cies of p53 variants at codon 72 are not different in Caucasian women in Sweden, the United Kingdom, and Austria.

Our results show that there is no difference in the allelic frequencies of the p53 gene at codon 72 between cervical carcinoma patients and the control group. Because we did not determine HPV status in our study group, we cannot directly compare our results with the data of Storey et al. (14). Storey et al. reported that individuals homozygous for the arginine form at codon 72 are approximately sevenfold more susceptible to HPV-associated cervical cancer than heterozygotes. However, our data are in accordance with the results of two other studies (15,16), one of which included only cases that were positive for HPV types 16 and 18. Both studies reported a lack of correlation between polymorphism at codon 72 of p53 and risk of cervical cancer. It cannot be ruled out that such a correlation exists in ethnic groups different from the ones investigated in these studies and ours because a significant difference for the prevalence of arginine homozygotes among various ethnic groups has been reported (6). Therefore, more epidemiological studies that include patients with different ethnic backgrounds should be undertaken. Detection of this p53 polymorphism with PCR and allele-specific hybridization on microtiter plates is amenable to automation and thus can be very useful for this purpose.

This study was supported by the Anniversary Fund of the Austrian National Bank for the Promotion of Scientific Research and Teaching (Project ÖNB 6054). We thank Andrea Wolf and Sabrina Zeillinger for isolating the DNA used in this study.

References


Recoveries of Phenylalanine from Two Sets of Dried-Blood-Spot Reference Materials: Prediction from Hematocrit, Spot Volume, and Paper Matrix, Barbara W. Adam, J. Richard Alexander, S. Jay Smith, Donald H. Chace, J. Gerard Loeber, L. H. Elvers, and W. Harry Hannon. (1) Centers for Disease Control and Prevention, Newborn Screening Quality Assurance Program, MS-19, 4770 Buford Hwy., Atlanta, GA 30341-3724; (2) NeoGen Screening, Inc., 110 Roessler Rd., Pittsburgh, PA 15220; (3) Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands; * author for correspondence: fax 770-488-4831, e-mail bwa1@cdc.gov)

Dried blood spots (DBSs) are used to screen newborns for phenylketonuria and other aminoacidopathies. The calibrators for this testing are usually DBSs with values for Phe. Two DBS reference materials have been prepared, the European Working Standard for Phe (EWS-Phe-01) (1) and the amino acid reference material (AARM) from the CDC (2). The two reference materials are not interchangeable because they differ in blood hematocrit, blood-spot size, and filter paper, each of which (3–6) affects analyte recovery. We measured quantitatively the effects of these differences on analyte recovery from DBSs and used results from our measurements to predict expected
Phe recoveries from tandem analyses of the two sets of materials.

In EWS-Phe-01 (1, 7), human blood with a 50.5% hematocrit and intact red cells was divided into five portions for enrichment with 0, 20, 40, 80, and 120 mg Phe/L blood (0, 120, 240, 480, and 720 μmol/L blood). The liquid added during enrichment (7) was sufficient to reduce the hematocrit to 50.1%. The Phe-enriched blood portions were dispensed in 35-μL aliquots (7) onto Schleicher & Schuell (S&S) Grade 2992 (lot no. 121576) filter paper (1).

The AARM was prepared (2) by dividing human blood with a 57% hematocrit and intact red cells into six portions for enrichment with pure amino acids to cover the usual analytic ranges of Phe, Tyr, Leu, Met, and Val. The Phe enrichments were 0, 40, 80, 120, 160, and 200 mg Phe/L blood (0, 240, 480, 720, 960, and 1200 μmol/L blood). The liquid added during enrichment was sufficient to reduce the hematocrit to 53%. The whole-blood pools were dispensed in 100-μL portions onto S&S Grade 903 (lot no. W941) filter paper with dashed-line 13-mm printed circles (2).

To examine the effect of blood hematocrit on Phe recovery, we prepared whole-blood portions with 40%, 45%, 50%, 55%, 60%, 65%, and 70% hematocrits from a single batch of packed erythrocytes and a single batch of clarified serum. We enriched the hematocrit-adjusted blood portions with 125I-labeled thyroxine, dispensed 25 100-μL aliquots of each portion onto S&S Grade 903 (lot no. W961) filter paper, and punched ~3 mm (1/8-inch) disks from the north, east, south, west, and center of each dried spot to determine the mean blood absorption volume per disk (8) at each hematocrit. We performed a simple linear regression analysis of blood volumes per disk vs hematocrit and used the resulting regression line slope to predict the blood volume of a ~3-mm disk from blood with hematocrits of 53% (the calculated hematocrit of the AARM) and 50.1% (the calculated hematocrit of the EWS-Phe-01).

The EWS-Phe-01 materials were dispensed in 35-μL spots (spot size range, 33–40 μL); the AARMs were dispensed in 100-μL (± 0.24%) spots. To compare Phe recoveries from spot volumes representative of the two sets of materials, we used a single batch of whole blood, adjusted to 55% hematocrit and enriched with 80 mg Phe/L blood, to dispense 35- and 100-μL blood volumes onto S&S Grade 903 (lot no. W941) filter paper. We punched an ~6 mm (1/4-inch) disk from the center of 20 dried spots of each blood volume and measured the Phe concentrations of all of the punched disks in a single HPLC run that was performed according to previously described protocols (2, 9, 10).

