concurrent with the attempt to adapt to and overcome the lowered oxygen tension. Of further interest is that no significant difference in circulating fetal DNA concentrations was discernable between ethnic Tibetans and recent migrant Han Chinese. This finding is rather unexpected because previous studies have supported the hypothesis that ethnic populations, such as Tibetans who have lived for almost 5 millennia at high altitude, have adapted to low oxygen concentrations and consequently have better pregnancy outcomes than do newly arrived migrant populations (16). We did not observe any influence of ethnicity concerning the release of fetal DNA, which suggests that the underlying placental alterations are similar in both groups. What does seem likely, however, is that residents who have lived for extended periods at high altitudes have developed optimal strategies for adapting their placental tissues to the underlying deficiencies to ensure an optimal pregnancy outcome (16). This may be reflected in the increased occurrence of intrauterine growth restriction in Han Chinese at high altitude, in contrast to native Tibetans, a trend we also observed in our study. i.e., babies born to Han Chinese women living at high altitude were smaller than those born to Han Chinese women living at sea level. We also observed some evidence of a further reduction in fetal weight for babies born to Han Chinese women with preeclampsia at high altitude, whereas no such difference was apparent in comparable Tibetan study groups. These findings will, however, need to be confirmed in larger studies.

In summary, analysis of circulating fetal DNA may not only be useful for the noninvasive prenatal assessment of fetal genetic traits; in the future, it may also be a unique tool for the study of anomalous placentation. In this context it will be interesting to determine whether circulating fetal RNA concentrations behave in a similar manner in pregnancies at high altitude.

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Genotyping of Hepatitis C Virus by Melting Curve Analysis: Analytical Characteristics and Performance, Doris M. Haverstick,^{*} Grant C. Bullock, and David E. Bruns (Department of Pathology, University of Virginia, Charlottesville, VA; * address correspondence to this author at: Department of Pathology, University of Virginia Medical School, PO Box 800214, Charlottesville, VA 22908-0214; fax 434-924-8060, e-mail dmh2t@virginia.edu)

Knowledge of the hepatitis C virus (HCV) genotype is important in guiding antiviral therapy (1). Viral pharmacogenomic studies have demonstrated that patients infected with genotype-1 HCV respond poorly to interferon-ribavirin therapy and may require a longer course of therapy (1–3). In the United States, where the most common HCV types are 1a/b, 2a/c, 2b, and 3a, it is particularly important to distinguish patients infected with type 1a/b from those infected with types 2 and 3. To meet the need for this information, we have developed a rapid-cycle, real-time PCR assay with melting-curve analysis for genotyping of HCV (4). This method uses reverse transcription-PCR performed in a block cycler followed by a seminested PCR with product identification using fluorescence resonance energy transfer (FRET) probes and DNA melting curves in a single tube. The FRET probes were designed to identify HCV types 1, 2a/c, 2b, 3a, and 3b/4. Other less common genotypes will likely either not be amplified (types 6b, 7b, and 11a) or will produce a product with a non-type 1 melting temperature (T_m) (4).

Real-time PCR has gained widespread use in clinical analyses since its introduction in 1991 (5), but little has been published on the performance characteristics of such assays over periods longer than a few days or weeks. The objective of the present study was to determine the analytical characteristics of the above HCV genotyping assay and its performance in routine use. The study period covered 23 months with 92 runs performed by six operators on four different LightCycler[®] instruments, using the exact assay described above (4).

Patient samples were analyzed in groups of 5 to 13. Each analytical run also contained three quality-control (QC) samples: serum negative for HCV, pooled serum previously analyzed and found to be type 1 HCV, and one of the following serum pools: type 2a/c, type 2b, or type 3a HCV. The $T_{\rm m}$ s of QC samples met our established criteria for acceptability in all 92 runs. Of the 184 QC samples, there was 1 amplification failure. For the QC samples that amplified, the $T_{\rm m}$ s segregated into discrete, nonoverlapping, type-specific intervals (Fig. 1A and Table 1). The within-type $T_{\rm m}$ range was ≤ 2 °C for all types (type 1, 1.7 °C; type 2a/c, 1.8 °C; type 2b, 1.6 °C; type 3a, 1.4 °C), giving a CV <1.0% for each type (type 1, 0.53%; type 2a/c, 0.66%; type 2b, 0.62%; type 3a, 0.66%).

