had Hb Hoshida (β43Glu→Gln). All 4 patients with abnormal peaks at the S window on HPLC (mean retention time, 0.91 min) had Hb Queens (α1 34Leu→Arg). All 10 patients with variant peaks were heterozygous, and they had abnormal bands within the Hb S/Hb G/Hb D area on cellulose acetate Hb electrophoresis.

Hb G Coushatta has been found in Koreans, Chinese, and in some Japanese families *(6)*. This variant usually leads to underestimation of Hb A_{1c}, as we noted in our patients. The abnormal peak between Hb A_{1c} and Hb A_0 seemed to be that of glycated Hb G Coushatta, as Ogawa et al. *(7)* have indicated, and Hb G Coushatta is thought to coelute with the normal Hb A_0 peak. Hb Hoshida has been reported in a few Japanese families and in 1 Yugoslavian family, and Hb Queens has been found in Koreans, Chinese, Japanese, and Vietnamese *(6)*.

We found increased Hb F concentrations in 15 patients. Gene dosage analysis revealed that the ratios of the β-globin gene to the albumin gene were ~0.5 in 2 patients, which suggested heterozygous deletion of the β-globin gene. One of the 2 patients had no phenotypic abnormality other than the increased Hb F (19.5%), suggesting deletional hereditary persistence of fetal Hb. The other patient had a history of chronic microcytic hypochromic anemia. The increased Hb A_2 concentration, the decreased osmolality fragility, and the typical findings on the peripheral blood smear suggested deletional β-thalassemia minor. The remaining 13 patients showed negative results for all molecular analyses on the β- and γ-globin genes. Only the *Xmn*I site sequence variation (c.−158C→T) on the promoter of the ^{G}γ-globin gene was noted in 10 patients, including 1 homozygote. This sequence variation has been shown to influence the Hb F concentrations in apparently healthy individuals *(8)*. Hereditary persistence of fetal Hb was suspected because no phenotypic or laboratory abnormalities other than the increased Hb F concentrations were seen.

In conclusion, the incidences of Hb^{var} and high Hb F concentrations were estimated to be 1 in 2700 and 1 in 1800, respectively. The most common Hb^{var} in Koreans were Hb G Coushatta and Hb Queens, which could be presumed from their characteristic HPLC patterns. The known sequence variations in the β-, ^{G}γ-, or ^{A}γ-globin genes that cause high Hb F are rare in Koreans. Considering that Hb^{var} and high Hb F concentrations are not uncommon, more effort should be made to estimate the correct Hb A_{1c} value in Korean patients with diabetes.

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Preparation of a Chimeric Armored RNA as a Versatile Calibrator for Multiple Virus Assays

To the Editor:

As with all diagnostic techniques, molecular testing requires careful quality control *(1–3)*. In detection of RNA viruses, which are often present at low concentrations and are prone to degradation, stringent monitoring is needed for all aspects of assay performance, including virus lysis, RNA isolation, reverse transcription, amplification, and detection steps. Among many proposed RNA control preparations *(4, 5)*, armored RNA is currently the most suitable for clinical applications as it carries the viral RNA target of interest in a form that is ribonuclease-resistant, noninfectious, and stable after prolonged incubation in clinical matrices, and the preparations are substantially less expensive to manufacture than virus-infected plasma *(6–8)*. Thus, armored RNA has been applied as a positive control for a variety of RNA viruses *(9)*.

Because most commercial armored RNA preparations contain exogenous sequences of <500 nucleotides *(9)*, separate armored RNA species are often prepared for calibration of each target in multiple virus assays. To reduce costs and simplify multivirus detection, we are seeking to produce a single chimeric armored RNA species that might be used as a positive control for multiple viral targets. We consider this task to be feasible because the inventors of armored RNA predicted that, theoretically, at least 2 kb of nonbacteriophage RNA sequence might be encapsulated *(8)*. As proof of this

