results are summarised in Figure 1. Of 88 samples that had been genotyped by LiPA, 58 belonged to genotype 1, 11 to genotype 2, 8 to genotype 3, 9 to genotype 4, 1 to genotype 5, 1 contained the 1 & 4 genotypes. Real-time PCR and LiPA gave concordant results in 86/88 samples (97.7%). The real-time PCR method correctly identified (at the subtype level) 56/58 samples (1a and 1b genotypes). LiPA identified the above samples as genotype 1. The correct subtype (1a or 1b) was attributed in only 39/58 cases. One case with mixed infection (1 & 4) was identified by both methods. One case attributed to genotype 1 by LiPA was given as indeterminate by real time PCR. One case was identified as genotype 4 by real-time PCR and as genotype 1 by LiPA. By sequencing the 5'UTR region it was shown to contain both genotypes 1 and 4.

Conclusions: Results of HCV genotyping by real-time PCR were in consistent agreement with LiPA results (97.7% of cases). The Abbott-Celera genotyping assay appeared to allow better discrimination of subtypes 1a and 1b ($p < 0.05$). The assay was fast and easy to perform and allowed to detect mixed infections.

P1883 Evaluation of a new combined hepatitis C antigen/antibody assay for routine HCV testing of patient samples

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Objective: To evaluate a new combined hepatitis C antigen/antibody assay (HCV Ultra, Bio-Rad) in patient samples that were borderline positive or positive with AxSYM anti-HCV EIA (version 3, Abbott) in a routine hospital setting. This test was shown to have a smaller window phase for the detection of acute HCV infection compared to anti-HCV assays that only detect antibodies.

Methods: The performance of HCV Ultra was determined in 257 sera that were borderline positive (S/CO = 0.8–1.0) or positive (S/CO > 1.0) on AxSYM. The group of positive sera consisted of 82 of the 2408 in-house sera tested over a 2.5 month period and 175 sera referred for confirmatory testing. We also tested 18 sera that were negative on AxSYM. All sera were tested with Monolisa Plus Anti-HCV EIA (version 2, Bio-Rad). Sera that were AxSYM S/CO > 1.0 and Monolisa S/CO > 3 were considered positive. Otherwise immunoblot analysis was performed with INNO-LIA HCV Score (Innogenetics). When INNO-LIA did not allow a conclusion (indeterminate), the sample was tested with PCR for the presence of HCV RNA when enough serum was available.

Results: All 118 sera that were positive with both AxSYM and Monolisa were positive with HCV Ultra. The results of the 111 other sera that were not positive with both AxSYM and Monolisa are shown in table 1. A significant number of sera were undetermined with INNO-LIA (13.5%). These 15 sera were excluded for the calculation of the performance of HCV Ultra. In 13 of these samples, HCV Ultra gave a correct result. The sensitivity and specificity of HCV Ultra on AxSYM borderline and positive sera was 99% and 95%, respectively. The 18 sera that were negative on AxSYM were also negative on Monolisa and Ultra. All sera that were HCV Ultra S/CO ⩾2.5 were from HCV-positive patients. The positive predictive value for in-house samples was 0% for AxSYM borderline positive sera and 54% for AxSYM positive sera. The only serum that was negative with HCV Ultra and positive with INNO-LIA was from a patient with normal liver enzymes and was negative with PCR. The 4 sera that were positive with HCV Ultra and negative with INNO-LIA could be from seroconverters as was confirmed by PCR in the only patient from which serum was available.

Table 1. Results in samples that were positive with AxSYM and Monolisa Plus

HCV Ultra\INNO-LIA	Negative	Positive	Undetermined
Negative	78	1	12
Positive	4	13	3

Conclusion: The performance of HCV Ultra in sera that were AxSYM borderline or positive was excellent. Confirmation testing with INNO-LIA has little or no added value in sera tested with HCV Ultra.