Patient results segregated into discrete, nonoverlapping intervals that were indistinguishable from those for the QC samples (Fig. 1B and Table 1). In addition, specimens from patients infected with HCV genotype 3b/4 produced a discrete, nonoverlapping T_m interval. Genotype 3b/4 QC material was not analyzed routinely because little serum was available to make a pool. As with the QC pools, the range of patient T_m s was $\leq 2 \,^{\circ}$ C within an individual genotype (type 1, 2.0 °C; type 2a/c, 1.4 °C; type 2b, 1.0 °C; type 3a, 0.9 °C; type 3b/4, 0.8 °C); for all types, the CV of T_m s was $\leq 1.0\%$ (range, 0.49–0.75%).

Among the 532 individual patient samples analyzed, genotyping was successful in 517 (>97%). There were 15 failures. One sample produced a $T_{\rm m}$ of 45.3 °C, which was below that of any other specimen analyzed; there was insufficient sample to repeat the melting curve analysis or obtain sequence information. Fourteen samples were reported as "none detected". Five of these samples had viral loads <10 000 IU/mL, which is below the lower limit for the assay (4). The most recent of these samples was successfully genotyped after fivefold centrifugal concentration (27 000g for 60 min) of the specimen. The remaining nine specimens had little or no clinical information available to confirm HCV infection of sufficiently high viral load for genotyping. Neither anti-HCV antibody status nor HCV quantification results were available for these specimens.

Six samples had co-infections (types 1 and 2a/c in two patients, types 1 and 2b in two patients, types 2a/c and 3b/4 in one patient, and types 2a/c and 2b in one patient); an example is shown in Fig. 1C. For the remaining 514 patient samples, the distribution of types was consistent with expected frequencies in the United States: 397 type 1, 26 type 2a/c, 39 type 2b, 47 type 3a, and 5 type 3b/4.

Because genotypes 3b and 4 are indistinguishable by our FRET sensor probe [see Table 1 of the online Data Supplemental from Bullock et al. (4)], samples that were genotyped as 3b/4 were sent for confirmatory genotyping by sequencing. Two of the six 3b/4 samples were confirmed as type 4 by sequencing. Two others were sequenced and found to be "aberrant" types; that is, sequence data did not allow precise assignment of a genotype. The remaining two of the six samples did not contain enough material for confirmation and were reported as type 3b/4. We have had no confirmed cases of HCV genotype 3b at our institution; we therefore have been unable to validate the performance of our assay by



Fig. 1. $T_{\rm m}$ s and first-derivative melting curves.

(A), T_m s for QC samples (n = 183); numbers indicate the mean temperature for each genotype. (*B*), T_m s for patient samples (n = 524); numbers indicate the mean melting temperature for each genotype. (*C*), first-derivative melting curves for serum from a patient co-infected with types 1 and 2a/c HCV (\square), serum from a patient with type 3a HCV (\blacktriangle), QC serum pools for types 1 and 2b (dashed lines), and a negative serum control (solid line).

use of a type 3b specimen or to compare type 4 with type 3b samples. We continue to verify the rare cases of type 3b/4.

During the earlier study (4), the current procedure was compared with the INNO-LiPa "line-probe" assay

Table 1. T _m values for HCV genotypes. ^a				
		<i>T</i> _m , °C		
Genotype	Sample type (n)	Mean (minimum–maximum)	SD	95% confidence interval
1	QC (92)	64.2 (63.2–64.9)	0.34	64.1-64.3
	Patients (399)	64.2 (63.0-65.0)	0.31	64.2-64.2
2a/c	QC (31)	59.5 (58.3–60.1)	0.39	59.3–59.6
	Patients (30)	59.5 (58.6–60.0)	0.34	59.4-59.7
2b	QC (30)	52.4 (51.4–53.0)	0.33	52.3–52.6
	Patients (42)	52.5 (52.1–53.1)	0.25	52.4–52.6
3a	QC (30)	50.3 (49.5–50.9)	0.33	50.1-50.4
	Patients (47)	50.4 (49.2–51.1)	0.38	50.3-50.5
3b/4	Patients (6)	54.6 (53.9–55.1)	0.41	Insufficient data
^a Method of analysis as in Bullock et al. (4).				