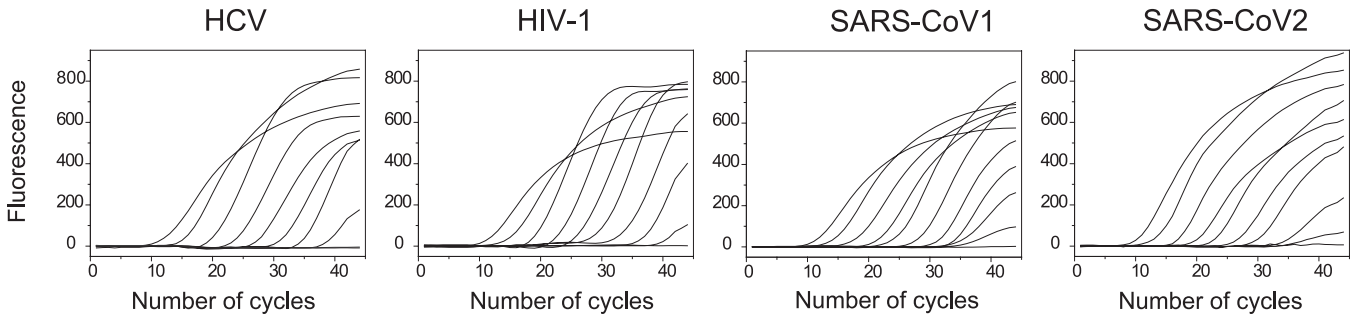

Fig. 1. Calibration of the real-time RT-PCR assay for HCV, HIV-1, SARS-CoV1, and SARS-CoV2.

We diluted purified and calibrated armored RNA with pooled normal human plasma supplemented with 1 g/L sodium azide and prepared 200-μL aliquots by 10-fold serial dilution to obtain samples containing 10^{10} to 10^{1} copies. From these materials, we isolated template RNA ranging from 10^{10} to 10^{1} copies (from *left* to *right*) for RT-PCR assays. Water was used as a negative control. All RNA templates were assayed in a single run using a diagnostic reagent set (Intec) for each individual virus. Real-time RT-PCR was conducted on an iCycler iQ thermal cycler (Bio-Rad).

principle, we tried to directly package a 1200-nucleotide–long foreign RNA sequence containing gene fragments of hepatitis C virus (HCV), HIV-1, severe acute respiratory syndrome coronavirus 1 (SARS-CoV1), and SARS-CoV2 into the original armored RNA production vector pAR-1 *(8)*.

We spliced the 4 target cDNA sequences by overlapping extension *(10)*. After cloning the 4-target chimeric sequence (see the Data Supplement that accompanies the online version of this letter at http://www.clinchem.org/content/vol52/issue7/ for the sequence information of the 4 fragments as well as the primers and probes used) into pAR-1, we used a simple but straightforward procedure to confirm the production of armored RNA and to purify it. Briefly, after induction of armored RNA production, we treated the supernatant of *Escherichia coli* cell lysate with RNase A and DNase I. On testing with agarose gel electrophoresis, if armored RNA was produced, a single DNA band of ~1.5 kb might be visible. We then cut the band from the gel and put in a dialysis bag for electroelution. Using this method, we successfully expressed and purified the chimeric armored RNA. We used a pure RNA transcript fragment of SARS-CoV2 (BNI) to calibrate the chimeric armored RNA, then used the chimeric armored RNA to prepare calibrators of the 4 real-time reverse transcription-PCR (RT-PCR) assays (Fig. 1; also see Fig. 1 in the online Data Supplement) based on displacing probes *(11)*. The linear range for each assay did not change when the calibrators were stored at 37 °C for 2 weeks, at 4 °C for 6 months, or at −20 °C for 1 year.

Our work indicates that multiple target sequences can be encapsulated into a single armored RNA species to serve as a common calibrator for detection of different RNA viruses. Chimeric armored RNA of even larger size may be prepared similarly, as indicated by our finding that by deleting some disposable sequences between the multiple cloning site and the transcription terminator, we were able to increase packaging capacity of the pAR-1 vector without affecting packaging efficiency (data not shown). Thus, the chimeric, multitarget approach for armored RNA preparation is practical and could reduce the labor and cost for quality control of multiplex RNA virus assays.