To compare the blood absorption characteristics of the S&S filter papers that were used to prepare the EWS-Phe-01 and AARM, we dispensed 100-μL spots of 125I-thyroxine-enriched blood with a 55% hematocrit onto clean, unprinted areas of 10 cards taken from each set of materials. We punched ~3-mm disks from the north, east, south, west, and center of one spot per card for gamma counting in a single analytic run and used a standardized method (8) to equate γ counts to the serum volume contained in each DBS disk. We used statistical analyses of the counting data to determine the mean serum-absorption volume per disk for each paper.

We used analyte recoveries computed from our examinations of hematocrit effects, blood-volume-per-spot effects, and filter-paper serum-absorption volumes to predict expected differences between Phe recoveries from the EWS-Phe-01 and the AARM. To evaluate the reliability of the Phe concentration values that were predicted from measurements of matrix variables, we analyzed the EWS-Phe-01 and the AARM in duplicate in each of five HPLC runs and compared the mean values of the measured Phe concentrations with the predicted Phe concentrations. We also compared regression slopes, derived from measured vs enriched Phe concentrations of each set of DBS materials, to show the ratio of their measured Phe recoveries.

Studies of hematocrit effects showed that the blood volume per ~3-mm disk was positively correlated with the hematocrit. The slope of the regression line, derived from measured blood volume per disk vs hematocrit, predicted that the EWS-Phe-01 materials, with a calculated hematocrit of 50.1%, should have a blood volume of 3.1 μL per ~3-mm disk, whereas the AARM, with a 53% calculated hematocrit, should have a blood volume of 3.2 μL per disk.

In studies of the effect of blood volume per spot on analyte recovery, the total Phe concentration of the blood used was equal to the endogenous Phe, which was not measured, plus the Phe enrichment. The mean of recovered Phe concentrations from the 35-μL spots (85.1 ± 4.7 mg/L blood) was lower than that from the 100-μL spots (95.5 ± 9.1 mg/L blood; P < 0.01, Student t-test).

The mean serum-absorption volume per ~3-mm disk punched from the S&S Grade 2992 paper used to prepare the EWS-Phe-01 was 1.250 ± 0.014 μL, whereas that from the S&S Grade 903 paper used to prepare the AARM was 1.502 ± 0.188 μL (P < 0.01).

The controlled comparisons of hematocrits, blood volumes per spot, and filter-paper serum-absorption volumes showed, in all cases, that analyte recovery per DBS disk was lower for the conditions used in the preparation of EWS-Phe-01 than from those used in the preparation of AARM. By summing these observed differences, we projected (Table 1) that analyte recovery from EWS-Phe-01 and AARM could be expected to differ by 30.8% when the two sets of materials were analyzed in tandem. By comparing the regression slopes derived from HPLC-measured vs enriched Phe concentrations of each set of DBS materials, we found that observed Phe recoveries from EWS-Phe-01 (regression slope, 0.698) were 31.6% lower than those from AARM (regression slope, 1.020) when the two sets of reference materials were analyzed in tandem against the same set of calibrators. Our HPLC calibration and data-reduction protocol was that provided by the manufacturer to newborn-screening laboratories in the United States. This protocol yields ~100% Phe recovery from 100-μL spots of intact-cell blood dispensed onto S&S Grade 903 filter paper; therefore, Phe recoveries from the
AARM were expected to equal the AARM target values (2). Conversely, in an analytic system with calibration and data-reduction protocols appropriate for European newborn-screening samples, Phe recoveries from the EWS-Phe-01 would be expected to equal the EWS-Phe-01 target values (1).

The effects of the differences in the blood hematocrit, blood volume per spot, and filter-paper sources used to prepare the EWS-Phe-01 and AARM yielded analytic recovery differences of 3.1%, 10.9%, and 16.8%, respectively. These relationships between blood-spot preparation variables and analytic recovery illustrate the importance of (a) preparing reference materials for newborn-screening tests from blood with a hematocrit typical of newborns and in spot sizes similar to those of the newborn-screening specimens and on the same filter paper grade and lot number used for collecting the newborn-screening samples, and (b) considering the blood-spot preparation variables when comparing different sets of blood-spot reference materials.

The EWS-Phe-01 and AARM were prepared from blood with hematocrits typical of newborns and with filter papers and blood-spot sizes that reflect newborn-screening practices in the regions in which they are used. Because of the observed matrix-related differences in analyte recoveries, we predicted that Phe recoveries from tandem analyses of the EWS-Phe-01 and AARM would be 30.8% higher from the AARM. In fact, measured Phe concentrations from the AARM were 31.6% higher than those from the EWS-Phe-01 when the materials were analyzed in tandem. We conclude that controlled measurements of blood-spot preparation variables can be used to reliably predict analyte recoveries from DBS materials, and we have shown that the Phe contents of the EWS-Phe-01 and AARM are concordant when the effects of their preparation variables are normalized; thus we verified the suitability of the materials as calibrators for their respective regions.

The effects of blood-spot preparation variables are not limited to phenylketonuria tests or to newborn screening. Investigators who use DBSs must be aware of the variables that affect blood-spot test results. Knowledge of the relationship between reference materials and DBS test samples and awareness of the relationships among different sets of reference materials are essential for evaluating test results, comparing data among laboratories, and evaluating laboratory performance in different areas of the world.

### References