(Bayer). To expand the information on rarer genotypes and to gather comparison data with a different method, the first 10 patient samples in the current study that were identified as genotype 2 (n = 9) or 3b/4 (n = 1) were genotyped by DupliType sequencing (Quest Diagnostics). In each case, the sequence data confirmed the genotype determined by the real-time PCR assay.

With the data generated during this study, it is now possible to calculate 95% confidence intervals for $T_{\rm m}$ determinations for genotypes 1, 2a/c, 2b, and 3a; additional data are needed for genotype 3b/4. The 95% confidence intervals (Table 1) demonstrate that the QC and patient samples for a given genotype are nearly identical, indicating that the sequence target of the FRET sensor probe is homogeneous within a given genotype and, moreover, does not change with storage of QC material at -20 °C. The type-specific 95% confidence intervals are completely nonoverlapping, allowing the confident assignment of genotype when the sample $T_{\rm m}$ falls within one of these intervals.

For the large majority of samples received during this study period, the proposed method allowed rapid screening and identification of the common genotypes seen in the United States and in much of Europe. Among 517 samples, only 7 required further testing, 6 because of genotyping as 3b/4 and 1 because of a T_m outside of the temperature intervals for T_m s that we have defined for each genotype or subtype. Operationally, this procedure has been integrated smoothly into our clinical laboratory. Extraction and reverse transcription-PCR set-up generally occur in the afternoon of day 1 (technologist hands-on times of ~20 and 15 min, respectively) with real-time PCR and data analysis occurring in the morning of day 2 (25 and 15 min of technologist time, respectively). Results are available to the clinicians by midmorning.

Taken together, the data provided in this study and the results published previously (4) confirm that real-time PCR with melting curve analysis using a single set of FRET probes is an accurate, precise, and robust approach to HCV genotyping for the majority of samples received by our laboratory. It easily distinguishes HCV genotype 1 infection from non-type 1 infections, using a fast and

relatively inexpensive format that has been reliable over time.

A preliminary report on this study was presented at the 2004 ACLPS meeting of the Academy of Clinical Laboratory Physicians and Scientists in Denver, CO (June 2004).

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ELISA for Specific Detection of PiZ-Related α_1 -Antitrypsin Deficiency, *Sten Gershagen*^{1*} and Sabina Jan*ciauskiene*² (¹ Wieslab AB, Ideon, Science Park, Lund, Sweden; ² Department of Medicine, University Hospital Malmö, Malmö, Sweden; * address correspondence to this author at: Wieslab AB, Ideon, Science Park, Sölvegatan 41, 22370 Lund, Sweden; fax 46-46-14-08-90, e-mail sg@ wieslab.se)

 α_1 -Antitrypsin (AAT) deficiency is a hereditary autosomal disorder resulting from a variety of mutations in the AAT gene. The most common severe deficiency variant of AAT is Z, which has been identified in most populations but occurs most frequently in northwest Europe. The frequency of the Z allele in the US population of European descent is between 0.01 and 0.02, with the homozygous deficiency affecting 1 in every 2000 to 7000 individuals. In Scandinavia, the frequency of the Z allele is considerably higher: at birth, 1 of every 1600 babies is homozygous for the Z allele. Individuals homozygous for the AAT Z allele have a high risk for developing early-onset pulmonary emphysema and/or abnormal liver function in infancy that may lead to complete liver failure. The Z allele is also suspected in patients with Wegener granulomatosis and panniculitis. Here we briefly describe a simple and accurate new ELISA-based test for identifying carriers of the AAT Z allele.

AAT is the main circulating and tissue serine protease inhibitor in humans (1). The mean concentration of AAT in serum or plasma in healthy individuals is estimated to be 1.3-1.7 g/L, with a half-life of 3-5 days (2, 3). Circu