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Two Assays for Urinary *N*-Acetyl-β-D-glucosaminidase Compared, *Pamela L. Drake,[1*] Edward Krieg,[2] Alexander W. Teass,[2] and Val Vallyathan[3]* ([1] National Institute for Occupational Safety & Health, Spokane Research Laboratory, 315 East Montgomery Ave., Spokane, WA 99207; [2] National Institute for Occupational Safety & Health, Division of Applied Research & Technology, 4676 Columbia Pkwy., Cincinnati, OH 45226; [3] National Institute for Occupational Safety & Health, Health Effects Research Laboratory, 1095 Willowdale Rd., Morgantown, WV 26505; * author for correspondence: fax 509-354-8099, e-mail pdrake@cdc.gov)

In the course of investigating the exposure of gold miners and other workers to mercury, investigators at the National Institute for Occupational Safety & Health (NIOSH) measured urine *N*-acetyl-β-D-glucosaminidase (NAG; EC 3.2.1.52) activity to monitor for renal injury *(1–3)*. Numerous methods are available for the urinalysis of NAG activity *(4)*. Most of the analyses for the NIOSH studies were performed by Pacific Toxicology Laboratories, using the Kamiya Biomedical Company reagent set, which was based on NAG-catalyzed hydrolysis of 6-methyl-2-pyridyl-*N*-acetyl-1-thio-β-D-glucosaminide to 6-methyl-2-pyridinethiol. However, while planning our most recent investigational visit to a gold mine, we learned that the Kamiya Biomedical reagent set was temporarily out of stock. Consequently, we decided to perform the analyses in-house using the reagent set manufactured by Boehringer Mannheim Biochemica, which we had used previously for the determination of NAG in rats. This reagent set is based on NAG-catalyzed hydrolysis of the sodium salt of 3-cresolsulfonphthaleinyl-*N*-acetyl-β-D-glucosaminide to give 3-cresol purple (3-cresolsulfonphthalein, sodium salt). Because the two assays are based on the hydrolysis of different substrates, and in light of the differences reported among four NAG assays (including the 3-cresol purple) *(5)*, we suspected that the 3-cresol purple assay might not give NAG activity results equivalent to those obtained by the 6-methyl-2-pyridinethiol assay used in previous investigations. To obtain data with which to compare the two assays, we applied both assays to 72 urine specimens.

The 6-methyl-2-pyridinethiol assay was performed with the K-Assay for NAG activity (cat. no. KAE-002) from Kamiya Biomedical Company adapted to the Cobas Fara II analyzer (Roche Diagnostic Systems) as a kinetic assay. The instructions supplied with the reagent set were followed, yielding a final solution at 37 °C in which the sample was at a 1:13 (1 part sample to 12 parts reagent) dilution and the final reagent concentrations were 123 mmol/L citrate buffer (original pH 4.5) and 4.6 mmol/L substrate. The increase in liberated 6-methyl-2-pyridinethiol was measured at 340 nm over forty-five 10-s intervals, and the rate of reaction was converted to NAG activity. The procedure was calibrated at 36 U/L (K-Assay NAG Calibrator KAE-003C) and monitored using a 13 U/L control material (K-Assay NAG Control KAE-004C), all prepared by reconstituting lyophilized bovine normal kidney preparations with distilled water. The mean results for the two control samples were 104% of the nominal concentrations.

The 3-cresol purple assay *(6)* was performed with the NAG assay from Boehringer Mannheim Biochemica (cat. no. 875406) adapted to the Cobas Fara II analyzer as an end-point assay. Sample was added to the reaction medium (original pH 5.14) at 37 °C, to obtain a 1:7 dilution (1 part sample to 6 parts reagent) of the sample and a final concentration of 36 mmol/L citrate buffer, 2.0 mmol/L substrate, and 2.2 mmol/L borax. After incubation for 15 min, the reaction was stopped by the addition of aqueous sodium carbonate (pH 10.86), and the absorbance of the solution at 580 nm was measured over 10 min. The procedure was calibrated at 27 U/L (Boehringer Mannheim NAG Standard 982962) and monitored with a 9 U/L control material (Boehringer Mannheim NAG Control 1164368), all prepared by reconstituting lyophilized bovine normal kidney preparations with distilled water. The mean results for the two control samples were 104% of the nominal concentrations.

In addition to analyzing standards and control samples as described above, we checked the operation of the Cobas Fara II analyzer routinely and also on changing reagents or adjusting the operation of the instrument. These checks involved replicate analyses of the Roche Precision Testing Set (cat. no. 44302); the instrument was deemed in control if the CV was $<2\%$.

The performance of the two assays was tested by 20 analyses each of control samples at three concentrations. The mean measured values (CVs) at each concentration for the two assays were as follows: 6-methyl-2-pyridinethiol assay, 3U/L (4%), 14 U/L (2%), 49 U/L (1%); 3-cresol purple assay, 2U/L (3%), 10 U/L (1%), 26 U/L (1%). Using the data for the lowest concentrations, we estimated the detection limits to be 0.35 U/L for the 6-methyl-2-pyridinethiol assay and 0.16 U/L for the 3-cresol purple assay.