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Agreement between K-*ras* Sequence Variations Detected in Plasma and Tissue DNA in Pancreatic and Colorectal Cancer

To the Editor:

Several studies have identified DNA alterations in circulating plasma DNA of cancer patients that match genetic changes in primary tumors, but the low sensitivity obtained has limited the clinical application of plasma analysis *(1, 2)*. A recent report in *Clinical Chemistry (3)* demonstrated that the method chosen to isolate plasma DNA, a modified guanidine/Promega resin (G/R) method, could increase detection of K-*ras* sequence variations in patients with colorectal disease. Using the same approach, another group *(4)* found no relationship between these isolation methods, nor between K-*ras* sequence variations found in DNA from plasma and tumor tissue in patients with non-small cell lung cancer. In a previous study *(5)*, we found that plasma K-*ras* analysis was a highly specific, low-sensitivity approach with prognostic significance in pancreatic carcinoma.

To evaluate the agreement rate between K-*ras* sequence variations in plasma DNA and corresponding tissue, we used the Qiagen method to isolate DNA from 112 plasma samples from patients with pancreatic disease and from 87 plasma samples from patients with colorectal disease. We also isolated DNA from corresponding pancreatic cytology samples and colorectal tissues. The restriction fragment length polymorphism–PCR method used to detect K-*ras* sequence variations has been described previously *(6)*. The concordant results between plasma and tissue are shown in Table 1.

In the patients with pancreatic adenocarcinoma, sensitivity for detecting K-*ras* sequence variations was 43% (19 of 44) in plasma samples and 87% (39 of 45) in fine-needle aspirate or pancreatic juice samples. No sequence variations were detected in plasma DNA from patients with chronic pancreatitis, acute pancreatitis, or other pancreatic neoplasms, giving a specificity of 100%. The agreement rate in pancreatic samples was 78% (19 positive and 67 negative; total, 86 of 110). Single-strand conformation polymorphism (SSCP) analysis allowed characterization of 11 of 19 positive plasma samples, and the spectrum was 8 GAT and 3 GTT. Concordant SSCP results were obtained in plasma and cytology samples. In the colorectal adenocarcinoma group, sensitivity for detecting K-*ras* sequence variations was 8.5% (7 of 82) in plasma samples and 41% (34 of 82) in resected tissue samples. In colorectal adenomas and diverticulosis, no variant sequences were detected in plasma or tissue. The agreement rate in colorectal plasma and tissue samples was 69% (7 positive and 53 negative; total, 60 of 87). Characterization was possible in 5 of 7 positive plasma samples (3 GAT, 1 GTT, and 1 CGT) and was concordant with results in tissue.

In addition, we performed our routine Qiagen assay method in parallel with the G/R method in a subset of 12 plasma samples (6 pancreatic and 6 colorectal adenocarcinomas) from the evaluated group (3 with the K-*ras* variant in each group). With the G/R

Table 1. Agreement rate between plasma and pancreatic or colorectal tissue.

		K-*ras* mutational analysis[a]		
Diagnosis	n	Plasma	Pancreatic (FNA[b] or PJ) or colorectal tissue	
Pancreatic adenocarcinomas	45	19 (+)		19 (+)
		25 (−)	5 (−)	20 (+)
		1 (NA)[c]	1 (−)	
Other pancreatic neoplasms[d]	8	8 (−)	8 (−)	
Chronic pancreatitis	53	52 (−)	48 (−)	4 (+)
		1 (NA)[c]	1 (−)	
Acute pancreatitis	6	6 (−)	6 (−)	
Colorectal adenocarcinomas	82	7 (+)		7 (+)
		75 (−)	48 (−)	27 (+)
Colorectal adenomas	3	3 (−)	3 (−)	
Diverticulosis	2	2 (−)	2 (−)	

[a] (+), DNA sample contains detectable codon 12 K-*ras* mutation; (−), DNA sample does not contain detectable codon 12 K-*ras* mutation.
[b] FNA, fine needle aspirate; PJ, pancreatic juice.
[c] NA, nonamplified samples (in 2 plasma pancreatic samples amplification was not possible).
[d] Including 3 cholangiocarcinomas, 3 pancreatic metastases, and 2 neuroendocrine tumors.