Urine specimens were obtained from 42 gold miners, 12 nonoccupationally exposed persons living in the vicinity of the mine, and 18 other nonoccupationally exposed persons. The protocol for the investigation was approved by the NIOSH Human Subjects Review Board, and the participants gave their informed consent. Nine of the specimens, seven from miners and two from nonoccupationally exposed persons living in the vicinity of the mine, were split and submitted as separate samples. Statistical analyses were performed using SAS® (SAS Institute). Samples from three workers gave "0" NAG activity by the 3-cresol purple assay and were excluded (the creatinine

concentrations in these three specimens were 0.39–0.68 g/L).

Simple linear regression for the 69 pairs of results gave an equation with a slope 1.07 (SE, 0.03) and an intercept 0.03 U/L (SE, 0.12 U/L; SD of the residuals, 0.68 U/L). The two assays also were compared using the Deming method *(7)*, in which the imprecision of both assays was taken into consideration. The slope of the resulting regression equation was 1.08, and the intercept was 0.01 U/L. The CVs for duplicate samples in the range 0.3–2.5 U/L were 5% for the 3-cresol purple assay (range, 0–12%; n = 8) and 12% for the 6-methyl-2-pyridinethiol assay (range, 0–28%; n = 9).

Chemists at Kamiya Biomedical performed a similar comparison of the two assays for 46 urine samples. Their simple linear regression of the 6-methyl-2-pyridinethiol assay results against the 3-cresol purple assay results yielded an equation with a slope of 1.24 (SE, 0.01) and an intercept of −0.4 U/L (SE, 0.11 U/L; SD of the residuals, 0.52 U/L).

The combined data sets were analyzed using a random coefficients model, giving the following equation for the relationship of the 6-methyl-2-pyridinethiol (MPT) assay results to the 3-cresol purple (CP) assay results:

$$\mathrm{MPT} = 1.16 \times \mathrm{CP} - 0.18\ \mathrm{U/L}$$

The data are shown in Fig. 1. Use of this equation enables the results for one assay to be estimated from the results obtained by the other, should the need exist to compare NAG activity results obtained by either of the methods. Some interlaboratory variation should be expected.

We are grateful to Joel W. Creekmore of Kamiya Biomedical Company (Seattle, WA) for arranging our special purchase of the NAG assay reagent set and to Colin E. Getty of Kamiya Biomedical Company for sharing with us assay comparison data.

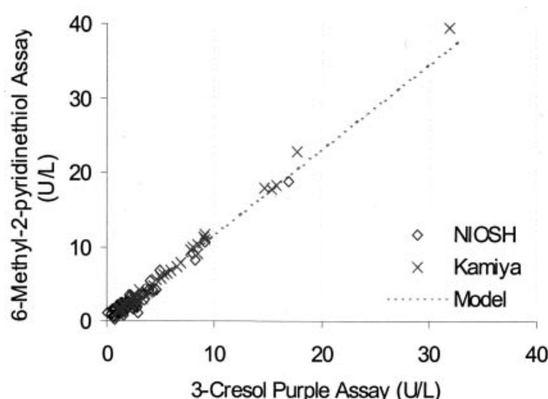

Fig. 1. Urinary NAG activity by two assays.

The range of urinary NAG activity measured in 18 healthy individuals was 0.6–8.0 U/L by the 6-methyl-2-pyridinethiol assay and 0.6–9.1 U/L by the 3-cresol purple assay.

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Single-Nucleotide Polymorphism Allele Frequencies Determined by Quantitative Kinetic Assay of Pooled DNA, *Ke Xu,* Robert H. Lipsky, Walid Mangal, Erica Ferro, and David Goldman* (Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852; * address correspondence to this author at: NIH/NIAAA/DICBR/LNG, 12420 Parklawn Dr., Park 5 Bldg., Room 451, MSC 8110, Rockville, MD 20852; fax 301-443-8579, e-mail ke@mail.nih.gov)

The identification of genes for complex traits relies on the detection of coinheritance of chromosomal regions in related individuals or the sharing of functional or marker alleles among phenotypically similar individuals. Single-nucleotide polymorphisms (SNPs) comprise the most abundant and accessible class of sequence polymorphisms in the genome and are valuable for high-throughput genotyping *(1)*. Genotyping individual DNA samples is expensive in time, reagents, labor, and DNA. These limitations can be overcome by applying a sample-pooling approach for allele frequency determination before genotyping *(2, 3)*. For quantitative allelotyping using pools, equivalent amounts of DNA from multiple individuals are combined in a single-tube assay. Most sample-pooling approaches involve post-PCR processing *(4–7)*. One pooled allelotyping method uses kinetic allele-specific PCR coupled with SYBR Green I fluorescence detection *(8)*. This method requires two separate PCR reactions for each DNA pool, each of which needs to be performed in parallel. Another pooled allelotyping method uses a bioluminometric assay coupled to a modified primer extension reaction. This method also involves multiple procedures *(9)*. Allelotyping by hybridization to DNA chips has been described *(10)*, but its quantitative accuracy has not been fully evaluated.

The 5′ nuclease assay (TaqMan®) uses fluorogenic allele-specific detection probes that allow PCR amplifica-